DELTA STATE UNIVERSITY ABRAKA, NIGERIA

CENTRE FOR DISTNACE LEARNING

General Medical Biochemistry

MBC 201

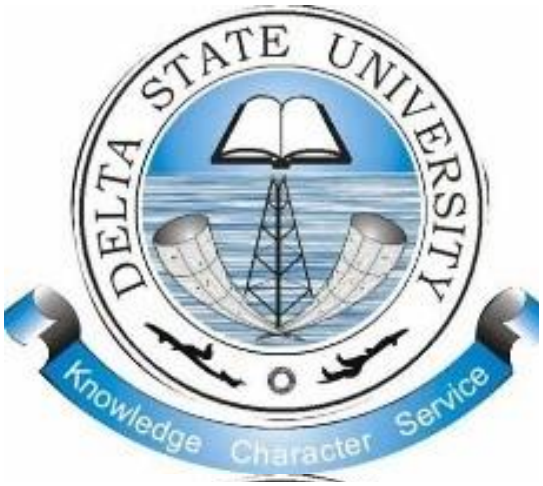
Course Material

DELTA STATE UNIVERSITY ABRAKA, NIGERIA
CENTRE FOR DISTANCE LEARNING

BACHELOR OF NURSING SCIENCE

MBC 201

INTRODUCTION TO MEDICAL BIOCHEMISTRY



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Vice Chancellor's Message

The Delta State University (DELSU), Abraka, was founded in April 1992. The dream of her founding fathers was to make the institution to not only cater for the higher education aspirations of young Nigerians, but to also ensure that in no time, the University will evolve and take her rightful place among other universities across the world. So far, despite the teething challenges confronting the world, DELSU has done well in her determination to realize her mission and vision. From the humble beginning of five faculties, DELSU has long come of age with thirteen faculties, a College of Health Sciences and a Postgraduate School. Our graduates are doing well in Nigeria and across the globe. The testimony to DELSU's sojourn so far attests to her realization of her vision as a centre for excellence in teaching, learning, research, and community service. This lofty vision has been well complemented with her mission of promotion of quality education, character and meeting the challenges of our time.

Three decades after, DELSU is once again taking on the challenge occasioned by the emergence of a New World Order by divesting from being a single mode university into a dual mode university as she is now set to run the Distance Learning system. What has confronted the university system in Nigeria in recent times is the inability of the conventional mode of learning to accommodate all qualified candidates. The Distance Learning mode has come in to fill the gap. What has now made it more compelling is the new role played by ICT in education in the world today. DELSU has taken on the challenge to provide learning opportunity for qualified candidates through the DL and also leverage on the use of ICT in learning. DELSU has thus come to harness the two factors for the good of humanity in promoting learning without borders.

DELSU is well placed to run a dual mode university in view of the phenomenal achievements we have recorded as among the best universities in Nigeria. Our array of courses, all fully accredited by the National Universities Commission, highly competent manpower, quality facilities, conducive environment and range of ICT facilities have combined to make DELSU the University of today and the future.

The course materials embedded here were authored by DELSU scholars after rigorous trainings in the technique of writing materials for DL. Remarkable efforts, quality time and rare expertise went into the production of the course materials in order for them to stand the test of global best practices. The content of the materials are lucid, interactive and up to date having been subjected to DELSU's quality assurance process. The course materials have been developed in multi-media formats which are available and accessible for use by learners.

It delightful to note that by running the DL, DELSU is offering those desirous of it an opportunity for lifelong learning. It is my pleasure to welcome our DL students to a new world of exciting learning.

Thank you.

Professor Andy Egwunyenga

Foreword

Delta State University has been committed to ensuring academic excellence in all her programmes for building knowledge, character and service among students. In consonance with this, the Centre for Distance Learning (CDL) is committed to the strive for excellence in the delivery of accessible, flexible and lifelong learning. Delta State University Centre for Distance Learning (DELSUCDL) is focused on the delivery of quality in all her academic and administrative activities. The centre is committed to providing quality learner support services to learners through provision of up-to-date information and guidance by the learner support unit.

Quality is entrenched in the admission process, development of course materials and appointment of facilitators and provision of learner support services. In consideration of the facts that learners are separated from their facilitators through space, the course materials have been developed in a manner that they are easily accessed by learners for their self-study and self-assessment at the approach of examination. In addition, the course materials are written as digestible bits that can be easily comprehended by learners.

As quality assurance measure in DELSU Distance Learning, the course materials were written by carefully selected brilliant and seasoned academics in their respective specialty and subjected to rigorous editorials by experts in English and open and distance learning to meet acceptable international standards. The course materials are learner-friendly, grouped into study sessions, with each study session having In-Text Questions (ITQs), In-Text Answers (ITAs), Self-Assessment Questions (SAQs), Self-Assessment Answers (SAAs), glossary of terms and references for learners' future study.

Learners are expected to take advantage of the worthy course materials and use them as guides in their study. In addition, learners are to source for other materials that are related and relevant to each course and use them as supplement. Some of these other course materials have been suggested by the course materials writers in each volume. Learners would find the direct Open Educational Resources (OERs) and references suggested by the course materials authors. You are advised to regularly be in touch with your e-tutors and e-facilitators assigned to you for help.

On behalf of the Vice Chancellor, Professor Andy Egwunyenga, I wish to appreciate all the course material writers and others for their contributions to the development of the course materials. To the learners, I wish you a treasurable ride as you read through the course materials and resounding progress as you navigate your academic journey.

Prof. (Mrs.) Romina I. Asiyai

Director, CDL

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COURSE GUIDE TO MBC 201:

Welcome Message

You are welcome to the course guide and the module on MBC 201: "Introduction to Medical Biochemistry". This course guide gives you the skills on how to navigate the concept of Medical biochemistry as a course of study. It is hoped you will have an interesting and educative period going through this guide and the module.

This course guide is particularly designed to help you develop the interest and relevant study skills required to successfully complete this course in order to accomplish your Degree programme. Perhaps you are in need of more clearance concerning any area of the module; do not hesitate to contact the facilitator. Wishing you success in your academic endeavours.

1.0 **INTRODUCTION**

This Course Guide is designed to provide you with details of the module. It has been divided into ten basic features to conveniently give you an understanding of the modalities in going through the module proper. A brief background to the content of the module has also been included for your basic understanding. The specific aim of the course guide is also highlighted for you while in other parts you are furnished with module outlines, general learning outcomes and study session topics to be covered during the course of studying the course. You shall as well be introduced to the learning support to be used, module delivery arrangement and expectations geared towards your preparations towards studying the module contents. However, details of the assessment techniques used in this module explaining the assessment components and their rationale and the Delta State University grading policy shall as well be explained to better equip you in going through this module.

a. Background of the Course

This course MBC 201 introduces the fundamentals of medical biochemistry which is the study of the biochemical changes that occur in the human system in terms the cell responses to diseases, the interaction by the drugs taken into the human system. It also emphasizes the biochemical functions of macromolecules in the human system.

b. Module Aims

2.0 MODULE OUTLINE

a. Learning Outcomes

On completion of this module you should be able to:

b. Study Sessions

This study guide should be read in conjunction with the Module in print, which will be provided to all learners. In addition to this module, learners are encouraged to consult additional recommended reading materials such as textbook and websites on the subject matter. The following topics are covered in the module:

Study Session 1:

Study Sessions 2:

Study Sessions 3:

Study Sessions 4:

Study Sessions 5:

Study Sessions 6:

Study Sessions 7:

Study Sessions 8:

Study Sessions 9:

Study Sessions 10:

c. Learning Supports

This course guide and the module are the relevant course materials for studying the study sessions. Each session provides you with an atlas to guide you through the module. You are also expected to attempt the online activities on the Learning Management System (LMS) as these will broaden your understanding of the individual topics. There will be an activity posted on the LMS for you at the end of every two weeks of study. You are also requested to participate in online discussions and collaborations as these will give you more opportunities to interface with your course mates to further help you in expressing yourself while gaining from the knowledge of your colleagues. Furthermore, you would benefit from the experiences and insights of your peers challenging their own perspectives and actions. Going through the textbooks referenced at the end of each study session will assist you in developing studying skills in addition to the course notes provided.

d. Module Evaluation

At the end of the session, you will be asked to provide feedback on this course guide through an online evaluation that will be sent to your email account. The gathering of such feedback is an

important part of our quality assurance and accreditation processes and we would encourage you to complete these evaluations.

3.0 MODULE DELIVERY SCHEDULE

a. Session Arrangements

The module contains ten study sessions and you are encouraged to spend at least two (2) hours on individual study session. The module is programmed for you to engage in prior preparation, to seek confirmation and clarification at appropriate periods, to practice each In-Text Questions (ITQs) and Self-Assessment Questions (SAQs) at the end of each study session and to be actively engaged during the session. The ITQs are short questions that give you immediate opportunity to assess yourself before going to study the next session. You are expected to study and be prepared for all sessions. The study sessions are divided into weekly format as will be seen on the LMS.

b. Preparation Required in Advance of Sessions

This is a 3-unit course which means that you are required to study and take all parts of the course serious. It is quite an interesting course and you can achieve great success by always participating in the online practice questions, module assignments and to have read the study sessions in advance of online facilitation. It will be of great benefit that you set out your study schedule so that you can plan your learning activities for the academic year ahead with the aim of striking a balance between study, work and family. Doing these will help you in achieving all set goals. You are expected to be fully familiar with the contents of the module at the end of each session before attempting the quiz and assignments.

c. Learner Engagement

As you study through the sessions you are expected to have a study note which will help you put down relevant points. Take part in all practice questions that will be posted on LMS. As stated earlier, do not hesitate to contact your facilitator or e-tutor should you run into any challenge. Also ensure you participate in class and online discussions in order to facilitate the formation of your critical judgments.

d. Preparatory Questions

There two types of Assessments in this module; the In-Text Questions (ITQs) and Self-Assessment Questions (SAQs). The In-Text Answers (ITAs) follow directly after the ITQs. Going through these gives you the opportunities to quickly assess yourself before moving to the next session. The ITQs come immediately after a sub-topic but you will find the SAQs at the end of each study session. Answering SAQs enable you to check your own progress in achieving the Learning Outcomes.

4.0 ASSESSMENT DETAILS

In every course undertaken there will always be a form of assessment. Assessment is to assist in evaluating the extent to which you have successfully completed in studying the content of the module. This module has three assignments with equal marks. Each will come after/during every five-week of facilitation with you.

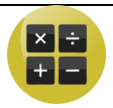
You will be exposed to other forms of assessments in form of Computer Marked Assessments (CMAs) and Tutor Marked Assessments (TMAs). Endeavour to submit all assignments via the relevant assignment upload link on the LMS which can be located under the 'Assignments' section of the course page before/ on the due date for submission. You are expected to complete all assignments ensuring that they are submitted by the specified date. Please take part in all TMAs and CMAs before the due dates. Do not wait until the last minute before participating in the assessment activities.

5.0 GRADING

Your programme is designed with courses which are weighted and classified into various levels. Courses are assigned units depending on the volume of work required to complete the course. This section is designed to acquaint you with the alphabets representing your final grade in this course. It is necessary to first recognize and be thoroughly familiar with certain ranges that are commonly used in arriving at your grade. These are defined as follows:

Grade	Percentage range	Grade point
A	(70-100%)	5 points
B	(60-69%)	4 points
C	(50-59%)	3 points
D	(45-49%)	2 points
E	(40-44%)	1 point
F	(0-39%)	0 point

6.0 List of Icons and Their Meanings

S/No	Icon	Meaning
1.		Activity
2.		Calculations
3.		Charts and Tables
4.		Experiments
5.		Group Activity
6.		IAG Information
7.		In-Text Questions
8.		In-Text Answers
9.		Introduction
10.		Learning Outcomes
11.		Summary
12.		E-Tutor

13.		Figure
14.		Key Terms

MODULE ONE

STUDY SESSION 1

INTRODUCTION TO THE STUDY OF BIOCHEMISTRY

INTRODUCTION

The scientific event that gave birth to modern biochemistry could be traced much further back to the early 17th century when scientists became more concerned in placing biological phenomena on firm chemical foundation. Such events started with the recognition of ORGANIC CHEMISTRY as a scientific field of specialization. Sequel to the recognition of organic chemistry as a distinct area of study, many scientists then, especially chemists with interest in living matters began to pursue intensified study on the isolation and characterization of various organic molecules. These studies thus, began to provide an understanding of the dynamic aspects of life.

Notable contributions to this unique field then, include the discoveries of Anthonie Lavoisier, who demonstrated the presence of Carbon and Hydrogen as the most essential and predominant elements in organic substances, through his combustion experiments done between 1771–1777. He also showed that chemical oxidation was similar to tissue respiration and Joseph Prestley, *et al*., discovered that respiration is essentially the reverse of photosynthesis. Scheele, a Swedish Chemist, during the 18th Century extracted Citric acid, Tartaric acid, Malic acid, Lactic acid and Nitric acid from lemons, grapes, apples, soured milk and urea, respectively.

However, before Organic Chemistry could sufficiently develop to serve as a tool for explaining these discoveries, some scientists have hurriedly postulated that elements obey some set of laws in non-living systems differently from what they obey in living systems, i.e. the organic and inorganic reactions obey quite different chemical laws, and consequently claimed that the generation, and to some extent the maintenance of the essential life processes were controlled by an unexplainable and unmeasurable vital force. Thus, vitalism became a popular biological theory until the latter part of the 19th Century when a German scientist, Frederick

Wolher, first prepared an organic compound, urea in the laboratory by heating an inorganic salt, ammonium cyanate.

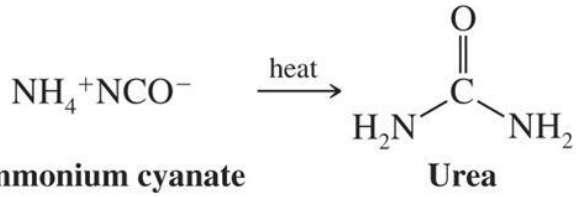


Fig. 1; Ammonium cyanate

Also, Justus von Liebig demonstrated that the heat of animal bodies was due to the combustion of the food substances they eat, which he classified into Carbohydrates, Fats and Proteins, and not as a result of a vital force. The vitalistic attitude was finally refuted in the 1860s, and this created a unified climate in the scientific community, that demands an alternative explanation for the living process. Although, the early years were not completely harmonious, as scientific history reveals that heated debate was frequent, nevertheless, the important occurrence was that the natural and physical sciences began to share a common scientific philosophy and methods. This interdisciplinary exchange is manifested today, more so than ever, by the emergence of hybrid areas of specialization such as BIOCHEMISTRY, BIOPHYSICS, and others. This hybridization does not represent a fragmentation in modern biology but each represents a specialized division with a specialized strategy and tactics.

Therefore, Biochemistry is a young scientific discipline, although it has been studied under guises such as Medical Chemistry, Physiological Chemistry, Agricultural Chemistry, Life Chemistry etc. From the middle of the 17th Century to the end of the 18th Century, scientists have made fundamental discoveries that were sufficient to stimulate biochemical thoughts , and so, the study of biochemistry became evident during the 20th century. However, what sparked off the study of modern biochemistry during the 19th Century was during the works on alcoholic fermentation by Yeast and its solution. Prior to this time, it had long been established that the yeast cell could ferment sugar to produce alcohol, the basis of brewing. However, this process was disputed by a group led by Liebig, who felt that it wrong to ascribe a chemical reaction to a living organism, rather argued that the fermentation process must have been brought about by chemical substances (inorganic ferments) in the yeast cell. This debate continued for a long time until 1897, when the Buchner brothers provided an experimental proof that there was no

difference between ferments and enzymes. This they did by showing that an extract of yeast cells was able to carry out fermentation. This discovery made it possible to work out a correct theory of fermentation and the glycolytic pathway (Chemical substances involved in the breakdown of glucose in the body)

Indeed, the term "Biochemistry" was first introduced by Carl Neuberg in 1903, and since then, outstanding contributions have been made towards the understanding, growth and development of the promising scientific discipline. These include the contributions of Emil Fischer, who demonstrated the specificity of enzymes, and pronounced the lock-and-key relationship between and enzyme and its substrate. A modification of this theory has however, been made by Kochland, who proposed the "induced-fit" hypothesis. Fischer also revealed that proteins were composed of a number of different small building blocks called amino acids.

Other early contributions were James B. Sumner, a one-handed scientist who crystallized the digestive enzymes Pepsin and Trypsin in the early 1930s. Sir Hans Kreb postulated the urea cycle and later, the Tricarboxylic acid (TCA) cycle, commonly called the Kreb's cycle. By Mid 20^{th} Century, the scientific strides made by the biochemists towards understanding the various aspects of the chemistry of life earned Biochemistry its identity as an independent and mature scientific discipline. Today, the discipline has grown so wide that it is divisible into so many areas, some of which have matured over the years into almost separate disciplines of their own. Important examples of such fast-growing sub-discipline include Molecular biology, Biotechnology and Genetic Engineering. Currently, researches involving genetic manipulation and gene cloning are making the fields of molecular biology famous and interesting. A lot has been done, and in most cases, our understanding has been quite detailed. However, there are still much to be known, and so if biochemistry does not disappear, it will engulf a good deal of areas that presently fit into chemistry and biology. Modern Biochemistry, though vast, remain the most fascinating and exciting scientific discipline.

Learning Outcomes for Study Session 1

After you have studied this study session, you should be able to:

1.1 Define Biochemistry. (SAQ 1.1)

1.2 Highlight the various scientists and their discoveries. (SAQ 1.2)

1.3 Describe the initial chemical reactions in biochemistry. (SAQ 1.3)

Key Terms: *enzyme, Properties, classification*

1.1 DEFINITION AND SCOPE OF BIOCHEMISTRY

Biochemistry, a laboratory science, is the study of the SUBSTANCES AND CHEMICAL PROCESSES which occur in LIVING SYSTEMS (organisms). It includes the identification and quantitative determination of the substances, studies of their structures, determining how they are synthesized and degraded in organisms, and elucidating their role in the operation of the organism. Some processes of special concern and interest are the conversion of foods to energy, respiration, the synthesis of nucleic acids and proteins, and the regulation of the chemical activities of cells and organisms.

1.2 THE CHEMICAL BASIS OF LIFE

It is almost impossible to understand cellular function without a reasonable knowledge of the structures and properties of the major types of biological molecules. Therefore, it is important to provide the necessary information about the chemistry of life in order to understand the basis of life. As a sequel, the types of bonds that atoms can form with one another would be considered.

1.2.1 Covalent Bonds:

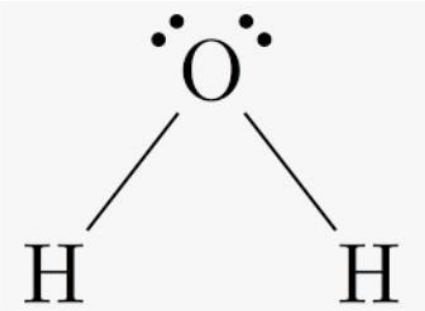
This is the band that joins together the atoms that make up a molecule; in which pairs of electrons are shared between the joint atoms. The formation of a covalent bond is governed by the basic principle: that an atom is most stable when its outermost electron shell is filled. The number of bonds an atom can form therefore, depends on the number of the electrons needed to

fill its outer shell. The outer shell is filled when it is in the duplet (for hydrogen and helium only) or octet (for other atoms) state. Thus, an oxygen atom with six outer-shell electrons can fill its outer shell by combining with two hydrogen atoms, forming a molecule of water (H-O-H). The oxygen atom is linked to each hydrogen atom by a single covalent bond, and this is usually accompanied by the release of energy which must be reabsorbed if the bond must be broken. The energy required to cleave C-H, C-K, or C-O covalent bonds is quite large (between 80 and 100 kcal/mol). As such, these bonds are stable under most conditions. Note that if two electron pairs are shared (as in molecular oxygen, O_2), the covalent bond is a double bond, and if three (as in molecular nitrogen N_2), it is a triple bond. Atoms joined by a single bond can rotate freely with respect to its neighbor, but stearic hindrance occurs if double or triple bonded. The type of bond between two atoms can therefore have important consequences in determining the shapes of molecules. When atoms of the same element bond to one another, as in H_2, the electron pairs of the outer shell are equally shared between the two bonded atoms. However, when two unlike atoms are covalently bonded, the positively charged nucleus of one atom exerts a greater attractive force on the outer electrons than the other. Consequently, the shared electrons tend to be located more closely to the atom with the greater attractive force, that is, the more electronegative atom. Among the atoms most commonly present in biological molecules, nitrogen and oxygen are strongly electronegative.

1.2.2 Polar and Non-Polar Molecules:

Water's single oxygen atom attracts electrons much more forcefully than either of its hydrogen atoms. As a result, the H-O bonds of a water molecule are said to be polarized, such that one of the atoms has a partial negative charge and the other partial positive charge as denoted:

negatively charged end



positively charged end

Fig. 1.2; **Water**

Molecules, such as water, that have an asymmetric distribution of charge are said to be polar molecules. Polar molecules of biological importance contain one or more electronegative atoms, usually O_2 N_2 S_2 and/or P. Molecules that lack polarized bonds, such as those that consist entirely of carbon and hydrogen atoms, are said to be nonpolar. The presence of polarized bonds is of the utmost importance in determining the reactivity of molecules. Molecules that lack electronegative atoms, such as waxes and fats, tend to be relatively inert. Some of the more interesting /biological molecules, including proteins and phospholipids contain both polar and nonpolar regions, and these behave very differently.

1.2.3 Ionization:

Some atoms are strongly electronegative that they can capture electrons from other atoms during a chemical reaction. For instance, when the elements: Sodium and chlorine are mixed, the single electron in the outer shell of each sodium atom migrates to the electron-deficient chlorine atom. As a result, these two materials are transformed into charged atoms, or ions.

$2Na + :CL: Či: — 2 Na:CL: — 2Na^+ + 2 :CL:^-$

The cation (Na^+) and anion (CL^-) generated above are relatively stable because they possess filled outer shells. A different arrangement of electrons within an atom can however, produce a highly reactive species, called free radicals. Free radicals are atoms of molecules that have orbitals containing a single unpaired election and are highly unstable. They may be formed when a covalent bond is broken such that each portion Keeps one -half of the shared electrons, or they may be formed when an atom or molecule accepts a single electron transferred during an oxidation-reduction reaction. For example, water can be converted into free radicals when exposed to radiation from the sun:

$H_2O \longrightarrow HO^O + H^O$

Free radicals are extremely reactive and capable of chemically altering many types of molecules, including proteins, nucleic acids, and lipids and have been implicated in a number of diseases. The formation of hydroxyl radical, H^O, is probably a major reason that sunlight is so damaging to skin.

1.2.4 NON-COVALENT BONDS:

Interactions between molecules or between different parts of a large biological molecule are governed by a variety of weak linkages called non-covalent bonds. Non-covalent bonds do not depend on shared electrons but rather on attractive forces between positively and negatively charged regions within the same molecule or on two nearby molecules. Even though individual non-covalent bonds are weak, when large numbers of them act in concert, their attractive forces are additive. Thus, taken as a whole, they provide the structure with considerable stability. Several of such bonds important in cells would be examined:

1.2.5 Ionic Bonds:

A crystal of table salt is held together by an electrostatic attraction between positively charged Na^+ and negatively changed Cl^- ions. This type of attraction between fully charged components is called an ionic bond or a salt bridge.

Ionic bonds within a salt crystal may be quite strong, but if a crystal is dissolved in water, each of the individual ions becomes surrounded by water molecules, which inhibit oppositely charged ions from approaching one another closely enough to form ionic bonds. Therefore, the strength of ionic bonds in a cell is generally weak, due to the presence of water, but deep within the core of a protein, where water is often excluded, such bonds can be influential.

1.2.6 Hydrogen Bonds:

When a hydrogen atom is covalently bonded to an electronegative atom, particularly an oxygen or nitrogen atom the single pair of shared electrons is greatly displaced toward the nucleus of the electronegative atom, leaving the hydrogen atom with a partial positive charge. As a result, the bare, positively charged nucleus of the hydrogen atom can approach closely enough to an unshared pair of outer electrons of a second electronegative atom to form an attractive interaction. This weak attractive interaction is called hydrogen bond.

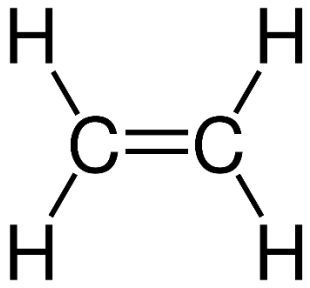


Fig. 1.2.6 Hydrogen bond

1.2.7 Hydrophobic Interaction:

Non-polar molecules, such as steroid or fat molecules, are essentially insoluble in water because they lack the charged regions that would attract them to the poles of water molecules. When non-polar compounds are mixed with water the non-polar, hydrophobic ("water fearing") substances are forced to into aggregates, which minimize their exposure to the polar Surroundings. This association of non-polar molecules is called a hydrophobic interaction.

This is why droplets of fat molecules rapidly reappear to the surface of a beef or chicken soup even after the liquid is stirred with a spoon. Again, this is the reason why non-polar groups tend to localize within the interior of most soluble proteins shielded from the surrounding water molecules. These types of hydrophobic interactions are not classified as true bonds because they do not result from an attraction between hydrophobic molecules. Also note that hydrophobic groups can form weak bonds with one another based on electrostatic attractions.

1.2.8 Van der Waals Forces:

Closer examination of the covalent bonds that make up a non-polar molecule (such as H_2 or CH_4) reveals that electron distributions are not always symmetric, even though the atoms share the electrons equally. These transient asymmetries in electron distribution result in momentary separations of charge (dipoles) within the molecule. If the molecules with transitory dipoles are very close to one another and oriented in the appropriate manner, they experience a weak attractive force, called van der Waals force, that bonds them together. The van der Waals forces has been demonstrated to be important in biological interactions.

Summary of Study Session 1

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 1

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

SAQ 1.1 **(Tests Learning Outcome 1.1)**

SAQ1.2 **(Tests Learning Outcome 1.2)**

What is

SAQ 1.3 **(Tests Learning Outcome 1.3)**

Define

SAQ 1.4 **(Tests Learning Outcome 1.4)**

What is the

SAQ 1.5 **(Tests Learning Outcome 1.5)**

What are the

Links to OERs

References/ Suggestions for Further Reading

.

Should you require more explanation on this study session, please do not hesitate to contact your e-tutor via the LMS.

Are you in need of General Help as regards your studies? Do not hesitate to contact the …… Center by e-mail or phone on:

MODULE ONE

SESSION 2

INTRODUCTION TO THE STUDY OF BIOCHEMISTRY

INTRODUCTION

You have learned the definition and scope of biochemistry in session one. You also learned the chemical basis of life, under which, we discussed the various bonds that are found in biological systems. In this session, we are going to be discussing the nature of biological molecules, functional groups and functions of biological molecules
fascinating and exciting scientific discipline.

Learning Outcomes for Study Session 1

After you have studied this study session, you should be able to:

discuss the nature of biological molecules

identify the different functional groups and

state the biological functions of certain molecules

2.1 Define Biochemistry. (SAQ 1.1) Define. (SAQ 1.2)

2.2 Define. (SAQ 1.3)

Key Terms: Molecules carbohydrates proteins fats and oils vitamins minerals water DNA and RNA

2.1 THE NATURE OF BIOLOGICAL MOLECULES.

A high percentage of the total weight of an organism is water and if evaporated, the remaining dry or weight consists of carbon-containing substances. When initially discovered, these molecules containing atoms of carbon were present only in living organisms and were then named organic molecules in order to distinguish them from inorganic molecules found in the inanimate world. However, this unique attribute of organic substances has been lost, because of the synthesis of more and more of these carbon-containing compounds in vitro (in the lab) by chemists. Today, Compounds produced by living organisms are called Biochemicals. The chemistry of life centers around the chemistry of the carbon atom and the essential quality of Carbon that has allowed it to play this role is the incredible number of molecules it can form. The size and electronic structure of carbon make it uniquely suited for generating large numbers of molecules, several hundred thousand of which are known.

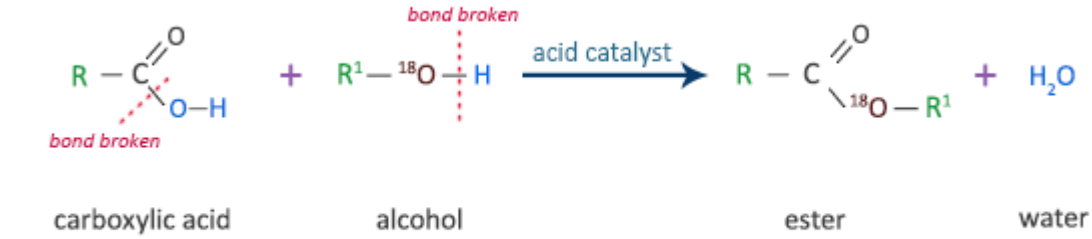
The simplest group of biomolecules, the hydrocarbons, Contain only carbon and hydrogen atoms. Ethane (C_2H_6), a simple hydrocarbon, consists of two atoms of carbon in which each carbon is bonded to the other carbon as well as to three atoms of hydrogen (as seen below). As more carbons are added, the skeletons of organic molecules increase in length and their structure becomes more complex. Note that hydrocarbons exhibit structural isomerism.

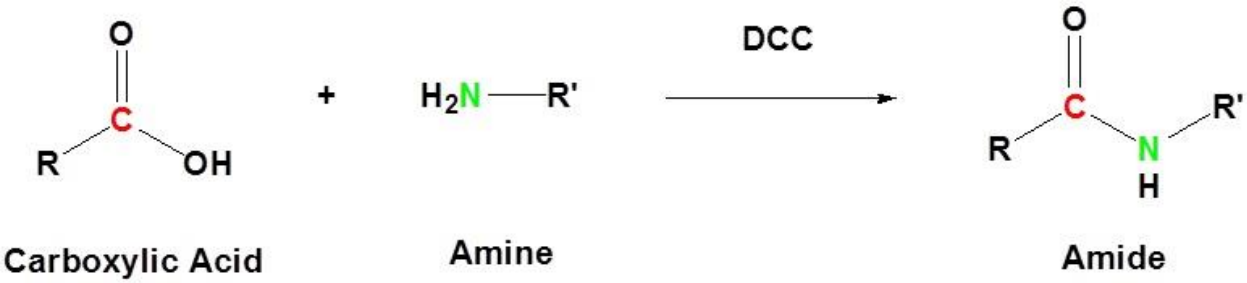
Fig. 2.1 Ethane

2.2 FUNCTIONAL GROUPS

Many of the organic substances that are important in biology contain chains of carbon atoms, like hydrocarbons, but certain of the hydrogen atoms are replaced by various functional groups. Functional groups are particular groupings of atoms that often behave as a unit and give organic molecules their physical properties, chemical reactivity, and solubility in aqueous solution. Some of such groups are: methyl (-CH3), hydroxyl (-OH), Carboxyl (-COOH), amino ($-NH_2$), phosphate ($-PO_2H_2$), carbonyl (C=0), Sulfhydryl (-SH). Most of the functional groups above,

contain at least one electronegative atom and the presence of such atom makes organic molecules more polar, more water Soluble, and more reactive. Two of the most common linkages between functional groups are ester and amide bonds.





2.3 FUNCTIONS OF BIOLOGICAL MOLECULES

Based on the functions performed, Biochemicals are classified into: Macromolecules, Monomers, Metabolites and Molecules of miscellaneous function.

Macromolecules, which contain from dozens to millions of Carbon atoms are highly organized and form the structure of cells. Macromolecules are divided into proteins, nucleic acids, polysaccharides, and certain lipids constructed from monomers. Macromolecules carry out the activities of cells, and so, the presence of macromolecules endows organisms with the properties of life.

Outside the cell's DNA, macromolecules are continually broken down and replaced by new ones in the cell. This means that cells must contain a pool (or supply) of low-molecular weight precursors (monomers) that are readily available to be incorporated into the growing macromolecules. These include sugars, the monomers of polysaccharides; amino acids, the

precursors of proteins: nucleotides, the precursors of nucleic acids (DNA & RNA); and fatty acids, which are incorporated into lipids.

Biomolecules are synthesized in successive steps: A→b→c→d→e →F. The cell starts with a specific Compound, A, and converts it to b then to c until it yields F. The end product, which can be utilized in some other reactions. This series of chemical reactions (transformations) is termed Metabolic pathway, and the compounds (b-e) formed along the pathway leading to the formation of the end product. F, are called metabolites (or metabolic intermediates).

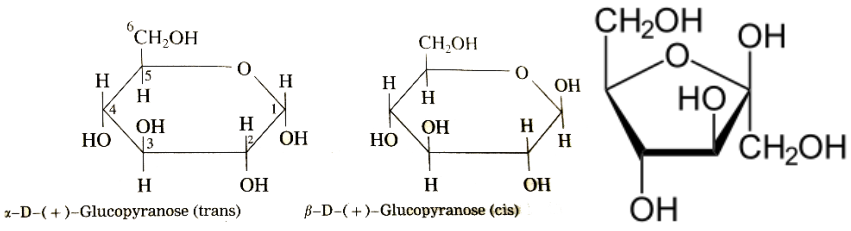
The molecules of miscellaneous function include such substances as vitamins, which function primarily as adjuncts to proteins; certain steroid or amino acid hormones; molecules involved in energy storage, like ATP or Creatine phosphate; regulatory molecules such as cAMP; and metabolic waste products such as urea.

2.4 SUBSTANCES IN LIVING ORGANISMS

The most essential substance for life is water, an inorganic molecule, and other major ones include macromolecules (Carbohydrates, proteins, lipids and nucleic acids: DNA & RNA); trace substances (vitamins, hormones and minerals) required in minute amounts.

2.4.1 CARBOHYDRATES:

Carbohydrates are a class of substances which includes simple sugars such as glucose, fructose, galactose, mannose and polysaccharides (e.g. starch, cellulose, and glycogen).



Structure of Some Simple Sugars

Polysaccharides

are polymers of simple sugars covalently linked by glycosidic bonds. They function mainly as nutritional sugar stores (eg [starch and glycogen), and as structural material (e.g. cellulose)

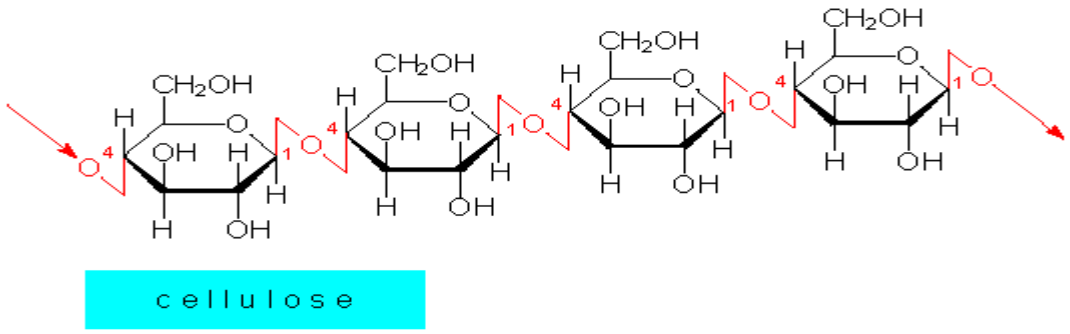
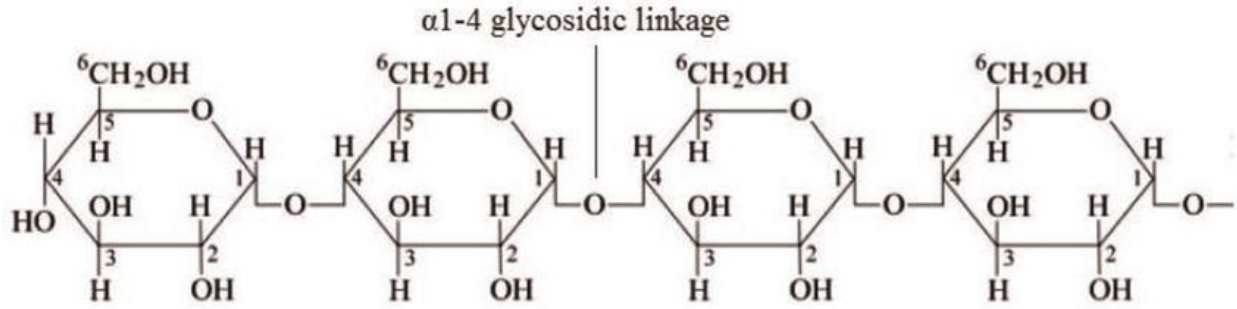


Fig 1b: The structure of cellulose, with $^{\beta}(1\rightarrow4)$ linkages



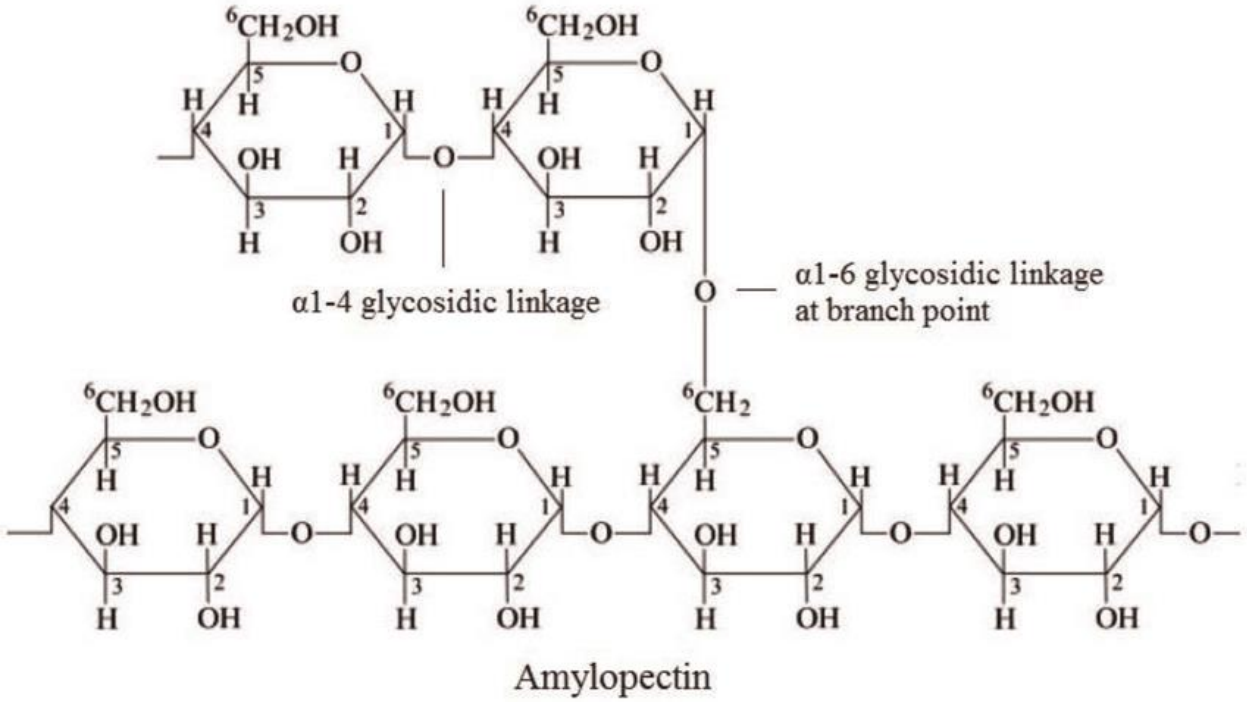


Fig 2.4: The structure of starch showing the ∝-amylose (linear) fraction with ∝ (1→4) linkages and the amylopectin (branched) unit with ∝ (1→6) linkages.

Polysaccharides could either be homoglycan, that is, homopolysaccharides - contain only one type of sugar unit, or heteroglycan (heteropolysaccharides) contain more than one type of sugar repeating unit. Starch and cellulose abundant in plants are homoglycans containing only glucose moieties as building units (Fig 1b and c). However, they differ in the way the glucose monomers are arranged. Cellulose is essentially a linear polymer because of the β (1-4) linkages, and is a major structural component of the plant cell wall. Chitin is found in fungal cell walls and in the exoskeleton of insects and crustacea. It is similar to cellulose, but the monomeric unit is N-acetylglucosamine. Starch, a storage form of glucose in plants contains two fractions (Fig. 1c), While glycogen, the form of glucose storage in some animal tissues (liver, muscle) and in fungi, has structural resemblance to starch, except that it is more profusely branched. Carbohydrates may also be found linked to proteins in glycoproteins, and linked to lipids in glycolipids. In addition, nucleic acids are composed partially of certain sugars, ribose and deoxyribose, which belong to the pentose class of sugars. Carbohydrates are important source of energy and structural materials in organisms.

2.4.2 PROTEINS:

Proteins are intimately involved in all life processes, and could be fibrous or globular. Proteins can fold into different levels of organizational structure comprising of primary secondary, tertiary and quaternary structures. Amino acids, the monomeric units of proteins are linked together by an amide bond, though called peptide bond when in proteins. The carboxyl (-COOH) group of one amino acid is covalently linked to the amino ($-NH_2$) group of the next amino acid by such peptide bond. (Fig. 1:1)

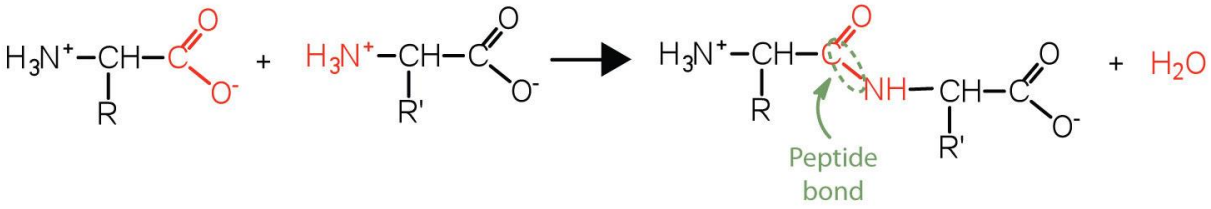


Fig. 1-1: Formation of a peptide bond between two amino acids to form a dipeptide

The amino acid at one end of the dipeptide has a free amino group, while the one at the other end has unattached carboxyl group. Thus, polypeptides (proteins) are directional with N and C.

termini. The linear sequence of amino acids from the N to the C terminus is the primary structure of the polypeptide. Typical sizes for a single polypeptide chain(s) are within the range of 100-1500 amino acids, but shorter or longer ones still exist.

Functions of proteins

Proteins, the largest fraction of living matter in most cell types, function as:

A. Enzymes:

With the exception of very few catalytically active RNA molecules (RNase D, E, F and P) called ribozymes, all enzymes are proteins. An enzyme is a soluble organic catalyst produced by a living organism which speeds up a thermodynamically feasible reaction which ordinarily proceeds slowly at the temperatures of living organisms, so that the rate of the reaction is compatible with the biochemical processes essential for cell maintenance. Binding of Substrate depends on specific non-covalent interactions (van der Waals forces, hydrogen bonds, salt bridges and hydrophobic forces), and being proteins, enzymes lose their catalytic prowess when subjected to heat, strong acids or bases, organic solvents or other conditions that denature proteins.

B. Signal Molecules:

Cell membrane proteins act as second messengers, and so, transfer the signals from first messengers (hormones), on cell surface into the interior. This is achieved when a ligand (hormones) binds to the receptor proteins.

C. Carrier Molecules:

Some proteins are carriers of materials, as in the case of haemoglobin, a carrier of oxygen in many animals. Albumin, another protein transports bilirubin to the liver the site of conjugation into Soluble and excretable form. Transferrin carries iron, again to the liver where it is stored bound to ferritin. Dietary fats are transported by a class of proteins called apoproteins.

D. Structural Material:

Certain proteins such as collagen, act as structural material, for instance, as portions of cellular membranes and as the major component of hair, horn, Skin, feathers and Connective tissues, while hair contains Keratin in addition. Contractile proteins, actin and myosin, in muscle form sliding filaments, and so are involved in muscular activity, including movement, and cell division.

E. Nutritional Component:

Casein and ovalbumin are the major proteins of milk and eggs, respectively. They provide the amino acids for growth and development of offspring: Amino acids derived from nutritional proteins is also required for the replacement of worn-out tissues and for the repair of damaged tissues.

F. Immunity:

The immune system depends on antibody proteins to combat viral or bacterial infections and other diseases caused by other alien (foreign) agents, also known as antigens.

G. Regulation:

Proteins could modify the functions of many other molecules by binding to them. For instance, the attachment of transcription factors, a small group of proteins to DNA modulates their activities. As earlier noted, proteins vary in size but all are composed of essentially the same 20 amino acids.

2.4.3 NUCLEIC ACIDS

There are two classes of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The nucleic acids are large molecules composed of long chains of primarily four different nucleotides. Nucleotides are composed of sugar phosphates bonded to organic nitrogenous bases; they exist as small molecules in addition to proteins the material for large nucleic acids. In a DNA or RNA molecule, deoxyribonucleotides or ribonucleotides are respectively joined into a polymer by the covalent, phosphodiester linkage.

