Uttar Pradesh Rajarshi Tandon
Open University

Bachelor in Science

UGBCH-101

Introduction To Biochemistry

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Introduction
To
Biochemistry

Block

1

Life history and Cell structure

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COURSE INTRODUCTION

The objective of this course is to provide the introductory of biochemistry. The aim is to provide brief introduction of biochemistry and biomolecules. The course is organized into following blocks:

Block 1 covers the basics of life history and cell structure

Block 2 deals the amino acids, proteins and carbohydrates

Block 3 describes in brief discussion of lipids, nucleic acids and vitamins

Introduction

This is the First block on Life history and Cell structure. It consists of the following three units:

Unit-1: In this unit we know about introduction to biochemistry. The origin of biochemistry and life history describes in brief. The role of biochemistry in career development also discuss in this unit. Water is import solvent and most useful element in biochemical reaction. The unique properties of water, weak interactions in aqueous systems, ionization of water and properties of buffer solution discuss in this chapter.

Unit-2: In this unit we cover the cell structure and function. Architecture of cells means, cell wall and cell membrane is briefly described. The short introduction of measurement units, microscopic and centrifugation techniques also discussed in this unit.

Unit-3: This unit covers the structure and function of cell organelles likes- mitochondria, nucleus, endoplasmic reticulum, chloroplast, Golgi apparatus, ribosomes, lysosomes, centrioles, and cytoskeleton.

UNIT-1

Introduction to biochemistry Structure

Structure

1.1. Introduction

This unit covers the introduction to biochemistry. Biochemistry is the simple combination of chemistry and biology. It not is a single subject; it describes whole life process, in other words it deals with biochemicals that present in living organism body. The living body groups of chemicals that undergo in metabolic processes and builds up our body structure. The organization of cell is called tissue, the association of tissues form organ and finally organ gives the shape of organism. Water plays a vital role in the metabolic process, growth and development of living body. Apart from that water is considered as universal solvent due to polar in nature, water make easily solubility to other substances. Water has several physical and chemical properties that make it suitable to living

organism. pH is also refers to hydrogen ion concentration, pH, which is physical parameter, plays important role in mentioning the nature of water that make it solubility. Water formed weak bonds with number of polar and non polar molecules. A buffer is a solution that resists a change in pH on addition of a small amount of acid [H^+] or base [OH^-] more effectively than an equal volume. Generally buffer is an aqueous solution consisting mixture of weak acid and it conjugated base vice versa.

Objectives:

- to understand the fundamental chemical principles that govern complex biological systems
- to learn about unity of life history and chemical organization of living organism
- to understands importance of water for living organism such as their physical and chemical properties
- to know buffer and pH role in biological reactions

1.2. Biochemistry overview

Biochemistry just a chemistry of living being. It defines the chemical process that occurs in living organisms. We know the body of organisms is made up of cells, thus the biochemistry has ultimate gol to understand the nature and behaviour of cell molecular organisation. In general, biochemistry define the combination of two subject viz. chemistry and biology, that means here in biochemistry principle and techniques of chemistry applied to understand the biology of living organisms.

Biochemistry deals with entire biochemical phenomenon such as growth, respiration, locomotion, fighting and illness. Thus biochemistry has structure, a set of unifying themes, and all the wonderful sequence of genetic control over the shape and function of protein.

Biochemistry is a central part of biology, or life science because it concerns with synthesis and structure of biomolecules that makeup living organism. With the reaction, molecules provide energy for growth and development and maintain metabolic process through chemical reaction. However, biochemisty helps in under print our understanding in all aspect of biology and biologist. biologist study the structure and properties of living cells.

Biochemistry is the study of biological phenomena at the molecular level. Its aim is to understand the fundamental chemical principles that govern complex biological systems. The program is an interdepartmental, major between biology and chemistry that emphasizes the importance of a solid foundation in the natural sciences, including mathematics and physics. The major focuses, however, on disciplines within biology and chemistry, ranging from cell biology and molecular biology to analytical chemistry and physical chemistry. Biochemistry,

involves the study of make large biomolecules, these molecules are polymer compound which are up long chain of identical or very small known monomer. Example glucose, the glucose combines together and makes long chain of starch molecule. Starch is example of polysaccharides. Polysaccharides has two main function in living cells, first to acts as store of energy and second is to make a structure like cellulose, Other class of large molecules that are protein, enzymes and nucleic acid.