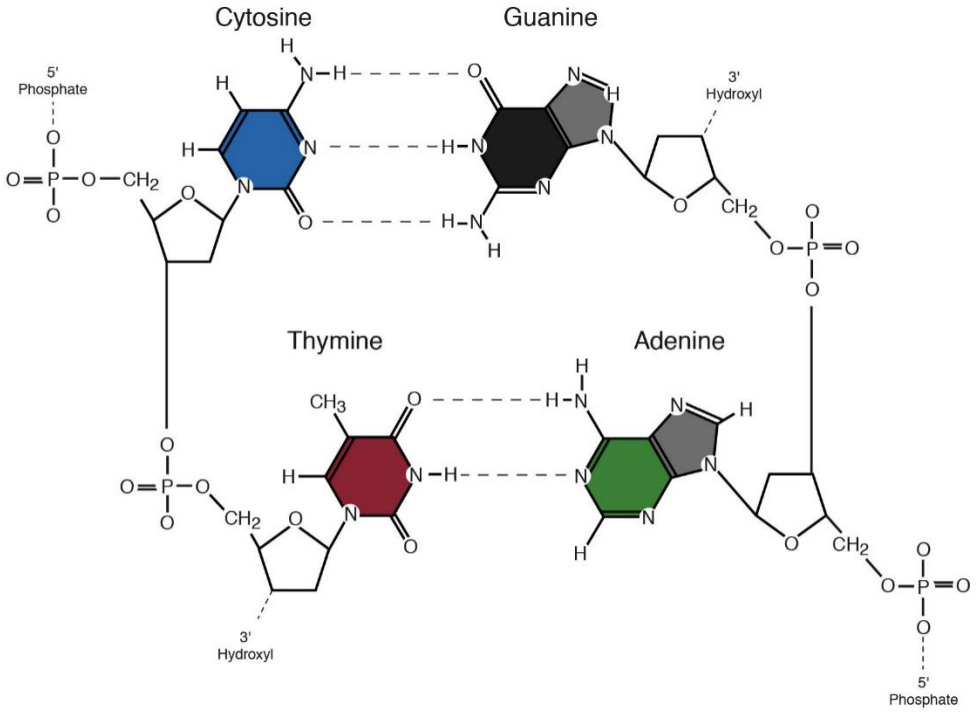


Fig. 1.5: The covalent structure of complementary DNA strands, showing the phosphodiester linkage in a circle.

The building nucleotides are bridged together via a phosphate group between the 5'-phosphate of the incoming block and the 3' hydroxyl end of the growing polymer. This kind of bridge is called a phosphodiester bridge because the phosphate is chemically in the form of a diester, hence a nucleic acid chain grows in the 5'-3' Direction (Fig. 1.5).

The sequential array of nucleotides is quite specific for each molecule and constitutes the form in which genetic information is stored processed and transferred, but the expression of such information requires proteins

2.4.4 LIPIDS

Lipids are a diverse group of "greasy" substances which are soluble in organic solvents but insoluble in water. Cells and tissues are composed predominantly of water, so cellular structures must be composed of materials which are insoluble in an aqueous environment. The most common lipids are the fats, which serve as insoluble stores of biological fuel (energy)

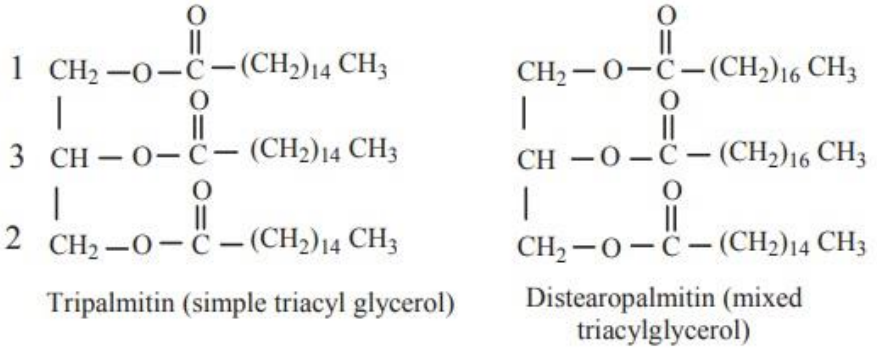


Fig. 2.4.4A: The structure of a typical fat, triacylglycerol: 1- palmitoyl, 2- stearoyl, 3-palmitoyl glycerol.

Animal fats (triacylglycerols) have saturated fatty acids, so the chains are linear and the molecules can pack tightly and the resulting fats are solids. On the other hand, plant "fats" (oils) contain unsaturated fatty acids with one or more double bonds which prevent close packing, so they tend to be liquid at room temperature. Cellular membranes are composed mainly of a class of lipids called phospholipids, which consists of glycerol esterified to two fatty acids and phosphate. The phosphate is usually attached (esterified) to a small molecule such as serine, ethanolamine inositol or choline.

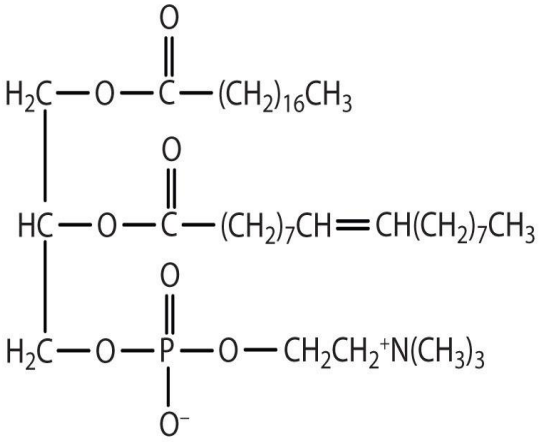


Fig. 2.4.4B: A phospholipid: Phosphatidylcholine, containing esterified oleic and stearic acids, and choline attached to the phosphate.

Membranes also contain sphingolipids e.g. sphingomyelin. Some other lipids are cholesterol and steroid hormones.

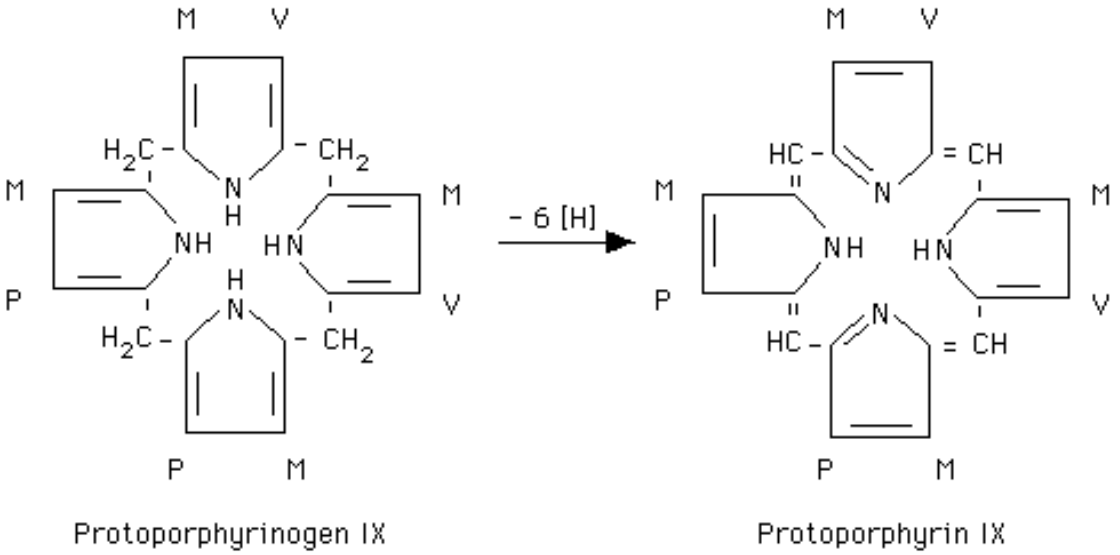
Note that proteins could interact with the other three macromolecules to form complexes that have wider range of function than the individual component parts: Examples include: Nucleoproteins, contain nucleic acid (DNA or RNA) and protein, e-g. Telomerase and ribonuclease P; glycoproteins (proteoglycans/mucoproteins), contains proteins covalently attached to carbohydrates; lipoproteins, are lipids with non-covalently attached protein component. The glycolipids, which have lipid and Carbohydrate parts is yet another group of complex macromolecules.

2.4.5 TRACE SUBSTANCES IN LIVING ORGANISMS

MINERALS:

Minerals such as salts of sodium, potassium, Calcium, and magnesium function in homeostasis (water and acid-base balance) and so contribute to the ionic environment within and around cells, and some too, participate in nerve conduction. In addition, calcium phosphate is an important constituent of bone, and iron (Fe^{2+}) is a necessary part of respiratory proteins such as haemoglobin and cytochromes.

M= -C



H_2 (methyl)

V=-CH=CH_2 (Vinyl)

P = -CH_2-CH_2-Coo (propionate)

Fig. 2.4.5A: Haem portion of haemoglobin showing the central location of iron.

Note that in haemocyanin of snails and in the chlorophyll pigment of plants, copper and magnesium, respectively occupy the central position. The oxidation of these central elements, iron, copper and magnesium produces the characteristic red, blue and green colours. In cyanocobalamin (vitamin B_{12}), Cobalt (Co) is present as an integral part of the molecule. Again, some trace metals like magnesium, selenium, and molybdenum have been found to be constituents of certain enzymes. Magnesium is the Co-factor for several kinases, energy (ATP) dependent enzymes. The precise role(s) of many trace metals is yet to be clearly established.

2.4.6 VITAMINS:

Vitamins are substances which are biologically effective in minute quantities. In living organisms, vitamins are converted into coenzymes, substance which collaborate with some enzymes in catalyzing reactions

Apoenzyme	+	Coenzyme	→	Holoenzyme
(Inactive enzyme)		(Vitamin part)		(Active enzyme)

2.4.7 HORMONES:

Hormones are made in and secreted by endocrine (ductless) glands, usually in small amounts, and they act in regulating the activities of other tissues. Hormone could be a protein hormone e.g. insulin or a steroid hormone e.g. oestrogen.

Summary of Study Session 1

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 1

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

SAQ 1.1 **(Tests Learning Outcome 1.1)**

SAQ1.2 **(Tests Learning Outcome 1.2)**

What is

SAQ 1.3 **(Tests Learning Outcome 1.3)**

Define

SAQ 1.4 **(Tests Learning Outcome 1.4)**

What is the

SAQ 1.5 **(Tests Learning Outcome 1.5)**

What are the

Links to OERs

References/ Suggestions for Further Reading

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Should you require more explanation on this study session, please do not hesitate to contact your e-tutor via the LMS?

Are you in need of General Help as regards your studies? Do not hesitate to contact the ……

Center by e-mail or phone on:

MODULE ONE

SESSION 3

INTRODUCTION TO THE STUDY OF BIOCHEMISTRY CONT…

INTRODUCTION

In the previous session, you learnt the nature of biological molecules, functional groups, functions of biological molecules and substances found in living organisms under which, you were introduced to carbohydrates proteins nucleic acids lipids, Trace substances and hormones. In this session of the introduction to biochemistry, we shall be discussing chemical processes in the living organisms, the living system, basic properties of cells and the subcellular organelles

Learning Outcomes for Study Session 1

At the end of this session, you should be able to

3.1. mention certain chemical processes in the living organism (SAQ 3.1)

3.2. list the properties of the living cell 2.1 (SAQ 3.2) and

3.3. know the function of the subcellular organelles. (SAQ 3.3)

Key Terms: Molecules carbohydrates proteins fats and oils vitamins minerals water DNA and RNA

3.1 CHEMICAL PROCESSES IN LIVING ORGANISMS

Special concern includes the chemical processes involved in the conversion of food materials to energy and the processing of the genetic information. programmed in DNA molecules, the cell's "store house".

Many of the chemical steps involved in the biological breakdown of sugars, fats, and amino acids are known. It is well established that living organisms capture the energy liberated from these reactions by forming a high-energy compound, adenosine triphosphate (ATP). In the absence of oxygen, some organisms and tissues derive ATP from an incomplete breakdown of glucose, degrading the sugar to an alcohol or an acid in the process. In the presence of oxygen many organisms degrade glucose and other foodstuff to carbon (IV) oxide, CO_2, and water. H_2O, producing ATP in a process Known as oxidative phosphorylation.

$C_6H_{12}O_6$ ----------$2CH_3CH(OH)COOH$ + ENERGY (***Absence of Oxygen***)

(Glucose) (Lactic acid)

$C_6H_{12}O_6$---------- $2C_2H_5OH + 2CO_2$ + ENERGY (***Absence of Oxygen***)

(Glucose) (Alcohol) carbon (iv)oxide

$C_6H_{12}O_6$ ---------- $6CO_2 + 6H_2O$ + ENERGY (***Presence of Oxygen***)

(Glucose) (water) carbon (iv) oxide

In spite of many years of intensive investigation, the chemical reactions on oxidative phosphorylation are not well understood.

3.2 THE LIVING SYSTEMS

The accumulated efforts of scientists in the field of cell biology over the years have culminated in the proposal of three tenets of the cell theory: (a) The cell is the structural unit of life (b) All organisms are composed of one or more cells, and (c) cells can arise only by division from a preexisting cell. Just as plants and animals are alive, so too are cells: Life, in fact, is the most basic property of cells, and cells are the smallest units to exhibit this property.

Whole cells can be isolated from a plant or animal and cultured in a laboratory where it is capable of continued propagation. Because of ease of study, cells grown in vitro (i.e. in culture outside the body) have become essential tool, and much of the information that have broaden our understanding of cell properties stemmed from in vitro investigations. A knowledge of cell characteristics provides an insight into the nature of the living systems.

3.3 BASIC PROPERTIES OF CELLS.

- Cells are highly complex but organized.
- Cells possess a genetic program and the means to use it.
- Cells are capable of producing more of themselves.
- Cells acquire and utilize energy.
- Cells carry out a variety of chemical reactions
- Cells engage in numerous mechanical activities.
- Cells are able to respond to stimuli
- Cells are capable of self-regulation

3.4 CLASSES OF CELLS

It has been discovered from electron microscopic studies of a wide variety of cells, that there are two basic classes of cells - Prokaryotic and Eukaryotic, distinguished by their size and the types of internal structures (organelles) they possess. The existence of two distinct classes of calls, without any known intermediates represents one of the most fundamental evolutionary discontinuities in the biological world.

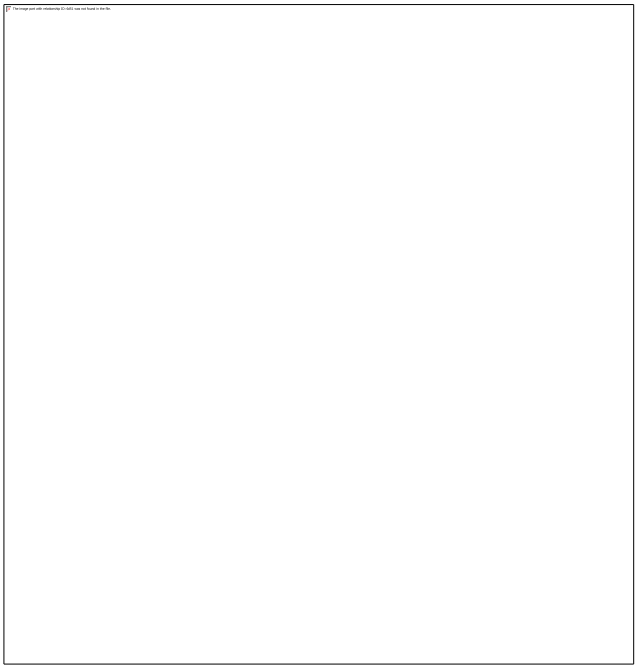


Fig 3.4a: A typical prokaryotic cell (schematic)

Adapted from: Turner, et al: (1998).

Eubacteria, structurally defined as prokaryotes, have a plasma membrane, usually enclosed in a rigid Cell wall, with no intracellular compartments. They have single, major circular chromosome, and May be unicellular or multi-cellular. Amongst the eubacteria, E. coli is the best studied (Fig. 1.9)

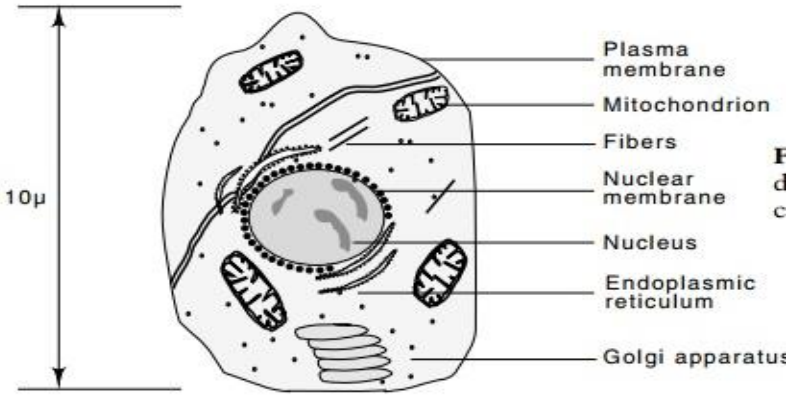


Fig3.4b: Schematic diagram of a typical eukaryotic cell

Source: Turner, et al. (1998).

The word prokaryote is from two Greek words pro (before) and karyon (nucleus) this shows that in a typical prokaryotic cell there is no defined nucleus the genome is stored and replicated in the nucleoid which contains circular DNA. The prokaryotic cell also has a store of circular DNA in the plasmids (extra chromosomal DNA element-responsible for antibiotic resistance in certain bacteria) while the ribosomes is the site of protein synthesis. The pili facilitates communication between cells/adherence to cells while the flagellum is for movement. There is a semi permeable plasma membrane and a cell envelope (made up of capsule, cell wall and plasma membrane) which gives the cell rigidity and separates the interior of the cell from its environment (protective filter). The cell capsule is antigenic, has antiphagocytic function (and so determines the virulence of many bacteria). It also plays a role in the attachment of the organism to the mucous membrane. The cell wall consists of a peptidoglycan in bacteria and acts as an additional barrier against external forces. It also prevents the cell from expanding and bursting (cytolysis) from osmotic pressure in hypotonic environment.

The word Eukaryote on the other hand is from two Greek words Eu (true) karyon (nucleus) which means that the eukaryotic cell has a well-defined nucleus covered by a nuclear membrane. Linear DNA molecules called chromosomes associated with histone proteins are stored in the nucleus and separated from the cytoplasm by the nuclear membrane. The mitochondria (the cell

power house) also contain circular DNA. There is presence of cilia in Eukaryotes which play roles in chemo sensation, mechano-sensation and thermo-sensation.

Taxonomically, eukaryotes are classified into animal, plant, fungi and protist Kingdoms. Structurally, eukaryotes are defined by their possession of membrane - enclosed organelles with specialized metabolic functions (fig. 1.10). These organelles, nuclei, mitochondria, endoplasmic reticulum etc., are the sites of distinct biochemical processes.

Daughter cells from the same ancestral origin may either be identical in every way, or they may change their patterns of gene expression to become functionally different from the parent cell. Spore formation by prokaryotes and some lower eukaryotes represents an example of such cellular differentiation. However, in complex multi-cellular eukaryotes, embryonic cells differentiate into highly specialized Cells e.g. muscle, nerve, liver and kidney. This differentiation, whether in prokaryotes or eukaryotes is regulated by developmental Control genes, and alterations (mutation) in such genes result in abnormality. Co-ordination of the activities of different cell types requires communication between them. Such communication involves signaling molecules such as neurotransmitters, hormones and growth factors which are secreted by one tissue and act upon another through specific Cell-surface receptors.

3.5 THE SUBCELLULAR ORGANELLES

Nuclei:

Most of the cellular DNA is found in the membrane-bound nucleus in multiple chromosomes. The Nucleus is also the site of transcription and RNA processing.

Mitochondria and Chloroplasts:

The mitochondrion is the site of ATP (energy) generation following the oxidation of nutrients (Cellular respiration) to Carbon (IV) oxide (CO_2) and water (H_2O). Basically, the structure of chloroplast is similar to mitochondrion, except that the chloroplast has a thylakoid membrane system containing the light-harvesting pigment, Chlorophyll.

Endoplasmic Reticulum:

In the cells, two forms are recognized, the Smooth ER and Rough ER. The Smooth ER is a cytoplasmic membrane system that harbours membrane bound enzymes. Some of such enzymes are involved in the biosynthesis of certain lipids and the oxidation/Conjugation (detoxication) of foreign compounds (xenobiotics). The Rough ER has attached ribosomes which are involved in

the Synthesis and secretion of membrane proteins e-g plasma membrane proteins. The (lipids) and proteins Synthesized on the rough ER are transported in specialized vesicles to the Golgi complex, which further modifies, sorts and directs them to their final destinations.

Microbodies:

Lysosomes, peroxisomes and glyoxysomes are collectively known as microbodies. The lysosomes bud off from the Golgi complex, and they contain a variety of digestive enzymes capable of degrading proteins, Nucleic acids, lipids and carbohydrates. The peroxisomes contain enzymes that destroy certain potentially dangerous free radicals and hydrogen peroxide (H_2O_2) arising from Some metabolic reactions.

$$H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

The glyoxyomes are specialized plant peroxiomes which carry out the reactions of the glycoxylate cycle.

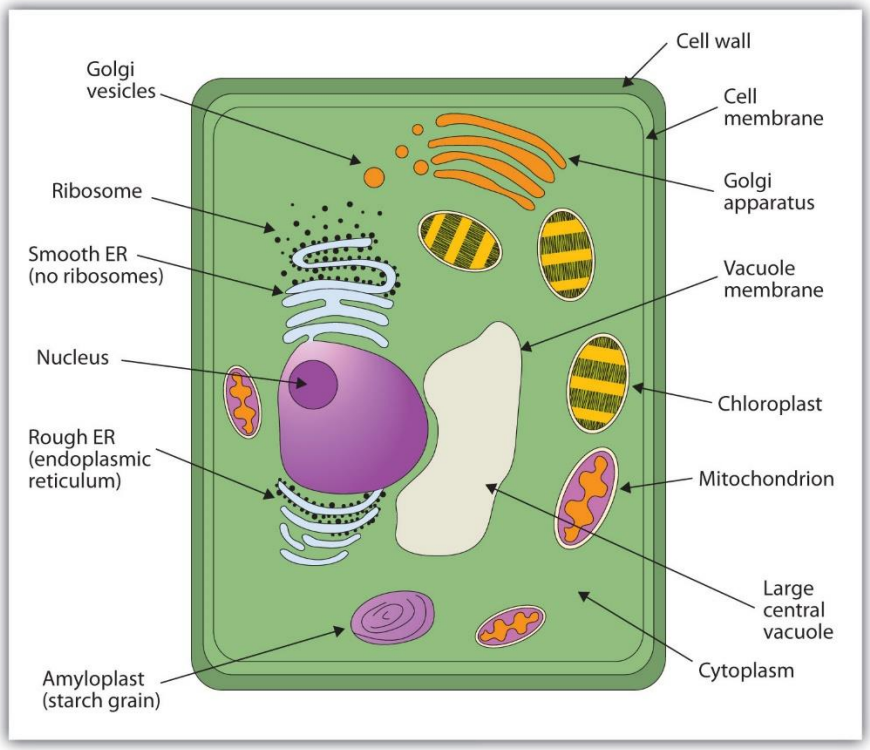


Fig. 3.5a; A Typical plant cell

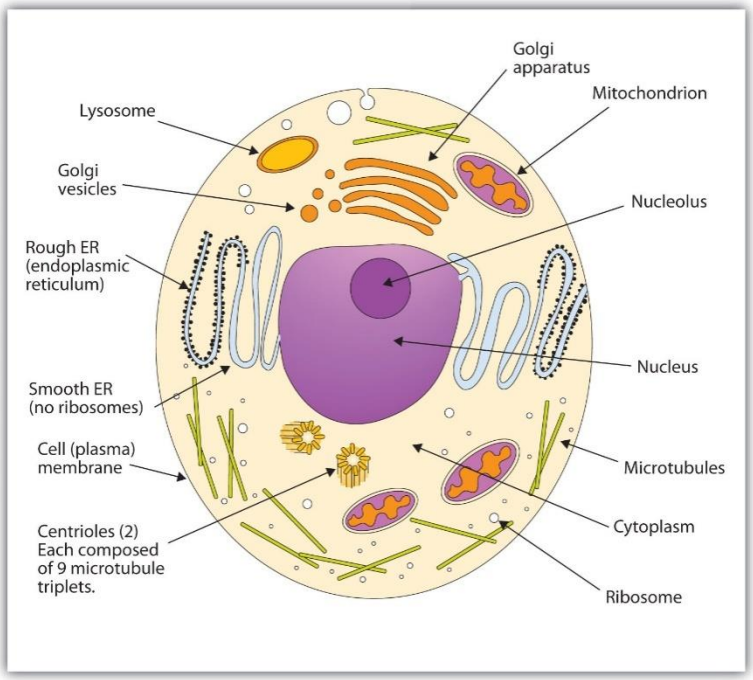
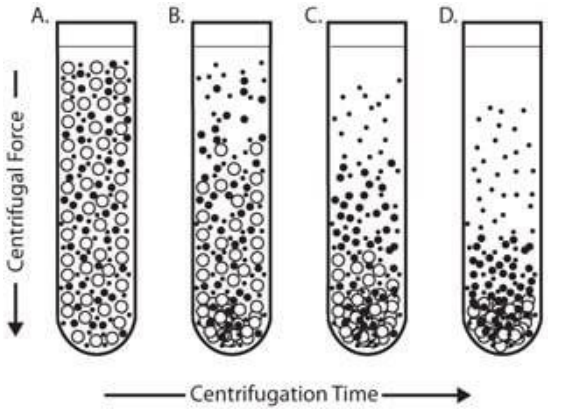


Fig. 3.5b; A Typical Animal cell

3.6 ORGANELLE ISOLATION

The plasma membrane of eukaryotes can be disrupted by various means including osmotic shock, controlled by mechanical shear or by certain nonionic detergents. Following disruption, the sub-cellular organelles can be Separated from each other and purified by a combination of differential Centrifugation and density gradient Centrifugation (both rate Zonal and isopycnic)



(a)

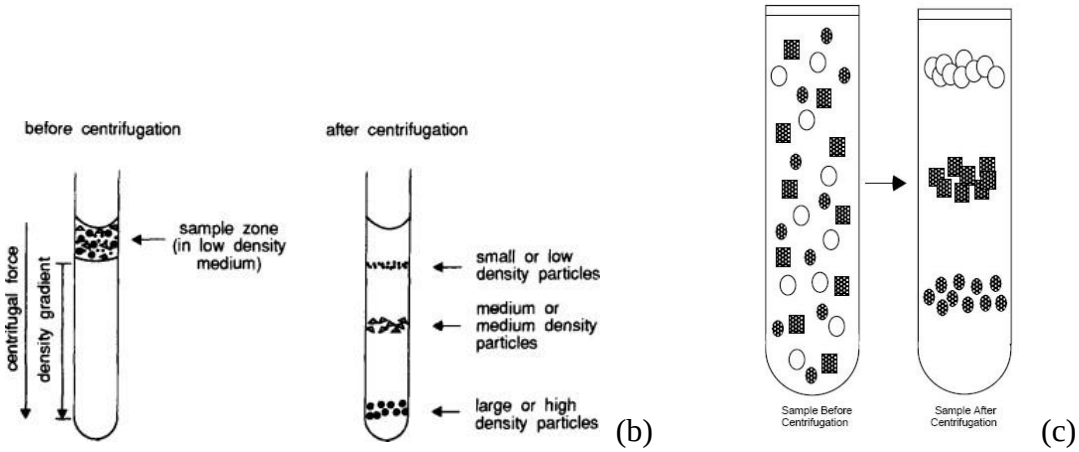


Fig3.6: Centrifugation techniques: (a) Differential (b) rate Zonal (C) isopycnic (equilibrium).

From: Turner, et al. (1998).

Purity of the isolated sub-cellular fractions could be determined by measuring the activities of organelle-specific enzymes.

3.7 VIRUSES

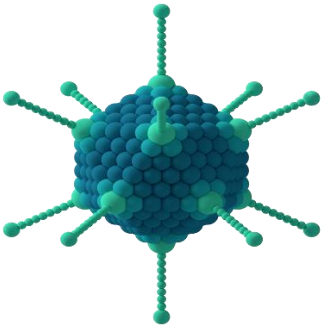
Early works, involving studies of tobacco mosaic disease in tobacco plants and hoof- and-mouth disease in cattle make investigators to recognize another type of infectious agent, smaller, and presumably simpler, than the smallest bacteria. They named these pathogens viruses.

Viruses are responsible for a good number of human diseases, including polio, influenza, cold sores, measles, a few types of cancer, AIDS and recently Severe acute respiratory syndrome, SARS. Viruses occur in a wide variety of very different shapes, sizes, and constructions, but all of them share certain common properties. All viruses are obligatory intracellular parasites, that is, they cannot reproduce unless present within a host cell, which depending on the specific views may be a plant, animal or bacterial cell. Outside of a living cell, the virus exists as virion, which contains a small amount of genetic material that, depending on the virus, can be single-stranded or double-stranded, RNA or DNA.

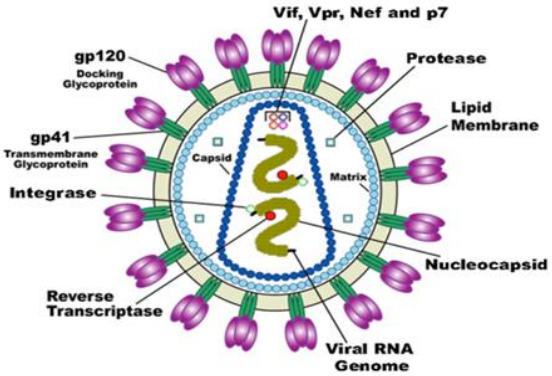
The genetic material of the virion is surrounded by a protein capsule or capsid. Many viruses have a capsid whose subunits are organized into a structure having planar faces (polyhedron) and the commonly encountered shape is the 20-sided Icosahedron. Adenovirus, which causes respiratory infections in mammals, has an icosahedral capsid (Fig3.7a). In many animal viruses,

including the Human Immunodeficiency Virus (HIV) responsible for AIDS, the protein capsid is surrounded by a lipid-containing outer envelope, derived from the modified plasma membrane of the host cell as the virus buds from the host cell surface (Fig. 1.12b). Bacteriophages (bacterial viruses) are among the most complex viruses (Fig.4.7c).

The T Bacteriophages consist of a polyhedral head containing DNA, a cylindrical stalk through which the DNA is injected into the bacterial cell, and tail fibres.



(a)



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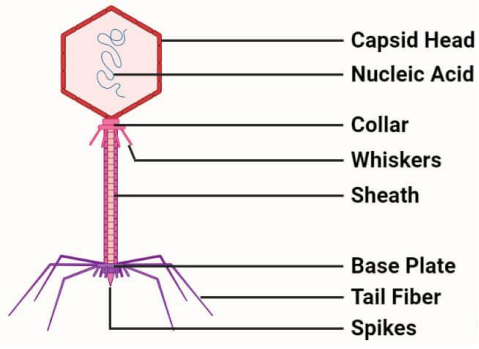


(c)

Fig3,7: Virus diversity. The structures of (a) an Adenovirus, (b) A Human Immunodeficiency Virus (HIV), and (c) a T-even bacteriophage

Each virus has on its surface a protein that is able to bind to a particular surface component of its host cell. For instance, the protein that projects from the surface of the HIV particle- labelled gp120 in Fig 1:12b, gp=glycoprotein interacts with a specific protein, called CD4 on the surface of certain white blood cells, facilitating entry of the virus into its host cell. Viral-host interaction exhibits cell and species specificity which could be narrow or wide range.

Virions, by themselves are unable to reproduce, metabolize, or carry on any of the other activities associated with life. As such, they are not considered to be organisms and are not described as being alive. Notwithstanding, once a virus has become attached to the outer surface

of a host cell and has passed through that cell's outer membrane, the genetic material of the virus contains the necessary information to alter the activities of the host cell: Such alterations favour viral proliferation and cause infections of which two basic forms have been described.

(1) In most cases, the virus arrests the normal synthetic activities of the host and redirects the cell to use its available materials to manufacture viral nucleic acids and proteins which assemble into new virions. Ultimately, the infected cell ruptures (lyses) and releases a new generation of viral particles, capable of infecting neighbouring cells. This is known as Lytic infection

(2) In other cases, the infecting virus does not lead to the rupture or death of the host cell, but instead, inserts its DNA into the DNA of the host cell's chromosomes. The provirus (inserted DNA) can have different effects, depending on the type of virus and host cell.

The fact that human viruses utilize host to carry out nearly all of their metabolic activities make it difficult to find drags that combat steps in the viral infection cycle that are not harmful to their human hosts.

Albeit, viruses are not without their virtues. Viruses have gene used to study the mechanism of DNA replication and gene expression in the much more complex hosts. In addition, viruses are now being used as a means to introduce foreign genes into human cells, a technique will serve as the basis for the treatment of human disease by gene therapy. Again, insect-killing viruses may play an increasing role in the future in the eradication of insect pests.

3.8 VIROIDS

It came as a surprise when Diener in 1971, discovered a pathogen which he named viroid, observed to be smaller than a virus. Viroid consist of a small circular RNA molecule that totally lacks a protein coat and they cause diseases by interfering with the Cell's normal path of gene expression. The effect on crops can be serious.

The discovery of a different type of infectious particle, prion (composed solely of proteins, no nucleic acid) even simpler and smaller than a viroid has been described in Creutzfeld-Jakob Disease (CJD), mad cows and human prion diseases.

MODULE TWO

STUDY SESSION 1

ACID-BASE BALANCE

Introduction

To achieve acid-base balance, there must be a balance between the intake or production of hydrogen ions and net removal of hydrogen ions from the body. The various mechanisms that contribute to the regulation of hydrogen ion concentration will be discussed in this module.

Learning Outcomes for Study Session 3

After you have studied this study session, you should be able to:

1. Define an acid, a base and a buffer (SAQ 1)
2. List 3 mechanism of regulation of hydrogen ions (SAQ 2)
3. Mention the different blood buffer systems (SAQ 3)

Key Terms: *acid, base, buffer*

1.1 ACIDS, BASES AND BUFFERS

• An acid is defined as a substance that releases protons or hydrogen ions (H +), e.g. hydrochloric acid (HCI), carbonic acid (H2CO 3).

$$HCl \longrightarrow H^+ + Cl^-$$

$$H_2CO_3 \longrightarrow H^+ + CO_3^-$$

A base is a substance that accepts protons or hydrogen ions, e.g. bicarbonate ion (HCO 3–), and HPO4–

$$HCO_3^- + H^+ \longrightarrow H_2CO_3$$

$$HPO_4^{--} + H^+ \longrightarrow H_2PO_4^-$$

Proteins in the body also function as bases, because some of the amino acids accept hydrogen ions, e.g.
haemoglobin in red blood cells and plasma protein especially albumin are the most important of the body's bases.

Buffer is a solution of weak acid and its corresponding salt which resists a change in pH when a small amount of acid or base is added to it. By buffering mechanism, a strong acid (or base) is replaced by a weaker one

1.2 NORMAL pH OF THE BODY FLUIDS

The normal pH of arterial blood is 7.4, whereas the pH of venous blood and interstitial fluids is about 7.35 because of the extra amounts of carbon dioxide (CO 2), released from the tissues to form H2 CO 3 in these fluids. Thus, the pH of blood is maintained within a remarkable constant level of 7.35–7.45. Normal pH of body fluids are shown in Table are shown in the table below

Normal pH of body fluids

Body fluid	pH
Extracellular fluid	
– arterial blood	7.40
– venous blood and interstitial fluid	7.35
Intracellular fluid	6.0–7.4
Urine	4.5–8.0
Gastric HCl	Gastri0.8

The maintenance of a constant pH is important because, the activities of almost all enzyme systems in the body are influenced by hydrogen ion concentration. Therefore, changes in hydrogen ion

concentration alter virtually all cell and body functions, the conformation of biological structural components and uptake and release of oxygen

1.3 Metabolic Sources of Acids and Bases Which Tend to Alter pH of the Body Fluids.

Metabolic Sources of Acids;

During metabolic processes two types of acids are produced:

Fixed acids or non-volatile acids.

Fixed acids are non-gaseous acids such as:

• Phosphoric and sulfuric acids, produced from the sulfur and phosphorus of proteins and lipoproteins.

• Organic acids such as pyruvic acid, lactic acid, keto acids (acetoacetic and β-hydroxybutyric acid), and uric acid.

Volatile acids

The physiologically important volatile acid is carbonic acid (H_2CO_3). It is equivalent to 36 liters of 1.0 N acid.

Metabolic Sources of Bases

Catabolism of few food materials produce bases. For example:

• Citrate salts of fruit juices may produce bicarbonate salt.

• Deamination of amino acids produces ammonia.

• Formation of biphosphate and acetate also contributes to alkalinizing effect

1.4REGULATION OF BLOOD pH

To maintain the blood pH at 7.35 –7.45, there are three primary systems that regulate the hydrogen ion concentration in the body fluids. These are:

1. Buffer mechanism: First line of defense.

2. The respiratory mechanism: Second line of defense.

3. Renal mechanism: Third line of defense.

The first two lines of defense keep the hydrogen ion concentration from changing too much until the more slowly responding third line of defense, the kidneys, can eliminate the excess acid or base from the body.

1.4.1 Buffer Systems and their Role in Acid-base Balance

The buffer systems of the blood, tissue fluids and cells; immediately combine with acid or base to prevent excessive changes in hydrogen ion concentration.

• Buffer systems do not eliminate hydrogen ions from the body or add them to the body but only keep them tied up until balance can be re-established.

Blood Buffers

The different buffer systems present in human body are given below.

1. Buffers of extracellular fluid present in plasma are

i. Bicarbonate buffer (NaHCO 3/H 2 CO3).

ii. Phosphate buffer (Na2HPO 4 /NaH 2PO 4).

iii. Protein buffer (Na protein/H protein).

2. Buffers of intracellular fluid present in RBCs

i. Bicarbonate buffer (KHCO 3 /H 2CO 3).

ii. Phosphate buffer (K 2HPO 4 /KH2PO 4).

iii. Hemoglobin buffer (KHb/HHb), (KHbO 2/H HbO2).

The Bicarbonate Buffer System

The bicarbonate buffer system is the most important extracellular buffer. Under physiological conditions, with a plasma pH 7.4, the ratio of bicarbonate to carbonic acid (HCO_3-/ H_2CO_3) is 20:1.

Mechanism Of Action Of Bicarbonate Buffer

• When a strong acid, such as HCI, is added to the bicarbonate buffer solution, the increased hydrogen ions are buffered by HCO3– .

• Thus, hydrogen ions from strong acid HCI react with HCO 3 to form very weak acid H_2 CO $_3$.

• The opposite reactions take place when a strong base such as sodium hydroxide (NaOH), is added to the bicarbonate buffer solution.

$$NaOH + H_2CO_3 \rightarrow NaHCO_3 + H_2O$$

• In this case, the hydroxyl ion (OH –) from NaOH combines with H 2CO 3 to form weak base HCO 3– . Thus, strong base NaOH is replaced by a weak base $NaHCO_3$

• In this case, the hydroxyl ion (OH –) from NaOH combines with H 2CO 3 to form weak base HCO 3– . Thus, strong base NaOH is replaced by a weak base NaHCO. Plasma bicarbonate is a measure of the base that remains after all acids, stronger than carbonic, have been neutralized. It represents the reserve of alkali available for the neutralization of such strong acids and it has been termed as the ***alkali reserve***.

The Phosphate Buffer System

• The phosphate buffer system is not important as a blood buffer, it plays a major role in buffering renal tubular fluid and intracellular fluids.

• Its concentration in both plasma and erythrocytes is low, i.e. only 8% of the concentration of the bicarbonate buffer. Therefore, the total buffering power of the phosphate system in the blood is much less than that of the bicarbonate buffering system.

Mechanism of action of phosphate buffer

• The main elements of the phosphate buffer system are HPO_4^{--} and $H2PO_4$. When a strong acid such as HCl is added to a phosphate buffer system, the H+ is accepted by the base HPO 4– – and converted to H2PO 4– and strong acid HCl is replaced by a weak acid H2 PO 4 and decrease in pH is minimized.

$$HCl + Na_2HPO_4 \rightarrow NaH_2PO_4 + NaCl$$

• When strong base, such as NaOH, is added to the buffer system, the OH– is buffered by the H 2PO 4– to form HPO4– – and water. Thus, strong base NaOH is replaced by weak base HPO 4– – causing slight increase in the pH. At a plasma pH of 7.4 the ratio HPO 4– – : H 2 PO 4– is 4:1

$$NaOH + NaH_2PO_4 \rightarrow Na_2HPO_4 + H_2O$$

Protein Buffer;

Plasma protein buffer (Na protein/H protein)

• In the blood, plasma proteins especially albumin act as buffer because:

– Proteins contain a large number of dissociable acidic (COOH) and basic (NH2) groups in their structure.

– In acid solution they act as a buffer in that, the basic amino group (NH2) takes up excess H+ ions forming (NH 3+).

– Whereas in basic solutions the acidic COOH groups give up hydrogen ion forming OH – of

alkali to water.

– Other important buffer groups of proteins in the physiological pH range, are the imidazole groups of histidine. Each albumin molecule contains 16 histidine residues.

Hemoglobin Buffer.

Hemoglobin is the major intracellular buffer of blood which is present in erythrocytes.

• It buffers carbonic acid (H 2CO 3) and its anhydride CO 2 from the tissues.

Action of hemoglobin buffer

Hemoglobin works effectively in cooperation with the bicarbonate system. The several reactions occur in regulation of body pH by hemoglobin are given below.

• In the tissues the CO_2 formed by metabolic processes diffuses into red blood cell and is converted to carbonic acid (H_2CO_3) by carbonic anhydrase (CA). The H 2CO 3 thus formed ionizes to form H+ and HCO 3– and results in decrease in blood pH.

• The deoxyhemoglobin (KHb) acts as a buffer and accepts these H + ions to form HHb (weak acid) and KHCO 3. Thus, H+ ions produced from H 2CO 3 does not cause any change in pH as show figure 1 below.

• Now, the increase in bicarbonate concentration in the erythrocyte leads to diffusion of these ions from the erythrocytes into the plasma where its concentration is low.

• The bicarbonate diffused from erythrocyte to plasma is transported to the lungs.

• In the lungs deoxyhemoglobin (HHb) carried from tissue is oxygenated to oxyhemoglobin (HHbO 2).

• Since, oxyhemoglobin (HHbO2) is a stronger acid results in the release of H + , which is buffered by KHCO 3– to give H 2 CO 3 and KHbO 2 . This buffering effect reduces the pH change as a result of the oxygenation of HHb

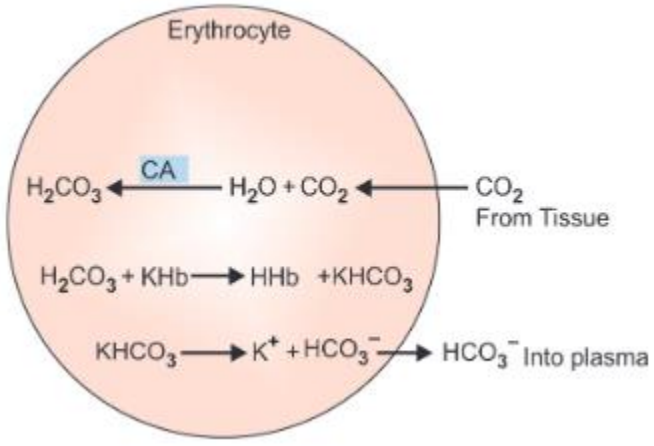
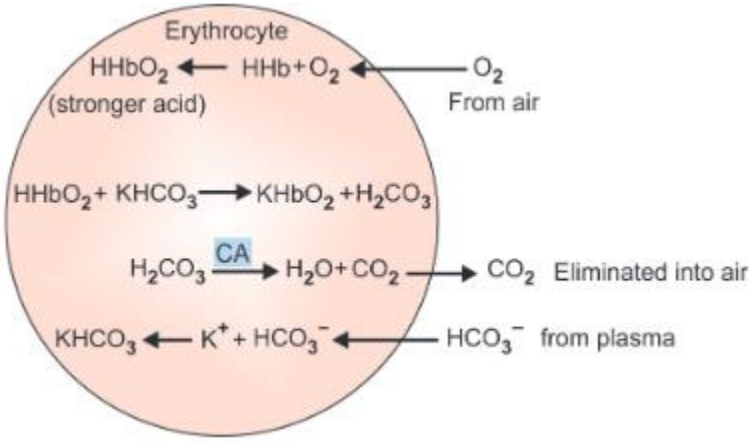


Figure 1.0: Action of hemoglobin buffer in tissuewhere, CA: Carbonic anhydrase

As the concentration of HCO3– in the erythrocytes is reduced, HCO 3– from the plasma where its concentration is higher entering into the erythrocyte.

The carbonic acid formed is converted quickly in the presence of the carbonic anhydrase (CA) to carbon dioxide and water which is eliminated by ventilation as in figure 2 below.



1). A 50-year-old male was admitted with a history of chronic obstructive airways disease for many years. On examination, he was found cyanosed, and breathless. Blood sample was analyzed with the following results:

Blood pH = below normal

pCO 2 = markedly elevated

(HCO3–) = markedly elevated.

Questions

1. Identify the nature of acid-base disorder.
2. What could be the cause of elevated pCO2 ?
3. What could be the cause of elevated (HCO 3–)?

acidosis.

Summary of Study Session 1 module 2

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 1, Module 2.

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting.