The program seeks to graduate biochemists who are conversant in concepts ranging from biological evolution to quantum chemistry. Understanding the molecular logic of life and being able to participate in the acquisition of this knowledge is integral to the liberal education. The chemical reaction occurs in living body, it occurs spontaneous and produce for further metabolic process.

Metabolism- that describe the chemical reaction that occurs in living organism. These reactions are traditionally divided into two broad groups.

Anabolism- this refers to these chemical reaction that build up large molecules form smaller ones.

Catabolism- this is a part of metabolism, that is devoted to break down of components in order to generate energy.

In otherworld, biochemistry is on inter disciplinary science which covers the range of scientific disciplines including genetic, microbiology, forensic science, biophysics, cell biology and molecular and molecular biology etc. A part from that some topics are also directly/indirectly reflect teh biochemistry, that are :

Organic chemistry focuses on molecules mainly composed of carbon and hydrogen, along with a handful of other elements - such as oxygen, nitrogen, silicon, sulphur, and phosphorus. Organic chemistry deals with synthetic methods, reaction mechanisms and kinetics, and uses analytical methods for reaction control and purification. *Biophysics* refers to the study of physical nature of bimolecular through physical techniques.

Medical research refers to understand the diseases which cover wide range of preclinical and clinical research. Most of researches in this field are carried out by biomedical scientist but significance contributions are made by other types of biologist. *Microbiology* which has shown that single celled organism and virus are ideally suited for studying many metabolic pathways regulating mechanism.

Nutrition refers to maintenance of health by dietary requirement of different types of food supplements. This is helpful to understanding of requirements and calorific values of dietary substances to promote health and manage diseases.

Physiology which investigates life process at the tissue and organism levels. Cell biology refers to study of physiology, structure and life process of cells. The biochemistry has central position in life science, because the biochemistry is concern with the synthesis and structures of macromolecules that build-up the living organism. Thus biochemistry, therefore, explain how the different atoms are combines together to make living entity. The biochemistry is underpinned of understanding of all aspect of biology. The structure and properties of living cells are govern by macromolecule; these macromolecules make up the structure of cell and are responsible for each properties of particular cell. That why we can say that biochemistry has complex relation with biology.

Biochemistry also explores the molecular biology that focuses on molecular understanding of the process of replication, transcription and translation of genetic materials. Biochemistry is a laboratory based science that relies on the availability of appropriate bio-analytical techniques. This bio-analytical techniques, define the nature and relationship between biological molecules especially protein and nucleic acids. In addition by using bioanalytial techniques, biochemist can also understand and solve the problems of physical environment in which organism live. Thus biochemistry is considered simple as well as complex form of science that reveals the complex physiological and metabolic process of living organism.

The biochemistry is interdisciplinary science, in the begging of 20 century, scientist ware combined, the physiology and biology to investigate the chemistry of living organism, form a cells, tissues and whole organisms. The basic principles of biochemisty are common to all living organism. We know that all life process is performed by organic molecules thus the biochemistry relies on heavy fundamental principles of organic chemistry as well as principles of other basic sciences.

How the macromolecules work together and produce most fascinating properties among the living species, deals by the biochemistry. A living being that can eat, grow appropriate that will be focus of basic understanding of biochemistry. It deals with the understanding of biological macromolecules such as protein, carbohydrate vitamin, lipids and nucleic acids.

To better understanding of biochemistry, the every student should have basic knowledge of

Genetics; Cell biology; Molecular biology,

Physiology and Immunology

Pharmacology and Pharmacy

Toxicology; Pathology; Microbiology

Zoology and Botany

Agriculture

Industrial applications

Environmental acceptability

The macromolecules like protein, carbohydrate, amino acid, lipids and nucleic acid etc are main components of living organism. The structural and functional roles of these macromolecules regulated by enzyme activity. The enzymes are also protein component and produce in living organism.

Enzymes are responsible of high complex reactions. The enzymes directly involved in the metabolic events, exhibit specificity toward substrates, and regulate the entire metabolism. Thus, they play key role in the degradation and synthesis of nutrients, biomolecules etc.