Links to OERs

References/ Suggestions for Further Reading

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MODULE TWO

SESSION 2

ACIDOSIS AND ALKALOSIS

Introduction

Acid-base balance depends on the ratio HCO 3– / H2CO 3 which is constant at 20:1 at physiological pH. Any alteration produced in the ratio between carbonic acid and bicarbonate results in an acid-base imbalance and leads to acidosis or alkalosis.

• Acidosis may be defined as an abnormal condition caused by the accumulation of excess acid in the body or by the loss of alkali from the body.

• Alkalosis is an abnormal condition caused by the accumulation of excess alkali in the body or by the loss of acid from the body.

Acidosis and alkalosis are classified, in terms of their immediate cause, as follows;

1.Metabolic acidosis: Decrease in bicarbonate (HCO3–) concentration

2. Respiratory acidosis: Increase in H 2 CO 3 concentration.

3. Metabolic alkalosis: Increase in bicarbonate (HCO 3–) concentration.

4. Respiratory alkalosis: Decrease in H 2 CO 3 concentration

Learning Outcomes for Study Session 3

After you have studied this study session, you should be able to:

1. Define metabolic acidosis and metabolic alkalosis(SAQ 1)
2. Define respiratory acidosis and respiratory alkalosis (SAQ 2)
3. Men (SAQ 3)

Key Terms: ***acidosis, alkalosis***

2.1 Metabolic Acidosis

A fall in blood pH due to a decrease in bicarbonate levels of plasma is called metabolic acidosis.

• Decrease in bicarbonate levels may be due to:

– Increased production of acids. In uncontrolled diabetes mellitus and starvation there is an excessive production of acetoacetic acid and β-hydroxybutyric acid. These acids are buffered by utilizing base component (i.e. HCO3–) of the bicarbonate buffer. Consequently, the concentration of bicarbonate ions falls, giving rise to bicarbonate deficit and results in metabolic acidosis (ketoacidosis).

– Excessive loss of bicarbonate occurs in the urine in renal tubular dysfunction and form GI tract in severe diarrhea.

Compensatory mechanisms

• Metabolic acidosis is compensated by:

1. Increasing rate of respiration to wash out CO 2 (hence H 2 CO 3) faster. Consequently, the ratio

   HCO 3– : H2 CO3 is elevated.

2. Increasing excretion of H + ions as NH4+ ions.

3. Increasing elimination of acid (H2PO 4–) in the urine.

All these compensatory mechanisms tend to reduce carbonic acid to keep the pH in the normal range and a compensated acidosis result.

2,2 Respiratory Acidosis

It results from an increase in concentration of carbonic acid (H2 CO 3) in plasma. An increase in concentration of H 2CO 3 is due to decrease in alveolar ventilation, and that leads to retention of CO2. Decreased alveolar ventilation may occur in following circumstances.

• Obstruction to respiration: This may occur in pneumonia, emphysema, asthma, etc.

• Depression of respiration: Administration of respiratory depressant toxic drugs, e.g. morphine depresses the respiratory center.

2.3 Metabolic Alkalosis

A rise in blood pH due to rise in the bicarbonate levels of plasma is called metabolic alkalosis. This is seen in the following conditions:

• Loss of gastric juice along with H + ions in prolonged and severe vomiting.

• Therapeutic administration of large dose of alkali (as in peptic ulcer) or chronic intake of excess antacids.

Compensatory mechanisms

1. Increased excretion of alkali (HCO_3^-) by the kidney

2. Diminished formation of ammonia

3. Respiration is depressed to conserve CO_2.

2.4 Respiratory Alkalosis

A rise in blood pH due to lowered concentration of CO_2 or H_2CO_3, due to hyperventilation. This occurs in the following conditions:

• Anxiety or hysteria

• Fever

• Hot baths

• At high altitude

• Working at high temperature, etc.

Compensatory mechanisms

1. Increase in renal reabsorption of bicarbonate.

2. Rise in urinary acid ($H_2PO_4^-$) and ammonia.

Compensatory mechanisms

1. Reduction of urinary ammonia formation

2. Increased excretion of bicarbonate.

2.5 Mixed Acid-base Disturbances

• Respiratory and metabolic disorders of acid-base balance can occur together and called mixed acid-base disturbances.

• For example, some patients with chronic renal failure (which causes a primary metabolic acidosis) may also have chronic obstructive airways disease, which causes a primary respiratory acidosis.

• Plasma (H+) will be increased in these patients, but the results for plasma CO 2 and concentration of(HCO3–) cannot be predicted. The history and clinical findings must be taken into account.

2.6 ANION GAP

• The concept of anion gap originally was devised as a quality control rule when it was found that if the sum of the Cl – and HCO 3– values was subtracted from the Na + and K+ values the difference or 'gap' averaged 16 mmol/L in healthy individuals.

• The concentration of anions and cations in plasma must be equal to maintain electrical neutrality.

Therefore, there is no real anion gap in the plasma. Anion gap is not a physiological reality.

• The anion gap is the difference between unmeasured anions and unmeasured cations and is estimated as:

Anion gap= ([Na +] + [K +]) – ([Cl –] + [HCO3–])

= (142 + 4) – (103 + 27)

= 146 – 130

= 16 mEq/L

• The most important unmeasured cations include calcium, magnesium, and the major unmeasured anions are albumin, phosphate, sulphate and other organic anions. The anion gap ranges between 8–16 mEq/L.

• Acid base disorders are often associated with alterations in the anion gap.

• In metabolic acidosis the anion gap can increase or remain normal depending on the cause of acidosis.

Summary of Study Session 2 of module 2

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 3

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting.

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MODULE THREE

STUDY SESSION 1

CARBOHYDRATES

Introduction

Definition:

Carbohydrates are defined chemically as aldehyde or ketone derivatives of the higher polyhydric alcohols, or compounds which yield these derivatives on hydrolysis or aldehyde or ketone with multiple hydroxyl groups. They have important structural and metabolic roles. Animals can synthesize carbohydrates from lipid glycerol and amino acids but most animal carbohydrate is derived ultimately from plants. Glucose is the most important carbohydrate. Most dietary carbohydrate is absorbed into the blood stream as glucose and other sugars are converted into glucose in the liver.

Learning Outcomes for Study Session 1, Module 3

After you have studied this study session, you should be able to:

Identify the; (SAQ .1)

List 5; (SAQ .2)

List 5 objectives; (SAQ .3)

Key Terms: carbohydrates.

1.1 USES OF CARBOHYDRATES

The uses of carbohydrates include

They serve as energy stores, fuels and metabolic intermediates. Starch in plants and glycogen in animals are the polysaccharides that can be rapidly mobilized to yield glucose, a prime fuel for the generation of energy.

I. Ribose and deoxyribose sugars form part of the structural frame work of RNA and DNA.
II. Polysaccharides are structural elements in the cell walls of bacteria and plants and in the exoskeleton of arthropods.
III. Carbohydrates are linked to many proteins and lipids. (Glycoproteins and glycoproteins and glycolipids)
IV. Studies have shown revealed that carbohydrate units on cell surfaces play important role in cell to cell recognition processes.
V. Degradation products act as "promoters" or 'catalysts'.
VI. Certain carbohydrate derivatives are used as drugs such as cardiac glycosides or antibiotics.
VII. Lactose is the principal sugar of milk—in lactating mammary gland.
VIII. They are utilized for synthesis of other substances such as fatty acids, cholesterol, amino acid, etc.
IX. Constituents of mucopolysaccharides form the ground substance of mesenchymal tissues.

1.2CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are divided into four major groups— monosaccharides, disaccharides, oligosaccharides and polysaccharides.

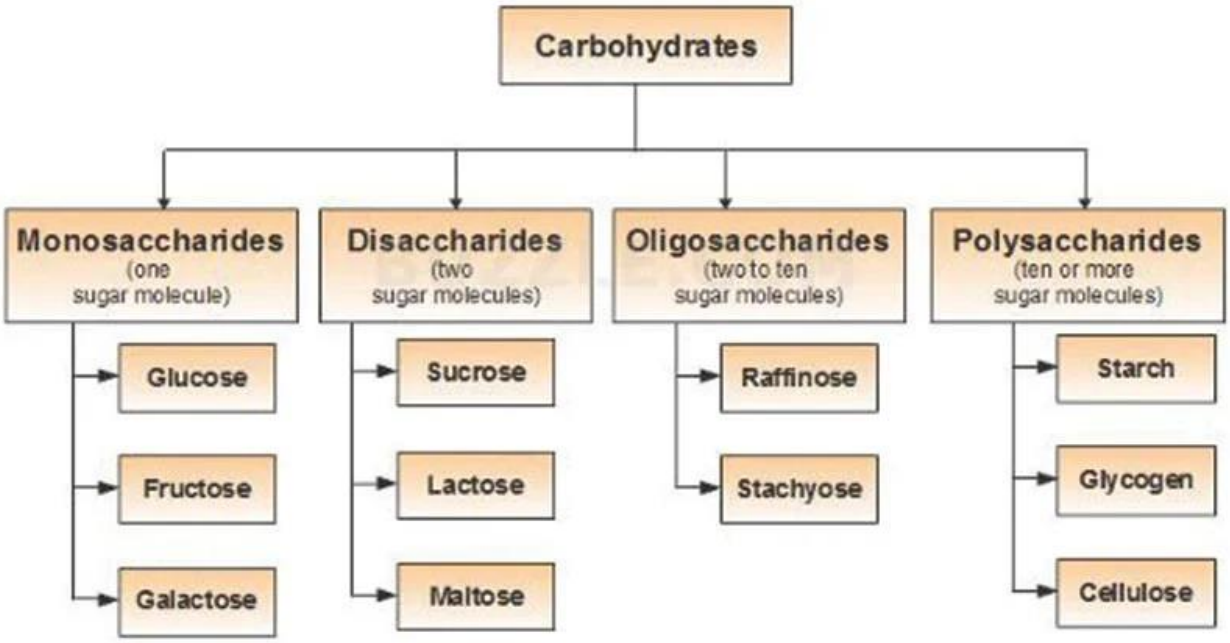


Fig. fig. 1.1: Summary of classification of carbohydrates

Credit. Microbenotes.com.

Monosaccharides (Simple sugars);

Are those which cannot be hydrolysed further into simpler forms. The empirical is (CH_2O) n. They can be subdivided further: (a) Depending upon whether ketone (– CO) or aldehyde (– CHO) groups are present as ketoses or aldoses. (b) Depending upon the number of carbon atoms they possess, as trioses, tetroses, pentoses, hexoses, etc.

TABLE T; SOME MONOSACCHARIDE CLASSIFICATION CATEGORIES

General formula	Aldosugars	Ketosugars
Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose

i. Disaccharides: These are sugars which yield two molecules of the same or different molecules of monosaccharide on hydrolysis.

ii. General formula: $Cn(H2O)_{n-1}$

Examples include;

• Maltose yields 2 molecules of glucose on hydrolysis.

• Lactose yields one molecule of glucose and one molecule of galactose on hydrolysis.

• Sucrose yields one molecule of glucose and one molecule of fructose on hydrolysis.

• Lactulose a ketodisaccharide

3. Oligosaccharides: These are sugars which yield 3 to 10 monosaccharide units on hydrolysis, e.g. Maltotriose.

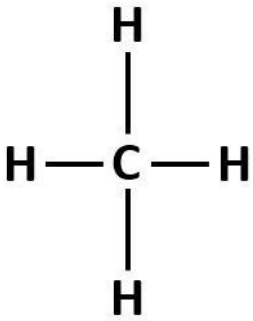
4. Polysaccharides (Glycans): These are sugars which yield more than ten molecules of monosaccharides on hydrolysis. General formula: $(C_6H_{10}O_5)_n$ Polysaccharides are further divided into two groups:

a. Homopolysaccharides (homoglycans): Polymer of same monosaccharide units. Examples—Starch, glycogen, inulin, cellulose, dextrins, dextrans.

b. Heteropolysaccharides (heteroglycans): Polymer of different monosaccharide units or their derivatives. Example—Mucopolysaccharides (glycosaminoglycans).

1.3.1 General Properties of monosaccharides in Reference to Glucose

Asymmetric carbon: A carbon atom to which four different atoms or groups of atoms are attached is said to be asymmetric.



Asymmetric carbon

Van't Hoff's rule of 'n':

The number of possible isomers of any given compound depends upon the number of asymmetric carbon atoms the molecule possesses. According to Van't Hoff's rule of 'n'; 2n equals the possible isomers of that compound, where, n = represents the number of asymmetric carbon atoms in a compound.

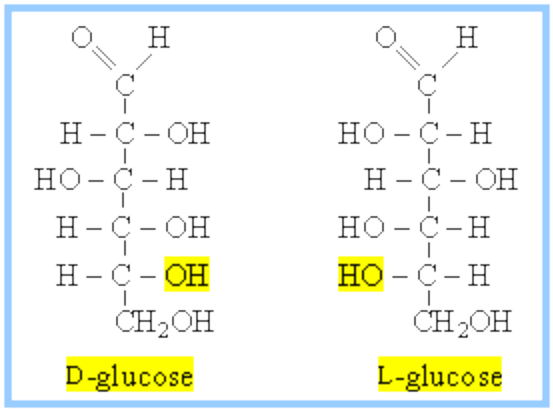
Stereoisomerism:

The presence of asymmetric carbon atoms in a compound gives rise to the formation of isomers of that compound. Such compounds which are identical in composition and differs only in spatial configuration are called stereoisomers.

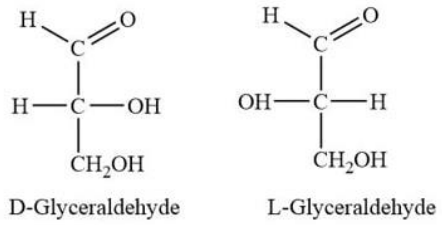
Two such isomers of glucose—*D-Glucose and L-Glucose are mirror image of each other.*

D-Series and L-Series:

The orientation of the H and OH groups around the carbon atom just adjacent to the terminal primary alcohol carbon, e.g. C-atom 5 in glucose determines the series. ***When the – OH group on this carbon is on the right, it belongs to D-series, when the – OH group is on the left, it is a member of L-series.***



L-glyceraldehyde and D-glyceraldehyde are the enantiomers of glyceraldehyde. These structures can be shown as,



Most of the monosaccharides occurring in mammals are D-sugars, and the enzymes responsible for their metabolism are specific for this configuration.

Optical activity:

Presence of asymmetric carbon atoms also confers optical activity on the compound. When a beam of plane-polarised light is passed through a solution exhibiting optical activity, it will be rotated to the right or left in accordance with the type of compound, i.e. the optical isomers or enantiomorphs; when it is rotated to right, the compound is called Dextrorotatory (D or + sign), when rotated to left, the compound is called Levorotatory (I or – sign).

Racemic:

When equal amounts of dextrorotatory and levorotatory isomers are present, the resulting mixture has no optical activity, since the activities of each isomer cancels each other. Such a mixture is said to be **Racemic.**

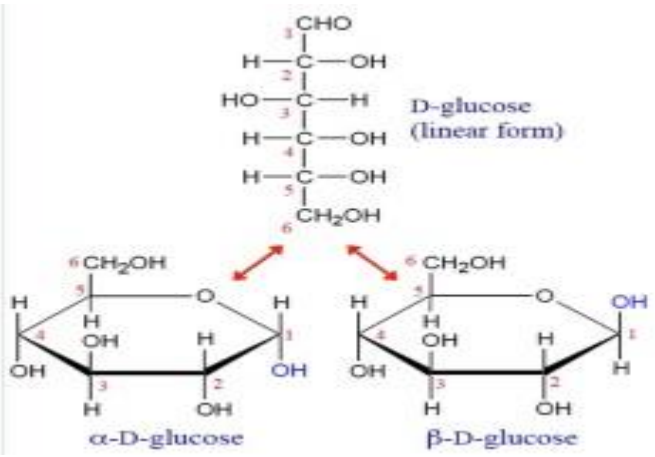
Resolution:

The separation of optically active isomers from a racemic mixture is called resolution.

1.4 CYCLIC STRUCTURES

As the two reacting groups aldehyde and alcoholic group belong to the same molecule, a cyclic structure takes place. If the open-chain form of D-Glucose, which may be called as Aldehydo-D-Glucose is taken, and condense the aldehyde group on carbon-1, with the alcoholic-OH group on carbon-5, two different forms of glucose are formed. ***When the OH group extends to right, it is α-D-Glucose and it extends to left, it is β-D-Glucose.***

Anomers and anomeric carbon: Carbon-1, after cyclization has four different groups attached to it and thus it becomes now asymmetric. The two cyclic compounds, α and β have different optical rotations, but they will not be same because the compounds as a whole are not mirror-images of each other. Compounds related in this way are called anomers and carbon-1, after cyclisation becomes asymmetric is called now anomeric carbon atom



MUTAROTATION

When an aldohexose is first dissolved in water and the solution is put in optical path so that plane polarized light is passed, the initial optical rotation shown by the sugar gradually changes until a constant fixed rotation characteristic of the sugar is reached. This phenomenon of change of rotation is called as mutarotation.

Explanation: Ordinary crystalline glucose happens to be in the α-form. The above change in optical rotation represents a conversion from α-Glucose to an equilibrium mixture of α and β-forms. The mechanism of mutarotation probably involves opening of the hemiacetal ring to form traces of the aldehyde form, and then recondensation to the cyclic forms. The aldehyde form is extremely unstable and exists only as a transient intermediate.

1.5HAWORTH PROJECTION

(a) Pyranoses:

Haworth in 1929 suggested that the six-membered ring forms of the sugars be called Pyranoses, because Pyran possesses the same ring of 5 carbons and oxygen.

(b) Furanoses:

Similarly, Haworth designated sugar containing 5-membered rings as the furanoses, because furan contains the same ring.

The Pyranose forms of the sugars are internal hemiacetals formed by combination of the aldehyde or ketone group of the sugar with the OH group on the 5th carbon from the aldehyde or ketone group. Similarly, the furanose forms of the sugars are formed by reaction between the

aldehyde or ketone group with the OH group on the 4th carbon from the aldehyde or ketone group. Harworth projection diagram????

1.6 Epimers and Epimerisation:

Two sugars which differ from one another only in configuration around a single carbon atom are termed Epimers. Eg Glucose and galactose are examples of an epimeric pairs which differ only with respect of C4 **Diagram** Similarly, mannose and glucose are epimers in respect of C2. **diagram**

Epimerisation:

Process by which one epimer is converted to other is called epimerisation and it requires the enzyme epimerase, e.g. conversion of galactose to glucose in liver.

Summary of Study Session 1 of module 3

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 3

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting.

Links to OERs

References/ Suggestions for Further Reading

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Are you in need of General Help as regards your studies? Do not hesitate to contact the …….

Center by e-mail or phone on:

MODULE 3

STUDY SESSION 2

MONOSACCHARIDES

Introduction

This is a continuation of the previous session and we are going to be discussing monosaccharides of biological importance

Learning Outcomes for Study Session 4

After you have studied this study session, you should be able to:

Identify the; (SAQ)

List 5; (SAQ)

List 5 objectives; (SAQ)

Give 2 reasons; (SAQ)

Key Terms:

1.1 MONOSACCHARIDES OF BIOLOGICAL IMPORTANCE

(a) Trioses:

Both D-glyceraldehyde and dihydroxyacetone occur in the form of phosphate esters, as intermediates in glycolysis. The aldehyde form is extremely unstable and exists only as a transient intermediate. They are also the precursors of glycerol, which the organism synthesises and incorporates into various types of lipids.

(b) Tetroses:

Erythrose-4-P occurs as an intermediate in hexosemonophosphate shunt which is an alternative pathway for glucose oxidation.

(c) Pentoses

• D-ribose is a constituent of nucleic acid RNA; also as a constituent of certain coenzymes, e.g. FAD, NAD, coenzyme A.

• D-2-deoxyribose is a constituent of DNA.

• Phosphate esters of ketopentoses—D-ribulose and D-xylulose occur as intermediates in HMP shunt.

• L-xylulose is a metabolite of D-glucuronic acid and is excreted in urine of humans afflicted with a hereditary abnormality in metabolism called pentosuria.

• L-fucose (methyl pentose): occurs in glycoproteins.

• D-Lyxose: It forms a constituent of lyxoflavin isolated from human heart muscle whose function is not clear.

(d) Hexoses

1. D-Glucose:

(Synonyms: Dextrose, Grape Sugar)

• It is the chief physiological sugar present in normal blood continually and at fairly constant level, i.e. about 0.1 per cent.

• All tissues utilise glucose for energy. Erythrocytes and Brain cells utilise glucose solely for energy purposes.

• Occurs as a constituent of disaccharide and polysaccharides.

• Stored as glycogen in liver and muscles mainly.

• Shows mutarotation.

2. D-galactose:

In combination it occurs both in plants and animals.

• Occurs as a constituent of milk sugar lactose and also in tissues as a constituent of galactolipid and glycoproteins.

• It is an epimer of glucose and differs in orientation of H and OH on carbon-4.

• It is less sweet than glucose and less soluble in water.

• It is dextrorotatory and shows mutarotation.

• On oxidation with hot HNO_3, it yields dicarboxylic acid, mucic acid; which helps in its identification, since the crystals of mucic acid are not difficult to produce and have characteristic shape.

D-fructose:

It is a ketohexose and commonly called as fruit sugar, as it occurs free in fruits.

• It is very sweet sugar, much sweeter than sucrose and more reactive than glucose. It occurs as a constituent of sucrose and also of the polysaccharide inulin. It is laevorotatory and hence is also called laevulose.

• Exhibits mutarotation. Biomedical Importance Seminal fluid is rich in fructose and sperms utilise fructose for energy. Fructose is formed in the seminiferous tubular epithelial cells from glucose.

D-mannose:

It does not occur free in nature but is widely distributed in combination as the polysaccharide mannan, e.g. in ivory nut. In the body, it is found as a constituent of glycoproteins.

Sedoheptulose:

It is a ketoheptose found in plants of the sedum family. Its phosphate is important as an intermediate in the HMP-shunt and has been identified as a product of photosynthesis.

1.2 IMPORTANT PROPERTIES OF MONOSACCHARIDES

1. Iodocompounds:

An aldose when heated with conc. HI loses all of its oxygen and is converted into an iodocompound.

2. Acetylation or ester formation:

The ability to form sugar esters, e.g. acetylation with acetyl chloride (CH3 – COCl) indicates the presence of alcohol groups. Due to alcoholic –OH groups, it can react with anhydrides and chlorides of many organic and inorganic acids, like acetic acid, phosphoric acid, sulphuric and benzoic acids to form esters of corresponding acids.

3, Osazone formation:

It is a useful means of preparing crystalline derivatives of sugars. Osazones have characteristic melting points, crystal structures, and precipitation time and thus are

valuable in identification of sugars. Preparation: They are obtained by adding a mixture of phenyl hydrazine hydrochloride and sodium acetate to the sugar solution and heating in a boiling water bath for 30 to 45 minutes. The solution is allowed to cool slowly by itself. Crystals are formed. A coverslip preparation is made on a clean slide and seen under the microscope.

Basis of reaction: The reaction involves only the carbonyl carbon (i.e. aldehyde or ketone group) and the next adjacent carbon. First, phenyl hydrazone is formed and then the hydrazone reacts with two additional molecules of phenyl hydrazine to form the osazones. The reaction with a ketose is similar.

Types of Crystals

• Glucosazone crystals: These are fine, yellow needles in fan-shaped aggregates or sheaves or crosses, typically described as Bundle of Hay. Glucose, mannose and fructose due to similarities of structures form the same osazones. But since the structure of galactose differs on C-4, that part of the molecule unaffected in osazone formation, it would form a different osazone. • Lactosazone crystals: These are irregular clusters of fine needles and look like a Powder puff.

• Maltosazone: These are star-shaped and compared to Sunflower petals.

4, Interconversion of sugars:

Glucose, fructose and mannose are interconvertible in solutions of weak alkalinity such as Ba(OH)2 or Ca(OH)2 These interconversions are due to the fact that all give the same Enediolform, which tautomerizes to all three sugars. This interconversion of related sugars by the action of dilute alkali is referred to as Lobry de Bruyn-Van Ekenstein reaction.

5, Oxidation to produce sugar acids:

When oxidised under different conditions, the aldoses may form: Monobasic Aldonic acids or Dibasic Saccharic acids or Monobasic uronic acids containing aldehyde groups thus possessing reducing properties.

6. Reduction of sugars to form sugar alcohols: The monosaccharides may be reduced to their corresponding alcohols by reducing agents such as Na-Amalgam. Similarly, ketoses may also be reduced to form ketoalcohol. Examples D-Glucose Yields D-Sorbitol, D-Galactose Yields D-Dulcitol, D-Mannose Yields D-Mannitol, Ketosugar D-Fructose Yields D-Mannitol and D-Sorbitol.

7, Action of acids on carbohydrates:

Polysaccharides and the compound carbohydrates in general are hydrolyzed into their constituent monosaccharides by boiling with dilute mineral acids such as HCl or H2SO4. • With conc. mineral acids the monosaccharides are decomposed.

• Pentoses yield the cyclic aldehyde "furfural" Twelve percent (12%) HCl has been found most satisfactory for decomposition.

Practical application

Hexoses are decomposed by hot strong mineral acids to give hydroxymethyl furfural, which decomposes further to produce laevulinic acid, formic acid, CO and CO2.

The furfural products thus formed by decomposition with strong mineral acid can condense with certain organic phenols to form compounds having characteristic colours. Thus it forms basis for certain tests used for detection of sugars. Eg

• Molisch's test: With α-naphthol (in alcoholic solution) gives red-violet ring. A sensitive reaction but non-specific, given by all sugars.

• Seliwanoff's test: With resorcinol, a cherry-red colour is produced. It is characteristic of D-fructose.

8, Action with alkalies:

With alkalies, monosaccharides react in various ways:

(a) In dilute alkali: The sugar will change to the cyclic α and β forms with an equilibrium between the two isomeric form.

• On standing: A rearrangement will occur which produce an equilibrated mixture of glucose, fructose and mannose through the common "enediol" form. • If it is heated to 37°C, the acidity increases, and a series of Enols are formed in which double bond shifts from the oxygen-carbon atoms

(b) In conc. alkali: The sugar caramelises and produces a series of decomposition products, yellow and brown pigments develop, salts may form, many double bonds between C-atoms are formed, and C bond C bonds may rupture.

9. Reducing action of sugars in alkaline solution: All the sugars that contain free sugar group undergo enolisation and various other changes when placed in alkaline solution. The enediol forms of the sugars are highly reactive and are easily oxidised by O_2 and other oxidising agents

and forms sugar acids. As a consequence they readily reduce oxidising ions such as Ag+. Hg+, Bi+++, Cu++ (cupric) and Fe (CN)6 – – –.

1.3 OTHER SUGAR DERIVATIVES OF BIOMEDICAL IMPORTANCE

1. Deoxy sugars:

Deoxy sugars represent sugars in which the oxygen of a –OH gr. has been removed, leaving the hydrogen. Thus, –CHOH or –CH2OH becomes –CH2 or –CH3. Several of the deoxy sugars have been synthesised and others are natural products. Deoxy sugars of biological importance are:

• 2-deoxy-D-Ribose is found in nucleic acid (DNA).

• 6-deoxy-L-Galactose is found as a constituent of glycoproteins, blood group substances and bacterial polysaccharides.

2. Amino sugars (hexosamines):

Sugars containing an –NH2 group in their structure are called amino sugars. Types: Two types of amino sugars of physiological importance are:

• Glycosylamine: The anomeric –OH group is replaced by an –NH2 group. Example: A compound belonging to this group is Ribosylamine, a derivative of which is involved in the synthesis of purines.

• Glycosamine (Glycamine): In this type, the alcoholic – OH group of the sugar molecule is replaced by – NH2 group. Two naturally occurring members of this type are derived from glucose and galactose, in which – OH group on carbon 2 is replaced by – NH2 group, and forms respectively Glucosamine and Galactosamine (Fig. 3.14). Biomedical Importance • N-acetyl derivative of D-Glucosamine occur as a constituent of certain mucopolysaccharides (MPS). • Glucosamine is the chief organic constituent of cell wall of fungi, and a constituent of shells of crustaceae (crabs, Lobsters, etc.), where it occurs as Chitin, which is made of repeating units of N-acetylated glucosamine. Hence Glucosamine is often called as Chitosamine. • Galactosamine occurs as N-acetyl-Galactosamine in chondroitin sulphates which are present in cartilages, bones, tendons and heart valves. Hence Galactosamine is also known as Chondrosamine. • Antibiotics: Certain antibiotics, such as Erythromycin, carbomycin, contain amino sugars. Erythromycin contains dimethyl amino sugar and carbomycin 3-amino-D-Ribose. It is believed that amino sugars are related to the antibiotic activity of these drugs.

4. Amino Sugar Acids

• Neuraminic acid: It is an amino sugar acid and structurally an aldol condensation product of pyruvic acid and D-Mannosamine. Neuraminic acid is unstable and found in nature in the form of acylated derivatives known as Sialic acids (N-acetyl Neuraminic acid —NANA).

• Muramic acid: Another amino sugar acid which is structurally a condensation product of DGlucosamine and Lactic Acid. Biomedical Importance

• Neuraminic acid and sialic acids occur in a number of mucopolysaccharides and in glycolipids like gangliosides.

• A number of nitrogenous oligosaccharides which contain neuraminic acid are found in human milk.

• Certain bacterial cell walls contain muramic acid.

•Neuraminidase is the enzyme which hydrolyses to split "NANA" from the compound.

1.4 Glycosides

Definition:

Glycosides are compounds containing a carbohydrate and a noncarbohydrate residue in the same molecule. In these compounds the carbohydrate residue is attached by an acetal linkage of carbon-I to the noncarbohydrate residue. The noncarbohydrate residue present in the glycoside is called as Aglycone. The aglycones present in glycosides vary in complexity from simple substances as methyl alcohol, glycerol, phenol or a base such as adenine to complex substances like sterols, hydroquinones and anthraquinones. The glycosides are named according to the carbohydrate they contain. If it contains glucose, forms glucoside. If galactose, it forms galactoside and so on.

Biomedical Importance

Glycosides are found in many drugs, spices and in the constituents of animal tissues. They are widely distributed in plant kingdom. • Cardiac glycosides: It is important in medicine because of their action on heart and thus used in cardiac insufficiency. They all contain steroids as aglycone component in combination with sugar molecules. They are derivatives of digitalis, strophanthus and squill plants, e.g. Digitonin 4 Galactose + Xylose + Digitogenin (Aglycone)

• Ouabain: A glycoside obtained from strophanthus sp. is of interest as it inhibits active transport of Na+ in cardiac muscle in vivo (Sodium Pump inhibitor).

• Phloridzin: A glycoside obtained from the root and bark of Apple tree. It blocks the transport of sugar across the mucosal cells of small intestine and also renal tubular epithelium; it displaces Na+ from the binding site of 'carrier protein' and prevents the binding of sugar molecule and produces glycosuria.

enzyme epimerase, e.g. conversion of galactose to glucose in liver.

Summary of Study Session 1 of module 3

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 3

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting.

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Center by e-mail or phone on:

MODULE THREE

STUDY SESSION 3

DISACCHARIDES

Introduction

They are the most abundant oligosaccharides found in nature. They are crystalline, water-soluble and sweet to taste. There are two types of disaccharides; *Reducing* disaccharides with free aldehyde or keto group e.g. lactose and maltose. *Non-reducing* disaccharides with no free aldehyde or keto group e.g. trehalose and sucrose. Three most common disaccharides of biological importance are: Sucrose, Lactose and Maltose. Their general molecular formula is $C_{12}H_{22}O_{11}$.The disaccharides are formed by the union of two constituent monosaccharides with the elimination of one molecule of water. The points of linkage, the glucosidic linkage varies, as does the manner of linking and the properties of the disaccharides depend to a great extent on the type of the linkage. If both of the two potential aldehyde/or ketone groups are involved in the linkage (the non-reducing type) the sugar will not exhibit reducing properties and will not be able to form osazones, e.g. sucrose. But if one of them is not bound in this way (the reducing type), it will permit reduction and osazone formation by the sugars, e.g. Lactose and Maltose.

Learning Outcomes for Study Session 4

After you have studied this study session, you should be able to:

Identify the; (SAQ 3.1)

List 5; (SAQ 3.2)

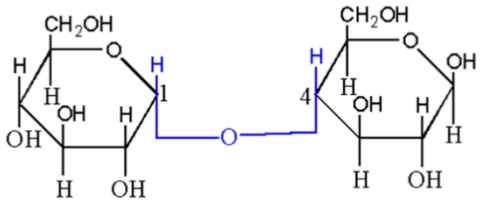
List 5 objectives; (SAQ 3.3)

Key Terms: Disaccharides, oligosaccharides

3.1 PROPERTIES OF DISACCHARIDES

Maltose:

Maltose or malt sugar is an intermediary in acid hydrolysis of starch and can also be obtained by enzyme hydrolysis of starch. In the human body, dietary starch digestion by Amylase in gut yields maltose, which requires a specific enzyme maltase to form glucose. It is a sweet sugar and is very soluble in water. Since it has one aldehyde 'free' or potentially free it has reducing properties, and forms characteristic osazones, which has characteristic appearance 'Sunflower'



Maltose

like.

As anomeric carbon of one glucose is free, can form α and β forms and exhibit mutarotation.

On hydrolysis Maltose yields two molecules of glucose.

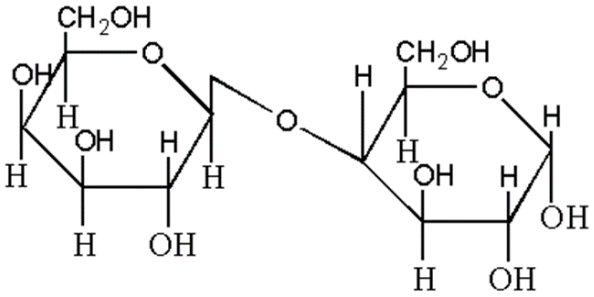
Lactose:

Lactose is milk sugar and found in appreciable quantities in milk and occurs at body temperature as an equilibrium mixture of the α and β forms in 2:3 ratio. It is not very soluble and is not so sweet. It is dextrorotatory.

Specific enzyme which hydrolyses is lactase present in intestinal juice.

On hydrolysis it yields one molecule of D-Glucose and one molecule of D-Galactose.

Because it contains galactose as one of its constituents, it yields mucic acid on being treated with Conc HNO3 after hydrolysis. As one of the aldehyde group is free or potentially free it has reducing properties and can form osazones. Lactosazone crystals have typical hedgehog shape or powder puff appearance. As anomeric carbon of glucose is free, can form α and β forms and exhibits mutarotation



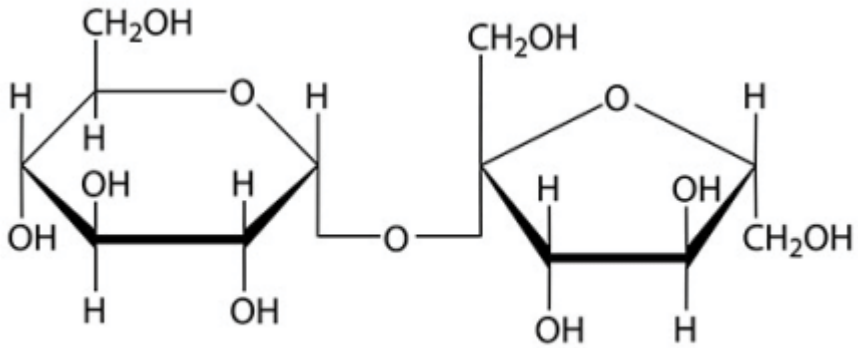
Lactose

Fearon's Test:

Serves to distinguish lactose simultaneously from sucrose and monosaccharides like glucose, galactose and fructose. A mixture of lactose and methylamine hydrochloride solution + NaOH solution, when heated in water bath at 56°C for ½ hour and then cooled by standing at room temperature, an intense red colour develops.

Sucrose:

This is ordinary table sugar. It is also called as 'Cane sugar', as it can be obtained from sugarcane. Also obtained from sugar beet, and sugar maple. Also occurs free in most fruits and vegetables, e.g. pineapples, and carrots. It is very soluble and very sweet and on hydrolysis yields one molecule of D-Glucose and one molecule of D-Fructose. The specific enzyme which hydrolyses sucrose is sucrase present in intestinal juice. As both aldehyde and ketone groups are linked together (α 1 → 2), it does not have reducing properties, and cannot form osazones. As both anomeric carbons are involved in 'linkage', it does not exhibit mutarotation



Glucose **Fructose**

Sucrose

Sucrose is dextrorotatory but its hydrolytic products are levorotatory because fructose has a greater specific levorotation than the dextrorotation of glucose. As the hydrolytic products invert the rotation, the resulting mixtures of glucose and fructose (hydrolytic products) is known as Invert Sugar and the process is called as Inversion. Honey is largely 'invert sugar' and the presence of fructose accounts for the greater sweetness of honey.

Lactulose:

This is a keto disaccharide. It is O-α-D-galctopyranosyl-(1→4)-β-D-fructo-furanose Heated milk (small amounts). Mainly obtained synthetically. It is not hydrolysed by intestinal enzymes, but fermented by intestinal bacteria. It is used clinically in medicine as an osmotic laxative.

Table x Differentiation of lactose from sucrose Lactose

S/N	Lactose	Sucrose
1	Known also as 'milk sugar'	Common table sugar (cane sugar)
2	Structurally one molecule of D-Glucose and one molecule of D-Galactose are joined together by (β 1 → 4) glucosidic linkage	Structurally one molecule of D-Glucose and one molecule of D-Fructose joined together (α 1→ 2) glucosidic linkage
3	Hydrolysed to give one molecule of glucose and one molecule of galactose	Hydrolysed to give one molecule of glucose and one molecule of fructose
4	Specific enzyme which hydrolyses is called lactase, which is present in intestinal juice	Specific enzyme which hydrolyses is called Sucrase (Invertase) which is present in intestinal juice
5	Dextrorotatory disaccharide but hydrolytic products are levorotatory	Also dextrorotatory Hydrolytic products are called invert sugars and process is called Inversion.
6	As anomeric carbon is free, can form α and β forms and exhibits mutarotation.	As both anomeric carbons are involved in linkage, cannot form α and β-forms and does not exhibit mutarotation
7	Can reduce alkaline copper sulphate solution like Benedict's qualitative reagent, Fehling's solution	Does not reduce alkaline copper sulphate solution.
8	Does not reduce Berfoed's solution	Does not reduce Berfoed's solution
9	Forms Osazone. Lactosazone crystals have typical hedge-hog shape or Powder puff	Cannot form osazones
10	Hydrolytic products on treatment with conc. HNO3 can form "mucic acid"	Cannot form mucic acid

11	Fearon's test is positive	Fearon's test is negative
12	Can be synthesised in lactating mammary gland	Cannot.
13	In lactating mother lactose may appear in urine, producing lactosuria	Does not

3.2 OLIGOSACCHARIDES:

Oligosaccharides contain 2 to 10 monosaccharide units. There are two types; the disaccharide and trisaccharide.

Examples of Disaccharides have been given above and an example of trisaccharide is Maltotriose.

Biomedical Importance:

Integral membrane proteins contain covalently attached carbohydrate units, oligosaccharides, on their extracellular face. Many secreted proteins, such as antibodies and coagulation factors also contain oligosaccharide units. Blood group substances also contain oligosaccharide sugar. These carbohydrates are attached to either the side-chain O_2 atom of serine or threonine residues by O-glucosidic linkages or to the side chain nitrogen of Asparagine residues by N-glucosidiclinkages. N-linked oligosaccharides contain a common pentasaccharide core consisting of three mannose and two N-acetyl glycosamine residues. Additional sugars are attached to this common core in many different ways to form the great variety of oligosaccharide patterns found in glycoproteins. Carbohydrates participate in molecular targeting and cell-cell recognition.

Summary of Study Session 3, module 3

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 3 module 3

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and

discuss them with your facilitator in your next study centre meeting. You can check your answers

Links to OERs

References/ Suggestions for Further Reading

MODULE 3

STUDY SESSION 4

POLYSACCHARIDE

Introduction

Polysaccharides are more complex substances. Some are polymers of a single monosaccharide and are termed as Homopolysaccharides (Homoglycans), e.g. starch, glycogen, etc.
Some contain other groups other than carbohydrates such as hexuronic acid and are called as Heteropolysaccharides (heteroglycans), e.g. Mucopolysaccharides.

Learning Outcomes for Study Session 5

After you have studied this study session, you should be able to:
4.1 Define theories of counselling; (SAQ 4.1)
4.2 List 4 theories of counselling; (SAQ 4.2)

4.3 Discuss 2 of the theories of counselling; (SAQ 4.3)
4.4 Explain the significance of counselling theories; (SAQ 4.4)

Key Terms: ***Counselling, Counselling theory, Significance***

4.1 HOMOPOLYSACCHARIDES (HOMOGLYCANS)

STARCH

This is a polymer of glucose, and occurs in many plants as storage foods. It may be found in the leaves, and stem, as well as in roots, fruits and seeds where it is usually present in greater concentration.

Starch granules: Appear under microscope as particles made up of concentric layers of material. They differ in shape, size and markings. Starchy foods are mainstay of our diet. It consists of two polymeric units of glucose called

(i) Amylose and

(ii) Amylopectin

But they differ in molecular architecture and in certain properties. Starch granules are insoluble in cold water, but when their suspension is heated, water is taken up and swelling occurs, viscosity increases and starch gels or pastes are formed. Both the granules and the colloidal solutions react with Iodine to give a blue colour. This is chiefly due to amylose, which forms a deep-blue complex, which dissociates on heating. Amylopectin solutions are coloured blue-violet or purple. Starches are capable of forming esters with either organic or inorganic acids.

Hydrolysis of starch:

It yields succession of polysaccharides of gradually diminishing molecular size. Both amylose and amylopectin are rapidly hydrolysed by α-amylase which is secreted by the salivary gland and the pancreas. α-amylase an endoglycosidase hydrolyses internal α-1,4 glucosidic linkages to yield maltose, maltotriose (an oligosaccharide) and α-dextrin. Maltose consists of two glucose residues in α-1,4 glucosidic linkages and maltotriose of three of such residues. **α -Dextrin** is made up of several glucose units joined by an α-1,6 glucosidic linkage in addition to α-1,4 glucosidic linkages. Maltose and Maltotriose are hydrolysed to glucose by Maltase, whereas α-dextrin is hydrolysed to glucose by α-dextrinase. **Dextran,** a storage polysaccharide in yeast and bacteria also consists only of D-glucose residues but differs from glycogen and starch in that nearly all linkages are α -1,6 glucosidic linkages. Occasionally branches are formed by α-1,2, α -1,3 and α -1,4 glucosidic linkages depending on the species. It is used as a plasma expander. When given via IV route in cases of blood loss it increases blood volume. It remains in the blood for many hours due to its high viscosity and low osmotic pressure. Its major disadvantage is that forms false agglutination (roleux formation) therefore blood sample for grouping and cross matching should be collected before administration of dextran in case of haemorrhage and blood loss.

Cellulose

Another major polysaccharide serves as a structural rather than a nutritional role. It is an unbranched polymer of glucose residues joined by β-1,4 glucosidic linkages. The β configuration allows cellulose to form very long straight chains which is optimal for the construction of fibres having high tensile strength. In contrast, the open helix formed by α- linkage is well suited to forming an accessible store of sugar. Heating cellulose with fairly high concentrations of acids yields, the disaccharide Cellobiose and D-Glucose. Cellobiose is made up of two molecules of D-Glucose linked together by β-Glucosidic linkage between C1 and C4 of adjacent glucose units. Mammals lack cellulase and therefore cannot digest wood and vegetable fibres (cellulose). But some ruminants harbor cellulose in their gut while fungi and protozoa secrete cellulose.

Glycogen

Glycogen is the carbohydrate reservoir in animals and is a polymer of D-glucose units and resembles amylopectin. Glucose units in main stem are joined by α1 → 4 glucosidic linkages and branching occurs at branch points by α1 → 6 glucosidic linkage. A branch point occurs for every 12 to 18 glucose units and serve to increase the solubility of glycogen and make its sugar more accessible. Its molecular weight varies from 1,000,000 to 4,000,000 and it's not readily soluble in water and it forms an opalascent solution.
Glycogen is not destroyed by a hot strong KOH or NaOH solution. This property is made use of in the method for determining it quantitatively in tissues. Glycogen gives a deep-red colour with iodine. In this respect it resembles erythrodextrin.

Inulin

Inulin is a polysaccharide of fructose (fructosan) and has a molecular weight of 5000. Its powder is white and tasteless. It gives no colour with iodine and is hydrolysed by the enzyme inulinase in plants only as the enzyme is absent in humans. Inulin is used in physiological investigation for the determination of glomerular filtration rate.

Agar

Agar is a homopolysaccharide made up of repeated units of galactose which is sulphated. It is used as laxative in humans because it cannot be digested and hence add bulk to the faeces and in microbiology it is used in agar plate for culture of bacteria

1. what is the difference between glycogen and starch?

2. state two uses of agar

4.2 HETEROPOLYSACCHARIDES (HETEROGLYCANS) — MUCOPOLYSACCHARIDES (MPS)

These group of substances are called Glycosaminoglycans (GAG). They are usually composed of amino sugar and uronic acid units as the principal components, though some are chiefly made up of amino sugar and monosaccharide units without the presence of uronic acid. The hexosamine present is generally acetylated. They are essential components of tissues, where they are generally present either in free form or in combination with proteins. Carbohydrate content varies. When carbohydrate content is > 4 per cent, they are called Mucoproteins and when < 4 per cent they are called as Glycoproteins.

Differentiate between mucoprotein and glycoprotein?

4.3 CLASSIFICATION

The nitrogenous heteropolysaccharides (mucopolysaccharides) are classified as follows:

Acidic Sulphate free MPS

Hyaluronic Acid

A sulphate free mucopolysaccharide. It was first isolated from vitreous humour of eye. Later it was found to be present in synovial fluid, skin, umbilical cord, haemolytic streptococci and in rheumatic nodule. It occurs both free and salt-like combination with proteins and forms so called ground substance of mesenchyme, an integral part of gel-like ground substance of connective and other tissues. It is composed of repeating units of N-acetyl glucosamine and D-Glucuronic acid.

On hydrolysis, it yields equimolecular quantities of D-Glucosamine, D-Glucoronic acid and acetic acid.

Hyaluronidase:

An enzyme presents in certain tissues, notably testicular tissue and spleen, as well as in several types of pneumococci and haemolytic streptococci. The enzyme catalyses the depolymerisation of hyaluronic acid and by reducing its viscosity facilitates diffusion of materials into tissue spaces. Hence the enzyme, sometimes, is designated as spreading factor.

Chondroitin

Another sulphate free acid mucopolysaccharide. Found in cornea and has been isolated from cranial cartilages. It differs from hyaluronic acid only in that it contains Nacetyl galactosamine instead of N-acetyl glucosamine.

Sulphate Containing Acid MPS

Keratan Sulphate (Kerato Sulphate)

A sulphate containing acid MPS. Found in costal cartilage, and cornea has been isolated from bovine cornea. It has been reported to be present in Nucleus pulposus and the wall of aorta. It is composed of repeating disaccharide unit consisting of N-acetyl glucosamine and galactose.

N-acetyl → Galactose → N-Acetyl

Glucosamine Glucosamine n

There are no uronic acids in the molecule. Total sulphate content varies, but ester SO4 is present at C6 of both N-acetyl glucosamine and galactose.

Types: Two-types have been described. They are found in tissues combined with proteins.

• Keratan *SO_4 I*: It occurs in cornea. In this type, linkage is between N-acetyl glucosamine and Asparagine residue to form the N-glucosidic bonding.

• Keratan *SO_4 II*: It occurs in skeletal tissues. In this type, the linkage to protein is by way of -OH groups on serine and threonine residues of the protein.

Chondroitin Sulphates

They are principal MPS in the ground substance of mammalian tissues and cartilage. They occur in combination with proteins and are called as Chondroproteins.

Four chondroitin sulphates have been isolated so far. They are named as chondroitin SO4 A, B, C and D.

(a.) Chondroitin SO4 A: It is present chiefly in cartilages, adult bone and cornea. It consists of repeating units of N-acetyl-DGalactosamine and D-Glucuronic acid. N-Acetyl galactosamine is esterified with SO4 in position 4 of galactosamine.

(b.) Chondroitin SO_4 B: It is present in skin, cardiac valves and tendons. Also isolated from aortic wall and lung parenchyma. It has L-iduronic acid in place of glucuronic acid which is found in other chondroitin sulphates. It has a weak anticoagulant property, hence sometimes it is called as β-Heparin. As it is found in skin, it is also called as Dermatan sulphate. It consists of repeating units of L-iduronic acid and N-acetyl galactosamine. Sulphate moiety is present at C4 of N-acetyl galactosamine molecule.

L-Iduronic acid: It is an epimer of D-Glucuronic acid. Metabolically it is formed in the liver from D-Glucose.

(c.) Chondroitin SO4 C: It is found in cartilage and tendons. Structure of chondroitin SO_4 C is the same as that of chondroitin SO_4 A except that the SO4 group is at position 6 of galactosamine molecule instead of position 4.

(d.) Chondroitin SO4 D: It has been isolated from the cartilage of shark. It resembles in structure to chondroitin SO_4 C except that it has a second SO_4 attached probably at carbon 2 or 3 of uronic acid moiety.

Heparin

It is also called α-Heparin. It is an anticoagulant present in liver and it is produced mainly by mast cells of liver (Originally isolated from liver). In addition, it is also found in lungs, thymus, spleen, walls of large arteries, skin and in small quantities in blood. It is a polymer of repeating disaccharide units of D-Glucosamine (Glc N) and either of the two uronic acids-D-Glucuronic acid (Glc UA) and L-Iduronic acid (IDUA). The -NH2 group at C2 and OH group at C6 of D-Glucosamine (Glc N) are sulphated. A few may contain acetyl group on C2 of D-Glucosamine. In addition, the OH group of C2 of uronic acids, D-Glucuronic acid and/or L-Iduronic acid, are sulphated. Initially, all of the uronic acids are D-Glucuronic acid (Glc UA), but "5-epimerase" enzyme converts approximately 90 per cent of the D-Glucuronic acid residues to L-Iduronic acid (IDUA) after the polysaccharide chain is fully formed. Hence, in fully formed Heparin molecule 90 per cent or more of uronic acid residues are L-Iduronic acid.

Properties:

It is strongly acidic due to sulphuric acid groups and readily forms salts. Molecular weight of Heparin varies from 17,000 to 20,000. It occurs in combination with proteins as proteoglycans. The protein molecule of heparin proteoglycan is unique, consisting chiefly Serine and Glycine residues. Approximately 2/3 of the serine residue contain GAG chains. Linkage with protein molecule is usually with GalN and serine/ sometimes with threonine.

Heparin antagonist: The anticoagulant effects of heparin can be antagonised by strongly cationic polypeptides such as protamines, which bind strongly to heparin, thus inhibiting its binding to antithrombin III.

Inherited Deficiency:

Individuals with inherited deficiency of antithrombin III have been reported who are prone to develop frequent and widespread clots.

1. What is the function of the enzyme hyaluronidase?
2. State the composition of heparin?
3. What is the clinical significance of heparin?

Heparitin Sulphate

Isolated from amyloid liver, certain normal tissues such as human and cattle aorta, and from the urine, liver and spleen of patients with gargoylism (Hurler's syndrome). This compound has negligible anticoagulant activity. It seems to be structurally similar to heparin, but has the following:

• Lower molecular weight,

• Some of the amino groups carry acetyl groups and percentage of –SO4 groups are smaller.

• Unlike heparin, its predominant uronic acid is D-Glucuronic acid (Glc UA). Recently it has been shown that it is present on cell surfaces as proteoglycan and is extracellular.

Neutral MPS

• Many of the neutral nitrogenous polysaccharides of various types are found in pneumococci capsule. Type specificity of pneumococci resides on specific polysaccharides present on capsule ("hapten"). Preparations of capsular polysaccharides from Type-1 pneumococci yield on hydrolysis glucosamine and Glucuronic acids.

• Blood group substances: These contain peptides or amino acids as well as carbohydrates. Four monosaccharides are found in all types of blood group substances regardless of source: Galactose, fucose, Galactosamine (acetylated) and acetylated glucosamine. Non-reducing end groups of acetyl glucosamine, galactose and fucose are associated with blood group specificities of A, B and H respectively. The amino acid composition of blood group substances is peculiar in that S-containing and aromatic amino acids are absent.

• Nitrogenous neutral MPS firmly bound proteins, e.g. ovalbumin (contains mannose and glucosamine).

4.4 PROTEOGLYCANS—CHEMISTRY AND FUNCTIONS

Chemistry

• Proteoglycans are conjugated proteins. Proteins called "core" proteins are covalently linked to glycosaminoglycans (GAGs).

• Any of the GAGs viz. hyaluronic acid (HA); keratan sulphates I and II, chondroitin sulphates A, B, C, heparin and heparan sulphate can take part in its formation.

• The amount of carbohydrates in proteoglycans is much greater (up to 95%) as compared to glycoproteins.

Linkages: Three types of linkages between GAG and core protein is observed.

• O-glucosidic linkage: Formed between N-acetyl galactosamine (GalNAc) and serine or threonine of the core protein. Example: Typically seen in keratan SO4 II.

• N-glycosylamine linkage: Formed between N-acetyl glucosamine (GlcNAc) and amide N of asparagine (ASn) of core protein. Example: Typically seen in keratan SO4I and N-linked glycoproteins.

• O-glucosidic linkage: Formed between xylose (Xyl) and serine of the protein. This bond is unique to proteoglycans.

Functions of Proteoglycans

• As a constituent of extracellular matrix or ground substance: Interacts with collagen and elastin

• Acts as polyanions: GAGS present in proteoglycans are polyanions and hence bind to polycations and cations such as Na and K. Thus attracts water by osmotic pressure into extracellular matrix contributing to its turgor.

• Acts as a barrier in tissue: Hyaluronic acid in tissues acts as a cementing substance and contributes to tissue barrier which permit metabolites to pass through but resist penetration by bacteria and other infective agents.

• Acts as lubricant in joints: Hyaluronic acid in joints acts as a lubricant and shock absorbant. Intraarticular injection of hyaluronic acid in knee joints is used to alleviate pain in chronic osteoarthritis of knee joints.

• Role in release of hormone: Proteoglycans like hyaluronic acid are present in storage or secretory granules, where they play part in release of the contents of the granules.

• Role in cell migration in embryonic tissues: Hyaluronic acid is present in high concentration in embryonic tissues and is considered to play an important role in cell migration during morphogenesis and wound repair.

• Role in glomerular filtration: Proteoglycans like hyaluronic acid is present in basement membrane (BM) of glomerulus of kidney where it plays important role in charges electiveness of glomerular filtration.

• Role as anticoagulant in vitro and in vivo: – In vitro, heparin is used as an anticoagulant. 2 mg/10 ml of blood is used. Most satisfactory anticoagulant as it does not produce a change in red cell volume or interfere with its subsequent determinations. – In vivo, heparin is an important anticoagulant. It binds with factor IX and XI, but its most important action is with plasma antithrombin III. Binding of heparin to lysine residues in antithrombin III produces conformational change which promotes the binding of the latter to serine protease thrombin which is inhibited, thus fibrinogen is not converted to fibrin. Four naturally occurring thrombin inhibitors in plasma are:

(i) Antithrombin III (75% of the activity)

(ii) α2-macroglobulin contributes remainder

(iii) Heparin cofactor II

(iv) α1-antitrypsin The last two shows minor activity.

• Role as a coenzyme: Heparin acts in the body to increase the activity of the enzyme Lipoprotein lipase. Heparin binds specifically to the enzyme present in capillary walls, causing a release of the enzyme into the circulation. Hence heparin is called as Clearing factor.

• As a receptor of cell: Proteoglycans like heparan sulphate are components of plasma membrane of cells, where they may act as receptors and can participate in cell adhesion and cell-cell interactions.

• Role in compressibility of cartilages: Chondroitin sulphates and hyaluronic acid are present in high concentration in cartilages and have a role in compressibility of cartilage in weight bearing.

• Role in sclera of eye: Dermatan sulphate is present in sclera of the eye where it has an important function in maintaining overall shape of the eye.

• Role in corneal transparency: Keratan sulphate I is present in cornea of the eye and lie between the collagen fibrils. It plays an important role in maintaining corneal transparency.

Mucopolysaccharidoses:

The mucopolysaccharidoses are a group of related disorders, due to inherited enzyme defect, in which skeletal changes, mental retardation, visceral involvement and corneal clouding are manifested to varying degrees.

Defect/defects in these disorders result in:

• Widespread deposits in tissues of a particular MPS

• Excessive excretion of MPS in urine

At least six types of mucopolysaccharidoses have been described.

1. List 5 functions of proteoglycans

Summary of Study Session 5

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 5

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

Links to OERs

References/ Suggestions for Further Reading

Should you require more explanation on this study session, please do not hesitate to contact your e-tutor via the LMS.

Are you in need of General Help as regards your studies? Do not hesitate to contact the ODL Center by e-mail or phone on:

MODULE FOUR

STUDY SESSION 1

AMINO ACIDS AND PROTEINS

Introduction

There are approximately 300 amino acids present in various animals, plants, and microbial systems, but only 20 amino acids are coded by DNA to appear in proteins.
Cells produce proteins with strikingly different properties and activities by joining the same 20 amino acids in many different combinations and sequences.
This indicates that the properties of proteins are determined by the physical and chemical properties of their monomer units, the amino acids.

Learning Outcomes for Study Session 1, MODULE 4

After you have studied this study session, you should be able to:

Define amino acids; (SAQ)

Classify amino acid; (SAQ)

Mention the physical and chemical properties of amino acids; (SAQ)

Define proteins and understand the components of a peptide bond

State the steps involved in protein synthesis

Differentiate between the primary, secondary, tertiary and quaternary structure of proteins

Understand the classification of proteins

State the physical and chemical properties of proteins

State the functions of proteins

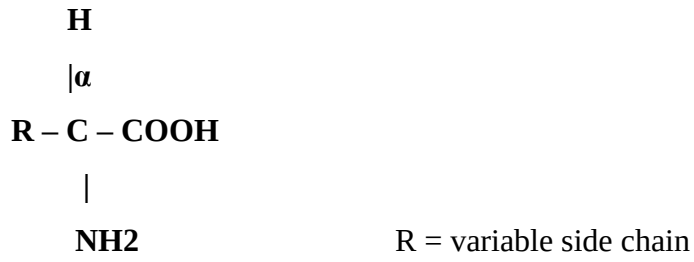
Key Terms: Definition of amino acids, Stereochemistry (Optical activity), classification, Social, Educational, Psychologist, Avenue

1.1 Definition

Amino acids are the basic structural units of proteins consisting of an amino group, (-NH2) a carboxyl (-COOH) group, a hydrogen (H) atom and a (variable) distinctive (R) group.

All of the substituents in amino acid are attached (bonded) to a central α carbon atom. This carbon atom is called α because it is bonded to the carboxyl (acidic) group.

The general formula for the naturally occurring amino acids would be:



In neutral solution or isoelectric point PI, both the α- amino and α carboxyl group are ionized resulting the charged form of an amino acids called zwitterion (dipolar) as shown in the figure below.

1.2 Stereochemistry (Optical activity)

Stereochemistry mainly emphasizes the configuration of amino acids at the α carbon atom, having either D or L- isomers.

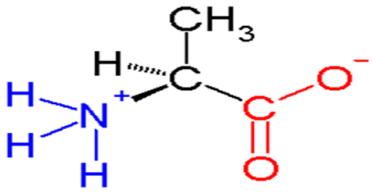
Out of the 20 amino acids, proline is not an α amino acid rather an α - imino acid.
Except for glycine, all amino acids contain at least one asymmetric carbon atom (the α-carbon atom). L-Amino acids are the building blocks of proteins.

1.3 Classification of Amino Acids

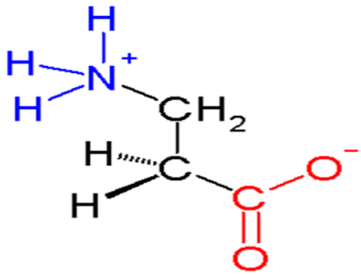
There are different mode of classification of amino acids.
They can be classified according to the core structural functional groups' locations as alpha- (α-), beta- (β-), gamma- (γ-) or delta- (δ-) amino acids.
Other categories relate to polarity, pH level, and side chain group type
(aliphatic, acyclic, aromatic, containing hydroxyl or sulfur, etc.).
On the basis of de novo synthesis, amino acids are classified into essential and non-essential amino acids too.
amino acid is designated by three letter abbreviation eg. Aspartate as Asp and by one letter symbol D.

Alpha and beta amino acids

In α amino acids, both the carboxylic acid group and the amino group are bonded to the same carbon center, termed the α carbon (because it is one atom away from the carboxylate group).
In β amino acids, the amino group is bonded to the β carbon (in the R-side chain) which is found in most of the 20 standard amino acids except glycine which lacks a β carbon, which means that β-glycine is not possible.



L-α-alanine



β-alanine

Polarity, ph and side chain

The variability in amino acids is due to the R group (side chain) and hence these can easily be classified on the basis of the type of R-group attached. Thus the following five groups of these amino acids can be recognized:

1. Non-polar aliphatic R-groups; Glycine, alanine, valine, leucine, isoleucine, methionine
2. Polar, uncharged R-groups; Serine, threonine, cysteine, proline, asparagine, glutamine
3. Aromatic R-groups; Phenylalanine, tyrosine, tryptophan
4. Positively charged R-groups; Lysine, arginine, histidine
5. Negatively charged R-groups; Aspartic acid, glutamic acid

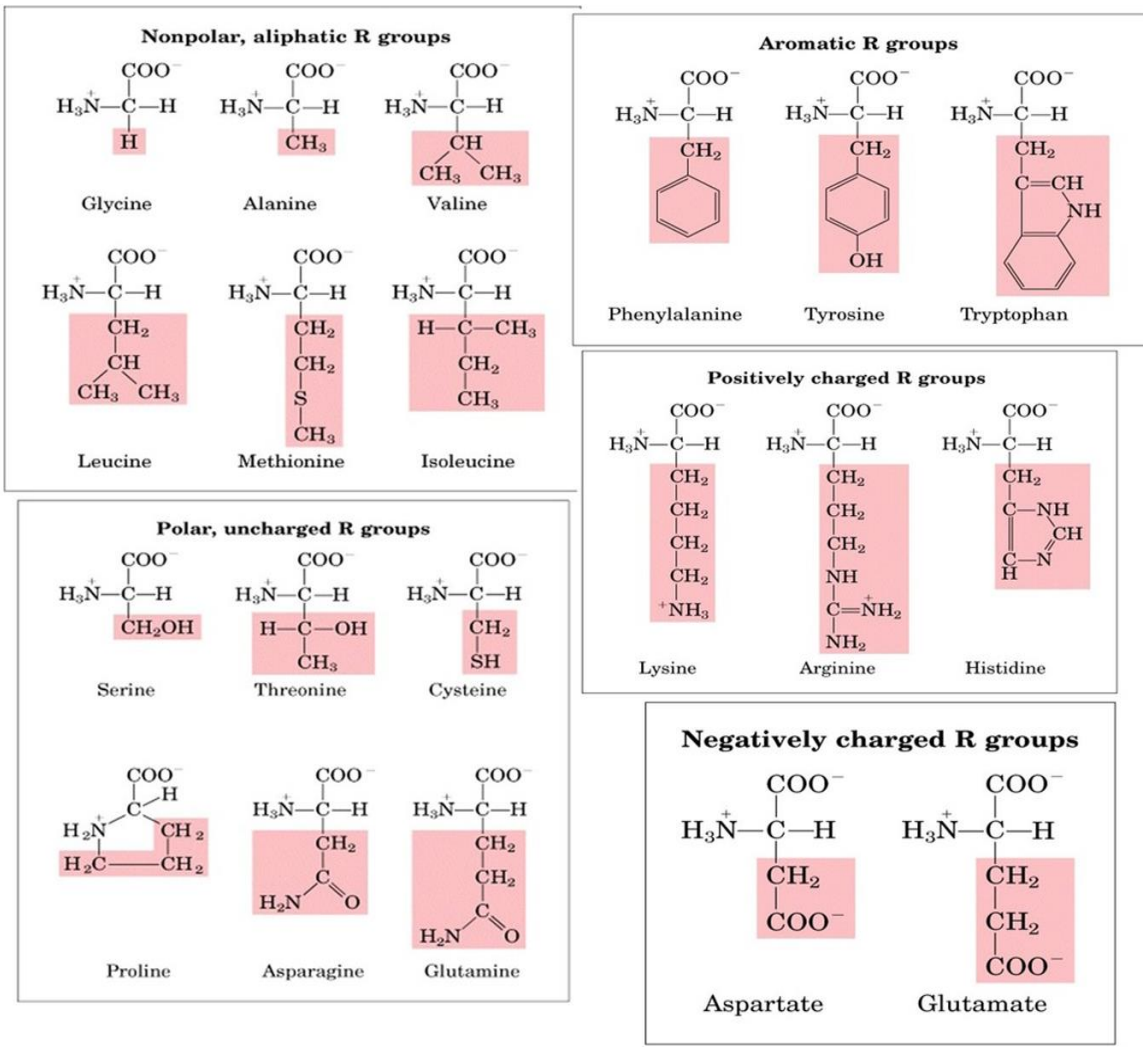


Fig. 1; classification of amino acid characteristics based on their side chain characeristics

Essential and non-essential amino acids

Nonessential amino acids can be made by the body, while essential amino acids cannot be made by the body so you must get them from your diet. All the amino acids have to be available before the body can synthesize the wide variety of proteins it needs.

Table 1; essential and non-essential amino acids

ESSENTIAL AMINO ACIDS	NON ESSENTIAL AMINO ACIDS
1. **Histidine**	10. Alanine
2. **Isoleucine**	11. Argenine
3. **Leucine**	12. Asparagine
4. **Lysine**	13. Aspartic acid
5. **Methionine**	14. Cysteine
6. **Phenylalanine**	15. Glutamic acid
7. **Threonine**	16. Glutamine
8. **Tryptophan**	17. Glycine
9. **Valine**	18. Proline
	19. Serine
	20. Tyrosine

The classification of amino acids as essential and non-essential is relative to their requirement under different conditions.

Those amino acids which can be synthesized through simple pathways are non-essential while those which are not synthesized or synthesized with complicated pathways are essential.

For example, tyrosine can be synthesized from phenylalanine by one step reaction, a simple pathway and is, therefore, treated as non-essential.

However, when the phenylalanine is not abundant this will need about 10 steps to be synthesized from glycolytic intermediate.

Hence under conditions of phenylalanine scarcity, tyrosine will be treated as essential amino acid.

Similarly, arginine is non-essential in adults but is essential in young animals.

Classification Based on the Fate of Each Amino acid in Mammals

Amino acids can be classified here as Glucogenic (potentially be converted to glucose), ketogenic (potentially be converted to ketone bodies) and both glucogenic and ketogenic.

I. Glucogenic Amino Acids

Those amino acids in which their carbon skeleton gets degraded to pyrurate, α ketoglutarate, succinyl CoA, fumrate and oxaloacetate and then converted to Glucose and Glycogen, are called as Glucogenic amino acids.

These include:- Alanine, cysteine, glycine, Arginine, glutamine, Isoleucine, tyrosine.

II. Ketogenic Amino Acids

Those amino acids in which their carbon skeleton is degraded to Acetoacetyl CoA, or acetyl CoA. then converted to acetone and β-hydroxy butyrate which are the main ketone bodies are called ketogenic amino acids.

These includes: - Phenylalanine, tyrosine, tryptophan, isoleucine, leucine, and lysine.

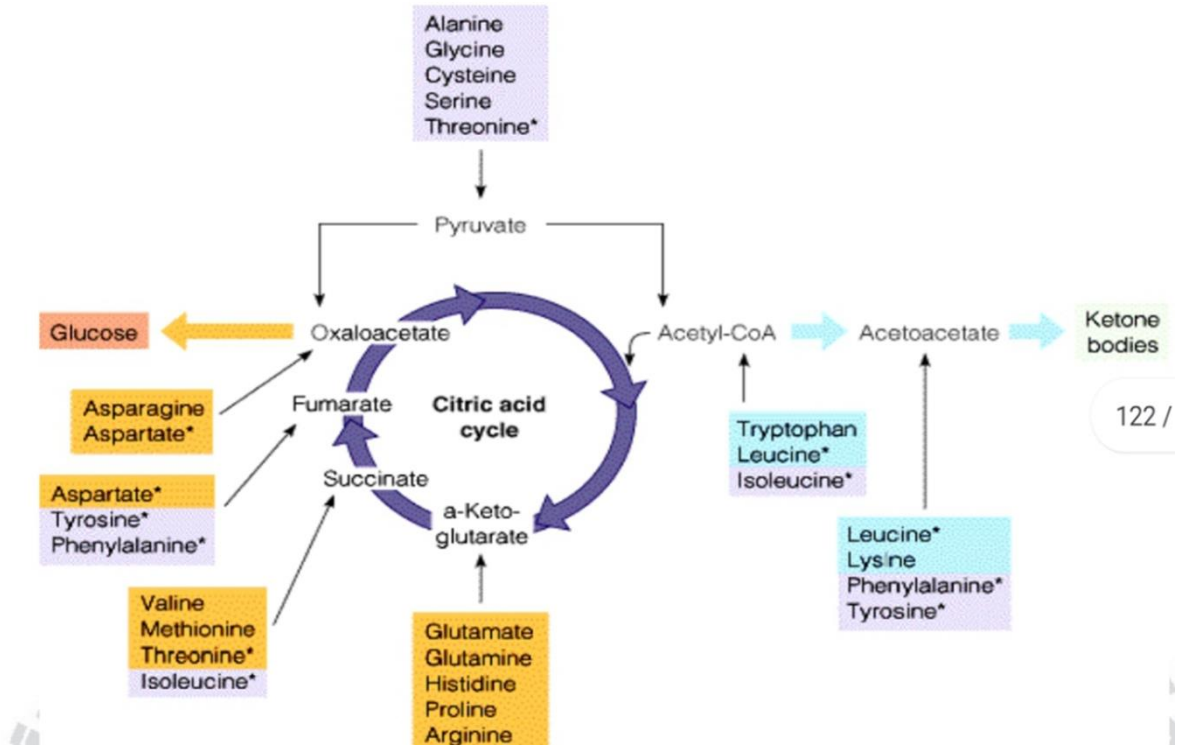
These amino acids have ability to form ketone bodies which is particularly evident in untreated diabetes mellitus in which large amounts of ketone bodies are produced by the liver (i.e. not only from fatty acids but also from ketogenic amino acids)

Degradation of Leucine which is an exclusively ketogenic amino acid makes a substantial contribution to ketone bodies during starvation.

III. Ketogenic and glucogenic Amino Acids

The division between ketogenic and glucogenic amino acids is not sharp for amino acids (Tryptophan, phenylalanine, tyrosine and Isoleucine are both ketogenic and glucogenic).

Some of the amino acids that can be converted in to pyruvate, particularly Alanine, Cysteine and serine, can also potentially form acetoacetate via acetyl CoA especially in severe starvation and untreated diabetes mellitus.



Classification Based on Participation in Protein Synthesis

Non-Standard Amino Acids

In addition to the 20 standard amino acids, proteins may contain non- standard (proteogenic) amino acids, which are normally components of proteins but created by modification of the standard amino acids. Among the non – standard amino acids are,

4 – hydroxyproline, a derivative of proline, 5- hydroxylysine, derivative of lysine.

Both are found in collagen, a fibrous protein of connective tissues.

6 N – methyllysine, a constituent of myosin, a contractile protein of muscle and γ-carboxy glutamate, a derivative of glutamate, which is found in the blood clotting protein prothrombin.

II. Non – Proteogenic Amino Acids

These amino acids occur in free or combined state, unlike in proteins, and play important roles in metabolism in plasma. Example;

Citrulline is an important metabolite of L. arginine and a product of Nitric Oxide synthase, an enzyme that produces nitric oxide, an important signaling molecule.

Antibiotics - gramicidin and antimycin D, γ-aminobutryric acid - which acts as an inhibitory neurotransmitter, D - Alanine - a component of vitamin, panthothenic acid, are some of the nonproteogenic amino acids.

 State 4 ways you can classify amino acids

1. They can be classified according to the core structural functional groups' locations as alpha- (α), beta- (β-), gamma- (γ-) or delta- (δ-) amino acids.
2. They can be classified based on the polarity, ph and R-side chain of the amino acid.
3. They can be classified as essential and non-essential amino acids based on the body's ability to synthesize them de-novo.
4. They can be classified based on the fate of each amino acid in mammals (glucogenic and ketogenic).
5. They can be classified based on participation in protein synthesis.

PROPERTIES OF AMINO ACIDS

The properties of α-amino acids are complex, yet simplistic in that every molecule of an amino acid involves two functional groups:

Carboxyl (-COOH) and amino (-NH2), Not forgetting the R-side chain which have variable features as shown above.

All these accounts for the different properties of amino acids.

Physical Properties

The solubility of amino acids reflects their ionic character

The charged functional groups of amino acids ensure that they are readily solvated by—and thus soluble in—polar solvents such as water and ethanol but insoluble in nonpolar solvents such as benzene, hexane, or ether.

Amino acids do not absorb visible light and thus are colorless.

However, tyrosine, phenylalanine, and especially tryptophan absorb high-wavelength (250–290 nm) ultraviolet light.

Because it absorbs ultraviolet light about ten times more efficiently than either phenylalanine or tyrosine, tryptophan makes the major contribution to the ability of most proteins to absorb light in the region of 280 nm.

Ionization States of Amino Acids

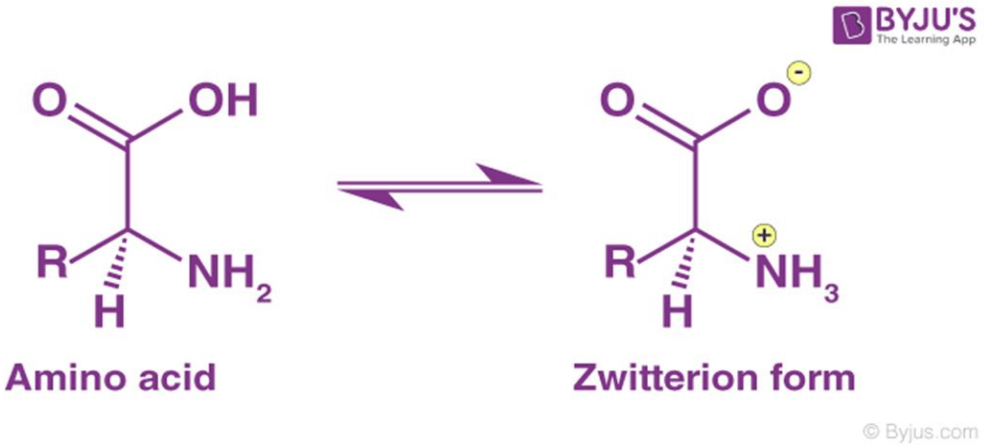
Amino acids are amphoteric molecules, that is, they have both basic and acidic groups. As such, are said to be zwitterions at neutral

Zwitterion

"A zwitterion is a molecule that has both positive and negative regions of charge."

In the solid state, amino acids exist as dipolar ions called zwitterions. While discussing whether a substance is zwitterionic or not, the pH range in which the information is required must be specified (because a sufficiently alkaline solution will change the zwitterion to an anion, and a sufficiently acid solution will change it to a cation).

At pH 7, the "zwitterions" H+ 3N – CH2 – COO- is the predominant species in solution and the overall molecule is electrically neutral.



At acidic pH the α amino (α -NH2) group is fully protonated and positively charged, yielding *H*_3^+N – CH2 – COOH, while at alkaline pH glycine exists primarily as the anionic H2N – CH2 – COO- species, (Negatively charged species).

Characteristics of Zwitterion

They can be formed from compounds like ampholytes which contain both acid and base groups in their molecules.

In this type of ion, the charged atoms are usually held together by one or more covalent bonds.

Zwitterionic compounds have stable, separated unit electrical charges on atoms.

These compounds contain quaternary ammonium cations.

Isoelectric Point

Another main property of a Zwitterion is that it has an isoelectric point (represented as pI.

This point is the pH value at which the charge in molecules is neutral.

Usually, the net charge on a molecule is greatly affected by the pH of its surrounding environment.

Molecules can become more charged (positively or negatively) as a result of gain or loss in the number of protons.

In amino acid, the amino group is a very effective proton acceptor and the carboxyl group is an effective proton donor.

In addition, the solubility of a molecule at a given pH is also affected by the pI value.

Calculation of the isoelectric pH

The pH value at the isoelectric point can be calculated from the equilibrium constants (acid and base) of the Zwitterion. It is represented by the formula;

$$PI = \frac{Pka1 + Pka2}{2}$$

Where,

pI = isoelectric point,

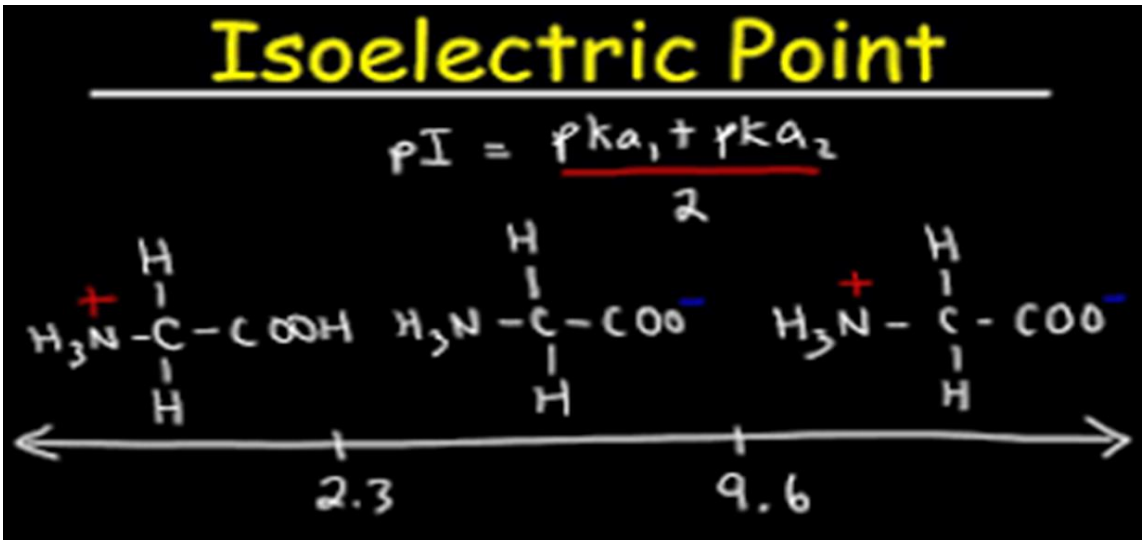
Ka1 = the equilibrium constant of the acid.

Ka2 = the equilibrium constant of the base.

The pKa value is one method used to indicate the strength of an acid. pKa is the negative log of the acid dissociation constant or Ka value.

Applications of Zwitterions

Zwitterions are widely applied in the process of separating protein molecules via SDS PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) method which is one of the most popular techniques used in molecular biology.



Typical Range of pKa Values for Ionizable Groups in Proteins

DISSOCIATING GROUP	pKa RANGE
α-Carboxyl	3.2–4.1
Non α- COOH of Asp or Glu	4.0–4.8
Imidazole of His	6.5–7.4
SH of Cys	8.5–9.0
OH of Tyr	9.5–10.5
α-Amino	8.0–9.0
ε-Amino of Lys	9.8–10.4
Guanidinium of Arg	~12.0

Acid Base Properties of Amino Acids

When a crystalline amino acid, such as Alanine is dissolved in water, it can act as either an acid (proton donor) or a base (proton acceptor).

According to Laury and Bronsted theory of acid and bases, and acid is a proton donor and a base is a proton acceptor.

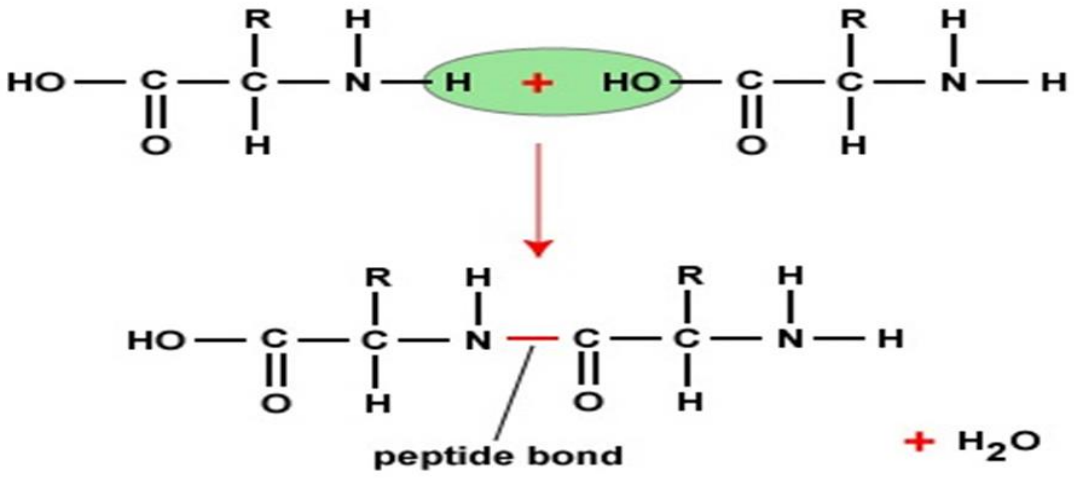
Example : Alanine acting as proton donor (Acid) H H | | H3N+ - C – COO- H2N – C - COO- + H+ | | CH3 CH3 Net charge (-1) Alanine acting as a proton acceptor (base) H | H+ + H3N+ - C – COO- H3N – C - COO- + H+ | | CH3 CH3 Net charge (+1) Substances having such dual nature are said to be Amphoteric and are often called Ampholytes.

Peptides

The peptide and their characteristics Proteins are macromolecules with a backbone formed by polymerization of amino acids in a polyamide structure.

These amide bonds in protein, known as peptide bonds formed by linkage of α - carboxyl group of one amino acid with α- amino groups of the next amino acid by amide bonds.

During the formation of a peptide bond, a molecule of water is eliminated as shown below: -



A peptide chain consisting of two amino acid residues is called a dipeptide, three amino acids tripeptide (e. g Glutathione) etc.

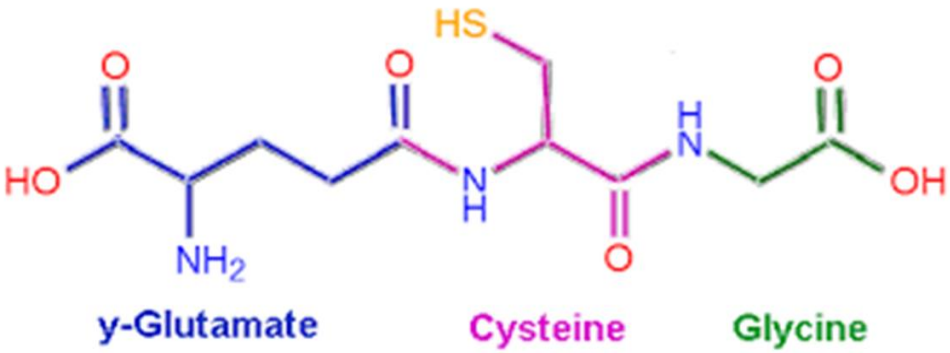
By convention, peptide structures are written with amino terminal residues on the left and with the carboxyl terminal residue at the right.

Peptide of physiological significance

Glutathione; A tripeptide formed from amino acids glutamate, cysteine and Glycine, linked together in that order.

The glutamate is linked to cysteine through the γ- carboxyl group and α - amino group of cysteine.

Here, the carboxyl group is first activated by ATP to form an acyl-phosphate derivative which is then attacked by cysteine amino group then undergoes condensation with glycine.



GENERAL REACTIONS OF AMINO ACIDS

Reactions due to carboxyl groups

1. Decarboxylation; amino acids undergo alpha decarboxylation to form corresponding amines.

Eg;

Histidine -- histamine +CO2

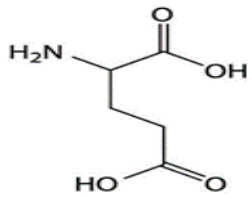
Tyrosine -- tyramine +CO2

Tryptophan -- tryptamine +CO2

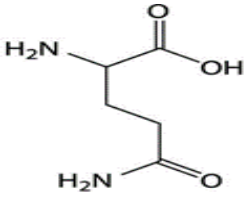
Glutamic acid -- gamma-aminobutyric acid (GABA) +CO2

2. Amide formation; the COOH group of dicarboxylic amino acid (other than the carboxyl group) can combine with ammonia to form the corresponding amides.

Amides are components of some protein structures. The amide group of glutamine serve as the source of nitrogen for nucleic acid synthesis



Glutamic acid

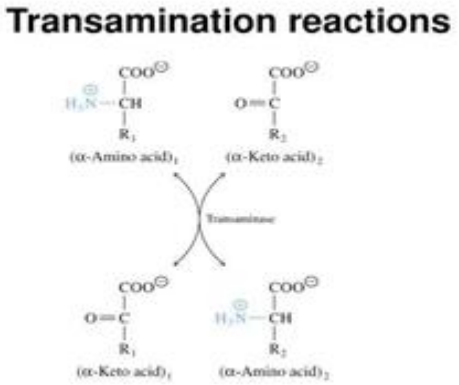


Glutamine

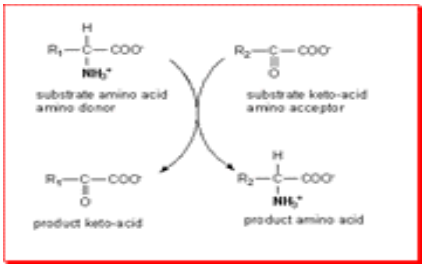
Reactions due to amino groups

1. Transamination; the alpha amino group of amino acids can be transferred to alpha keto acid to form the corresponding new amino acid and alpha keto acid.

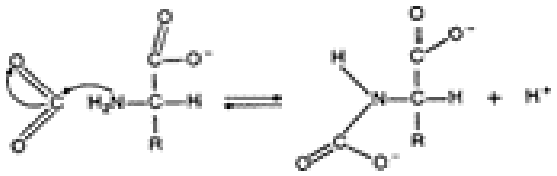
This is an important reaction in the body for the interconversion of amino acids and for the synthesis of non-essential amino acids.



2. Oxidative deamination; the alpha amino group is removed from the amino acid to form the corresponding keto acid and ammonia. In the body, glutamic acid is the most common amino acids to undergo oxidative deamination.



3. Formation of carbamino compounds; carbon dioxide adds to the alpha amino group of amino acids to form carbamino compounds. The reaction occurs at alkaline PH and serves as a mechanism for the transportation of carbon dioxide from tissues to the lungs by hemoglobin.



Reactions due to side-chain

1. Transmethylation; the m group of m Union after activation may be transferred to an acceptor which becomes methylated

2. Ester formation by OH group; the hydroxyl amino acid form esters with phosphoric acid. In this manner, the serine and threonine residues of protein are involved in the formation of phosphoproteins. Similarly, these hydroxyl group can form a glycosidic bond with carbohydrate residues to form glycoproteins

Other reactions of the sidechain includes disulfide Bond formation and reactions of the amino group.

FUNCTIONS OF CERTAIN AMINO ACIDS AND THEIR DERIVATIVES.

Histamine synthesized from histidine is the mediator of allergic reactions

Cycloserine a derivative of serene is an anti-tuberculosis drug azaserene inhibit reactions where amide groups are added and so act as an anti-cancer drug.

Thyroxine from tyrosine is an important thyroid hormone

Gamma-aminobutyric acid (GABA: a derivative of glutamic acid) and dopamine (derived from tyrosine) are neuro transmitters. Gaba pentin can pass the blood-brain barrier and can form GABA in the brain.

Histidine residues are important in the buffering activity of proteins.

Ornithine and citrulline are derivatives of arginine and are essential for urea synthesis

Glycine (G/Gly).

Slices DNA and produces different proteins.

One of the three most important glycogenic amino acids.

Needed for the synthesis of the antioxidant glutathione

Alanine (A/Ala).

Important source of energy for muscle.

One of the three most important glycogenic amino acids.

The primary amino acid in sugar metabolism.

Boosts immune system by producing antibodies.

Leucine (L/Leu).

Beneficial for skin, bone and tissue/wound healing

Isoleucine (I/Ile).

Necessary for the synthesis of hemoglobin.

Proline (P/Pro).

Critical component of cartilage, aids in joint health, tendons and ligaments.

Keeps heart muscle strong.

Phenylalanine (F/Phe).

Beneficial for healthy nervous system.

It boosts memory and learning.

Tyrosine (Y/Tyr).

Precursor of dopamine, norepinephrine and adrenaline.

Increases energy, improves mental clarity and concentration, can treat some depressions.

Tryptophan (W/Trp).

Necessary for a synthesis of neurotransmitter serotonin.

Effective sleep aid, due to conversion to serotonin.

Reduces anxiety and some forms of depression.

Treats migraine and headaches.

Stimulates growth hormone

Serine (S/Ser).

One of the three most important glucogenic amino acids, the others being alanine and glycine.

Maintains blood sugar levels, and boosts immune system.

Myelin sheaths contain serine.

Threonine (T/Thr).

Required for formation of collagen.

Helps prevent fatty deposits in liver.

Aids in antibodies' production

Cysteine (C/Cys).

Protective against radiation, pollution and ultra-violet light.

Detoxifier, necessary for growth and repair of skin.

Methionine (M/Met).

An antioxidant.

Helps in breakdown of fats and aids in reducing muscle degeneration

Asparagine (N/Asn).

Helps in the metabolism of ammonia

One of the two main excitatory neurotransmitters.

Glutamine (Q/Gln).

Essential for helping to maintain normal and steady blood sugar levels.

Stimulate IgA production

Help produce glutathione

6 Regulates nitric oxide formation

Helps muscle strength and endurance.

Gastrointestinal function, provides energy to small intestines.

Lysine (K/Lys).

Component of muscle protein and collagen, needed in the synthesis of enzymes and hormones.

It is also a precursor for L-carathine, which is essential for healthy nervous system function.

Arginine (R/Arg).