Different types of macromolecules makeup the intricate internal structure of living cells. These living cells extract, transfer and use the energy from environment which is useful in to build-up and maintenance of intricate structure of macromolecules. These are some basic point discuss why study of biochemistry is useful in our life.

- All the biological process performed by structure and function of macromolecules that present in living cells
- Biochemistry may be used to study the properties of biological molecules, for a variety of purposes. For example, a biochemist may study the characteristics of the keratin in hair so that a shampoo may be developed that enhances curliness or softness. Biochemists find uses for biomolecules. For example, a biochemist may use a certain lipid as a food additive.
- Alternatively, a biochemist might find a substitute for a usual biomolecules. For example, biochemists help to develop artificial sweeteners. Biochemists help cells to produce new products. Gene therapy is within the realm of biochemistry. The development of biological machinery falls within the realm of biochemistry.
- Biochemistry is used to learn on modelling of nucleic acid with computers. The molecular structure and properties of molecule are defined by using computational chemistry and graphical techniques.

1.3. Origin of biochemistry

The term biochemistry is first coined by German chemist Carl Neuberg in 1903. German Neuberg was fist scientists that observe dead cells after invention of microscope. Before him, later in 1674, Anton van Leeuwenhoek saw live plant cells under a simple microscope. In 18^{th} century the French scientist Antoine Lavoisier was the first scientist who studied plant cell and proposed the reaction mechanism of photosynthesis.

He also was the first person to investigate the process of cell respiration, the process of making the energy molecule, adenosine triphosphate (ATP), in the mitochondria of the cell.

French biologist Louis Pasteur observed the role of yeast and bacteria in fermentation and he give the process for pasteurization. Later in 1877 Pasteur's fermentation take place due to activity of were designated as enzymes. When first crystalline enzyme was first isolated in 1926 reveals the chemical nature of enzymes. Later enzyme proved to be made up of proteins which are form of high weight molecular chain of amino acids. These amino acids are considering the building blocks of proteins.

In 19th century discover of jelly-like substance inside the cells that is called protoplasm. Protoplasm was found to carry out all of the process involved. That time it was believed that the chemistry of living organism was inherently different form of non living ones. But in earlier, the experiment was carried out by Eduard Buchner has change this view. Eduard Buchner extracts zymase from the yeast. The zymase did not contain any living cells; it could still ferment glucose to produce carbon dioxide and ethanol. After this experiment zymase was used as a multiple enzymes.

In the 20 century, a German scientist Hens Krebs observed a series of chemical reactions during cellular respiration where glucose is converted in to ATP, carbon dioxide and water. In this century, the DNA becomes known as the genetic material of the cell and its structure was identifying by James Watson and Francis Crick from previous research done by Rosalind Franklin. In present time in, biochemistry, the number of technology has been appearing such as recombinant DNA, gene splicing and radio isotopic labelling DNA finger printing etc.

1.4. Unity of life-History

Our nature consists a lots of inanimate matter founds in air soil, air water and rocks. Some of elements are part of living organism. The chemical and physical laws describe the behavior of these inanimate matters. When these elements become the part of living organism, they possess extra ordinary attributes as compare to inanimate. If we examine some of these special properties we can approach the study of biochemistry.

If we can see the body of any living organism, we found that the body of living organism has well organized cell structure. The cells which are the unit structure of body, consisting different kinds of complex molecules. Each components of living organism have specific purpose or functions made up of different kinds of cells. The cells make internal structure as well as external structure of organism. The macromolecules that present in cell for example lipids; protein and nucleic acid have specific functions. The specific functions found both in macroscopic

structure (e.g. leaves, steams or heard, year legs) and microscopic intracellular structure (e.g. nucleus and protoplasm) components of living organism. Specific functions refers to change among chemical organization, is dynamic in components causes coordinating or compensating change in other in all set of organic components.