It is a precursor for the synthesis of nitric oxide (NO) making it important in the regulation of blood pressure

Important in cell division, wound healing, removing ammonia from the body, immune function, and the release of hormones

May increase endurance and decrease fatigue.

Detoxifies harmful chemicals.

Histidine (H/His).

Found in high concentrations in hemoglobin. Treats anemia, has been used to treat rheumatoid arthritis

proton buffer, metal ion chelation, scavenging of reactive oxygen and nitrogen species

a precursor for several hormones (e.g., thyrotropin-releasing hormone)

Aspartate (D/Asp).

Increases stamina and helps protect the liver; DNA and RNA metabolism, immune system function.

Glutamate (E/Glu).

Neurotransmitter that is involved in DNA synthesis.

 ASSIGNMENT; State 5 chemical properties of amino acids

MODULE FOUR

STUDY SESSION 2

PROTEIN

Introduction

Proteins are macromolecules formed by amino acids.

A total of 20 different amino acids exist in proteins and hundreds to thousands of these amino acids are attached to each other by the peptide linkage in long chains to form a protein.
Amino acids can be released from proteins by hydrolysis. (Hydrolysis is the cleavage of a covalent bond by addition of water in adequate conditions.)
A linear chain of amino acid residues is called a polypeptide. A protein contains at least one long polypeptide.
Short chains of amino acids, containing less than 20–30 residues, are rarely considered to be proteins and are commonly called peptides, or sometimes oligopeptides
The sequence of amino acid residues in a protein is defined by the sequence of a gene, which is encoded in the genetic code.
In general, the genetic code specifies 20 standard amino acids.
Shortly after or during synthesis, the amino acid residues in a protein can be chemically modified in a process known as post-translational modification, which alters the physical and chemical properties, folding, stability, activity, and ultimately, the function of the proteins.
Sometimes proteins have non-peptide groups attached, which can be called prosthetic groups or cofactors.

Learning Outcomes for Study Session 2

After you have studied this study session, you should be able to:

3.1 Define proteins and understand the components of a peptide bond

3.2 State the steps involved in protein synthesis

3.3 Differentiate between the primary, secondary, tertiary and quaternary structure of proteins

3.4 Understand the classification of proteins

3.5 State the physical and chemical properties of proteins

3.6 State the functions of proteins

Key Terms: Primary, secondary, tertiary and quaternary structure of proteins

2.1 Synthesis of Proteins

Proteins are assembled from amino acids using information known as genetic codes (encoded in genes).

The genetic code is a set of three-nucleotide sets called codons and each three-nucleotide combination designates an amino acid, for example AUG (adenine-uracil-guanine) is the code for methionine and TGG (thymine, guanine, guanine) is the code for tryptophan.

Protein syn3.1thesis starts with transcription; Genes encoded in DNA are first transcribed into pre-messenger RNA (mRNA) by proteins such as RNA polymerase.

Then translation; which is the actual synthesis.

It is divided into three parts; initiation, elongation and termination

Initiation;

in the cytoplasm, protein synthesis is actually initiated by the AUG codon on mRNA.

The AUG codon signals both the interaction of the ribosome with m-RNA and also the tRNA with the anticodons (UAC).

The tRNA which initiates the protein synthesis has N-formyl-methionine attached.

The formyl group is really formic acid converted to an amide using the -NH2 group on methionine.

The next step is for a second tRNA to approach the mRNA (codon - CCG), this is the code for proline.

The anticodon of the proline tRNA which reads this is GGC.

The final process is to start growing peptide chain by having amine of proline to bond to the carboxyl acid group of methinone (met) in order to elongate the peptide

Elongation

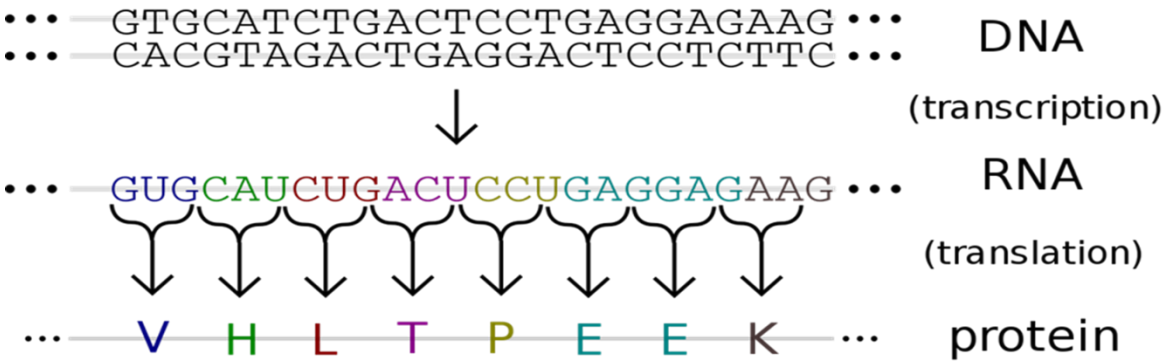
Elongation of the peptide begins as various tRNA's read the next codon

Termination:

When the stop signal on mRNA is reached, the protein synthesis is terminated and the last amino acid is hydrolyzed from its t-RNA.

The peptide chain leaves the ribosome. The N-formyl-methionine that was used to initiate the protein synthesis is also hydrolyzed from the completed peptide at this time.

The ribosome is now ready to repeat the synthesis several more times.



2.2 Structure Of Proteins

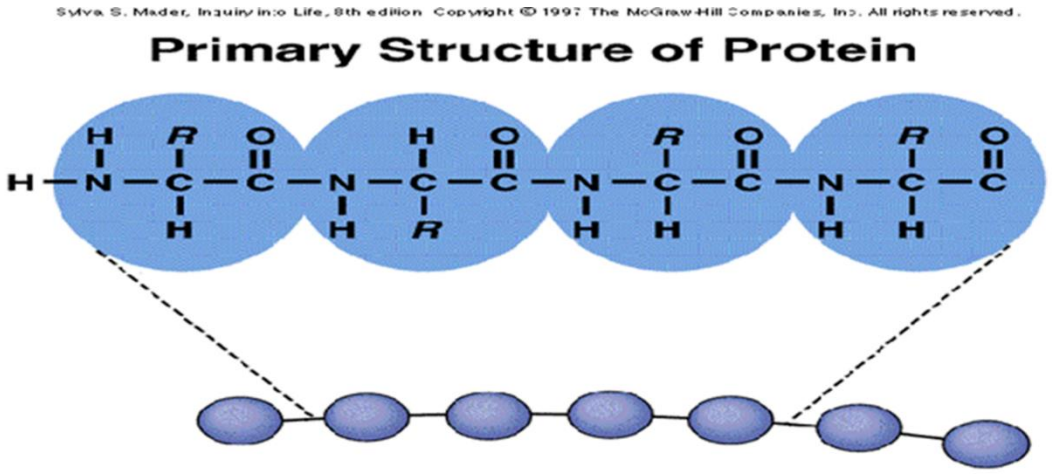
There are four levels of protein structures and these are distinguished from each other by the degree of complexity in the polypeptide chain.

A single protein molecule may contain one or more of the protein structure types which are: primary, secondary, tertiary, and quaternary structure.

Primary structure:

This is the amino acid sequence. A protein is a polyamide.

The primary Structure describes the unique order in which amino acids are linked together to form a protein.



Secondary structure:

Regularly repeating local structures stabilized by hydrogen bonds.

It refers to the coiling or folding of a polypeptide chain that gives the protein its 3-D shape.

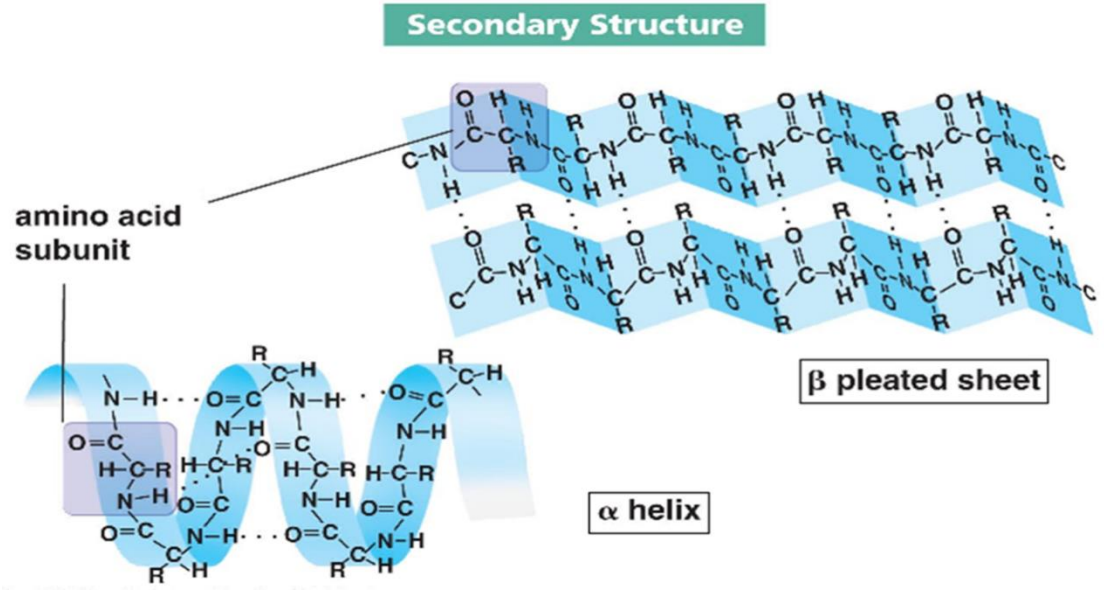
There are two types of secondary structures observed in proteins.

alpha (α) helix structure.

This structure resembles a coiled spring and is secured by hydrogen bonding in the polypeptide chain.

beta (β) pleated sheet.

This structure appears to be folded or pleated and is held together by hydrogen bonding between polypeptide units of the folded chain that lie adjacent to one another.



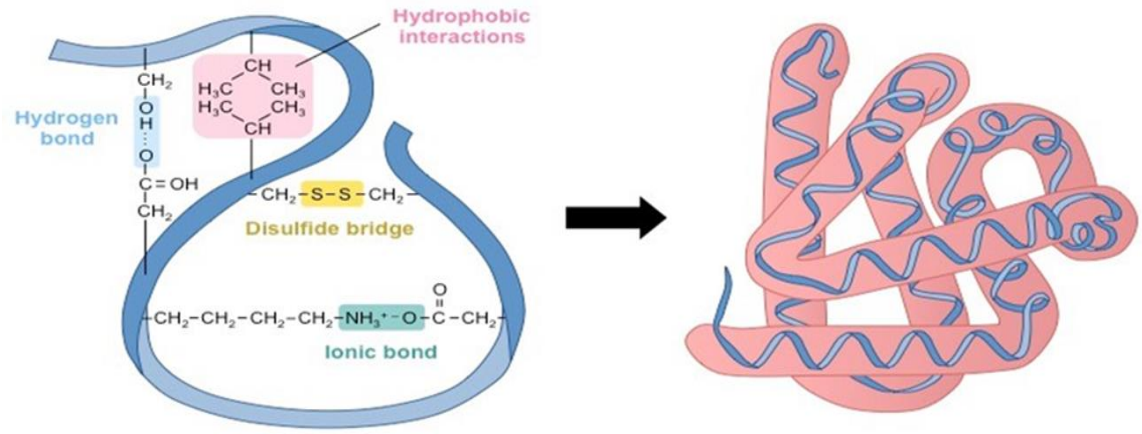
Tertiary structure:

This is the overall shape of a single protein molecule.

It is formed by the spatial relationship of the secondary structures to one another.

Tertiary Structure refers to the comprehensive 3-D structure of the polypeptide chain of a protein.

There are several types of bonds and forces that hold a protein in its tertiary structure.



Types of side chain interactions

Overall 3D shape (3° Structure)

Bonds and Forces Found in Tertiary Protein Structures

Hydrophobic interactions

Hydrophobic interactions greatly contribute to the folding and shaping of a protein.

The "R" group of the amino acid is either hydrophobic or hydrophilic.

The amino acids with hydrophilic "R" groups will seek contact with their aqueous environment, while amino acids with hydrophobic "R" groups will seek to avoid water and position themselves towards the center of the protein.

Hydrogen bonding

Hydrogen bonding in the polypeptide chain and between amino acid "R" groups helps to stabilize protein structure by holding the protein in the shape established by the hydrophobic interactions.

Due to protein folding, ionic bonding can occur between the positively and negatively charged "R" groups that come in close contact with one another.

Covalent bonding

Folding can also result in covalent bonding between the "R" groups of cysteine amino acids.

This type of bonding forms what is called a disulfide bridge.

van der Waals forces

Interactions called van der Waals forces also assist in the stabilization of protein structure.

These interactions pertain to the attractive and repulsive forces that occur between molecules that become polarized.

The term "tertiary structure" as often used is synonymous with the term fold.

The tertiary structure is what controls the basic function of the protein.

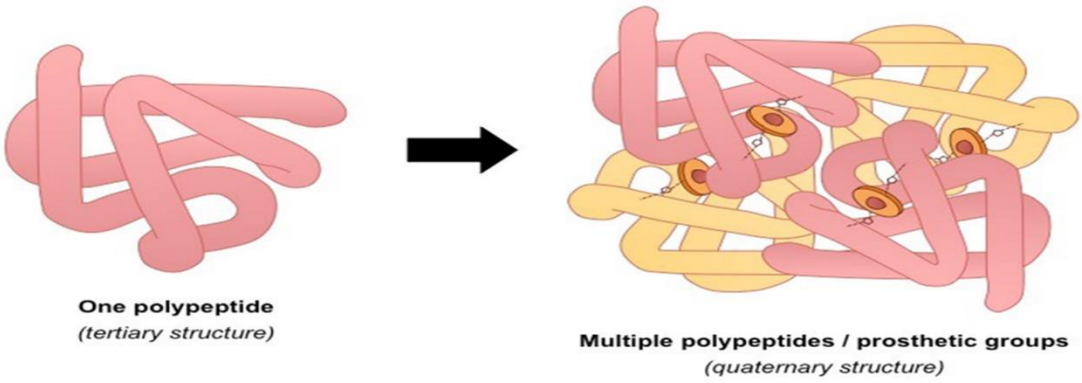
Quaternary structure:

This refers to the structure of a protein macromolecule formed by interactions between multiple polypeptide chains.

Each polypeptide chain is referred to as a subunit.

Proteins with quaternary structure may consist of more than one of the same type of protein subunit.

They may also be composed of different subunits.



Fig; Quaternary structure of protein

Hemoglobin is an example of a protein with quaternary structure.

Hemoglobin, found in the blood, is an iron-containing protein that binds oxygen molecules.

It contains four subunits:

two alpha subunits and two beta subunits which function as a single protein complex.

Another example is protein existing as a multienzyme complex

Proteins are not entirely rigid molecules.

In addition to these levels of structure, proteins may shift between several related structures while they perform their functions.

In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as "conformations", and transitions between them are called conformational changes.

Such changes are often induced by the binding of a substrate molecule to an enzyme's active site, or the physical region of the protein that participates in chemical catalysis.

In solution, proteins also undergo variation in structure through thermal vibration and the collision with other molecules

2.3 CLASSIFICATION OF PROTEINS

Proteins perform several duties in the cell as they function as structural proteins, enzymes, pigments, transporters etc.

As a result, there are different criteria for their classification and these are;

2.3.1 Classification Based on Structure

Proteins are classified into fibrous, globular and intermediate proteins based on their structural appearance in the body

Fibrous proteins;

These are linear in shape, long, parallel, tough, insoluble in water and usually do not have tertiary structures as their function rest mainly on their secondary structure.

Examples are silk, myosin, collagen and keratin.

Globular proteins;

Proteins in this group are spherical in shapes.

Their polypeptide chain is tightly folded into spheres.

They are soft and readily soluble in water.

Their tertiary structure is the most important functional position.

They function as enzymes, antibodies and hormones.

Examples include insulin, haemoglobin, DNA polymerase etc.

Intermediate proteins;

These proteins have characteristics that are intermediate to fibrous and globular proteins.

Unlike fibrous proteins, they are soluble in water, short and less linear.

They function in the clotting of blood. Eg fibrinogen

2.3.2 Classification Based on Function

based on their biological functions, proteins are classified into;

Structural proteins;

these form the structures of the body such as bones, connective tissues, cartilages, nail, hair, skin, etc.

They are mainly fibrous proteins hence, insoluble in water.

Examples are keratin and collagen.

Hormones;

mainly protein hormones. Eg ACH, insulin and glucagon

Enzymes;

biological catalyst

Transport proteins;

components of blood. Eg haemoglobin for transport of oxygen and carbon iv oxide,

Transmembrane proteins forming transport channels in the membranes eg Na +k + ATPase

Contactile proteins;

tissue contration eg myosin and actin for muscle movement.

Antibodies;

igG, igM, igE

Toxins;

snake venom, antigens

Storage proteins;

Eg ferretin etc

2.3.3 Classification based on composition

two category exist under this classification

Simple proteins;

These have simple structural organization and are mainly made up of amino acids

eg; actin, myosin, keratin

Conjugated proteins;

these exist in conjugation with other non-protein components.

The non-protein components are called **prosthetic group**.

The prosthetic group could be a lipid, CHO, metal, phosphate group or nucleic acid group.

Conjugated proteins are globular in shape and the prosthetic group aid the biological function of that protein.

They function mainly as enzymes and co-enzymes.

Conjugated Proteins

Class	Prosthetic Group	Example
Nucleoprotein	Nucleic acids	Viruses
Lipoprotein	Lipids	Serum lipoproteins
Glycoprotein	Carbohydrates	Mucin in saliva
Phosphoprotein	Phosphate groups	Casein in milk
Hemoprotein	Heme	Hemoglobin, cytochormes
Metalloprotein	Iron, zinc	Ferritin, hemoglobin

Metalloproteins: These are proteins conjugated with metal like iron, copper, zinc, ……

a- Iron-containing proteins: Iron may present in heme such as in
- hemoglobin (Hb)
- myoglobin (protein of skeletal muscles and cardiacmuscle),
- cytochromes,
- catalase, peroxidases (destroy H2O2)
- tryptophan pyrrolase (desrtroy indole ring of tryptophan).

Iron may be present in free state (not in heme) as in:
- Ferritin: Main store of iron in the body. ferritin is present in liver, spleen and bone marrow.
- Hemosidrin: another iron store.
- Transferrin: is the iron carrier protein in plasma.

2.4 PROPERTIES OF PROTEINS

2.4.1 Physical Properties of Proteins

Proteins are colourless and usually tasteless.

They are homogeneous and crystalline.

Proteins range in shape from simple crystalloid spherical structures to long fibrillar structures.

Molecular Weight

Proteins generally have large molecular weights ranging between 5 × 103 and 1 × 106.

It might be noted that the values of molecular weights of many proteins lie close to or multiples of 35,000 and 70,000.

Colloidal Nature

Because of their giant size, proteins exhibit many colloidal properties, such as;

Their diffusion rates are extremely slow and they may produce considerable light-scattering in solution, thus resulting in visible turbidity (Tyndall effect).

Denaturation

Denaturation refers to the changes in the properties of a protein.

In other words, it is the loss of biologic activity.

In many instances the process of denaturation is followed by coagulation— a process where denatured protein molecules tend to form large aggregates and to precipitate from solution.

Amphoteric Nature

Like amino acids, the proteins are amphoteric, i.e., they act as acids and alkalies.

These migrate in an electric field and the direction of migration depends upon the net charge possessed by the molecule.

The net charge is influenced by the pH value.

Each protein has a fixed value of isoelectric point (pl) at which it will move in an electric field.

Ion Binding Capacity

The proteins can form salts with both cations and anions based on their net charge.

Solubility

The solubility of proteins is influenced by pH.

Solubility is lowest at isoelectric point and increases with increasing acidity or alkalinity.

This is because when the protein molecules exist as either cations or anions, repulsive forces between ions are high, since all the molecules possess excess charges of the same sign.

Thus, they will be more soluble than in the isoelectric state.

Optical Activity

All protein solutions rotate the plane of polarized light to the left,

i.e., they are levoratotory.

2.4.2 Chemical Properties of Proteins

Hydrolysis

Proteins are hydrolyzed by a variety of hydrolytic agents.

A. By acidic agents:

Proteins, upon hydrolysis with conc. HCl (6–12N) at 100–110°C for 6 to 20 hrs, yield amino acids in the form of their hydrochlorides.

B. **By alkaline agents**:

Proteins may also be hydrolyzed with NaOH.

Reactions involving COOH Group

A. Reaction with alkalies (Salt formation)

B. Reaction with alcohols (Esterification)

C. Reaction with amines

Reactions involving NH2 Group

A. Reaction with mineral acids (Salt formation):

When either free amino acids or proteins are treated with mineral acids like HCl, the acid salts are formed.

B. Reaction with formaldehyde:

With formaldehyde, the hydroxy-methyl derivatives are formed.

C. Reaction with benzaldehyde:

Schiff 's bases are formed

D. Reaction with nitrous acid (Van Slyke reaction):

The amino acids react with HNO2 to liberate N2 gas and to produce the corresponding α-hydroxy acids.

E. Reaction with acylating agents (Acylation)

F. Reaction with FDNB or Sanger's reagent

G. Reaction with dansyl chloride

Reactions involving both COOH and NH2 Group;

A. Reaction with triketohydrindene hydrate (Ninhydrin reaction)

B. The alpha COOH group can be decarboxylated to give a primary amine

D. Reaction with phosgene: With phosgene, N-carboxyanhydride is formed

E. Reaction with carbon disulfide: With carbon disulfide, 2-thio-5-thiozolidone is produced

Reactions involving R Group or Side Chain

A. Biuret test

B. Xanthoproteic test

C. Millon's test

D. Folin's test

E. Sakaguchi test

F. Pauly test

G. Ehrlich test

Reactions involving SH Group

Nitroprusside test: Red colour develops with sodium nitroprusside in dilute NH4.OH.

The test is specific for cysteine.

Sullivan test:

Cysteine develops red colour in the presence of sodium 1, 2-naphthoquinone- 4-sulfonate and sodium hydrosulfite.

2.5 Functions of Proteins

Receiving and sending chemical signals

Responding to stimuli

Providing structural support

Catalyzing chemical reactions

Synthesizing and repairing DNA

Transporting materials across the cell

Movement

Fight infections etc

discuss the classification of proteins based on their composition.

Summary of Study Session 2 of module 4

In this study session, you have learnt that:

Proteins are polymers of amino acids linked adjacently by peptide bonds.

Protein structure can be defined and studied at four levels and these are primary secondary tertiary and quaternary.

Quaternary structure is present in proteins with more than one polypeptide chains examples of illegal marik proteins with quaternary structure of haemoglobin and creatinine kinase.

cysteine forms disulfide linkages between two polypeptide chains in oligomeric proteins.

The primary structure determines the biological activity of a protein alterations leads to loss of functional capacity as same as in sickle cell disease secondary structure of protein is stabilized by hydrogen bonds ionic bonds hydrophobic interactions and van der waals forces.
Secondary structure could be an alpha helix and beta pleated sheets. A beta pleated sheet may be parallel or anti-parallel.
The tertiary structure of a protein is the most thermodynamically stable configuration.
Protein folding into its 3D configuration is assisted by chaperones.
Incorrect folding has been proposed as a mechanism in the origin of prion disease.
The solubility of proteins depends on the ionic concentration of the medium hence protein may be salted in or salted out.

Self-Assessment Questions (SAQs) for Study Session 3

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting.

Links to OERs

References/ Suggestions for Further Reading

Should you require more explanation on this study session, please do not hesitate to contact your e-tutor via the LMS.

Are you in need of General Help as regards your studies? Do not hesitate to contact the Center by e-mail or phone on:

MODULE 4

STUDY SESSION 3

ISOLATION AND PURIFICATION OF PROTEINS

Introduction

Proteins can be gotten from different sources. Proteins for diagnostic purposes, may be obtained from a patient's blood or tissues, while proteins for experimental purposes may be gotten from microorganisms or from cell lines derived from insects, vertebrate animals, or plants. In any case, endogenous proteins that we do not want are present in a much greater quantity than the proteins we do want and as such, isolation and purification becomes necessary

Learning Outcomes for Study Session 3 of Module 4

After you have studied this study session, you should be able to:

1. Describe most common methods of protein isolation and purification
2. Compare between different methods of protein purification
3. Construct a purification algorithm based on your knowledge in protein purification

Key Terms: homogenization, intracellular), extracellular, chromatography
Electrophoresis, immunoblotting

3.1 Isolation of Extracellular Proteins

No need for cell disruption

Secreted soluble proteins can be collected in the cell supernatant after centrifugation

Membrane-bound proteins might be released from the cell simply using detergents

3.2 Isolation of Intracellular Proteins

Isolation of intracellular proteins need cell disruption breaking cells and tissues.

The first step in the purification of most proteins is to disrupt the tissues and cells in a controlled fashion using gentle mechanical procedures called homogenization.

The plasma membrane of the cells is ruptured so that the cell's contents are released.

The resulting thick soup (called a homogeneous or an extract) contains large and small molecules from the cytosol such as enzymes ribosomes and metabolites as well as all the membrane-bound organelles.

When homogenization is carefully applied it leaves most of the membrane-bounded organelles intact.

Seven commonly used procedures are shown here.

3.2.1 Detergents lysis

This uses a mild detergent to make holes on the cell membrane thereby, releasing the content of the cell.

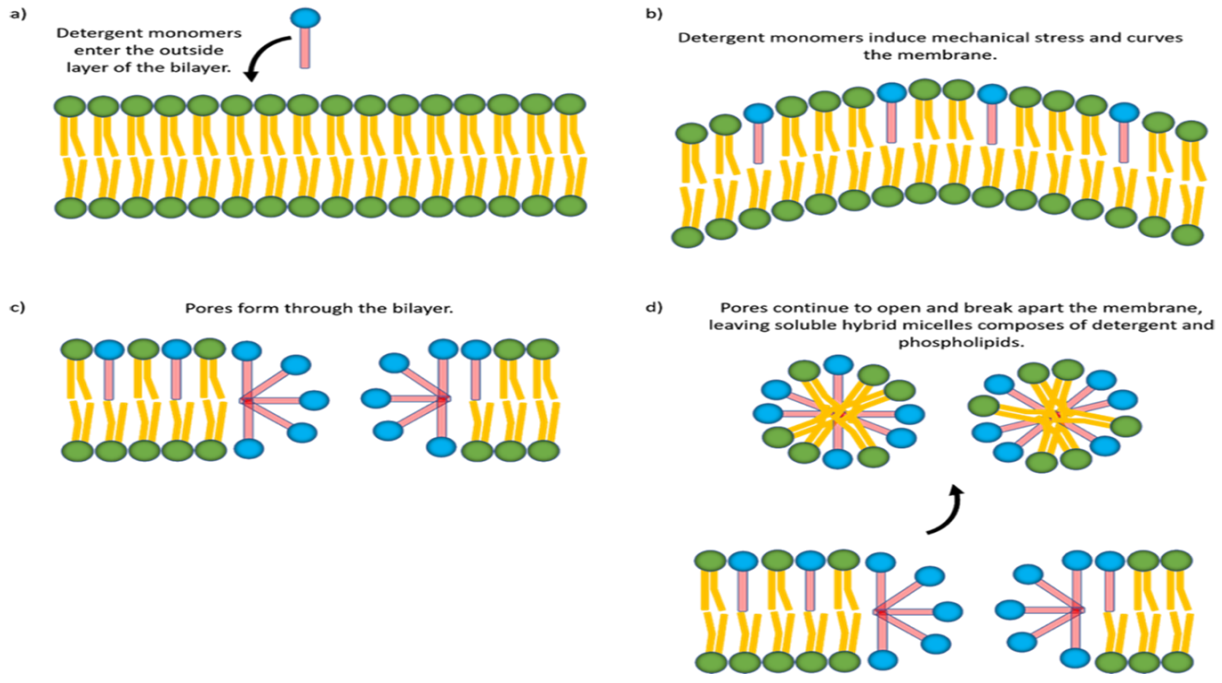
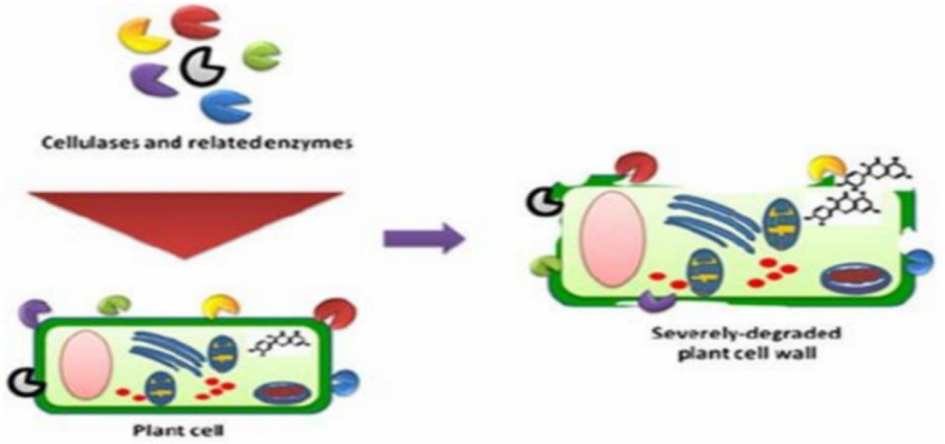


Fig. formation of micelles by the lipid bilayer as a result of detergent lysis

3.2.2 Enzymatic lysis

Uses digestive enzymes which will decompose the microbial cell wall. Different cell types and strains have different kind of cell walls and membranes, and thus the used enzyme depends on microbe.



Example of enzymatic lysis

lysozyme is the commonly used enzyme to digest cell wall of gram positive bacteria.

Lysozyme hydrolyzes β-1-4-glucosidic bonds in the peptidoglycan (Crapisi et al. 1993).

The cell wall of gram negative bacteria differs from the cell wall of gram positive bacteria so lysozyme is not very efficient in the case of gram negative cell wall.

The cell wall of yeast and fungi differs significantly from the cell wall of bacteria.

One commonly used enzyme mixture for degradation of cell wall of yeast and fungi is Zymolyase.

It has for example β-1,3 glucanase and β-1,3-glucan laminaripentao-hydrolase activities (Zymolyase | Yeast lytic enzyme).

In addition, the enzymes that are commonly used for degradation of cell wall of yeast and fungi include different cellulases, pectinases, xylanases and chitinases.

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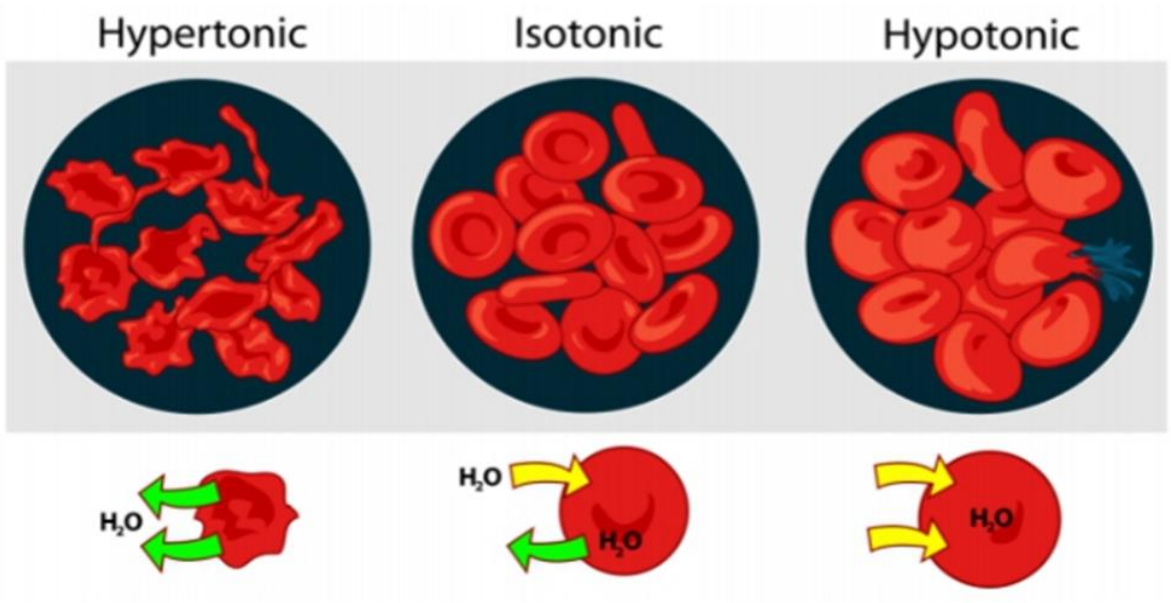
3.2.3 Osmotic lysis



Cells have an ability to actively control the internal conditions but sudden and major changes in cell's surrounding environment might lead to extreme shock which results in cell disruption and death.

Osmotic shock is a technology which can be utilized in biotechnical applications to cause cell lysis.

In this technology, cells are first exposed to either high or low salt concentration. Then the conditions are quickly changed to opposite conditions which leads to osmotic pressure and cell lysis.

The reason for that is that water quickly flows from low salt concentration conditions towards conditions with high salt concentration.

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3.2.4 Freeze-thaw cycles;

This technique involves **freezing a cell suspension in a dry ice/ethanol bath or freezer and then thawing the material at room temperature or 37°C**.

This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing.

Freezing and thawing of a cell can cause the cells to burst due to the formation and melting of ice crystals.

Gradual freezing, leading to the formation of larger crystals, can cause an extensive damage to the cell.

By combining this method with cell grinding, this technique has shown great results.

Disadvantages;

It is very costly, and restricted to small-scale laboratories.

Some reports have also shown loss of enzyme activity. (Harrison S., 1991)

3.2.5 Ultrasonication

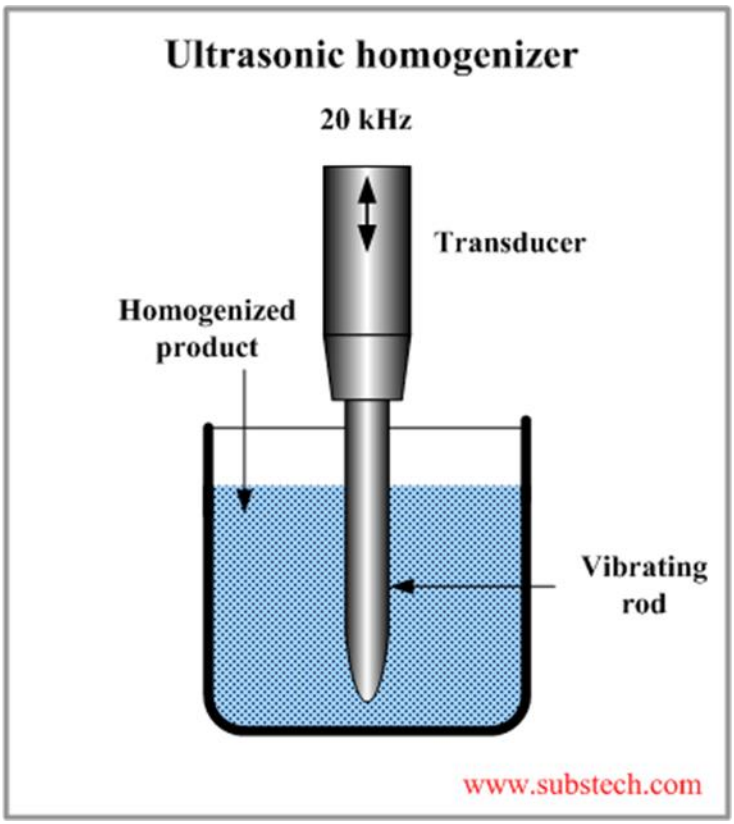
Ultrasonic disruption is caused by ultrasonic vibrators that produce a high frequency sound with a wave density of about 20 kHz/s.

A transducer then converts the waves into mechanical oscillations through a titanium probe, which is immersed into the cell suspension.

Such a method is used for both bacterial and fungal cell disruption.

Bacterial cell can be disrupted in 30 to 60 sec, and yeast between 2 and 10min.

This method is usually used in combination with a chemical method (mostly lysis). (Harrison S., 1991)



ultrasonic disruption

3.2.6 Solvents

One additional method for chemical cell disruption is the utilization of chemical solvents. Solvents which can be used for cell lysis include for example some alcohols, dimethyl sulfoxide, methyl ethyl ketone or toluene (Stanbury et al. 2016).

These solvents extract cell wall's lipid components which leads to release of intracellular components.

This method can be used with wide range of production organisms but the problem can be that some proteins are denatured.

However, the advantage is that by the choice of solvent, it might be possible to select the relished product.

This method is not generally applied in large scale processes.

In addition to solvents, cell lysis can be achieved by hydrolysing the cell wall by alkali compound (pH 10.5-12-5).

Disadvantage of this method is that chemical costs for neutralization of alkali are high. In addition, the product may not be stable in alkali conditions.

3.2.7 Decompression

During explosive decompression, the cell suspension is mixed with pressurized subcritical gas for a specified time, depending on the cell type.

The gas enters the cell and expends on release, causing the cell to burst.

Decompression has been used in small scale laboratories for the disruption of E.coli.

The technique has shown promising results with yeasts, where it has the advantage that supercritical CO2 is able to extract off flavours that are caused by lipid components.

This technique is proving to be promising, being gentle on the cells, resulting in large debris that are easier to remove in order to obtain the desired product.

The disadvantages, however, include its low efficiency and its high dependency on pressure release and time of contact between the cell suspension and the gas.

Decompression chamber

French press and high pressure homogeniser

In a French press, or high pressure homogenization, the cell suspension is drawn through a valve into a pump cylinder.

Then it is forced under pressure of up to 1500 bar, through a narrow annular gap and discharge valve, where the pressure drops to atmospheric pressure.

Cell disruption is achieved due to the sudden drop in pressure upon the discharge, causing the cells to explode.

This method is one of the most widely known and used methods

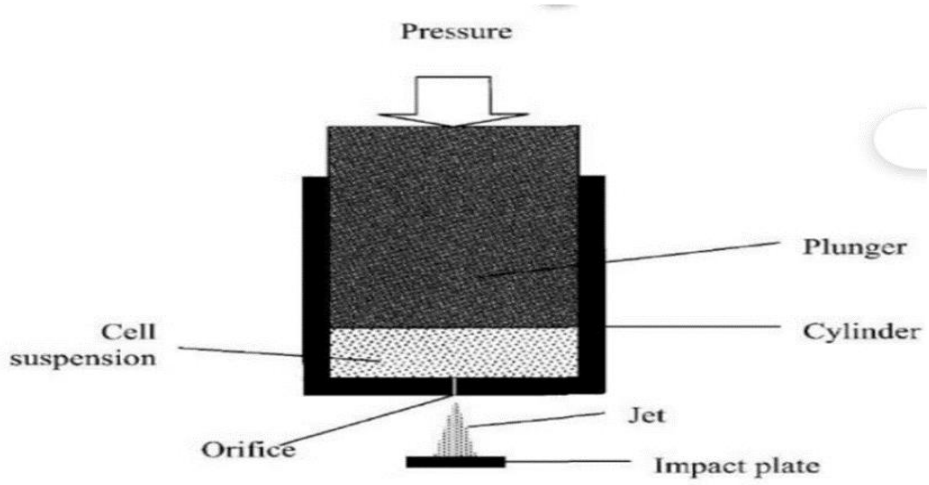
It is mostly used for yeast cells.

It is a vital unit in the dairy production industry, for milk homogenization. (Middleberg A., 1995)

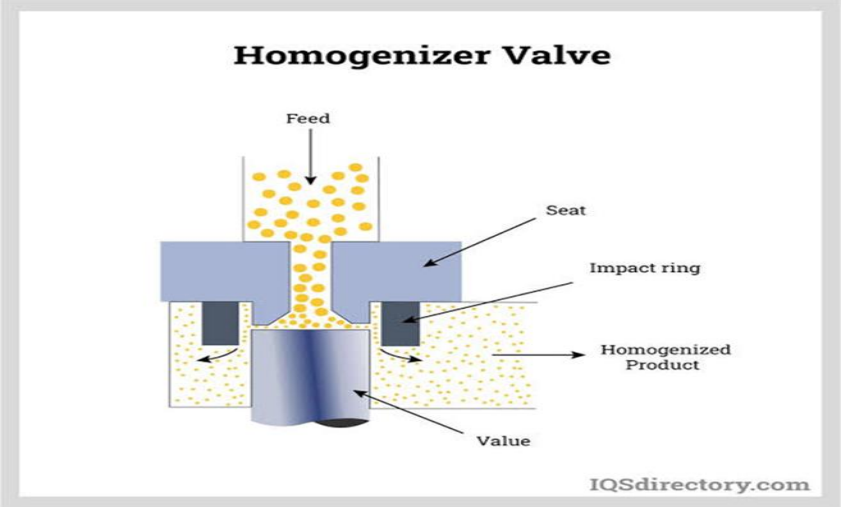
The French press is a small scale method, whereas the homogenizer can be applied to a large scale production.

Homogenisers can vary in design and has a high amount of solids, up to 50% of the feed.

Heat generation is also high – 1.5ºC/1000 psi. (Geciova J., 2002)



Schematic representation of the basic
principle of a French press



Schematic representation of the homogenizer

3.2.8 Thermolysis :

Thermolysis has shown potential in becoming more common in large scale production.
Periplasmic proteins in G (-) bacteria are released when the cells are heated up to 50ºC.
Cytoplasmic proteins can be released from E. coli within 10min at 90 ºC.
Improved protein release has been obtained after short high temperature shocks, then when at longer temperature exposures at lower values.

Disadvantages

Unfortunately, the results are highly unreliable, as the protein solubility changes with temperature fluctuations. (Middleberg A., 1995)

3.3 PURIFICATION OF PROTEINS

There are different methods for the purification of proteins and more than one method can be used.

These methods include differential centrifugation, differential salt precipitation, differential solvent precipitation, preparative electrophoresis and column chromatography.

However, these methods depend heavily upon being able to isolate and purify the desired proteins so that their physical and chemical properties can be understood, along with their tertiary structures and interactions with ligands and substrates.

The intensity to which this purification process is pursued depends upon the use of the protein. For instance, pharmaceutical and food proteins need to be brought to a high grade of purity, and pass through several sequential steps, and as few as possible, since at each step some protein will inevitably be lost.

There are 3 steps in protein purification and these are

Step 1: Creating a Crude Protein Extract;

Crude extracts of intracellular proteins are prepared by lysing the cell using chemical or mechanical processes.

The debris is then removed by centrifugation.

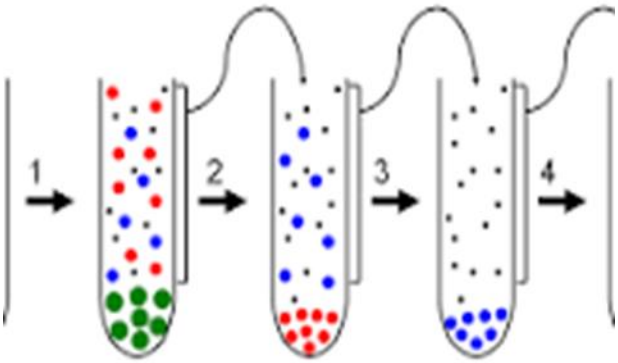
Differential Centrifugation

Separation of proteins (or any material) on the basis of their size, mass, and density

It is a function of the size of the protein and the speed of

centrifugation

It is gives us a rough separation. It is basically fractioning!



The resulting supernatant is a far from pure form, being mixed with many other macro and micromolecules.

Extracellular proteins are obtained by centrifuging the solution and removing the cells.

A specific method to obtain a crude extract of thermostable enzymes is to heat the mixture so as to denature other proteins, and then cool it to reform the thermostable proteins of interest, finally centrifuging it to remove the denatured proteins.

Step 2: Intermediate Purification:

Salting-out

Proteins in a crude extract are next purified by precipitating them in a highly concentrated salt solution (salting out), such as ammonium sulfate.

This works on the basis of the lower solubility of protein at high concentrations of salt.

However, all proteins do not precipitate at the same concentration of salt, which means salting out also helps to fractionate proteins.

It can also be used to concentrate proteins in solution.

This step increases the purity three times and 92% of the protein in the solution is recovered

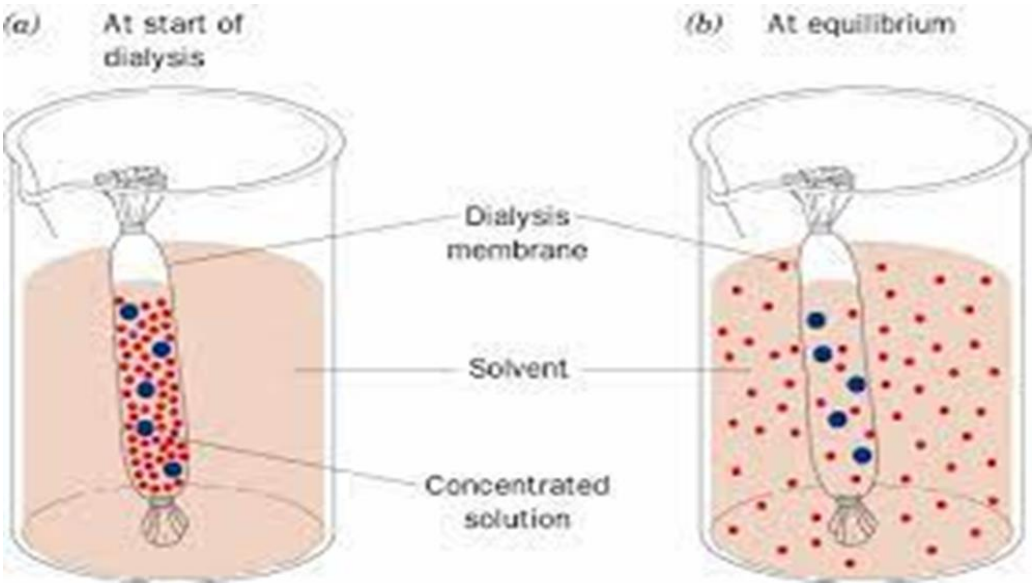
Next in step 2 is Dialysis

Dialysis

Proteins are large molecules, and this means the proteins will be retained by passing the solution (protein-salt mixture) through a semipermeable membrane.