Living organism has capacity to synthesize, complex compound by using inanimate matter and convert into different kinds of macromolecules such as proteins, nucleic acid and lipids etc. Furthermore, the living organism can extract or transfer energy from their environments that not only use in maintenance and repairing of own structure but also utilize in other works. Self replication or self assemble means is the most extra ordinary attributions of living organism such as billions of daughter cells can carry a faithful copy of the genetic material of their parental cells. When we see the nature of living organisms we found that there are lots of question arises in our mind such as i) how the living matter different from the non living matter, which also consists of inanimate molecules, ii) Why the living organism does appears to be more than sum of its inanimate part. iii) How different biomolecules maintain the all process in living organism. To answer these quantities, the modern science has central goal to determine how the collection of inanimate interact with each other to contribute maintain and perpetuate living state.

The basic concept of organization of biology can be understood with their regularity structure. The biological system is biologically active structure and characterized by a definite arrangement of their components, these components interact themselves, resultant give biological function to the structure. Their for it for is resemble to consider the organization of living matter as unity of structure and function. On the other hand biological organization is a set of elements or sub systems of lower rank. The mechanism of elements in biological system made the hierarchy in biological system.

Although, the hierarchy nature of biological system is generally recognized which based on structured approach such as low molecules-weigh compounds- biomolecules- cells- tissues-organ system-organism-populations- biogeocenosis and biosphere. Therefore biological organization is also defined as the unity of structure, function and regulation of biological system. The biological system contains the different types of chemicals with their hierarchical level. The organization includes the level of the matter organization starting with the cell, lower subcellular and supramolecules structure are considered as distinct levels in the organization of biosynthesis.

1.5. Scope and current prospective of biochemistry

The biochemistry deals with the chemical combinations and reactions that take place in biological processes such as growth,

reproduction, metabolism, and physiological and psychological behaviour of living organisms. Even biochemistry is the main subject to better understand the life science. As initial biochemistry was just focusing as topic of life science that deals the chemical reactions that occurs in living organism but later it has been considered as subject that deals every aspects of life directly or indirectly. Recently, the biologist focusing our research on the biochemistry nothing. The people who how work on chemical phenomenon that deals the chemical reaction occurs in the living organism called biochemist. The biochemist has mainly focused their research work on enzymes, proteins, carbohydrates, fats, process of metabolism and the molecular basis of the action of gene. As a field biochemistry has seen unprecedented growth because of its significant contribution towards the illumination and grasping of the DNA Structure. Recently, biochemistry extent their area. It is not limited to as practical subject but also has lot of professional significance because biochemistry provides number of opportunity in both public and private sectors. Thus the biochemist might be obtained number of opportunity such as

- She/he may involved as research scientist work in field of biochemistry
- She/he can may stand their career as professor or subject expert who leads the practical and subjective knows to student and society.
- Biochemist may be worked with other physics, chemist, health care professional, policy maker and biotechnologist.
- Biochemist can initiate their work with coordination of different public or private sector research units.
- Lot of organization such as hospitals, universities, agriculture, food institutes, education, cosmetics, forensic crime research, drug discovery and much higher research professional. Beyond such organization number of other public sector organization also provide career as follows
    - Drug manufacturing companies
    - Public health entities
    - Blood service
    - Industrial laboratories
    - Cancer research institutes
    - Departments of educational institutes
    - Environmental pollution control etc.

1.6. Water

Water is the simple molecule that forms by the combination of hydrogen and oxygen in 2:1. Water considered as universal solvent. It has molecular formula H_2O, water is reactive substance with usual properties. Water present in nature in three form liquid, gas, and solid on the basis of temperature. Our body contain about 70 to 90% water because it familiar and iniquitous in nature. Water found in our body in the association with macromolecules and cell organelles that require to biological properties of proteins, nucleic acid as well as membrane, ribosomes and many other components. Water recognize as good solvent because it is polar in nature. Water ionizes in two forms as hydronium ions and hydroxide ions. Water as molecular form has both partial positive (due to presence of hydrogen in one side of water molecules) and partial negative charge (due to presence of oxygen in one side of water molecules). This nature of water makes it more reactive to with other molecules.