Cellulose is a typical dialysis membrane.

Dialysis cannot be used to separate proteins of different molecular weights.



Why use laboratory dialysis?

Many Advantages:

- Simple, easy & quick set-up
- Inexpensive & disposable
- Very gentle separation (no pressure or shear stress)
- Doesn't require monitoring
- Dissolved solutes remain in solution
- Solutes are unaltered or damaged
- Maintains macromolecule concentration
- Purify low MW solutes
- Total product recovery

Only 2 Major disadvantages:

- Very slow (1 – 2 days)
- Molecules should be 100 X size difference

Chromatography

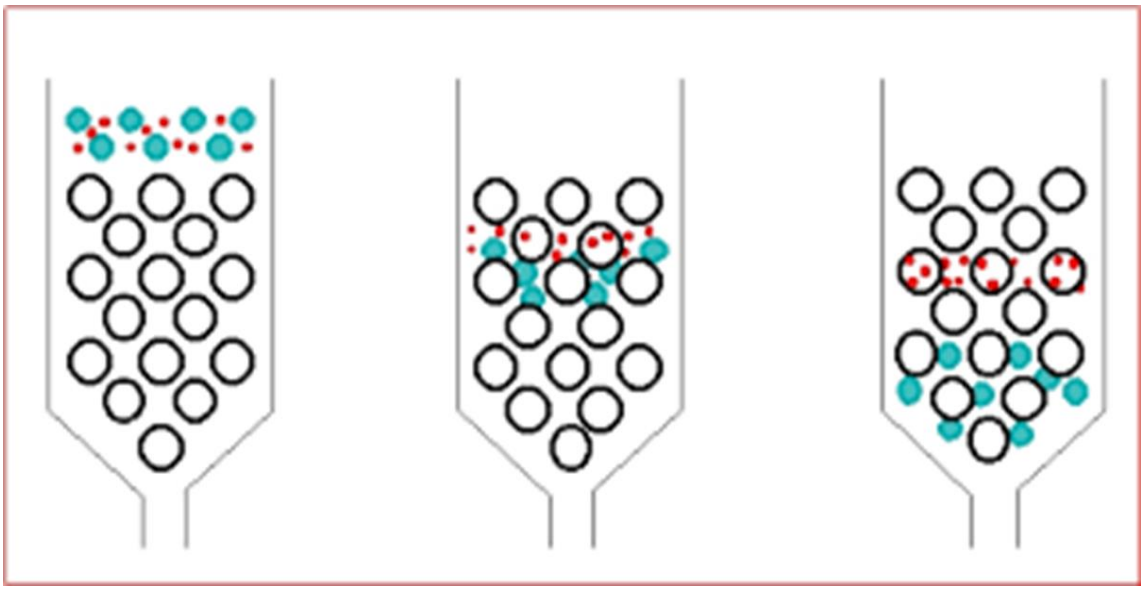
Other techniques used to remove salted out proteins include gel exclusion chromatography and filtration.

These are now available as preformed kits for many standard proteins, and are often suitable for large-scale processes.

Gel filtration works on the basis of size separation through a column of porous polymer beads, such as dextran or agarose.

The large molecules can flow only through the spaces between the beads, while the smaller ones occupy both these spaces and the space inside the beads, slowing them down.

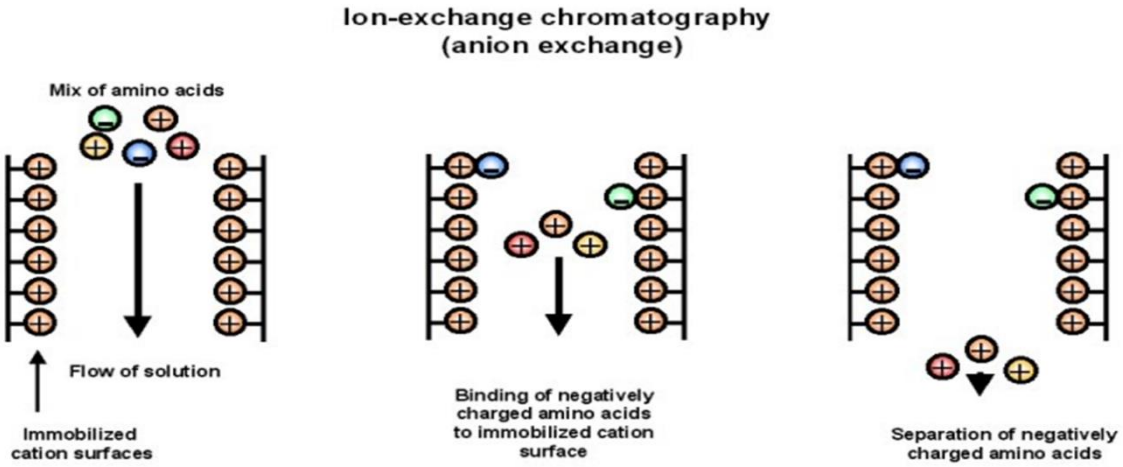
Gel filtration chromatography



Thus the eluent contains molecules emerging in order of their size, from largest to smallest.

Reverse-phase or ion-exchange techniques of chromatography

It operates on the basis of differential hydrophobic properties and charge respectively.



Principle is to separate on basis of charge “adsorption”

Highest resolving power

Highest loading capacity

Widespread applicability

Most frequent chromatographic technique for protein purification

Ion exchange/Reversed-phase chromatography may be limited in its application due to possible protein denaturation by organic solvents.

Dialysis and ion-exchange result in a solution which is 9 times as pure, but with only 77% of the original protein being now available.

After gel exclusion chromatography, the yield is only 50% but the purity is 100-fold.

Step 3: Final Purification Affinity Chromatography

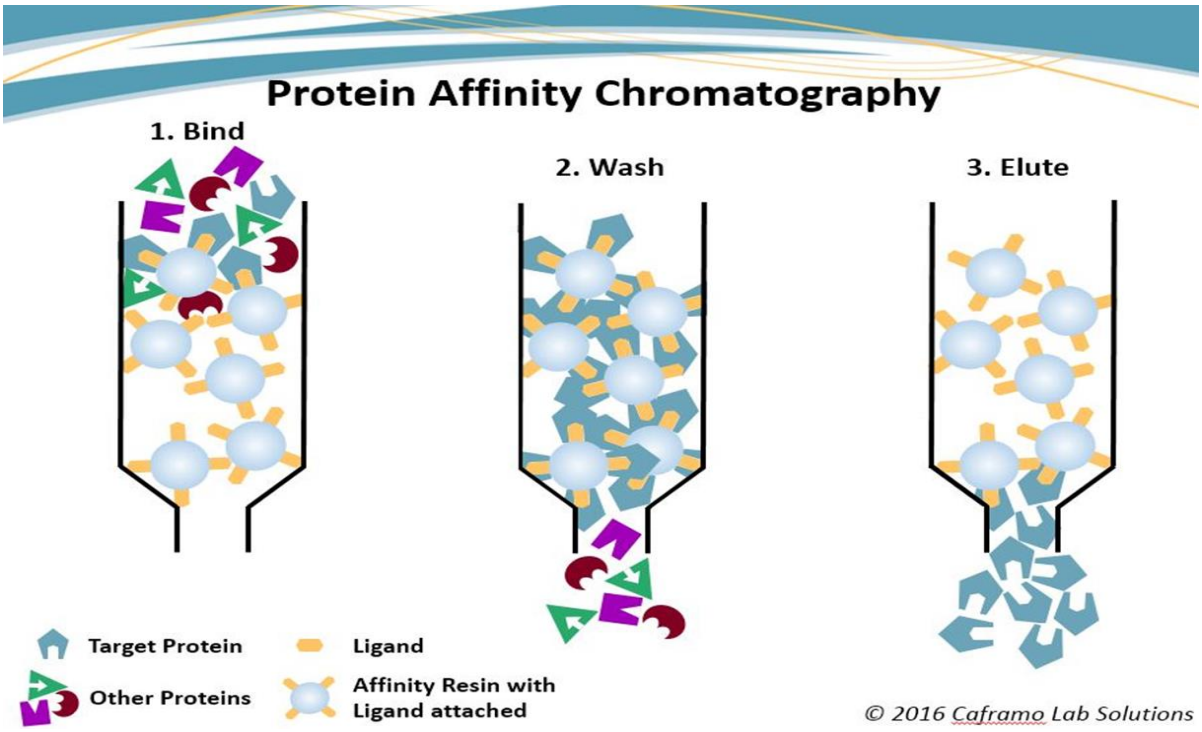
This process depends upon using ligands bound to beads which bind specifically to the protein of interest which can then be rinsed out with another solution of free ligands.

Adsorptive separation in which the molecule to be purified specifically and reversibly binds (adsorbs) to a complementary binding substance (a ligand) immobilized on an insoluble support (a matrix or resin)

Purification is 1000X or better from a single step (highest of all methods)

Preferred method if possible

This results in extremely pure protein samples which have the highest specific activity among all techniques in common use.

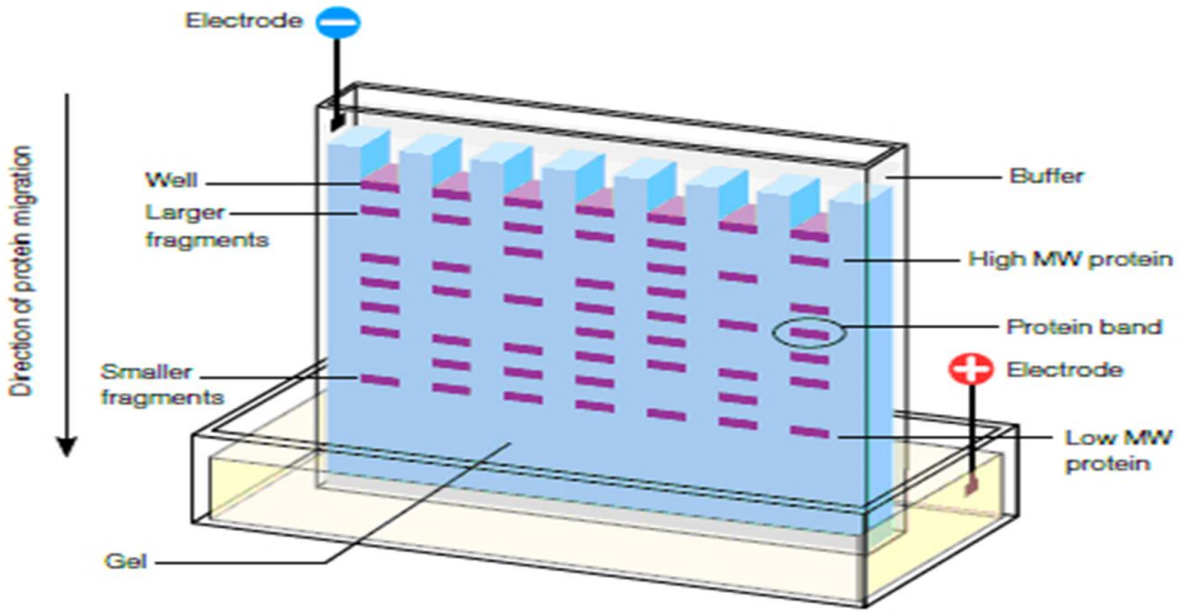


Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis is used to detect the purity of the protein sample after each step based on the size.

The net charge on the molecule makes it move down the gel column or sheet in an electric field, making it possible to separate the proteins based on their velocity of migration, which in turn depends upon their charge, as well as the friction and the field strength.

The gel acts as a chemically inert and easily formed filter, with protein molecules being almost immobile in the column because they are stuck between the much smaller pores between the molecules of the gel. Initially a series of bands is displayed which represent different proteins in the mixture, which gradually reduce in number till the final step shows only one band.



Immunoblotting

Immunoblotting techniques use antibodies (or other specific ligands in related techniques) to identify target proteins among a number of unrelated protein species.

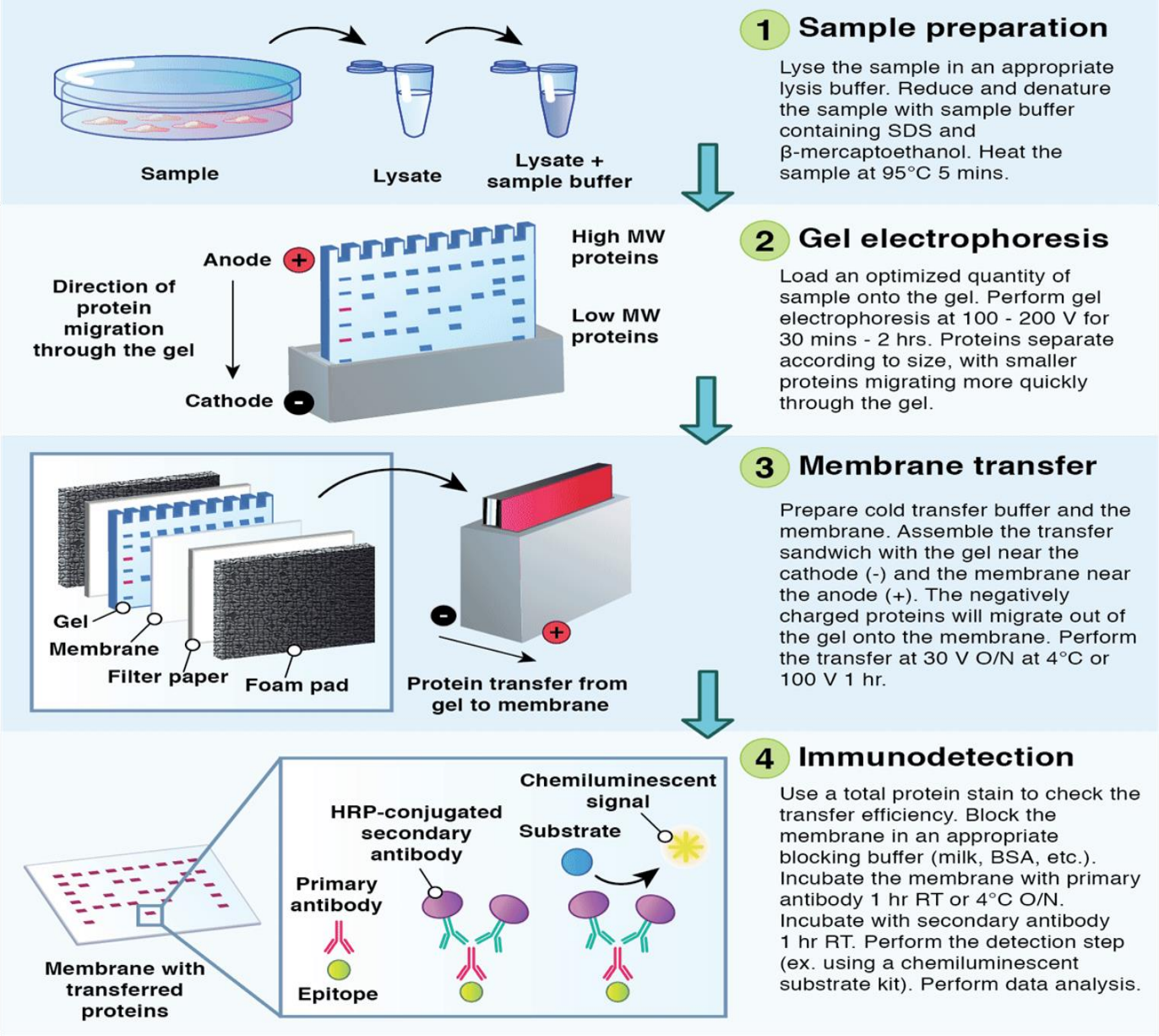
They involve identification of protein target via antigen-antibody (or protein-ligand) specific reactions.

Proteins are typically separated by electrophoresis and transferred onto membranes (usually nitrocellulose).

The membrane is overlaid with a primary antibody for a specific target and then with a secondary antibody labeled, for example, with enzymes or with radioisotopes.

When the ligand is not an antibody, the reaction can be visualized using a ligand that is directly labeled. Dot blot is a simplified procedure in which protein samples are not separated by electrophoresis but are spotted directly onto membrane.

Immunoblotting is now widely used in conjunction with two-dimensional polyacrylamide gel electrophoresis, not only for traditional goals, such as the immunoaffinity identification of proteins and analysis of immune responses but also as a genome-proteome interface technique.



high-performance liquid chromatography (HPLC).

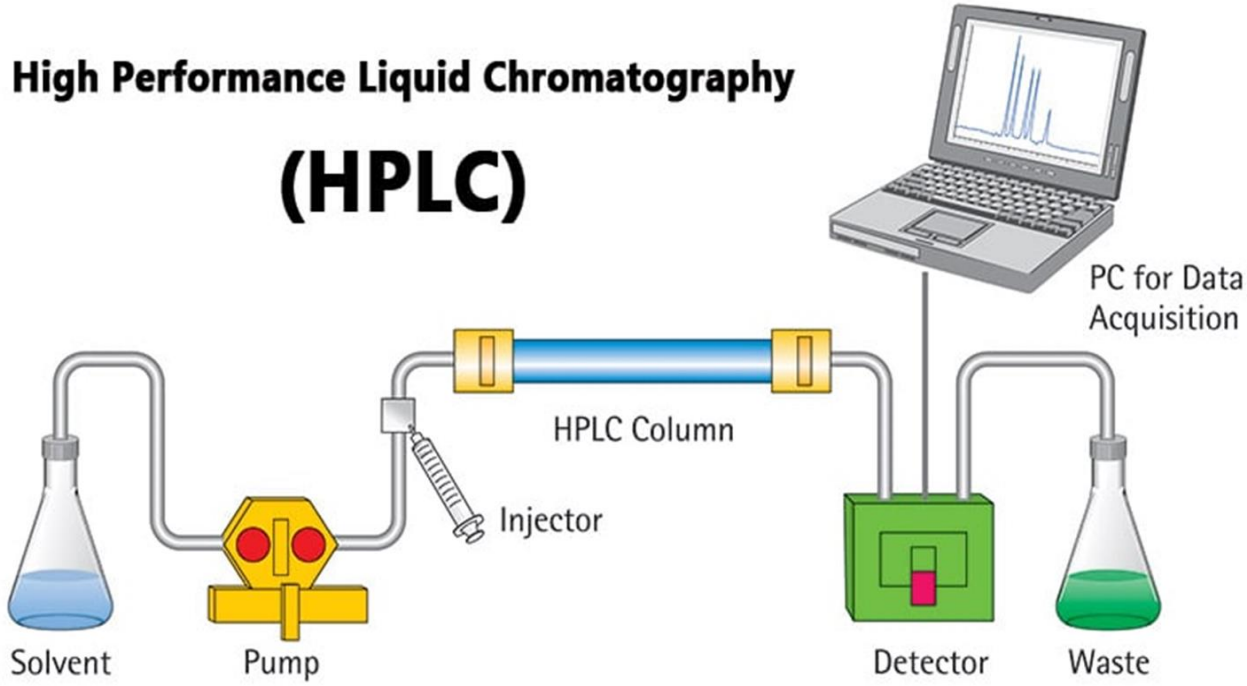
Any chromatographic technique is improved by using pressure to force the solution through a column of finely divided materials, whether charged or ligand-bonded beads.

The increased surface area results in greater interaction which pushes up the resolution and speed of the technique.

This is referred to as high-performance liquid chromatography (HPLC).

High-performance liquid chromatography is an analytical technique used to separate, identify or quantify each component in a mixture.

The mixture is separated using the basic principle of column **chromatography** and then identified and quantified by spectroscopy.



Summary of Study Session 2 of module 4

In this study session, you have learnt how to;

Describe most common methods of protein isolation and purification

Compare between different methods of protein purification

Construct a purification algorithm based on your knowledge in protein isolation and purification

Self-Assessment Questions (SAQs) for Study Session 3

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting.

1. What is homogenization?
2. State 4 protein isolation methods
3. Differentiate between high performance liquid chromatography and affinity chromatography
4. Describe the working principle of dialysis as highlighted above

Links to OERs

References/ Suggestions for Further Reading

Should you require more explanation on this study session, please do not hesitate to contact your e-tutor via the LMS.

Are you in need of General Help as regards your studies? Do not hesitate to contact the Center by e-mail or phone on:

MODULE 5
STUDY SESSION 1

ENZYMOLOGY

THE MERCHANT THEORY

Introduction

In the last will of a rich merchant he put aside his 17 white horse for horse for his 3 sons to be shared as following ½ for the first son, 1/3 for the second son, 1/9 for the third son. After his death the sons started to quarrel, as the division could not produce a whole number. Then their brother in-law donated his black horse to be included in the sharing.

Thus now they had 17+1=18 horses, and so the division was possible. First son got ½ or 9 horses, the second son got 1/3 or 6 horses and the last son got 1/9 or 2horses. Now all 17 white horses were correctly divided by the sons. The remaining black horse was taken back by the brother in-law. Catalysts are similar to the black horse.

Catalysts are substances that accelerate the rate of chemical reactions, but don not change the equilibrium of the reaction.

Learning Outcomes for Study Session 1

After you have studied this study session, you should be able to:

4.5 Define Enzymes. (SAQ 1.1)

4.6 Define (SAQ 1.2)

4.7 Define. (SAQ 1.3)

Key Terms: *enzyme, Properties, classification*

1.1Brief Historical Overview

Berzelius 1835. ------ Put forward the theory of enzyme catalysis. In 1850s, Louis Pasteur studied fermentation of beer and found that yeasts have something in it which converts sugar into alcohol. He

named the unknown thing as 'ferments'. According to Louis Pasteur, that we cannot separate those ferments from yeasts.

Wilhelm Frederick Kuhne 1878 ---- Coined the word enzyme. (Greek – 'In yeast'). Discovery of enzymes and its role in the sustaining life opened the new branch of Life Science called Biochemistry.

In 1897, Eduard Buchner break open the yeasts cells releasing the yeast extract. He showed that yeast extract can also ferment sugar to alcohol. Hence, he successfully separated the 'ferments' from the yeasts proving that fermentation can also be carried out even when after separating ferments from the cells, hence disapproving the concept postulated by Louis Pasteur. Eduard Buchner has received the Nobel Prize for discovering cell free fermentation.

James Sumner, and John Northrop and Moses Kunitz crystallized urease, pepsin and trypsin respectively. The crystallization of enzymes found that chemically they are proteins. It was Haldance who postulated catalytic ability of enzymes. He suggested that enzyme interacts with its substrate with weak and non-covalent bonds forming the product. This is the fundamental principle of enzymology till date.

Oswald 1909 --- Rate of chemical reactions equilibrium and catalysis.

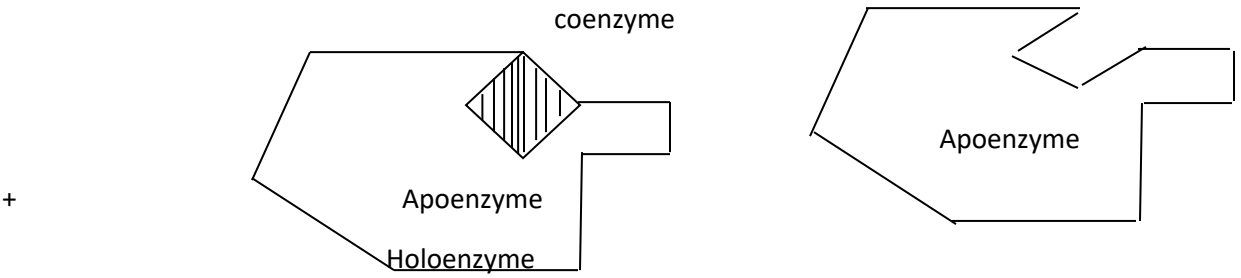

What are enzymes?


enzymes are

1.2Chemical nature and Properties of enzymes

- Enzymes are biological catalysts, a kind of protein formed by the living cells which catalyze a particular reaction or a group of closely related reaction.
- All enzymes are proteins having three (3) dimensional structure.
- Many enzymes contain a non-protein component called prosthetic group, which may be covalently or non-covalently linked to the enzyme molecule.
- The enzyme and the prosthetic group (Coenzyme) together form a holo enzyme. If the cofactor is dissociated from the enzyme molecule, it loses its catalytic activity and is called Apo enzyme

.

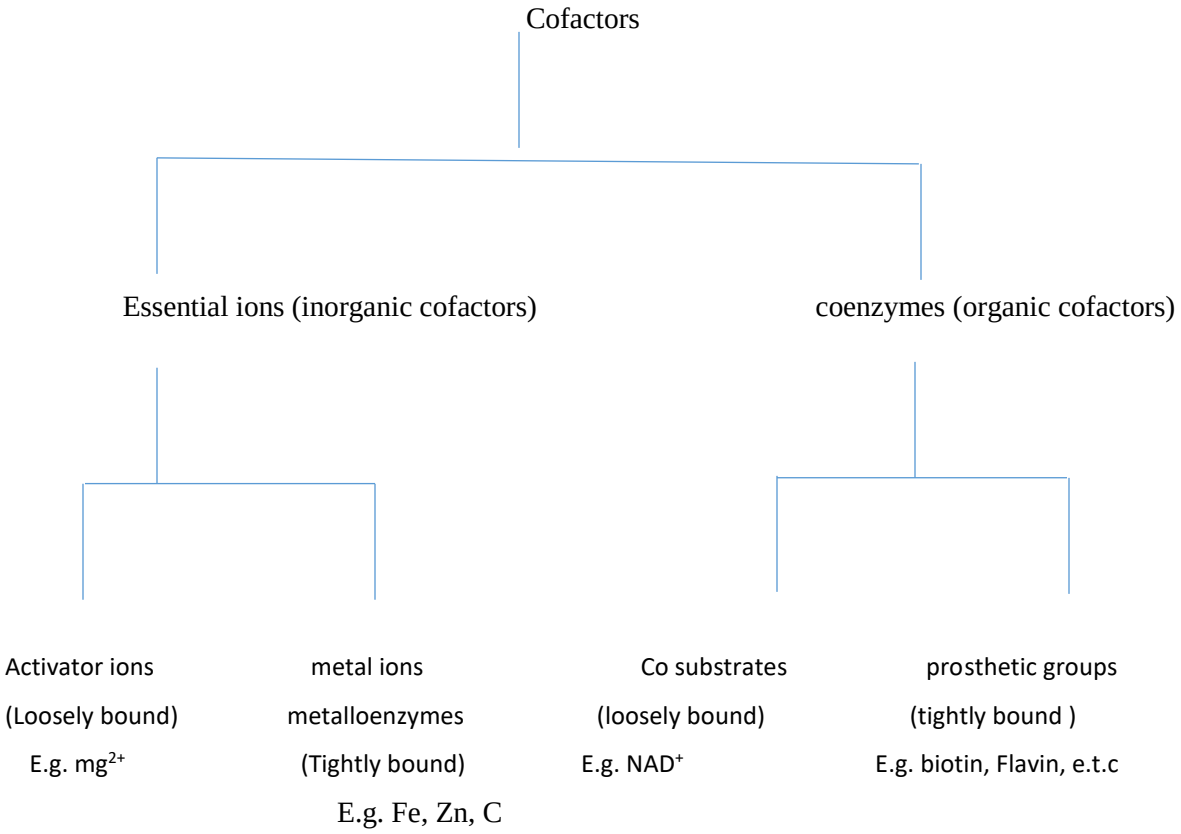


Holoenzyme

Is composed of a cofactor (coenzyme) and an apoenzyme molecule. Cofactor is an essential component for catalytic function.

The substance on which the enzyme acts is called a substrate. Most enzyme have specific substrates whose specificity is determined by the protein or the apoenzyme. The enzyme usually converts the substrate to the product(s). Lack of enzymes will lead to block in metabolic pathways causing in born errors of metabolism. E.g Albinism, etc.

1. Many enzymes contain a non-protein component called cofactors and are defined as small non-protein molecules that is bound (either tightly or loosely) to an enzyme and is required for catalysis. The catalytic activity of many enzymes depend on the presence of cofactors.



2. They are specific in their action. Enzyme specificity is its ability to act selectively on one substance or a small no of chemically similar substances, the enzyme substrate. This property distinguishes enzymes from other inorganic catalysts.

Four types of specificity

Absolute specificity:

Only one reaction is catalyzed by the enzymes in this group. e.g. glucokinase, lactase etc.

Group Specificity:

In this type, enzymes will react with molecules with specific functional groups (example of such groups include: aromatic groups, methyl groups, etc.). Example of enzymes in this group include: Trypsin and Chymotrypsin. Trypsin hydrolyses only lysine and arginine residues while chymotrypsin hydrolyses aromatic amino acids.

Linkage Specificity (bond specificity).

The enzymes in this group acts on particular types of chemical bonds. E.g. Amylase and Pepsin. Amylase hydrolyses glycosidic bonds in Starch, dextrin and glycogen while Pepsin hydrolyses peptide bonds.

Stereo chemical Specificity:

Enzymes here are sensitive to the substrates optical activity of orientation. Stereochemical molecules differ in the way they rotate plane polarized light when in solution. E.g. L-amino acid oxidase acts on L-amino acid only.

3. Enzymes are saturable: The effect of enzyme on substrates increases until equilibrium when the rate of binding is equal to dissociation rate.
4. The chemical equilibrium of an enzyme catalyzed reaction remain unchanged.
5. They are sensitive. (Sensitivity): Enzymes are sensitive to temperature, Ph, ionic strength, inhibitors, e.t.c.
6. They have high catalytic efficiency. Catalytic efficiency of an enzyme is the number of substrates chemically changed to products per unit time per unit enzyme. It is measured in K-cat.
7. Enzymes can be regulated. Enzymes can be regulated by hormonal or non-hormonal components.

1.3 Characteristics of Enzymes

1. Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.
2. They are heat labile
3. They are water soluble
4. They can be precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid)
5. They contain 16% weight as nitrogen.
6. They are required in minute amounts.
7. They speed up chemical reactions.

1.4 Classification of enzymes;

There are hundreds of biochemical reactions that take place simultaneously in the living system, all under the control of enzyme systems. Arising from this is the fact that there must be very many enzymes to be found in living cells. It is therefore necessary that some rational system of classification that emphasize relationships and similarities should exist. Early attempt at classification was based on the addition of the suffix, -ase to the substrate acted upon. For example, enzymes that act on lipids, proteins, starch (amylon) were called lipases, proteinases or proteases, and amylases respectively. Such Classes as oxidases, glucosidases, dehydrogenases, decarboxylases were also introduced. In order to have a uniformity and unambiguity, the international union of Biochemistry (IUB) adopted a nomenclature system based on chemical reaction type and reaction mechanism. According to this system, enzymes are grouped into six main classes:

Each enzyme is characterized by a code number (enzyme code number or EC number) comprising four figure (digits) separated by points, the first being that of the main class (one of the six).

The second figure indicates the type of group involved in the reaction.

The third figure denotes the reaction more precisely indicating substrates on which the group acts.

The fourth figure is the serial number of the enzyme.

In summary, the four digits characterize; class, sub class, sub-sub-class and serial number of a particular enzyme.

The enzymes are grouped into the following six major classes:

$AH_2 + B \rightarrow A + BH_2$

Class1: Oxidoreductases;

They are involved in the oxidation and reduction reactions of their substrates.

$$AH_2 + B \rightarrow A + BH$$

Examples here include;

1. Dehydrogenases: They catalase the removal of two atoms of hydrogen.
2. Oxidases: These catalyze reduction of O_2
3. Oxygenases: Catalyze incorporation of molecular O_2 into substrate.
4. Oxidative deaminases: Catalyze the oxidation of amino compounds with the formation of NH_3
5. Hydroxylases: They introduce OH groups
6. Peroxidases: They use H_2O_2 as oxidants.

Class 2: Transferases

These class of enzymes transfers one group (other than hydrogen) from one substrate to another substrate. $A\text{-}R + B \rightarrow A + B\text{-}R$

Examples here include;

1. Aminotransferases: Catalyze exchange of amino and keto group between amino and keto group.
2. Kinases: Catalyze the transfer of a PO_4 radical.
3. Acyl Transferases: Catalyze the transfer of acyl/acetyl group to a suitable acceptor.
4. Glycosyltransferases. They transfer glycosyl groups.

CLASS 3: Hydrolases;

They catalyze hydrolysis reactions. These classes of enzymes can hydrolyze ester, either, peptide or glycosidic bonds by adding water and then breaking the bond.

$$AB + H_2O \rightleftharpoons A\,OH + HB$$

Examples include:

1. Peptidases: catalyze hydrolysis of peptide bonds

2. Glycosidases: catalyze glycosidic bonds
3. Esterases: carry hydrolysis of carboxylic esters
4. Phosphatases: hydrolyse phosphoric acid esters
5. Phosphodiesterases
6. Deaminases: catalyze hydrolysis of amines
7. Deamidases: catalyze hydrolysis of amides.

Class 4: Lyases

These enzymes can remove groups from substrates or break bond by mechanisms other than hydrolysis.

Examples include:

1. Decarboxylases
2. Aldolases
3. Dehydratases

Class 5: isomerases.

These enzymes catalyze isomerization of substances (substrates). They can produce optical, geometrical or positional isomers of substrates.

Examples include:

1. Racemases.
2. Epimerases.
3. Cis and trans isomerases.

Class 6; Ligases

These enzymes link two substrates together usually with the simultaneous hydrolysis of ATP.

Examples include:

1. Synthases: They bring about the formation of C-O, C-S, C-N or C-C bonds. Reactions require expenditure of energy with simultaneous cleavage of ATP.

2. Acetyl Carboxylase.


what is enzyme specificity?

Summary of Study Session 1

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 1

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

SAQ 1.1 **(Tests Learning Outcome 1.1)**

Define enzymes

SAQ1.2 **(Tests Learning Outcome 1.2)**

What is

SAQ 1.3 **(Tests Learning Outcome 1.3)**

Define

SAQ 1.4 **(Tests Learning Outcome 1.4)**

What is the

SAQ 1.5 **(Tests Learning Outcome 1.5)**

What are the

Links to OERs

References/ Suggestions for Further Reading

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MODULE 6

STUDY SESSION 1

VITAMINS

Introduction

Vitamins are organic compounds occurring in natural food either as such or as usable precursors which are required in small amounts for normal growth maintenance and reproduction that is, for normal nutrition and health.

They are different from other organic food substances because they do not enter into tissue structures unlike proteins and they do not undergo degradation for providing energy unlike carbohydrates and lipids. They are also different from hormones in not being produced within the organism and most of them are provided in the diet.

Learning Outcomes for Study Session 1 of module 6

After you have studied this study session, you should be able to:

Define VITAMINS; (SAQ 1.1)

List the different types of vitamins and classify them based on their solubility; (SAQ 8.2)

Key Terms: ***vitamins, water soluble vitamins, fat soluble vitamins***

1.1 Classification of Vitamins.

Vitamins are classified into two major classes based on their solubility. These are;

1. Fat soluble vitamins;

Vitamin A, Vitamin D, Vitamin E, and Vitamin K

2. Water Soluble vitamins:

(a) Vitamin C (ascorbic acid),

(b) Vitamin B complex group includes:

- Vitamin B1 (thiamine)
- Vitamin B2 (riboflavin)
- Niacin (nicotinic acid)
- Vitamin B6 (pyridoxine)
- Pantothenic acid
- α-Lipoic acid
- Biotin
- Folic acid group
- Vitamin B12 (cyanocobalamine).

Define Vitamins.

List the different types of vitamins and classify them based on their solubility

Summary of Study Session 8

In this study session, you have learnt that:

- Vitamins are organic compounds occurring in natural food either as such or as usable precursors which are required in small amounts for normal growth maintenance and reproduction that is, for normal nutrition and health.
- Vitamins are classified into two major classes based on their solubility

Self-Assessment Questions (SAQs) for Study Session 8

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

SAQ 8.1 **(Tests Learning Outcome 8.1)**

Define vitamins

SAQ 6.2 **(Tests Learning Outcome 8.2)**

List the different types of vitamins and classify them based on their solubility

Links OERs

References/ Suggestions for Further Reading

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MODULE 6

STUDY SESSION 2

FAT SOLUBLE VITAMINS

Introduction

Fat-soluble vitamins are organic compounds that are essential for the proper functioning of the body. Unlike water-soluble vitamins, they are stored in the body's adipose tissue and liver and are not easily excreted. The four fat-soluble vitamins are vitamins A, D, E, and K. In this teaching module, we will discuss each of these vitamins in detail, including their scientific names and alphabetic names, their sources, chemistry, metabolism, and metabolic roles, including their deficiency diseases.

Learning Outcomes for Study Session 9

After you have studied this study session, you should be able to:

what are fat soluble vitamins (SAQ 1.1)

list 4 fat soluble vitamins; (SAQ 1.2)

List the stages of perception; (SAQ 1.3)

Identify four the factors affecting perception; (SAQ 1.4)

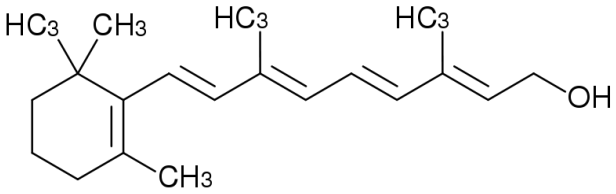
State five educational significance of perception; (SAQ 1.5)

Key Terms: vitamins, fat soluble vitamins, ADEK

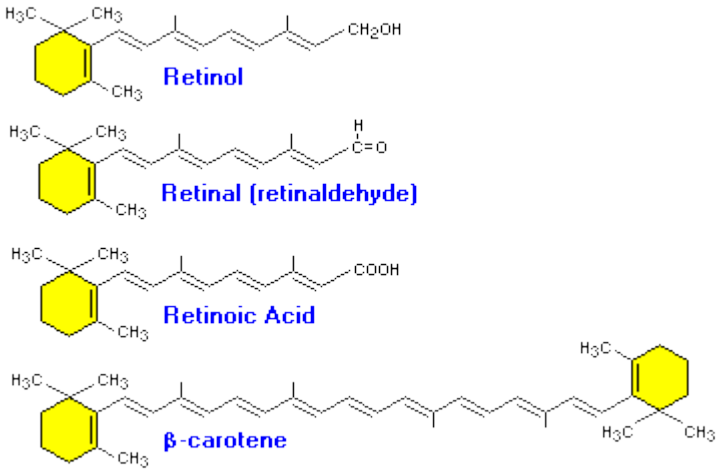
2.1 Vitamin A

Chemistry:

Vitamine A



Retinol



Vitamin A is a fat-soluble vitamin that is stored in the body's adipose tissue and liver. It is also known as retinol because of its role in the formation of the retina in the eye. There are three forms of Vitamin A.

Retinol→ vitamin A alcohol

Retinal → vitamin A aldehyde also called retinene

Retinoic Acid → Vitamin A acid

The above three forms are sometimes called retinoids. They are derivatives of certain carotenoids called provitamin A which are red or yellow pigments that are ubiquitous in nature. They are α, β and ϒ carotenes. Two molecules of vitamin A are formed by symmetrical oxidative cleavage of β-carotene while only one molecule of vitamin A is obtained from α and γ carotenes. Beta-carotene is composed of 40 carbon atoms and has a symmetrical structure. It consists of two connected rings, each containing multiple double bonds. The molecule has an extended, linear shape and is hydrophobic. At each end of the

molecule, there is a cyclohexene ring, and in the center, there is a chain of 11 conjugated double bonds. Beta-carotene is a precursor to vitamin A and has antioxidant properties.

Occurrence.

Two different forms of Vitamin A exist in nature.

Vitamin A1, which is the usual form occurs in nature more than the other type except in fresh water fish, where another form called **Vitamin A2** predominates. Vitamin A1 is found more in major species of animals while Vitamin A2 is predominantly present in fresh water fish liver and other tissues. Vitamin A1 can be gotten from β-carotene and is more potent than Vitamin A2 which cannot be obtained from β-carotene.

Dietary Sources;

• Animal sources: Egg yolk, Liver oil, milk, butter, cheese.

• Plant sources: In the form of pro vitamin carotene, carrots, sweet potatoes, spinach, Spirulina species tomatoes and other dark leafy green-yellow vegetables, fruits like, papayas and mangoes, corn and sweet potatoes.

Adult female and male require about 3000 IU daily, but a recommended allowance is about 5000 IU daily dose. It is required more in growing children, lactating mothers and pregnant women. The requirement is also more in liver disease.

Metabolism:

Vitamin A is absorbed in the small intestine and is transported to the liver where it is converted to retinyl esters. It is then transported to other tissues where it is converted to retinol, which is the active form of vitamin A.

Functions/Metabolic roles:

Vitamin A is essential for proper vision, immune function, and the growth and development of bones and teeth. Deficiency in vitamin A can lead to night blindness, dry skin, and increased susceptibility to infections.

Role in Vision Perhaps the only function of vitamin A which is clearly understood to its molecular details is its role in vision. The overall mechanism through which vitamin A functions in visual system is known as Wald's visual cycle or "Rhodopsin cycle" discovered by Wald George. Retina contains two types of receptor cells: (i) Cones: Specialized for

colour and detail vision in bright light contains iodopsin. (ii) Rods: Specialised for visual activity in dim light (night vision), contains rhodopsin.

2. Role in Reproduction; Experimental work with rats shows that vitamin A deficient male rats do not develop their testes properly in that they are oedematous and sperm cells do not develop to state of maturity. When such male rats are allowed to mate with normal fertile females, no conception takes place. In contrast, vitamin A deficient female rats maintain normal oestrous cycle and do conceive, but are unable to carry the pregnancy to full-term.

3. Role in Epithelialisation; The epithelial structures of skin and mucous membrane show gross structural changes in deficiency.

• Skin: Skin becomes dry, scaly and rough. These changes are called keratinisation.

• Lacrimal glands: Similar changes occur in these glands leading to dryness of conjunctivae and cornea, a condition described as xerophthalmia.

• Cornea: White opaque spots called Bitot's spots appear in the conjunctiva on either side in each eye. Corneal epithelium becomes Keratinised, opaque and may become softened and ulcerated, condition described as keratomalacia.

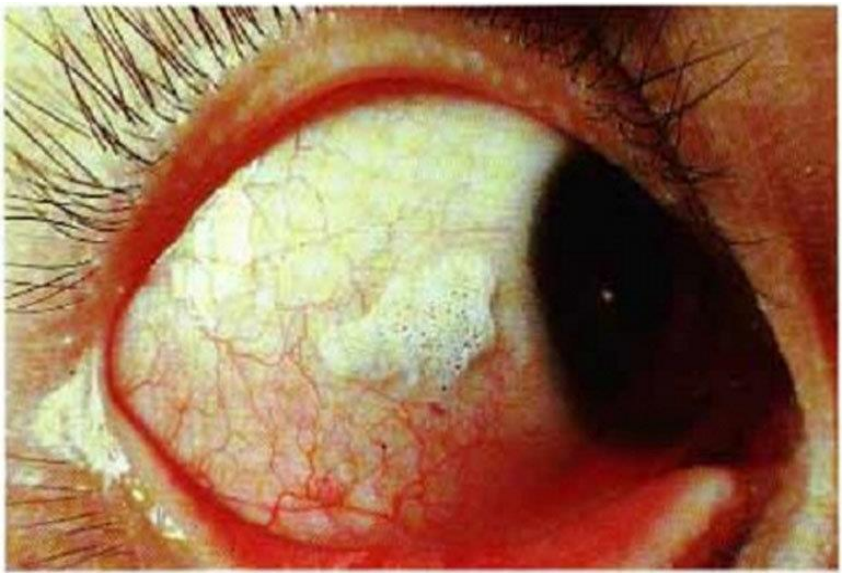


Image by Rui Ribeiro; Science Direct

• Respiratory tract: Keratinisation occurring in the mucous membrane of respiratory tract leads to increased susceptibility to infection and lowered resistance to disease.

• Urinary tract: Keratinisation of UT leads to calculi 'formation'.

4. Role in Bone and Teeth Formation: It plays a role in construction of normal bone. Deficiency results in slowing of endochondral bone formation and decreased osteoblastic activity, the bone becomes cancellous losing their fine structural details. Mechanical damage to the brain and cord due to arrested limits of bony frame work and cranium and vertebral column in which it has to grow. Teeth become unhealthy due to thinning of enamel and chalky deposits on surface.

5. Growth: Vitamin A alongside other vitamins is principally involved in growth. Its role in cell differentiation and cell division has been proved beyond doubt.