1.6.1. Properties of water

The water molecules have special physical properties. The water molecule as liquid phase has high boiling point, high melting point, high vaporization, specific heat capacity and surface tension compare to other hydride molecules. Water molecules are formed by equal sharing of electron by covenant bonding. It has sp^3 hybridization and hydrogen tetrahedral arrangement, this type of arrangement result occur net dipole in water in which the end of molecule containing the unshared electron has partially positive charge character and the end containing the two hydrogen has partially negative charge character shown in Fig. . In addition, hydrogen oxygen bonding as dipolar character due to unequal sharing of electrons between hydrogen and oxygen. However, due to presence of equal partially positive and negative charge in both side, the resulting net charge in the molecule in zero. Thus, it is known as dipole molecule. The spectroscopic and X rays studies define the precise H-O-H analysis is 104.5˚ a hydrogen oxygen intra-atomic distance is 0.0965 nm.

Due to presence of partial positive charge on hydrogen atom and partially negative charge on oxygen atom, two water molecule approach to closely attached together by electrostatic attraction. Where positive charge of one water molecule (hydrogen atom) attract toward negative charge of other water molecule (oxygen), same as the partially negative charge of one water molecule oxygen atom attract toward positive charge hydrogen atom of other water molecule. This accompanied by redistribution of the electronic range in both molecules. Thus, this is association of both two hydrogen atom is called hydrogen bonding. This is only properties that responsible for the great internal cohesion of liquid water. The hydrogen bonding is relatively weak than covalent bonds. The each water molecules have hydrogen bonding to 4 neighbour molecules.

Since, water molecule arrange in tetrahedral arrangement same the neighbour molecule is also have tetrahedral arrangement. The ice which is the form of water also have tetrahedral crystalline lattice in which water molecules attract by hydrogen bonding. The cohesion of water molecules contain as high melting point and high boiling point in inspite of low molecular weight (18 g/mole). The high special heat and high evaporation make it excellence thermal for actively metabolizing cells and tissue.

1.6.2. Weak interactions in aqueous systems

Water is polar in nature and a good solvent. Water forms weak bonds with number of polar and non polar molecules. The interaction of water with substances would be understood as follows:

Hydrogen bonding between water and solvent:

We know the water have properties to form hydrogen bonding due to presence of partially positive and partially negative charge. However, hydrogen atom also covalently bonded to carbon atom, even it do not participate in hydrogen bonding because carbon does not has negative charge. For example butanol ($CH_3CH_2CH_2CH_2OH$) has relatively higher boiling point (117 ˚C) as compare to butane ($CH_3CH_2CH_2CH_3$), which has boiling point only -0.5˚C because, butanol has hydrogen bonding. The butanol has hydroxyl group and formed hydrogen bond with other molecules.

However, hydrogen atom has stabilizing effect with some polar molecules that's why sugar dissolves readily in water. Hydrogen of water forms that form hydrogen bonding between with hydroxyl groups or carbonyl group of the sugar. The other polar water molecules, such as alcohol, aldehyde and ketones all form hydrogen bonding with water.

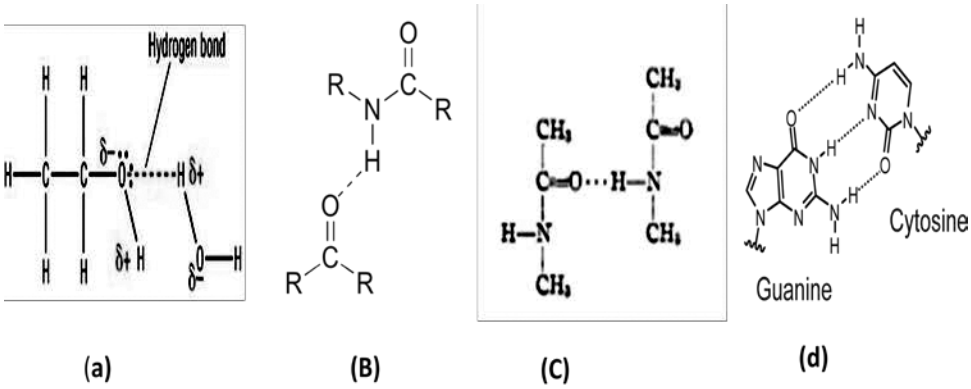

Fig: 1.1 Hydrogen bond between (a) water molecules, (b) carbonyl compound, (c) polypeptide chain, (d) nucleotides**.**

a. Interaction between water and charge solutes or nonpolar gas:

The molecules that are easily dissolved in water (polar) are generally charged or polar molecules are known as hydrophilic molecules.

However, non polar solvent are poor solvent for polar molecules but they readily dissolve nonpolar molecules such as lipids and wax etc. For example, NaCl (polar) is readily dissolved in water by hydrating and stabilising the Na^+ and Cl^- ions. Polar molecules such as glucose, glycine, aspartic acid and lactic acid etc easily dissolve in water. O_2, N_2 and CO_2 gas are non polar in nature, but CO_2 gas forms carbonic acid (H_2CO_3) in aqueous solution. The two the gas NH_3 and H_2S dissolve in water and have biological role in organism.

b. Van der Waal interactions:

it is weak and non specific intrinsic, attractions. It formed when two charge atoms closely stand at 3 to 4 A˚. They are weak and less specific than electrostatic and hydrogen bonding and also have importance in biological system. All types of molecules exhibit van der Waal forces which arise from the attraction of the bounding electron of one atom for the molecules of other. When two atoms are for apart, this is very weak attraction which becomes stronger as the atom more close together.

1.6.3. Ionization of water:

It must be know that lots of properties of water molecules is governs by it uncharged form as (H_2O). While to understand their properties we should know the ionization of water as to hydrogen ion (H^+) and hydroxide ion (OH^-). In this ionization, we can see through reversible ionization of water molecules that yield a hydrogen ion and a hydroxide ion:

$$H_2O \leftrightharpoons H^+ + OH^- \quad \text{(i)}$$

This reversible ionization is crucial to the role of water in cellular functions. In this reversible reaction, the equilibrium of H_2O and it's ionize formed is determine by it equilibrium constants as shown in Eq. (ii).

$$A + B \leftrightharpoons C + D \quad \text{(ii)}$$

Equilibrium constant can be defined in terms of concentration of reactant (A & B) and product (C & D) present in equilibrium (iii).

$$K_{eq} = \frac{[C][D]}{[A][B]} \quad \text{(iii)}$$

Where keq is equilibrium constant and bracket [] represent concentration of reactant and product. The equilibrium constant is fix and characteristic for given chemical reaction at specific temperature. The H_2O also concentrated at given tem 25 ˚C, about only one of every 10^7 molecule in pure water is ionized at any instant. The equilibrium constant for the reversible ionization of water is

$$K_{eq} = \frac{[H^+][OH\text{-}]}{[H_2O]} \quad \text{(iv)}$$

In pure water at 25 ˚C, the concentration of water is 55.5 Mole. Thus, value is essentially constant in reaction to the very low concentration of H^+ and OH^- normally 1 x 10^7 M.

$$K_{eq} = \frac{[H^+][OH^-]}{[55.5\ M]} \quad \text{(v)}$$

$$(55.5M)\ (k_{eq}) = [H^+] + [OH^-] = k_w$$

Where k_w denotes the product (55.5 M), (k_w) the ion product of water is 25 ˚C. The value of k_w is 1.8 x 10^{-16} at 25 ˚C. Put the value of k_w in Eq (v) we found.

$$(55.5M)\ 1.8\ x\ 10^{-16}M = [H^+] + [OH^-] = k_w$$

$$99.9\ x\ 10^{-16}M^2 = [H^+] + [OH^-] = k_w$$

$$1.0\ x\ 10^{-14}M^2 = [H^+] + [OH^-] = k_w$$

Thus, the product $[H^+] + [OH^-]$ in aqueous solution at 25 ˚C always equal to 1 x 10^{-14} M^2, where are exactly equal concentration of both product $[H^+] + [OH^-]$.

In pure water, the solution is said to be neutral pH. At this pH, the concentration$[H^+]\ and\ [OH^-]$ can be calculated from the ion product of water as follows

$$k_w = [H^+] + [OH^-] = [H^+]^2$$

Solution of $[H^+]$ gives

$$H^+ = \sqrt{k_w}\sqrt{1\ x\ 10^{-14}M^2} = 1\ x\ 10^{-7}M$$

$$OH^- = 1\ x\ 10^{-7}M$$

1.7. pH Scale

pH is also refers to potential of hydrogen. pH is a symbol and donates the relative concentration of hydrogen ion in solution of extend form of value 0 to 14, the lower the value, the higher the acidity or the more hydrogen ion concentration. In solution, water at 25^0 C has a concentration of hydrogen ion of 10-7, the pH therefore, is 7.