6. Metabolism: It may be involved in protein synthesis and may play a role in metabolism of DNA.

Vitamin A deficiency

Nyctalopia (Night blindness): This is one of the earliest signals of vitamin A deficiency which is impairment of dark adaptation. Therefore, continual supply of retinol is essential for normal visual function. Vit A deficiency depresses the re-synthesis of rhodopsin and interferes with the function of rods resulting in night blindness. Vitamin A affects growth and differentiation of epithelial cells leading to defective epithelization, a condition affecting the cornea of the eye. It produces softening and opacity. Severe Vit A deficiency leads to progressive keratinization of the cornea and possibly permanent blindness. Another form, retinoic acid, induces differentiation of epithelial cells. Vitamin A deficiency also predisposes to gastrointestinal and respiratory tract infections. Plasma Vitamin A may be decreased in states of severe protein deficiency, due to lack of its carrier protein. Low plasma Vitamin A has been shown to be associated with an increased risk of developing cancer. Failure of bone formation (Thick, solid bones) is associated with Vitamin A deficiency. Also abnormal Keratin forms in the mucosal cells in Vitamin A deficiency, to cause keratomalecia in the eye. The deficiency causes dryness and roughness of skin developing keratosis of hair follicles with concomitant of Vitamin-B complex deficiency. Bone growth is markedly impaired, osteoclastic activity is also hampered, causing defective bone formation.

Hypervitaminosis:

Excessive intake of vitamin A, in humans will cause hypervitaminosis A syndrome characterized by the following symptoms; head ache, nausea, vomiting and dizziness which might be as a result of increased spinal fluid pressure. Other manifestations include; dry itchy

skin, alopasia, cracking of lips e.tc. The management is simply withdrawal of vitamin A. It is important to note that it is impossible to develop Vitamin A toxicity by ingesting natural foods. When people consume supplements, there might be hypervitaminosis A.

Summary of Study Session 2 of Module 6

In this Study Session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 7

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

Links to OERs

References/ Suggestions for Further Reading

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MODULE 6

STUDY SESSION 3

VITAMIN D

Introduction

Vitamin D is the second of the fat soluble vitamins (ADEK). It is responsible for increasing intestinal absorption of calcium, magnesium, and phosphate. It also has many other biological effects such as increasing the bone tensile strength and maintaining the immunes system.

Learning Outcomes for Study Session 10

After you have studied this study session, should be able to:

Key Terms: vitamin D, fat soluble vitamin

3.1 Chemistry

This Vitamin has two variants also called provitamins which are inactive precursors. They are;

• Provitamin D2 (Ergosterol) found in plants. • Provitamin D3 ((7-dehydrocholesterol) found in the skin. All the provitamins D possess a certain essential structural characteristic. • OH group at C3 • Two conjugated double-bonds between C5-C6 and between C7-C8. • A hydrocarbon chain at C17. Ultraviolet rays transform inactive provitamin to the active vitamin.

3.2 Dietary Sources

Fish liver oil is the richest source of vitamin D. Egg-yolk, margarine, lard, also contain considerable quantity of vitamin D. Some quantity is also present in butter, cheese, etc. Ergosterol is widely distributed in plants. It is not well absorbed hence is not of nutritional importance. Calciferol is readily absorbed. 7-dehydrocholesterol is formed from cholesterol in the intestinal mucosa, and principally liver, then passed on to the skin where it undergoes activation to vitamin D3 by the action of solar Ultra Violet rays. Daily Requirement (1 USP unit = IU = 0.025 μg of vitamin D3). About 100 IU or 2.5 μg of vitamin D3 is the daily requirement in adult man. Pregnant and lactating mother as well as infants and children require about 220 IU per day. Vitamin is easily supplied by cutaneous synthesis in sunlight in tropical countries.

3.3 Absorption and Transport

Bile salts help in absorption of vitamin D from duodenum and jejunum just like most other fat-soluble vitamins. After absorption, it is carried in chylomicron droplets of the lymph in combination with serum globulin in blood plasma.

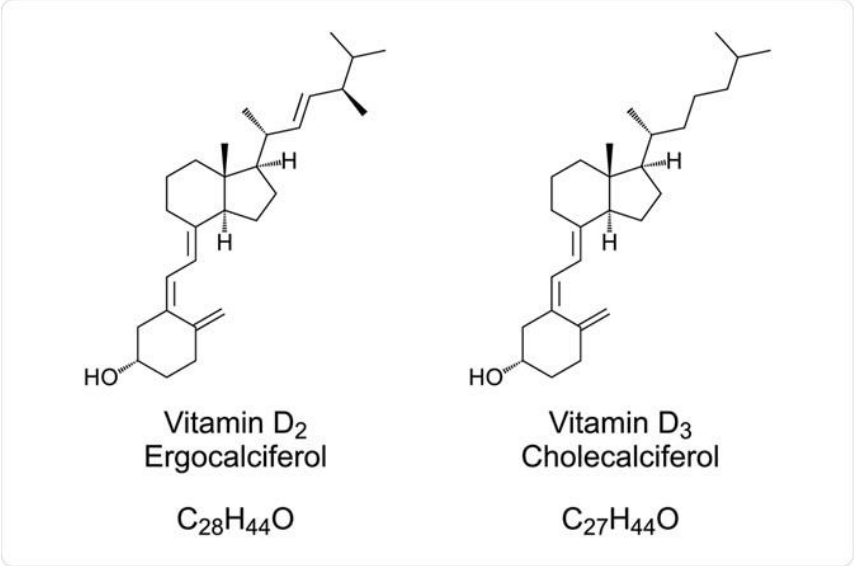


Image Credit: Peter Hermes Furian / Shutterstock.com

Biologically Active Form of Vitamin D is called Calcitriol

3.4 Formation of Calcitriol

Calcitriol is produced in the kidneys and the liver.

(a) Synthesis of 25-OH-D3 in Liver (Calcidiol)

• Vitamin D2 and/or D3 binds to specific D binding protein and is transported to liver.

• It undergoes hydroxylation at 25 position, by the enzyme 25-hydroxylase, in the endoplasmic reticulum of the mitochondria of liver cells.

• Coenzyme/cofactors required are:

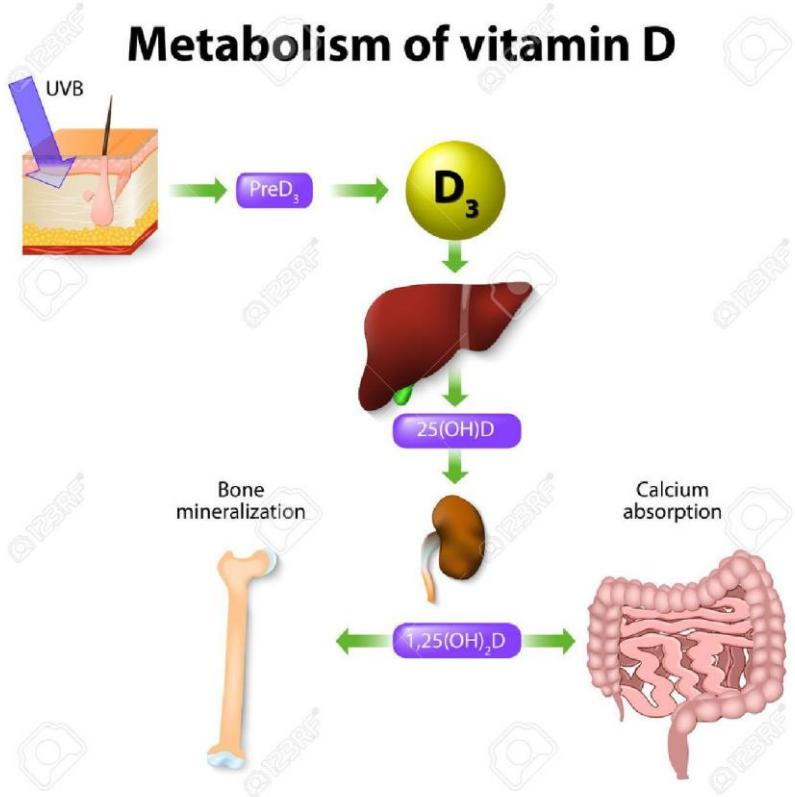
• Mg++

• NADPH, and

• Molecular O2

A cytoplasmic factor is also required, the exact nature not known. Two enzymes, an NADPH-dependant cytochrome P450 reductase and a cytochrome P450 are involved also.

• 25-OH-D3 (calcidiol) is the major storage form of vitamin D in liver and found in appreciable amount in circulation. The blood level of 25-OH-D3 exerts feedback inhibition on the enzyme 25-hydroxylase.



(b) Synthesis of 1, 25-di –OH-D3 (Calcitriol) in Kidneys

• 25-OH-D3 is bound to a specific vitamin D binding protein and is carried to kidneys.

• It undergoes hydroxylation at 1-position, by the enzyme 1 α-hydroxylase, in the endoplasmic reticulum of mitochondria of proximal convoluted tubules of kidney.

• The reaction is a complex three component monooxygenase reaction requiring Mg++, NADPH and molecular O2 as coenzymes/cofactors.

• In addition, at least three more enzymes are required.

They are:

• Ferrodoxin reductase

• Ferrodoxin, and

• Cytochrome P450

This system produces 1,25-di-OH-D3 (calcitriol) which is the most potent metabolite of vitamin D.

It is pertinent to note that renal hydroxylation at position C1 is the most significant, but recently similar hydroxylation has been found to occur in placenta and bone also.

3.5 Regulation of Vitamin D Metabolsm:

Regulation of calcitriol synthesis is done by:

• Its own concentration—by feedback inhibition of 1 α-hydroxylase.

• Parathyroid hormone (PTH)

• Serum phosphate level.

Hypocalcaemia leads to marked increase in 1 α hydroxylase activity, the effect requires PTH.

As stated above, calcitriol regulates its own concentration since high levels of calcitriol inhibit "1 α-hydroxylase" and stimulates the formation of 24,25-di-OH-D_3 which is not potent as calcitriol and now supposed to be a storage form.

3.6 Mode of action of calcitriol

Calcitriol acts in a similar way as the steroid hormone receptors. This binding is specific and reversible. The receptor has a specific binding site on DNA that appears to contain Zinc-finger motif characteristic of other steroid receptors.

Vitamin D as prohormone:

Since calcitriol is synthesised in the body and acts like steroid hormone and has a basic sterol nucleus in its structure, it is now regarded as a hormone and vitamin D as a prohormone.

Vitamin D is considered as a "Prohormone" and calcitriol (1,25-di-OH-D_3) as a hormone.

3.7 Functions/Metabolic Roles

Vitamin D is found to act on target organs like bones, kidneys, intestinal mucosa to regulate calcium and phosphate metabolisms.

1. Mineralization of bones: Mineralization of bones is promoted by 1, 25, (OH)2D_3 as well as 24, 25(OH)2D3. It is believed that the synthesis of Ca++-binding proteins like osteocalcin and alkaline phosphatase is promoted which increases calcium and phosphate ions in the bone. These ions enhance the mineral deposition in the bone. 24, 25(OH)2D_3 helps the deposition of hydroxyapatite in bone. Vitamin D is also believed to promote bone resorption and calcium mobilization to raise the levels of Ca and P in blood in association with PTH.
2. Intestinal absorption of calcium and phosphate: It binds to the chromatin of target tissue and expresses the genes for calcium binding protein as well as Ca++ ATPase in intestinal cells. This increases the Ca++ absorption by actively transporting Ca++ across the plasma membrane against electrochemical gradients.

Other Functions

• Renal reabsorption of calcium and phosphorus is also done by 1,25(OH)2D_3 in similar way.

• It lowers the pH in certain parts of the gut such as colon and produces increase in urinary pH.

• It counteracts the inhibitory effect of calcium ions on the hydrolysis of phytate. In adequate amounts and in case of high calcium intake, it suppresses the anticalcifying and rachitogenic effect of phytate.

• In physiologically compatible intake it is found to increase the citrate content of bone, blood, tissues and urinary level.

3.8 Deficiency:

Usually deficiency of Vitamin D is due to insufficient exposure to sunlight, inadequate dietary intake, GI disorder, obstructive jaundice and Partial gastrectomy. This can lead to rickets in children and osteomalacia in adults.

Rickets is characterized by the production of soft pliable bones due to defective mineralization secondary to calcium deficiency. Vitamin D deficiency is also characterized by low concentration of calcium in blood in association with increased serum alkaline phosphatase.

Type I vitamin D dependent rickets Is caused by an inherited defect in the conversion of 25(OH)- D3 to calcitriol

Type II Is a vitamin D-resistant rickets caused by absence of calcitriol receptor. In adults the deficiency produces Osteomalacia due to decreased absorption of calcium and phosphorous, maintains a low plasma level resulting in weak mineralization of bones.

Renal Osteodystrophy can also result due to Vit D deficiency. There is loss of renal parenchyma or the renal parenchyma could be severely diseased and is not able to form calcitriol and calcium absorption is impaired. Hypocalcemia leads to increase in PTH which acts on bone to increase Ca++. As a result, there is excessive bone turnover and structural changes. This condition is known as renal osteodystrophy

3.9 Hypervitaminosis D

Excess Vitamin D level enhances calcium absorption leading to hypercalcemia and metastatic calcium deposits. There is a tendency to develop kidney stones from the hypercalciuria, secondary to hypercalcemia.


differentiate between vitamin D dependent and vitamin D resistant ricket

Summary of Study Session 3 of module 6

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 8

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

SAQ

Links to OERs

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MODULE 6

STUDY SESSION 4

VITAMIN E (TOCOPHEROLS) AND VITAMIN K

Introduction

Vitamin E is a fat soluble vitamin made up of 8 compounds known as tocopherols and tocotrienols (4 tocopherols and 4 tocotrienols). It is highly distributed in nuts, seeds and vegitables and it functions majorly as an antioxidant.

Vitamin K is also a fat-soluble vitamin found in foods and certain drugs as dietary supplements. It is required by humans for post-translational modification of some proteins needed for blood coagulation and the control of calcium binding in bones and other tissues.

Learning Outcomes for Study Session 4 Of Module 6

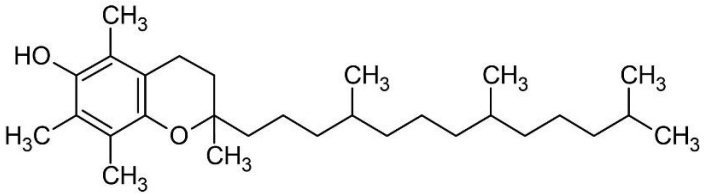
After you have studied this study session, you should be able to:

Key Terms: vitamin E, fat soluble vitamin, alpha tocopherol

4.1 Chemistry of Vitamin E

There are four variants of tocopherols and they differ from each other in the number or position of methyl groups.

- α-tocopherol: 5, 7, 8 trimethyl tocol (most active in vitamin E activity)
- β-tocopherol: 5, 8 dimethyl tocol
- γ-tocopherol: 7, 8 dimethyl tocol
- δ-tocopherol: 8 methyl tocol



Vitamin E α-tocopherol

4.1.2 Dietary Sources and Recommended Allowance

Cottonseed oil, corn oil, sunflower oil, wheat germ oil and margarine are the richest sources of vitamin E. It is also found in fair quantities in dry soybeans, cabbage, yeast, lettuce ,apple seeds, peanuts. Normal blood level = 1.2 mg/dl.

4.1.3 Recommended Allowance

• Adults: 20–25 IU/day Special attention has to be given to the dietary intake of unsaturated fatty acids in which case the daily requirement is increased. Requirement is also more in pregnancy and lactation.

• Children: 10–15 IU/day

4.1.4 Absorption, Distribution and Excretion Vitamin E

Free tocopherols and their esters are readily absorbed in small intestine with the help of bile acids. Absorbed vitamin E is transported to liver where it gets incorporated into lipoproteins and carried by blood to muscle tissues and to adipose tissue for storage. The normal value of blood level is around 1 mg/dl and it is transported chiefly in the α lipoprotein fraction. Under normal dietary conditions, there is no significant excretion of tocopherols in urine or faeces as it rapidly and extensively undergoes destruction in the GI tract and in tissues.

4.1.5 Functions/Metabolic Roles.

1. Acts as an antioxidant. This property is due to the presence of phenolic-OH group on the 6^{th} carbon of the chromane ring and is the most important function of vitamin E. Vitamin E removes free radicals from the system and also prevents the production of free radicals from lipid peroxidation acting as a radical trapping agent.

2. Role in Reproduction in Rats; Vitamin E helps in maintaining seminiferous epithelium intact.

3. Other Functions;

• Tocopherol derivative tocopheranolactone is involved in synthesis of coenzyme Q or ubiquinone.

• Vitamin E have some role in nucleic acid synthesis.

4.1.6 Deficiency of Vitamin E

Its deficiency leads to irreversible degenerative changes leading to permanent sterility in rats. Motility of sperms is lost and spermatogenesis is impaired in male rats. In female rats the ovary is unaffected by vitamin E deficiency, but the foetus does not develop normally, dying in utero undergoing resorption.

• Muscular dystrophy: Vitamin E deficiency leads to the increased oxidation of polyunsaturated fatty acids in the muscle with a consequent rise in O_2 consumption and peroxide production, peroxides may then cause an increase in intracellular hydrolase activity by affecting the lysosomal membranes. Those hydrolases may then catalyse breakdown in muscle and produce muscular dystrophy. The muscle creatine is low and creatinuria occurs.

• Hemolytic anemia: Low tocopherol diet will produce low plasma tocopherol, increased susceptibility to hemolysis due to peroxides and dialuric acid. This is the reason of hemolytic or macrocytic anemia.

• Dietary hepatic necrosis: Diets low in cystine and rich in polyunsaturated fatty acids can cause hepatic necrosis. Fall in acetate utilisation and in respiration of necrotic liver is more effectively cured or prevented by tocopherols.

VITAMIN K

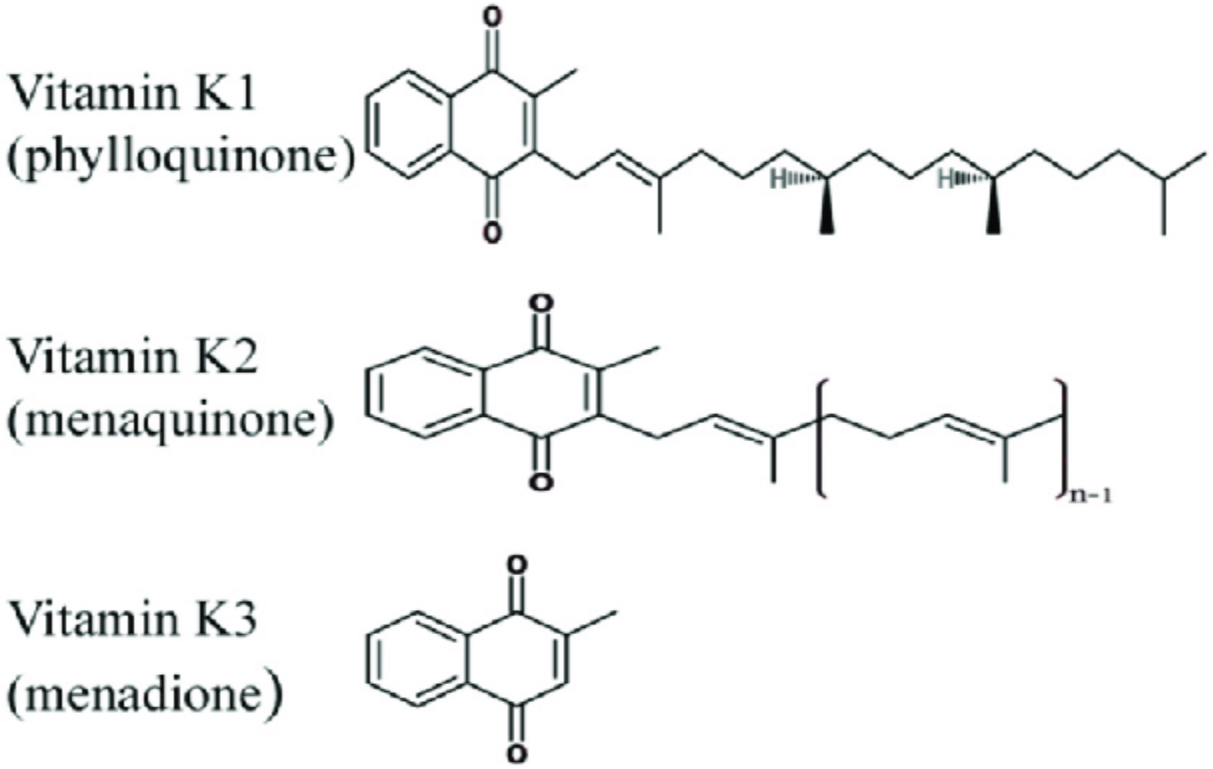
4.2 Chemistry:

There are two main variants of Vitamin K. Vitamins K1 and K2 are the two naturally occurring forms of vitamin K that have been identified. A third form vitamin K3 is the synthetic analogue.

4.2.1 Types of Vitamin K

1. Vitamin K1 It is phylloquinone or phytonadione isolated from alfalfa leaves. Also called Mephyton. Thus vitamin K1 is 2 methyl, 3 phytyl-1,4 naphthoquinone. It is a light yellow oil.
2. Vitamin K2 Also known as farnoquinone, it was isolated from putrid fish meal synthesised by bacteria. Vitamin K2 (farnoquinone) is 2 methyl-3-difarnesyl-1,4 naphthoquinone. It is also a yellow oil.

3. Vitamin K3 Vitamin K3 is 2 methyl, 1, 4 naphthoquinone without any side chain or OH group (Also known as menadione), is the synthetic analogue of vitamin K. It is three times more potent than natural varieties. It is water-soluble and can be given parenterally.



The chemical structures of vitamin K

4.2.2 Dietary sources and daily requirement:

Both vitamin K1 and K2 are mainly found in plants and synthesised by bacteria respectively. Vitamin K1 is present chiefly in green leafy vegetables, such as alfalfa, spinach, cauliflower, cabbage, soyabeans, tomatoes, e.t.c.

4.2.3 Absorption and excretion:

It is absorbed from the small intestine in presence of bile salts. It is not stored to any appreciable extent. It can cross the placental barrier and is available to the foetus. Vitamin K is not excreted in the urine or bile. Faeces contain large quantities.

4.2.4 Functions/Metabolic roles.

1. Blood Coagulation. Promotes blood coagulation by helping in the post transcriptional modifications of blood factors such as prothrombin, and factors II, VII, IX, X.

2. Calcium Binding Proteins: Vitamin K carboxylates specific glutamate residues of calcium binding proteins of bones, spleen, placenta and kidneys. This enhances the capacity of these proteins to deposit calcium in the tissues concerned.

3. Role in Oxidative Phosphorylation Vitamin K is a necessary cofactor in oxidative phosphorylation being associated with mitochondrial lipids.

4.2.5 DEFICIENCY OF VITAMIN K;

Deficiency of vitamin K is very rare, since most common foods contain this vitamin. In addition, intestinal flora of microorganisms also synthesise vitamin K. However, a deficiency may occur as a result of:

• Prolonged use of antibiotics and sulfa drugs: This suppresses the growth of vitamin K2 producing bacteria thus making vitamin K2 not available.

• Malabsorption and biliary tract obstruction: Sprue, steatorrhoea and coeliac disease can lead sometimes to vitamin K deficiency. Vitamin K being a fat soluble vitamin, is absorbed with the help of bile salts. The biliary obstruction impairs the delivery of bile hence vitamin K is not able to get absorbed. • Spoilt Sweet-clover hay: When consumed by cattle, causes a bleeding disease. In such cases fall in O2 consumption, poor oxidative phosphorylation, low prothrombin, proconvertin and stuart factor activities are observed. Spoilt sweet-clover hay contains dicumarol-vitamin K antagonist.

• Short circuiting of the bowel: As a result of surgery short-circuiting of the bowel may also foster deficiency which may not respond even to large oral doses of vitamin K. Water-soluble form of vitamin K, i.e. vitamin K3 alone is useful in such cases.

• In immediate post-natal infants: Hypoprothrombinemia and bleeding in many tissues occurs in vitamin K deficiency. Relatively small amounts of vitamin K are obtained from the mother through placental membranes and also because the intestinal microflora has not yet been established, this leads to vitamin K deficiency and its consequent effects. If prothrombin is significantly low this may result in hemorrhagic disease of the newborn. Note: Hypoprothrombinemia can be prevented by administering vitamin K to the mother before parturition or by giving the infant a small dose of vitamin K.

In summary Vitamin K deficiency will lead to bleeding diastasis.

Summary of Study Session 4 of module 6

In this study session, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 9

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

SAQ

Links to OERs

References/ Suggestions for Further Reading

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MODULE 7

STUDY SESSION 1

WATER SOLUBLE VITAMINS

Introduction

As the name implies, they are water soluble. They are nine in number and they include the B vitamins -- folate, thiamine, riboflavin, niacin, pantothenic acid, biotin, vitamin B6, and vitamin B12 -- and vitamin C. they are widely distributed in nature and can be found in fruits and vegetables. Deficiency of any can result in debilitating ailment or may even lead to mortality.

Learning Outcomes for Study Session 12

After you have studied this study session, you should be able to:

Key Terms: water soluble vitamins, vitamin C and the B- vitamins

VITAMIN C (ASCORBIC ACID)

1.1 Chemistry of Vitamin C

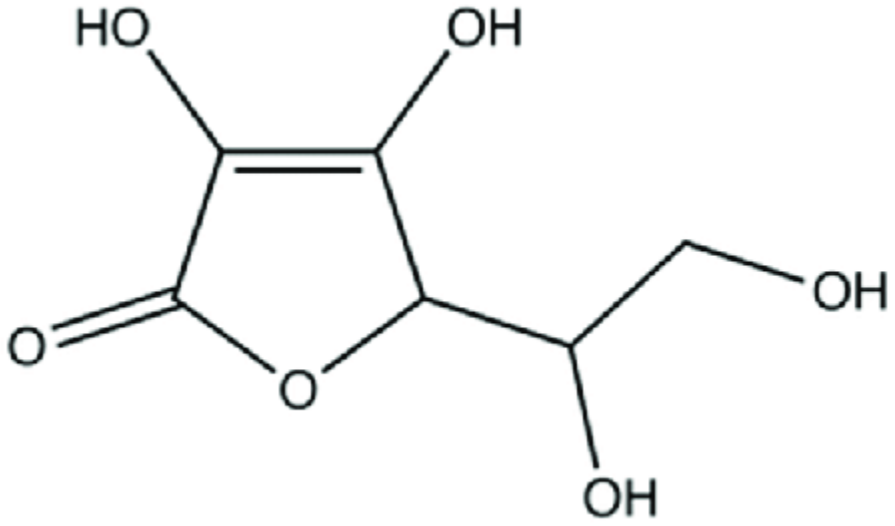
• Ascorbic acid is an enediol-lactone of an acid with a configuration similar to that of the sugar L-glucose.

• It is a comparatively strong acid, stronger than acetic acid, owing to dissociation of enolic H at C2 and C3 positions.

• D-forms are generally inactive as anti-scorbutic agent. Naturally occurring vitamin C is L-Ascorbic acid.

• Strong reducing property: Depends on the liberation of the H-atoms from the enediol –OH groups, on C2 and C3; the ascorbic acid being oxidized to dehydroascorbic acid, e.g. by air, H_2O_2, $FeCl_3$, methylene blue, ferricyanide, 2:6-dichlorophenol indophenol, etc. The reaction is readily reversible by reducing agents in vitro by H2S and in vivo by –SH compounds, such as 'Glutathione'.

• It is stable in solid form and in acidic solutions, but is rapidly destroyed in alkaline solutions. Oxidative destruction of ascorbic acid is accelerated by increasing pH. Silver (Ag++) and cupric (Cu+++) ions accelerate process.



Chemical structure of vitamin C

1.2 Synthesis and absorption

Man, monkey and guinea pigs lack the enzymes necessary for the synthesis of ascorbic acid. They cannot convert ketogulonolactone to ascorbic acid. Hence the entire human requirement must consequently be supplied by the diet. It is absorbed readily from the small intestine, peritoneum and subcutaneous tissues. It is widely distributed throughout the body. Some tissues contain high concentrations as compared to others. Local concentration roughly parallels the metabolic activity, found in descending order as follows: Pituitary gland, adrenal cortex, corpus luteum, Liver, brain, gonads, thymus, spleen, kidney, heart, skeletal muscle, etc. From maternal blood, it can cross the placental barrier and supplies the foetus.

1.3 Distribution and RDA.

Normal human blood plasma: It contains approx. 0.6 to 1.5 mg of ascorbic acid per 100 ml. The vitamin exists in the body largely in the 'reduced' form, with reversible equilibrium with a relatively small amount of "dehydroascorbic acid" (oxidised form). Both forms are physiologically and metabolically active.

Requirement:

A daily intake of about 100 mg is quite adequate in normal adults. Official recommended minimal daily intakes are: • Infants: 30 mg per day • Adults: 75 mg per day • Adolescence: 80 mg per day • Pregnant women: 100 mg per day • Lactating women: 150 mg per day Requirement is increased in presence of infections.

1.4 Metabolism and Excretion

• Under normal dietary intake (of 75 to 100 mg) 50 to 75% are converted to inactive compounds while 25 to 50% is excreted in urine as such.

• It is also secreted in milk.

In human beings, the chief terminal metabolites are oxalic acid and diketogulonic acid, which are excreted in urine. Conversion of ascorbic acid to oxalate in man may account for the major part of the endogenous urinary oxalate.

1.5 Occurrence and food sources:

It is widely distributed in plants and animal tissues. In animal tissues, no storage, contains small amount, but highest concentration in metabolically highly 'active' organs, e.g. adrenal cortex, corpus luteum, liver, etc.

Dietary sources:

These are chiefly vegetable sources. Good sources are citrous fruits—orange/lemon/lime, etc; other fruits like papaya, pineapple, banana, strawberry. Amongst vegetables—leafy vegetables like cabbage and cauliflower, germinating seeds, green peas and beans, potatoes, and tomatoes. Alma is the richest source. Considerable amount of vitamin C activity is lost during cooking, processing and storage, because of its water-solubility and its irreversible oxidative degradation to inactive compounds.

1.6 METABOLIC ROLE AND FUNCTIONS

1. Role in Cellular Oxidation-Reduction

The fact that vitamin C is very sensitive to reversible oxidation, Ascorbic acid ← Dehydroascorbic acid, suggests that it may be involved in cellular oxidation reduction reactions, perhaps serving as hydrogen transport agent.

1. **Role in Collagen Synthesis**

Hydroxyproline and hydroxylysine are important constituents of mature collagen fibres. Precollagen molecules contain the amino acids proline and lysine. They are hydroxylated by corresponding hydroxylases in presence of vitamin C, Fe++ and molecular O2.

2. **Functional Activity of Fibroblasts/Osteoblasts**

Ascorbic acid is required for functional activities of fibroblasts, and osteoblasts, and consequently for formation of MPS of connective tissues, osteoid tissues, dentine and intercellular cement substance of capillaries.

3. **Role in Tryptophan Metabolism**

Vitamin C is required as a cofactor for hydroxylation of tryptophan to form 5–OH derivative, in the pathway of biosynthesis of serotonin (5–HT).

4. **Role in Tyrosine Metabolism**

It is required as a cofactor with the enzyme p-OH phenyl pyruvate hydroxylase, which is necessary for hydroxylation and conversion of p-OH phenyl pyruvate to Homogentisic acid.

5. **Formation of Active FH4 (Tetrahydrofolate)**

Ascorbic acid in combination with folic acid helps the maturation of the RB cells. Vitamin C regulates the conversion of folic acid to folinic acid (so-called "citrovorum factor"). It has been suggested that vitamin C by maintaining the folic acid reductase in its "active" form keeps the folic acid in the reduced tetrahydrofolate FH4 form.

6. **Formation of Ferritin**

Ascorbic acid is necessary for the formation of tissue "ferritin". ATP, NAD+ and NADP+ stimulate the process.

7. **Absorption of Fe**

Ascorbic acid in food helps in the absorption of Fe by converting the inorganic ferric iron to the ferrous form. Also by forming water-soluble Fe-ascorbate chelate. It also helps in

mobilisation of Fe from its storage form 'Ferritin'. Disturbances of these functions may contribute to the development of hypochromic microcytic anaemia in scurvy. Absorption of Fe both in normal or Fe-deficient patients is increased by over 10 per cent after administration of vitamin C.

8. Role in Electron Transport Systems

Ascorbic acid seems to take part in electron transport system of mammalian 'microsomes'.

9. Action on Certain Enzymes

Activation/Inhibition Vitamin C is capable of both activating and inhibiting different groups of enzymes. Arginase and papain are activated, whereas, activity of the enzymes like urease and β-amylase from plants is inhibited.

10. Role in Formation of Catecholamines

Vitamin C is required as a coenzyme with the enzyme dopamine hydroxylase which catalyses the conversion of dopamine to norepinephrine.

11. Role in Formation of Carnitine

Formation of 'carnitine' in liver by hydroxylation of γ-butyrobetaine is helped by vitamin C, αketoglutarate, Fe++ and a dioxygenase.

12. Role in α-Oxidation of Fatty acid

Vitamin C helps in the action of the enzyme α-hydroxylase (a mono-oxygenase) which catalyzes the α-oxidation of long-chain F.A. to form α-OH-FA.

14. Effect on Cholesterol Level

Relation of ascorbic acid with hypocholesterolaemia in man and guinea pigs has been reported.

15. Role in Stress

The adrenal cortex contains a large quantity of vitamin C and this is rapidly depleted when the gland is stimulated by ACTH. A similar depletion of adrenocortical vitamin C activity is noted when experimental guinea pigs are injected with large quantities of diphtheria 'toxin'. Increased losses of the vitamin accompany infection and fever. Circulating vitamin C level has been found to be low in acute infectious diseases, congestive heart failure, in renal and hepatic diseases and malignancies. All of the above suggest that the vitamin C may play an important role in the reaction of the body to stress.

16. coenzyme

Vitamin C has also been reported to act as coenzyme for cathepsins and liver esterases.

17. Depleted in leucocytes by OCPs;

Ascorbic acid in both leucocytes and platelets found to be lowered significantly in women taking oral contraceptive pills.

1.7 DEFICIENCY AND ITS MANIFESTATIONS:

Vitamin C deficiency produces a disease called Scurvy. The main defect is a failure to deposit intercellular cement substance. Capillaries are fragile and there is tendency to hemorrhages: Petechial, subcutaneous, subperiosteal and even internal hemorrhages can occur. Wound healing is delayed due to deficient formation of collagen. There is poor dentine formation in children, leading to poor teeth formation. The gums are swollen and becomes spongy and bleeds on slightest pressure–Hyperemia, swelling, sponginess and bleeding of gums are seen. In severe scurvy, may lead to secondary infection and loosening and falling of teeth. Osteoid of bone is poorly laid and mineralization of bone is poor. The bones are weak and readily fractures. Hemorrhages occurring below the periosteum and into the joints may cause extremely painful swellings of bones and joints. Anemia may be associated which is hypochromic microcytic type. Bachelor Scurvy: Elderly bachelors and widowers who may prepare their own foods are particularly prone to development of vitamin C deficiency.

1.8 Hypervitaminosis (Effects of Excess Ascorbic Acid)

Administration of large amounts of ascorbic acid are not known to produce any effects in humans.

1.9 Empirical Uses of Vitamin C

Aside treatment of scurvy, Ascorbic acid has been used empirically in many other conditions viz. in the control and treatment of infectious diseases. Has been found to help wound healing, in ulcer, trauma and burns, in allergic conditions, common cold and coryza, during

labour: vitamin C given in doses of 150 to 250 mg produces an oxytocic action, increasing both the frequency and intensity of uterine contractions, in methaemoglobinaemia may be used for its reducing property.

Summary of Study Session 1 of module 7

In this study, you have learnt that:

Self-Assessment Questions (SAQs) for Study Session 1

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

Links to OERs

References/ Suggestions for Further Reading

Should you require more explanation on this study session, please do not hesitate to contact your e-tutor via the LMS.

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MODULE 7

STUDY SESSION 2

B-COMPLEX VITAMINS

Introduction

The B complex vitamin is composed of eight B vitamins and these are B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folic acid) and B12 (cobalamin). They are all water soluble and widely distributed in nature.

Learning Outcomes for Study Session 12

After you have studied this study session, you should be able to:

Key Terms: water soluble vitamins, the B- vitamins

2.1 THIAMINE (VITAMIN B1)

This is also called; Antiberiberi factor, antineuritic vitamin, aneurin.

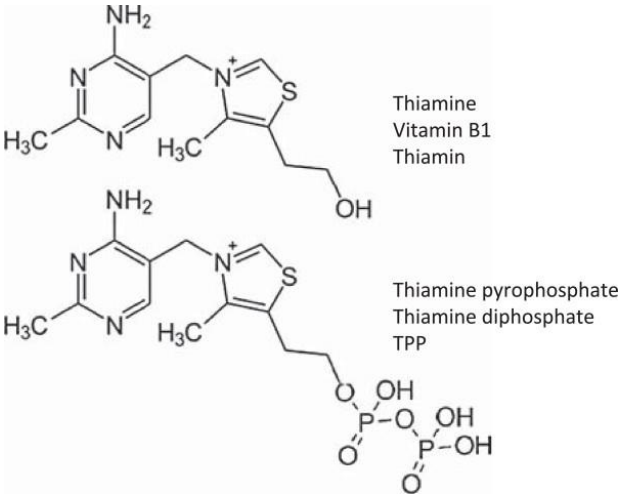
The active form is Thiamine pyro phosphate (TPP)

2.1.1Chemistry

Free thiamine is a basic substance and contains (a) a pyrimidine, and (b) a thiazole ring. It contains Sulphur (Sulphur containing vitamin). Generally prepared as a chloride-hydrochloride.

Soluble in water (1 gm/ml) and 95 per cent alcohol (1 gm/100 ml). Not soluble in fat solvents. Resistant to heat (boiling/autoclaving) in solution < pH 3.5 but loses activity at pH > 5.5. Thiamine content of vegetables is well preserved by freezing and by storage below 0°C. Rapidly destroyed in alkaline medium.

Chemically it is 2,5-dimet hyl, 4-methyl-6-amino pyrimidine 5-OH ethyl thiazole, Thiamine pyrophosphate (thiamine diphosphate).



Chemical structure of thiamine and the coenzyme TPP

Vitamin B1 is not synthesised by human beings, hence should be supplied in diet. Intestinal bacterial flora can synthesise the vitamin.

2.1.2Metabolism

Absorption:

Free thiamine is absorbed readily from the small intestine, but the pyrophosphate (ester-form) is not. Bulk of the dietary vegetable thiamine is in the "free" form. In tissues, it is actively phosphorylated to form Thiamine pyrophosphate (TPP) in Liver, and to a lesser extent in other tissues like muscle, brain and nucleated RB Cells.

2.1.3 Metabolic roles;

1. Energy Production: Thiamine is a coenzyme in the metabolism of carbohydrates, particularly glucose. It plays a vital role in converting glucose into usable energy in the form of adenosine triphosphate (ATP) through a process called glycolysis. Thiamine is necessary for the function of several enzymes involved in this process.
2. Citric Acid Cycle (Krebs Cycle): Thiamine is involved in the citric acid cycle, also known as the Krebs cycle or the tricarboxylic acid (TCA) cycle. This cycle is a central metabolic pathway that generates energy by breaking down acetyl-CoA derived from carbohydrates, fats, and proteins. Thiamine-dependent enzymes are crucial for the proper functioning of this cycle.

3. Nervous System Function: Thiamine is essential for the normal functioning of the nervous system. It is a vital coenzyme for various enzymes involved in the synthesis and metabolism of neurotransmitters, including acetylcholine, serotonin, and gamma-aminobutyric acid (GABA). Thiamine deficiency can lead to neurological symptoms such as peripheral neuropathy, muscle weakness, and cognitive impairments.
4. Amino Acid Metabolism: Thiamine is involved in the metabolism of certain amino acids, including the breakdown of branched-chain amino acids (leucine, isoleucine, and valine) and the synthesis of neurotransmitters like GABA. Thiamine-dependent enzymes participate in these processes and are essential for proper amino acid metabolism. B1 is also required in amino acid Tryptophan metabolism for the activity of the enzyme Tryptophan pyrrolase.
5. Pentose Phosphate Pathway: TPP acts as a coenzyme with the enzyme Transketolase in transketolation reaction in PPP of glucose metabolism. Thiamine is required for the normal functioning of the pentose phosphate pathway, a metabolic pathway that generates essential molecules like ribose-5-phosphate and reducing agents like nicotinamide adenine dinucleotide phosphate (NADPH). These molecules are crucial for various cellular processes, including nucleotide synthesis and protection against oxidative stress.

6. Fatty Acid Metabolism: Thiamine is involved in the metabolism of fatty acids, which are essential for energy production and various physiological functions. Thiamine-dependent enzymes play a role in the breakdown of fatty acids through a process called beta-oxidation. This process generates acetyl-CoA, which enters the citric acid cycle to produce energy.

7. Red Blood Cell Formation: Thiamine is necessary for the synthesis of DNA and RNA, which are crucial for the formation of red blood cells. It plays a role in the metabolism of nucleic acids, ensuring the proper replication and synthesis of genetic material.

8. Detoxification: Thiamine is involved in the detoxification of certain compounds in the body. It participates in the metabolism of xenobiotics, including alcohol and drugs.

Thiamine-dependent enzymes aid in the breakdown and elimination of these substances, contributing to the body's detoxification processes.

9. Brain and Cognitive Function: Thiamine is essential for brain health and cognitive function. It supports the production and release of neurotransmitters, which are chemical messengers involved in communication between brain cells. Thiamine deficiency can lead to cognitive impairments, memory loss, and neurological disorders such as Wernicke-Korsakoff syndrome.

10. Antioxidant Defense: Thiamine helps protect cells against oxidative stress, which occurs due to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses. Thiamine-dependent enzymes, such as transketolase, are involved in the generation of reducing agents like NADPH, which play a role in neutralizing ROS and maintaining cellular redox balance.

2.1.4Deficiency

The deficiency of thiamine produces a condition called beriberi. There are two types; **Dry beriberi,** when it is not associated with oedema and **Wet beriberi**, when Oedema is associated. It is characterised by the following manifestations.

Manifestation of Beriberi:

Cardiovascular features include; palpitation, dyspnoea, cardiac hypertrophy and dilatation, which may progress to congestive cardiac failure.

Neurological features: These are predominantly those of ascending, symmetrical, peripheral polyneuritis. When these neurological features are accompanied by an acute haemorrhagicpolioencephalitis is then known as Wernicke's encephalopathy.

Gastro intestinal symptoms: anorexia, gastric atony, with diminished gastric motility and nausea; fever and vomiting occur in advanced stages.

Deficiency in Animals:

Pigeons: Develop a characteristic rigid retraction of head-opisthotonos.

Chastek paralysis: Observed in foxes eating raw fish. It is characterized by extreme, board-like rigidity with retraction of head. Reason: Raw fish contains heatlabile thiamine-splitting

enzyme thiaminase which destroys thiamine. Bracken disease: A similar condition which has been reported in grazing animals feeding on ferns and related species of plants which contain the enzyme thiaminase.

2.1.5 Sources are:

cereal grains, (e.g. rice polishings), peas, beans, whole cereal grains, bran, nuts, prunes, Liver, meat, eggs, Ham/pork meats are particularly rich. Milk has low concentration.

2.2 RIBOFLAVIN (VITAMIN B2)

Also called: Lactoflavin, ovoflavin, hepatoflavin.

2.2.1 Chemistry

It contains; – A ribose alcohol: D-Ribitol – A heterocyclic parent ring structure Isoalloxazine (Flavin nucleus). 1-Carbon of ribityl group is attached at the 9 position of isoalloxazine nucleus. Ribityl is an alcohol derived from pentose sugar D-ribose.

It is stable to heat in neutral acid solution but not in alkaline solutions. Aqueous solutions are unstable to visible and UV light. Riboflavin undergoes reversible reduction readily in presence of a catalyst to a colourless substance Leucoriboflavin.

Biological Active Forms: There are two biological active forms, in which riboflavin serves as the prosthetic group (as coenzyme) of a number of enzymes;

They are: FMN (Flavin mononucleotide) and FAD (Flavin adenine nucleotide). Thus, FMN and FAD are two coenzymes of this vitamin. The acidic properties given by phosphoric acid group influence their capacity for combining with proteins apoenzyme-forming flavoproteins (Holoenzyme).

Thus, FP (holoenzyme) = FMN/FAD + Protein (coenzyme) (Apoenzyme) FP may also unite with metals like Fe and Mo thus forming Metalloflavoproteins.

2.2.2Biosynthesis

All higher plants can synthesise riboflavin. In nature, it occurs both as “free form” and also as “nucleotide” form or as flavoproteins. Human beings and animals cannot synthesise and hence solely dependant on dietary supply. In man, considerable amounts can be synthesised by intestinal bacteria, but the quantity absorbed is not adequate to maintain normal nutrition.

2.2.3 Metabolism Absorption:

Flavin nucleotides are readily absorbed in small intestine. It is excreted via urine faeces and also secreted in milk.

Occurrences and Food Sources It is widely distributed in nature, present in all plant and animal cells. It is present in yeasts, whole grains, dry beans and peas, nuts, green vegetable, germinating seeds, e.g. grams/Dals are very good source. Other sources include: Liver, kidney, milk, eggs and crab meat which has high content.