H^+ ion of water, k is the basis for the pH scale, it show the actual concentration of hydrogen ion in aqueous solution in range blew 1.0M H + and 1.0 M OH^-.All the biological reactions defined this pH scale.

The pH also defines that pH of a solution as the negative logarithm of concentration of hydrogen ion, thus:

$$pH = \log\frac{1}{H^+} = -\log H^+$$

At mention solution at 25 C the concentration of H^+ ion found 1 x 10^{-7} M. The pH can be calculated as

$$pH = \log\frac{1}{1\ x\ 10^{-7}} = -\log(1\ x\ 10^{7})$$

$$= \log 1.0 + \log 10^{7}$$

$$= 0 + 7.0$$

$$= 7.0$$

Solutions in which pH = 7.0 are defined as neutral. Solutions with pH < 7.0 are called "acidic," and solutions in which pH > 7.0 are called "basic." **1.2**

$[H^+]$ M	pH	$[OH^-]$ M	pOH*
$10^{-0(1)}$	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-2}	1
10^{-14}	14	$10^{-0(1)}$	0

1.8. Buffer solution

A buffer is a solution is one that resists a change in pH on addition of a small amount of acid $[H^+]$ or base $[OH^-]$ more effectively than an equal volume. Generally buffer solution consists of mixture of acid and conjugated substance. For example mixture of acidic acid conjugate base, and sodium acetate or of ammonium hydrogen hydroxide and ammonium chloride are buffer solution. If see the titration curve of acetic acid, the pH of a solution undergoes change at near the midpoint (where pH = pKa, and $[CH_3COOH]=[CH_3COO]$) of the curve. However the buffering region extends about 1 pH unit on either side of the pKa. In this pH range, buffering power is best because the concentrations of both buffering species, HA and A, are the highest. Buffers are selected based on their pKa values and the range of pHs to be buffered. There are

some basic factors which determine the effectiveness or capacity of buffer solution.

Molecules of the buffer components

Relative concentration of the conjugate base and weak acid.

The hydrogen ion concentration of most body fluids and secretion in on the alkaline side.

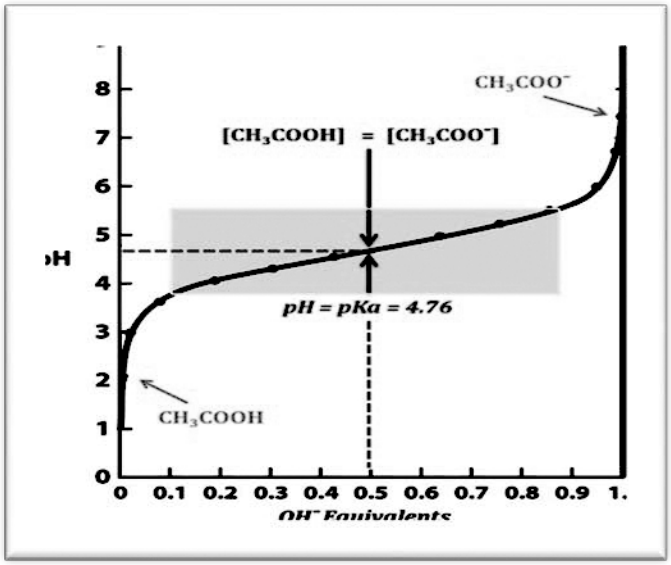

Fig : 1.2 Buffer region of acidic acid (5.76 50 3.76)

Biological buffer system:

Biological process depends on the pH, however, small change in pH, a large change in the role of process. Biological process affected directly or indirectly by concentration of hydrogen ions. Enzymes and many other molecules on with the act, contain ionizable groups with characteristic pk_a values.