2.2.4 METABOLIC ROLE

FMN and FAD act as coenzymes to various enzymes in various H-transfer reactions in metabolism these enzymes include: FMN; Warburg's yellow enzyme, Cytochrome-C-reductase and L-amino acid oxidase. FAD; Xanthine oxidase (Xanthine → uric acid), D-amino acid oxidase, Aldehyde oxidase, Fumarate dehydrogenase (Succinate → Fumarate), Glycine oxidase, Acyl-CoA dehydrogenase and Diaphorase.

2.2.5 Deficiency Manifestations

There is no definite disease entity. Deficiency is usually associated with deficiencies in other B-vitamins. In human beings lesions of the mouth, tongue, nose, skin and eyes with weakness, and lassitude reported. They include:

Lips: Redness and shiny appearance of lips.

Cheilosis: Lesions at the mucocutaneous junction at the angles of the mouth leading to painful fissures are characteristic.

Tongue: Painful glossitis, the tongue assuming a red-purple (magenta) colour.

Seborrhoeic dermatitis: Scaly, greasy, desquamation chiefly about the ears, nose and naso-labial folds.

Eyes: May lead to corneal vascularisation and inflammation with cloudiness of cornea, watering, burning of eyes, photophobia, scleral congestion and cataract has also been reported. **Protein synthesis**: This is impaired in severe riboflavin deficiency;

2.3 NIACIN (VITAMIN B3)

This vitamin is also called: Nicotinic acid, P-P factor, Pellagra-preventing factor of Goldberger.

2.3.1 Chemistry:

Nicotinic acid (niacin) is chemically Pyridine3-carboxylic acid. In tissues: Occurs principally as the amide (nicotinamide, niacinamide). In this form it enters into physiological active combination. Biological "Active" Forms in tissues, nicotinamide is present largely as a "dinucleotide", the pyridine 'N' being linked to a D-ribose residue. Two such nucleotide active forms are known: Nicotinamide adenine dinucleotide (NAD+) Other names are: DPN+, coenzyme-I, cozymase, or codehydrogenase. The compound contains: – One molecule of nicotinamide, – Two molecules of D-ribose, – Two molecules of phosphoric acid, and – One molecule of adenine. The reduced form is NADH. The second one is Nicotinamide adenine dinucleotide phosphate (NADP+), also called coenzyme II. It differs from NAD+ in that it contains an additional molecule of phosphoric acid attached to 2-position of D-ribose attached to N-9 of adenine. The reduced form is NADPH

2.3.2 Biosynthesis

Amino acid tryptophan is a precursor of nicotinic acid in many plants, and animal species including human beings. Pyridoxal-P is required as a coenzyme in this synthesis. It can be synthesised also by intestinal bacteria. Bacteria in addition to synthesis from tryptophan, can also synthesise from other amino acids, e.g. glutamic acid, proline, ornithine and glycine.

In humans: – In addition to dietary source, – It is synthesised in tissues from amino acid tryptophan, and – To a limited extent supplemented by bacterial synthesis in intestine. Applied Aspect in high corn diet, requirement of dietary niacin increases, as synthesis from tryptophan cannot take place. The reason is the maize protein Zein lacks the amino acid tryptophan. Hence pellagra is more common in persons whose staple diet is maize.

2.3.3 Metabolism Absorption:

Nicotinic acid and its amide are absorbed from the small intestine.

2.3.4 Occurrence and Food Sources

Both nicotinamide and coenzyme forms are distributed widely in plants and animals. Important food sources are: Animal source: Liver, kidney, meat, fish. Vegetable source: Legumes (peas, beans, lentils), nuts, certain green vegetables, coffee and tea. Poor sources are: Fruits, milk and eggs.

2.3.5 Metabolic Role

• The coenzymes NAD+ and NADP+ operate as hydrogen and electron transfer agents by virtue of reversible oxidation and reduction. The mechanism of the transfer of Hydrogen from a metabolite to oxidised NAD+, thus completing the oxidation of the metabolite and the formation of reduced NAD (NADH + H+) is shown in box. Reduction of NAD+ occurs in para position; one H loses an electron and enters the medium as H+.

• Function of NADP+ is similar to that of NAD+ in hydrogen and electron transport. The two coenzymes are interconvertible. The important enzymes to which NAD+ and NADP+ act as coenzyme are given below in the box. Other Role of NAD+ In addition to its coenzyme role, NAD+ is the source of ADP-ribose for the ADP-ribosylation of proteins and poly-ADP ribosylation of nucleoproteins involved in the DNA-repair mechanism. Niacin antagonists are: (a) Pyridine-3-sulfonic acid

(b) 3-Acetyl pyridine.

2.3.6 Deficiency Manifestations:

Nicotinic acid deficiency produces a disease called pellagra (Pelle = skin; agra = rough) The cardinal features of pellagra are known as "3 D's" and they are; Dermatitis, Diarrhoea, and Dementia.

The precipitating factors of pellagra are:

(a) High-corn diet and

(b) Alcoholism

The clinical features of the deficiency include:

(a) Skin lesions: Typically involves areas of skin exposed to sunlight and subjected to pressure, heat or other types of trauma or/irritation. These include face, neck, dorsal surfaces of the wrist, forearms, elbows, breasts and perineum. The skin becomes reddened, later brown, thickened and scaly.

(b) GI manifestations: Include anorexia, nausea, vomiting, abdominal pain, with alternating constipation/ diarrhoea. Gingivitis and stomatitis with reddening of the tip and margin of the tongue, which become swollen and cracked. Achlorhydria present in about 40% cases. Thickening and inflammation of the colon, with cystic lesions of the mucosa, which later becomes atrophic and ulcerated.

(c) Cerebral manifestations: These include headache, insomnia, depression and other mental symptoms ranging from mild psychoneuroses to severe psychosis.

(d) General effects: These include: Inadequate growth, loss of weight and strength, anaemia which may be due to associated deficiency of other vitamins, dehydration and its consequences resulting from diarrhoea. Simultaneous deficiency of riboflavin (Vit B2) or pyridoxine (B6) can contribute to the etiology of pellagra. (Both of these are required for synthesis of niacin from tryptophan.)

Incidence of pellagra is more in women than men, reason is oestrogen metabolites can inhibit tryptophan metabolism and prevents synthesis of niacin from amino acid tryptophan.

2.3.7 Niacin toxicity:

Excessive dosage can produce toxic effects: – Dilatation of blood vessels and flushing. – Skin irritation – Can produce liver damage.

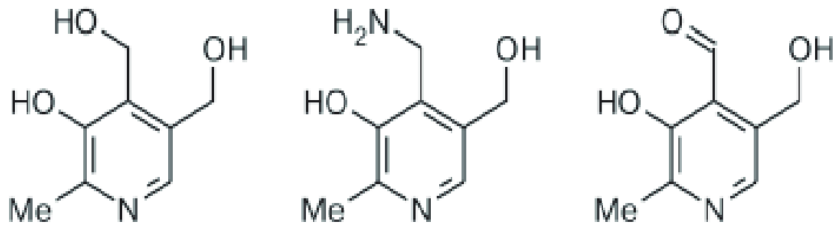
2.4 PYRIDOXINE (VITAMIN B6)

This vitamin is also called: Rat antidermatitis factor.

2.4.1Chemistry

Pyridoxol (Pyridoxine): also called as Adermin is chemically 2-methyl-3-OH-4, 5-di (hydroxymethyl) pyridine. It occurs in association, perhaps in equilibrium, with an aldehyde-Pyridoxal and an amine Pyridoxamine form. All three forms exhibit vitamin B6 activity. The biological 'Active' forms of the vitamin are:

Pyridoxal-PO4, and Pyridoxamine-PO4. The active forms are the phosphorylated derivatives, phosphorylation involves the hydroxymethyl group –CH2OH at position 5 in the pyridine ring.



Pyridoxine Pyridoxamine Pyridoxal

2.4.2 Biosynthesis:

Vitamin B6 can be formed by many microorganisms and probably also by plants. Human beings cannot synthesise the vitamin, hence has to be provided in the diet. Intestinal bacteria can synthesise the vitamin.

2.4.3 Metabolism Absorption:

Dietary vitamin B6 is readily absorbed by the intestine. Pyridoxal and pyridoxamine are excreted in urine in small amounts. Major urinary metabolite, is the biologically inactive form 4-pyridoxic acid.

2.4.4 Occurrence and food sources:

The vitamin is distributed widely in animal and plant tissues. Rich sources of the vitamin are yeast, rice polishings, germinal portion of various seeds and cereal grains and egg-yolk. Moderate amounts are present in liver, kidney, muscle, fish. Milk is a poor source. Highest concentration occurs in royal jelly (bee).

2.4.5 Metabolic Role

Pyridoxal P acts as a coenzyme, it is principally involved with metabolism of amino acids.

Cotransaminase: It acts as a coenzyme for the enzyme transaminases (aminotransferases) in transamination reaction.

Codecarboxylase: It acts as coenzyme for the enzyme decarboxylases in decarboxylation reaction. Amino acids are decarboxylated to form corresponding amines. E.g. Tyrosine → Tyramine + CO2, Histidine → Histamine + CO2, Glutamic acid → G A B A + CO2

Acts as coenzyme for deaminases (dehydrases): Catalyses non-oxidative deamination of OH-amino acids viz., serine, threonine, etc.

Coenzyme for kynureninase: In tryptophan metabolism, pyridoxal-P acts as a coenzyme for the enzyme kynureninase which converts 3-OH-kynurenine to 3-OHanthranilic acid which ultimately forms nicotinic acid. Thus in B6-deficiency niacin synthesis from tryptophan does not take place. In B6 deficiency, kynurenine and 3-OH kynurenine levels increases and they are converted to xanthurenic acid in extrahepatic tissues, which is excreted in urine. xanthurenic acid index is a reliable criterion for B6 deficiency. Examination of urine for xanthurenic acid after the feeding of a test dose of tryptophan has been used to diagnose B6-deficiency.

Transulfuration: It takes part in transulfuration reactions involving transfer of –SH group, e.g. Homocysteine + Serine → homoserine + cysteine.

As coenzyme for desulfhydrases: It catalyses non-oxidative deamination of cysteine in which H2S is liberated.

In interconversion of glycine and serine by serine hydroxy methyl transferase:

In this both F.H4 and B6 are required as coenzymes.

Pyridoxal-P is required as a coenzyme in the biosynthesis of arachidonic acid from linoleic acid.

Synthesis of Sphingomyelin: Pyridoxal-P is required as a coenzyme for activation of serine which is required for synthesis of sphingomyelin.

Required as a coenzyme for amino acid racemases: D-Glutamic acid → L-Glutamic acid D-Alanine → L-Alanine

Intramitochondrial FA synthesis: Required as a coenzyme with condensing enzyme for chain elongation of FA in intramitochondrial FA synthesis.

Required for "active transport" of amino acids through cell membrane and intestinal absorption of amino acids.

Muscle phosphorylase: As a constituent of muscle phosphorylase: 4 molecules of pyridoxal-(P) per molecule of enzyme (tetramer).

Transport of K+: Vitamin B6 has been reported to promote transport of K+ across the membrane from exterior to interior.

As coenzyme for aminoacetone synthetase which is required for formation of aminoacetone from acetyl-CoA and glycine.

Synthesis of CoA-SH (Coenzyme A): Vitamin B6 is involved in synthesis of coenzyme A form pantothenic acid. In B6 deficiency, coenzyme A level in Liver is reduced.

In porphyrin synthesis: Pyridoxal-P is required for conversion of α-amino-β-ketoadipic acid to δ-ALA, an important step in haem synthesis. In B6-deficiency haem synthesis suffers and leads to anaemia.

Hypercholesterolaemia: Relationship of B6deficiency, hypercholesterolaemia and atherosclerosis has received considerable attention, although the exact role of vitamin B6 is not clear.

Immune response: In vitamin B6 deficiency, immune response is impaired. Oxaluria: Vitamin B6 deficiency has been observed to produce oxaluria in experimental animals.

2.4.6 Deficiency Manifestations

No deficiency disease has been described. But following clinical manifestations are attributed to vitamin B6 deficiency. Epileptiform convulsions in infants: have been attributed to pyridoxine deficiency. It is related to lowered activity of Glutamic acid decarboxylase, for which pyridoxal P is a coenzyme. As a result, there occurs lowering of γ-amino butyric acid (GABA) in the brain which causes convulsions.

Pyridoxine responsive anaemia: A hypochromic microcytic anaemia called as sideroblastic (sideroachrestic) anaemia, with high serum Fe level and haemosiderosis of Liver, spleen and bone marrow may occur with B6-deficiency.

Pyridoxal-P is required as a coenzyme in the reaction by which α-amino-β-ketoadipic acid is decarboxylated to form δ-ALA in heme synthesis. In B6-deficiency heme synthesis suffers and Fe cannot be utilised.

Isonicotinic acid hydrazide treatment in tuberculosis: A syndrome resembling vitamin B6 deficiency has been observed in humans during the treatment of tuberculosis with high doses of tuberculostatic drug Isonicotinic acid hydrazide or Isoniazid (INH). Few percentages of patients receiving conventional doses of isoniazid, 2 to 3 mg/kg, developed neuritis. 40 per cent receiving 20 mg/kg developed neuropathy.

Tryptophan metabolism was also altered, there was increased Xanthurenic acid excretion in urine. Signs and symptoms were alleviated by administration of pyridoxine to these patients. 50 mg of pyridoxine per day completely prevented the development of neuritis and neuropathies. Mechanism of Action: It is believed that isoniazid forms a ‘hydrazone complex’ with pyridoxine, resulting in incomplete activation of the vitamin. Like INH, the drug penicillamine (β-dimethyl cysteine) has been incriminated to produce deficiency of Vit B6. The drug is used in treatment of Wilson’s disease to chelate copper. The drug reacts with pyridoxal-P to form inactive thiazolidine derivative. During penicillamine treatment B6 should be given as a supplement to prevent its deficiency.

Therapeutic uses: Vitamin B6 has been found empirically to be of value in treatment of: Nausea and vomiting of pregnancy (“morning sickness”), Radiation sickness, Muscular dystrophies, Treatment of hyperoxaluria, and recurring oxalate stones of kidney, and Mild

forms of pyridoxine deficiency have been reported to occur sometimes in women taking oral contraceptives containing oestradiol. Vitamin B6 may cut risk of Parkinson's Disease Recently it has been claimed by Dutch researchers and reported in neurology that a higher intake of vitamin B6 may decrease the risk of Parkinson's disease. It could lower Parkinson's disease risk by protecting brain cells from damage caused by free radicals. Pyridoxine Status Vs Hormone Dependant Cancer Increased sensitivity to steroid hormones action may be important in the development of hormone-dependant cancer of the breast, prostate and uterus;

MODULE 7

STUDY SESSION 3

LIPOIC ACID (THIOCTIC ACID)

Introduction

This vitamin is also called: Protogen, Acetate replacement factor (ARF), Pyruvate oxidation factor (POF).

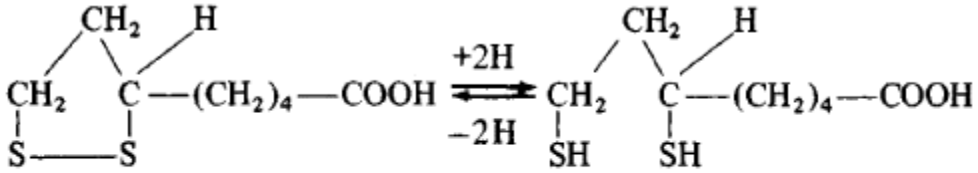
Learning Outcomes for Study Session 12

After you have studied this study session, you should be able to:

Key Terms: water soluble vitamins, the B- vitamins

3.1.1Chemistry

It is a Sulphur containing fatty acid called 6, 8-dithiooctanoic acid (α-lipoic acid or thioctic acid). It contains eight carbons and two Sulphur atoms.



Structure of Lipoic acid

3.1.2 Deficiency:

No known deficiency manifestations.

3.1.3 Metabolic roles

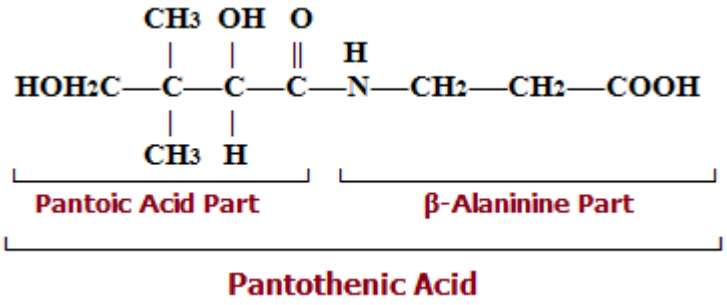
1. Energy production: Lipoic acid is a crucial component of the multi-enzyme complex called the pyruvate dehydrogenase complex (PDH). PDH converts pyruvate, a product of glucose metabolism, into acetyl-CoA, which enters the citric acid cycle (also known as the Krebs cycle or TCA cycle) for further energy production. Lipoic acid acts as a coenzyme that enables the enzymatic reactions within the PDH complex, facilitating the oxidative decarboxylation of pyruvate into acetyl-CoA.
2. It also acts as a coenzyme of alpha ketoglutarate dehydrogenase in the conversion of ketoglutarate to succinyl CoA.
3. Antioxidant activity: Lipoic acid is a powerful antioxidant that helps protect cells from oxidative stress. It can neutralize harmful free radicals and regenerate other antioxidants such as vitamins C and E, glutathione, and coenzyme Q10. By reducing oxidative damage, lipoic acid helps maintain the proper functioning of various metabolic pathways.
4. Glucose metabolism: Lipoic acid plays a role in glucose metabolism by enhancing insulin sensitivity and glucose uptake. It helps regulate blood sugar levels by facilitating the transport of glucose into cells and promoting its conversion into energy.
5. Metabolism of amino acids: Lipoic acid is involved in the metabolism of amino acids, which are the building blocks of proteins. It participates in the oxidative decarboxylation of certain amino acids, such as alpha-keto acids, to generate energy.
6. Detoxification: Lipoic acid contributes to the detoxification process by assisting in the metabolism and elimination of harmful substances, including heavy metals and xenobiotics (foreign compounds). It acts as a cofactor for enzymes involved in detoxification reactions, aiding in the removal of toxins from the body.
7. Mitochondrial function: Lipoic acid supports mitochondrial health and function. Mitochondria are the energy-producing organelles within cells, and lipoic acid plays a vital role in their optimal functioning. It helps regulate mitochondrial energy production and protects against mitochondrial dysfunction, which is associated with various metabolic disorders.

3.2 PANTOTHENIC ACID (VITAMIN B5)

This vitamin is also called filtrate factor or Chick antidermatitis factor.

3.2.1 Chemistry

Pantothenic acid consists of β-alanine in peptide linkage with a dihydroxy dimethyl butyric acid ('Pantoic' acid). β-alanine + Pantoic acid → Pantothenic acid



This vitamin is soluble in water and is easily hydrolysed by acids or alkalis. It is destroyed by heat and so it is thermolabile.

The active form is Coenzyme A. In body tissues it is present in the form of CoA and bound to proteins (apoenzyme).

This vitamin cannot be synthesized by humans and must be supplied in the diet. It can be also being synthesized by intestinal bacteria.

3.2.2 Excretion:

The waste products of pantothenic acid are not known. Under ordinary dietary conditions, It is excreted via urine, sweat, and milk.

3.2.3 Occurrence and Food Sources

It is widely distributed in plants, animal tissues and food materials. Sources include kidney, Liver, egg-yolk and yeasts, cereals and legumes, skimmed milk, chicken, certain fishes, sweet potatoes, molasses, vegetables and fruits. The richest known source of pantothenic acid is Royal Jelly (also rich in Biotin and pyridoxine).

3.2.4 Metabolic Role.

Only demonstrated metabolic function of pantothenic acid is as a constituent of coenzyme A. As a constituent of CoA, pantothenic acid is essential to several fundamental metabolic reactions which includes;

Formation of active acetate (Acetyl-CoA). In the form of active acetate, it participates in a number of important metabolic reactions, e.g.

Utilised directly by combination with oxaloacetate (OAA) to form citric acid, which initiates TCA cycle.

Acetylcholine formation.

For acetylation reactions.

Synthesis of cholesterol.

Formation of ketone bodies.

Acetyl-CoA and Malonyl-CoA are used in the synthesis and elongation of fatty acids.

Formation of active succinate (Succinyl-CoA): Product of oxidative decarboxylation of α-oxoglutarate in TCA cycle is a coenzyme derivative called Active succinate (Succinyl-CoA), Succinyl-CoA is involved in certain important metabolic reactions as follows:

Haem synthesis: In haem synthesis, "active" succinate and glycine combines to form δ-ALA, the first step in the pathway of heme formation.

Clinical correlate: Anaemia may occur in pantothenic acid deficiency probably due to deficiency in formation of succinyl-CoA. Due to non-availability of the substrate the heme synthesis suffers.

Degradation of ketone bodies by extrahepatic tissues.

Role in lipid metabolism: – Oxidation of FA (β-oxidation): First step in oxidation of FA catalysed by Thiokinases (acyl synthases) involves the activation of the FA by formation of CoA derivatives.

Biosynthesis of FA: Pantothenic acid is a constituent of a compound called "Acyl-carrier protein" (ACP) and also a constituent of "multienzyme complex" in mammals, which is used in the extramitochondrial de Novo fatty acid synthesis. Role in Adrenocortical function: Pantothenic acid appears to be involved in adrenocortical activity, being essential to the formation of adrenocortical hormones from "active" acetate and cholesterol.

Activation of some amino acids may also involve CoASH: Occur among the branched chain amino acids such as valine and leucine.

3.2.5 Deficiency Manifestations:

No deficiency disease has been recognized in man.

3.3 BIOTIN (VITAMIN B7)

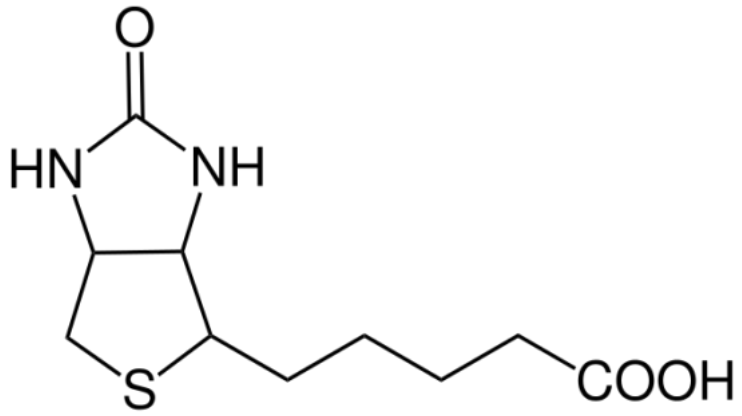
This vitamin is also called vitamin H, Co-enzyme R, Anti egg-white injury factor.

3.3.1Chemistry

Biotin is a heterocyclic monocarboxylic acid; it is a Sulphur-containing water-soluble B-vitamin. It consists of two fused rings, one imidazole and the other thiophene derivative. Biotin (C10 H16 O3 N2S) is chemically, Hexahydro-2-oxo-1-thieno-3,4-imidazole-4 valeric acid.

There are two forms with essentially identical biological activities: α-Biotin (egg-yolk) and β-Biotin (Liver)-differing only in the nature of the side chain.

Biotin is said to occur both as free form and bound form in tissues and foods.



Structure of Biotin

3.3.2 Biosynthesis and Metabolism

Biotin can be synthesised by many bacteria, yeast, and fungi. In green plants, it may be formed in leaf and root. Coenzyme-R is a growth essential for the nitrogen fixing organisms, Rhizobium, in the root nodules of Leguminous plants. Coenzyme R had been proved to be Biotin.

Excreted in urine, faeces and milk.

3.3.3 Occurrence and food sources:

sources of biotin include: Egg yolks, Liver, kidney, nuts, seeds, almonds, peanuts, walnuts, sunflower seeds, legumes such as lentils, chickpeas, soybeans, fish and seafood: e.g. salmon, tuna, trout, mussels, and oysters. Others include: Milk, Cheese, beef, chicken, and poultry, such as turkey, some vegetables contain biotin, examples include spinach, cauliflower,

carrots, and sweet potatoes. Fruits like bananas and avocados. Whole grains, such as oats, barley, and wheat.

3.3.4 Metabolic Roles

1. Coenzyme for carboxylation reactions: Biotin serves as a coenzyme for several carboxylase enzymes, which are involved in various metabolic pathways. These enzymes include acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, and methyl crotonyl-CoA carboxylase. Biotin acts as a carrier of activated carbon dioxide, which is added to specific molecules during these carboxylation reactions.
2. Energy metabolism: Biotin is involved in the metabolism of macronutrients, including carbohydrates, proteins, and fats. It assists in the conversion of glucose from carbohydrates into usable energy in the form of adenosine triphosphate (ATP). Biotin also helps in the breakdown of fatty acids and the synthesis of amino acids.
3. Fatty acid synthesis: Biotin is necessary for the synthesis of fatty acids, which are essential components of cell membranes and play a role in energy storage. Biotin acts as a cofactor for acetyl-CoA carboxylase, an enzyme that catalyzes the first step in fatty acid synthesis.
4. Amino acid metabolism: Biotin is involved in the metabolism of several amino acids, including leucine, isoleucine, and valine. Biotin-dependent enzymes participate in the breakdown of these amino acids and the removal of toxic by-products from their metabolism.
5. Gene expression: Biotin plays a role in regulating gene expression by affecting the activity of certain transcription factors. It helps in the activation of specific genes involved in various cellular processes.
6. Skin, hair, and nail health: Biotin is often associated with promoting healthy skin, hair, and nails. It is involved in the synthesis of keratin, a protein that is a major component of these structures.

3.3.5 Deficiency of Biotin

Human volunteers: Deficiency has been produced by excluding dietary biotin and feeding large amounts of raw egg white. Such individuals developed following symptoms beginning after 5 to 7 weeks: Dermatitis of the extremities, pallor of skin and mucous membranes,

anorexia and nausea, muscle pains and hyperaesthesia, depression, Lassitude and somnolence, anaemia, and hypercholesterolaemia. Prompt relief of symptoms occurred when biotin concentrates was given.

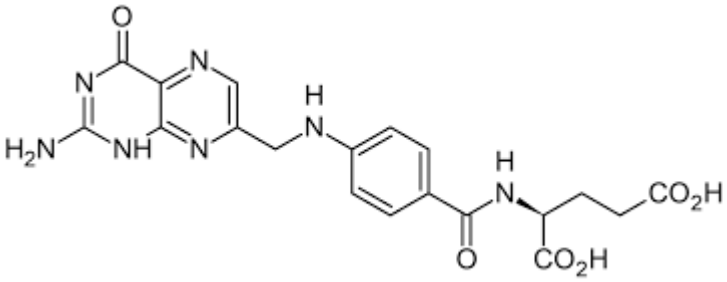
3.3.6 DEFICIENCY DISEASES:

There is no definite deficiency disease.

3.4 FOLIC ACID GROUPS (VITAMIN B9)

This vitamin is also called lactobacillus Caseifactor, vitamin M, Streptococcus lactis R (SLR) factor, vitamin Bc, Fermentation residue factor, pteroyl glutamic acid (PGA). The biologic active form of the vitamin is the reduced tetra-hydro derivative, **Tetrahydrofolate F.H4**.

3.4.1 Chemistry:



Structure of Folic acid (pteroyl glutamic acid (PGA))

The name "folic acid" is applied to a number of compounds which contain the following groups: A pteridine nucleus (pyrimidine and pyrazine rings), Para-aminobenzoic acid ("PABA") and Glutamic acid.

There are at least three chemically related compounds of nutritional importance which occur in natural products, all may be termed pteroyl glutamates. These three compounds differ only in the number of glutamic acid residues attached to pteridine PABA complex (pteroic acid). Monoglutamate: Having one glutamic acid. It is synonymous with vitamin Bc., Triglutamate: Having three glutamic acid residues. This substance once designated as "fermentation factor "and Heptaglutamate: Having seven glutamate residues

Pteroyl glutamic acid is liberated from these conjugates by enzymes called conjugases.

Before folic acid, functions as a coenzyme, it must be reduced first to 7, 8-dihydrofolic acid (F.H2) and then to 5, 6, 7, 8 tetrahydrofolate (F.H4). Both the reactions are catalysed by Folic

acid reductases enzyme, which use NADPH as hydrogen donor. Also requires vitamin C (ascorbic acid) as cofactor.

3.4.2 Biosynthesis and Metabolism

Many microorganisms, those inhabiting the intestinal tract inclusive can synthesise folic acid. Some of them cannot synthesise PABA, which has to be supplied. In presence of ATP and CoA-SH, PABA reacts with glutamic acid to form "p-amino-benzoyl glutamic acid". The latter then reacts with a "Pterin" to produce "pteroyl monoglutamic acid, PGA" (folic acid); pterin moiety is probably derived from Guanosine. Sulphonamides and antibiotics inhibit their growth by blocking the incorporation of PABA in the synthetic pathway (by competitive inhibition). Higher animals including human beings cannot synthesize folic acid and it has to be supplied in diet. In human beings, intestinal bacteria can synthesise and is a good source.

Absorption: Occurs along the whole length of mucosa of small intestine. Polyglutamates ingested in diet are converted to monoglutamates and dihydrofolates are reduced to tetrahydrofolates by folate reductase. Tetrahydrofolates are then converted to methyl tetrahydrofolates, which enter the portal blood and then carried to liver. It is transported in blood as methyl tetrahydrofolate bound to a specific protein. It is excreted in urine and faeces.

3.4.3 Occurrence and Food Sources

It is widely distributed in nature being present in many animal and plant tissues and in microorganisms. It is present in the liver, yeast, kidney and green leafy vegetables. Spinach and cauliflower are good vegetable sources. Other good sources are: meat, fish, wheat, milk, fruits.

3.4.4 Metabolic Role

They function as coenzymes in metabolic reactions eg tetrahydrofolate

Folic acid and Inositol when given in pregnancy may prevent formation of neural tube defects (NTDs), such as spina bifida. About 70% of NTDs can be prevented by taking folic acid.

Folic acid antagonists have been used successfully in treating various ailments. Most of them work by their ability to inhibit cell division and multiplication, they have been used in treatment of conditions where there is unrestricted cell growth, e.g. In Leukemia, Erythraemias, and malignant growths. Methotrexate inhibits "dihydrofolate reductase" and has been used as anticancer drug. Trimethoprim or septran: Inhibits dihydrofolate reductase and formation FH4 is decreased. The drug has been used as antibacterial agent. 4. Pyrimethamine: It is used as antimalarial drug. It also inhibits dihydrofolate reductase.

3.4.5Deficiency

Deficiency of this vitamin results to Megaloblastic anemia.

Macrocytic anemia.

3.4.6 Recommended dietary daily allowance:

Adults: 400 to 500 µg daily , Infants: 50 µg , Children: 100 to 300 µg, Pregnant women: 800 µg and Lactating women: 600 µg Folic Acid and Inositol

3.4.7Risks of Excess Folic Acid intake.

Dosage over 1 mg may cause aggravation of vitamin B12 deficiency and may precipitate irreversible nerve damage.

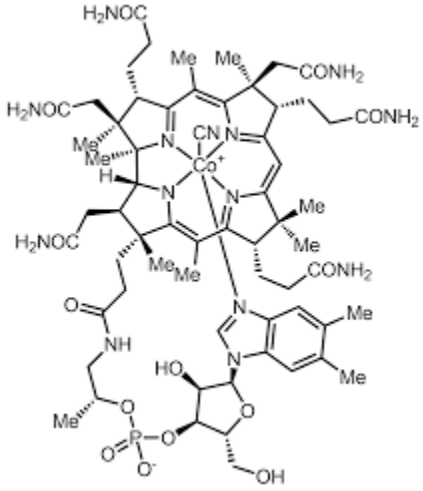
Antagonism between folic acid and the anticonvulsants used in the treatment of epilepsy observed.

Solubility of folic acid is low, hence large doses of folic acid if given parenterally there is risk of crystallization in kidney tubules leading to renal damage.

3.5 VITAMIN B12 (CYANOCOBALAMINE)

This vitamin is also called: Antipernicious anaemia factor, extrinsic factor of Castle, animal protein factor.

3.5.1 Chemistry



(a) Structure of vitamin B12:

1. Central portion of the molecule consists of four reduced and extensively substituted pyrrole rings, surrounding a single cobalt atom (Co). This central structure is called as Corrin Ring system.

2. The above system is similar to porphyrins, but differ in that two of pyrrole rings (Rings I and IV) are joined directly.

3. Below the corrin ring system, is DBI ring –5, 6- dimethyl Benzimidazole riboside which is connected: – At one end, to central cobalt atom, and – At the other end from the riboside moiety to the ring IV of corrin ring system.

4. One PO4 group connects ribose moiety to aminopropanol (esterified), which in turn is attached to propionic acid side chain of ring IV.

5. A cyanide group is coordinately bound to the cobalt atom and then is called as cyanocobalamine.

(b) Various forms of Vitamin B_{12}

1. When cyanide is bound to cobalt atom it is called as **cyanocobalamine**, but if cyanide group is removed, then it is called as cobalamine. Cyanocobalamine is identical with originally isolated vitamin B_{12}. B_{12} which occurs in natural materials does not contain cyanide group. In the original isolation, cyanide group was added only to promote crystallization.

2. –OH group, NO2, Cl– and SO4 = may replace cyanide group, in which case, it is called respectively as:

• Hydroxycobalamine (B_{12a}), (Hydroxocobalamine)

• Nitritocobalamine (B_{12c}),

• Chlorocobalamine, and

• Sulphatocobalamine, in that order.

3. Biologic actions of these derivatives are similar to cobalamine, but Hydroxycobalamine (B12a) is superior as:

• It is more active in enzyme systems

• It is retained longer in the body when given orally.

Hence, B12a is more useful for therapeutic administration of B12 by mouth.

3.5.2 Occurrence and Sources of Vitamin B_{12}

This vitamin is present in foods of animal origin only e.g., include; liver, eggs, fish, meat, kidney, milk and dairy products.

3.5.3 Metabolism

Absorption and Excretion

1. Vitamin B_{12} is absorbed from Ileum (small intestine); for its proper absorption it requires the presence of HCl and intrinsic factor (IF) of Castle, a constituent of normal gastric juice which is secreted by parietal cells. Intrinsic factor is found in 'Cardiac' end and fundus of stomach, but not in the pylorus. Atrophy of fundus of stomach and a lack of free HCl (achlorhydria) is usually associated with pernicious anaemia, caused by B_{12} deficiency.

3. For the proper absorption of this vitamin, the following are reqired; Cobalophilin: A binding protein secreted in the saliva, Intrinsic factor (IF), a glycoprotein secreted by parietal cells of gastric mucosa, Gastric acid (HCl) and pepsin release the vit B_{12} from protein binding in food and make it available to bind to salivary protein, cobalophilin. In the duodenum, cobalophilin is hydrolyzed, releasing the vitamin for binding to "Intrinsic factor" (IF).

• Vitamin B12 is absorbed from the distal third of the ileum via specific binding site (receptors) that binds the "B_{12}-IF complex". The removal of B_{12} from 'intrinsic factor' (IF) in presence of Ca++ ions and a releasing factor (RF) secreted by duodenum take place and B_{12} enters the ideal mucosal cells for absorption into the circulation.

Transport in the blood:

Vitamin B12 is transported in blood in association with specific proteins named Transcobalamine I and Transcobalamine II and III. Physiologically Transcobalamine II is more important.

3.5.4 Deficiency of Vitamin B_{12}

Results to Pernicious anemia which produces macrocytic anemia which is sometimes combined with neurological features (subacute combined degeneration of the cord, an auto immune disease where antibodies to intrinsic factor and parietal cells are found).

Summary of Study Session 3 of module 7

In this study session, you have learnt that

Self-Assessment Questions (SAQs) for Study Session 3

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting.

MODULE 7

STUDY SESSIONS 4

HORMONES

Introduction

Definition:

It is a chemical substance which is produced in one part of the body, enters the circulation and is carried to distant target organs and tissues to modify their structures and functions.

They are stimulating substances and act as body catalysts.

Similarities of hormones and enzymes

Hormones have several characteristics in common with enzymes.

1. They act as body catalysts resembling enzymes in some aspect.
2. They are required only in small quantities.
3. They are not used up during the reaction.

Dissimilarities of hormones and enzymes

They differ from enzymes in the following ways:

1. They are produced in an organ other than that which they ultimately perform their action
2. They are secreted in blood prior to use.
3. Structurally they are not always proteins. Few hormones are protein in nature, few are small peptides, and some hormones are derived from amino acids while some are steroid in nature.

Learning Outcomes for Study Session 4 OF MODULE 7

After you have studied this study session, you should to be able to:

Key Terms: ***Hormones, functions, classification,***

4.1. Function of hormones

1. They control growth and development
2. Regulate operation of reproductive systems
3. Help regulate;

I. Chemical composition and volume of internal environment [extra cellular fluid]
II. Metabolism and energy balance.
III. Biological clock (circadian rhythms)
IV. Glandular secretion
V. Some immune system activities.
VI. Contraction of smooth and cardiac muscle fibers

Major hormone secreting glands are;

Pituitary, Thyroid, Parathyroid, Adrenal, Pancreas, Ovaries and Testes.

Several other glandular tissues are considered to secrete hormones viz;

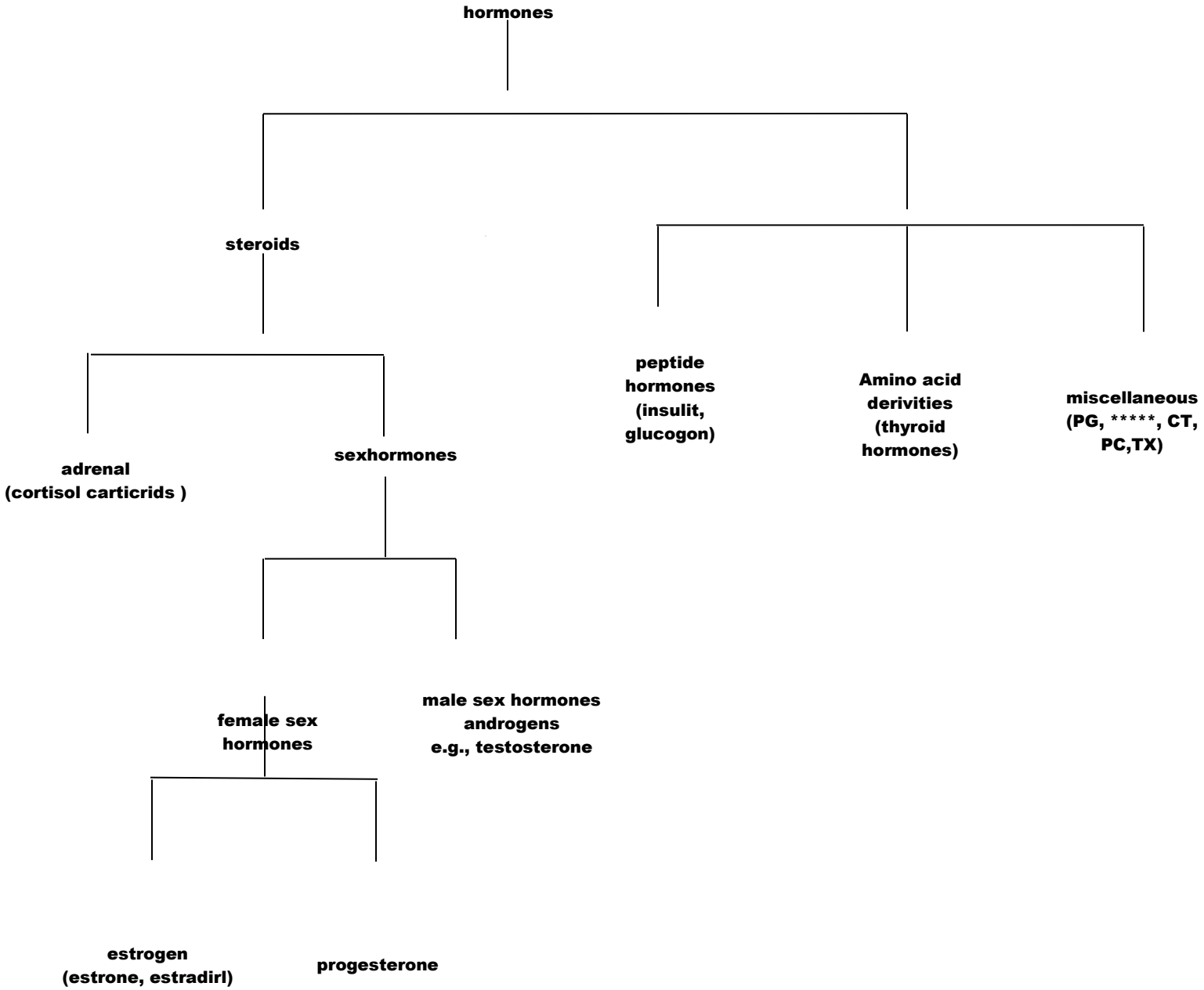
1. Juxta glomerular cells of the kidney; may produce erythropoietin hormone which regulate erythrocyte maturation and erythropoiesis.
2. Thymus; this produces a hormone that circulates from this organ to stem cells in lymphoid organ inducing them to become immunologically competent lymphocytes.
3. Pineal gland; it produces a hormones that antagonizes the secretion or effects of adrenocortico trophic hormone (ACTH) it also produces factors called glomerulotrophins that regulates the adrenal secretion of aldosterone.
4. Gastro Intestinal tract; Few hormones are also produced by certain specialized cells of GI tract and they are called GI hormones.

4.2 CLASSIFICATION OF HORMONES;

CHEMICAL COMPOSITION (MAIN CLASSIFICATION)

1. Steroid hormones; these are steroid in nature such as adrenocorticosteroid hormones, androgens, estrogens and progesterone.

2. Amino acid derivatives; these are derived from amino acid tyrosine e.g. epinephrine, norepinephrine and thyroid hormones.
3. Peptide/protein hormones; these are either large proteins or small or medium size peptides e.g. insulin, glucagon, parathormone, calcitonin, pituitary hormones, e.t.c.
4. Fatty acid derivatives; e.g. Eicosanoids. (Prostaglandins)



4.3. Other Methods of Classification of Hormones.

Classification based on solubility in aqueous medium in cells.

A. Hydrophilic hormones (Lipophobic Hormones); These hormones;

I. Are soluble in aqueous, medium.

II. Cannot cross the cell membrane.

III. They bind to receptor molecules on the outer surface of target cells, initiating reactions within the cell that ultimately modifies the functions of the cell e.g. Anterior pituitary hormones, HGH, TSH, ACTH, FSH,LH, prolactin, MSH, parathyroid hormone, pancreas hormones, (somatostatin, insulin, glucagon), Epinehrine, (catecholamines). Peptides/proteins; All hypothalamic releasing hormones, oxytocin, ADH.

A. Lipophilic Hormones, (Hydrophobic hormones); These hormones;

I. Are not soluble in aqueous medium, but soluble in lipid

II. Can easily cross the cell membranes

III. Can enter target cells that bind to intracellular receptors to carry out their action, example: thyroid hormones, steroid hormones.

B. Classification based on proximity of site of synthesis to site of action.

There are 3 classes of hormones based on proximity of site of synthesis to site of action;

I. Autocrine hormones; those that act in the same cells that synthesize them.

II. Paracrine hormones; those that are synthesized very close to their site of action.

III. Endocrine hormones; those that are synthesized by endocrine glands and transported in the blood to target cells that contain the appropriate receptors.

4.4Factors regulating hormone action

1. Rate of synthesis and secretions; the hormone is storied in the endocrine gland.
2. In some cases, specific transport systems in plasma.
3. Hormone-specific receptors in target cell membranes which differ from tissue to tissue.
4. Ultimate degradation of the hormone usually by the liver or kidney.

classify hormones based on their chemical composition

List 4 factors regulating hormonal actions

4.5 Steps in Hormonal Signaling.

1. Biosynthesis of a particular hormone in a particular tissue.
2. Storage and secretion of the hormone. Hormones exit their cell of origin via exocytosis or other means of membrane transport.
3. Transport of the hormone to the target cells.
4. Recognition of the hormone by an associated cell membrane or intracellular receptor protein.
5. Relay and amplification of the received hormonal signal via a signal transduction process. This leads to cellular response. The reaction of the target cells may then be recognized by the original hormone-producing cells, leading to a down-regulation in hormone production. This is an example of a homeostatic negative feedback loop.
6. Breakdown of the hormone.

Summary of Study Session 2

In this study session, you have learnt the:

Definition of hormones

Similarities between hormones and enzymes

Differences between hormones and enzymes

Functions of hormones

Hormones secreting glands

Classification of hormones

Factors affecting hormonal actions and

Steps in hormone signaling

Self-Assessment Questions (SAQs) for Study Session 2

Now that you have completed this study session you can assess how well you have achieved the learning outcomes by answering the following questions. Write the answers in your jotter and discuss them with your facilitator in your next study centre meeting. You can check your answers at the end of this course material.

Links to OERs

References/ Suggestions for Further Reading

Should you require more explanation on this study session, please do not hesitate to contact your e-tutor via the LMS.

Are you in need of General Help as regards your studies? Do not hesitate to contact the DLI IAG Center by e-mail or phone on:

APPENDIX I: ANSWERS TO ALL SELF-ASSESSMENT QUESTIONS

APPENDIX II: GLOSSARY OF TERMS