Extracellular fluids	Bicarbonate buffer
Extracellular fluids	Protein buffer Phosphate buffer
Erythrocytes	Protein Homological buffer

Cell and organism mentain "specific and constant cytosolic pH. Keeping biomolecules in their optimum ionic sate, usually pH 7. The optimum pH for biological process mentioned by the biological buffers. In the blood, the CO2-carbonic acid-bicarbonate system is used for buffering. Inter and intracellular and extracellular fluids of living organism contains

conjugated acid base which act as buffer and maintain the normal pH of these fluids.

1.9. Summary

The biochemistry has central position in life science, because the biochemistry is concern with the synthesis and structures of macromolecules that build-up the living organism. Thus biochemistry, therefore explain how the different atoms are combines together to make living entity. The biochemistry helps in understanding of all aspect of biology. The term biochemistry is first coined by German chemist Carl Neuberg in 1903. Each components of cell of living organism have specific purpose or functions. Water found in our body in the association in macromolecules and cell organelles that require to biological properties of proteins, nucleic acid as well as membrane, ribosomes and many other components.Water forms weak bonds with a number of polar and non polar molecules.pH of a solution is the negative logarithm of concentration of hydrogen ion. In the blood, the CO_2-carbonic acid-bicarbonate system is used for buffering.

1.10. Terminal Questions

Q.1. What is biochemistry?

Answer:----------

Q.2. How biochemistry is interdisciplinary subjects.

Answer:----------

Q.3. Define the scope of biochemistry in career development?

Answer:----------

Q.4. What is pH and pH scale? How it useful in biochemical reactions?

Answer:----------

Q.5. Discuss about weak interaction of water with other substance.

Answer:----------

Q.6. Write short notes on

- **a.** Properties of water
- **b.** Role of pH in biochemical reaction
- **c.** Hydrogen bonding
- **d.** Buffer solutions

Answer:----------

1.11. Further readings

1. Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Publishers and Distributors, Delhi.
2. Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company, seven editim
3. Cell Biology (Cytology, Biomolecules and Molecular Biology): P S Verma and V K Agarwal.
4. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

UNIT -2

Cell structure and functions

Structure

2.1. Introduction

This unit covers the cell structure and functions. The body of organism is consists of cells. and the cell is basic unit of life. The organization of cell is called tissue, the association of tissues form organ and finally organ gives the shape of organism. Cellular macromolecules are the part of interaction and reactions taking place inside the cell and it's fundamental to all living being. Any organism, mainly contain either Prokaryotic cell or Eukaryotic cell. The organisms having many cells in their body are called multi-cellular organisms for example most of plants and animals are multi-cellular organisms. The cells have different structure and functions in plant and animal cell. The brief structure and functions of animal and plant cells are discussed in this unit. Microscopy is important techniques in the study of cell size and structure, the light and

electron microscope discuss in this unit reveals that the different types of microscopes are used in study of cell biology. This microscope is very useful in detection of very small living and non living organism. The phase contrast, dark and fluorescence microscope is used for detection of different specimen.

Objectives

- to learn about the basic concept cell biology
- to learn how the plant and animal cell are differ and which component make the cell structure and cell wall.
- to understand the role of light and electron microscope in determination of biological specimen
- to learn about cell function and it chemical organization and size in different organism.

2.2. What is cell?

Cells are very small, we can't see it with our naked eyes limited by the fact that we cannot resolve two points separated by less than 0.1 mm, the cell can see with the help of microscope. Cells are smaller and could be at the range of 1 micrometer for bacteria and 30 micrometers for epithelial cells. Cellular components are still smaller and we need an electron microscope to see them. Cell is basic unit of life that involved in structure and function of body of living organism. Thus we can say that body of living organism is cellular organisation. Various chemical reactions occur in it to keep the cell in the 'living state'.

Mostly cell in a human being have diameters 10-20 nm. The three principle components of cell are

- Cell membrane
- Nucleus and its chromosomes
- Cytoplasm and its organelles

2.3. Cell theory

- The German botanist Mathias Jacab Schleiden (1838) first time observed the cell in plants.
- The term "Cell" was first coined by Robert Hooke in 1655. He used magnifying glass to observe the structure of cork and used the term "cell".
- Brown (1831) stated that all cells have a nucleus and the cell content was called protoplasm.

- Theodore and Schwann (1839) also studied and applied the theory of Schwann in different types of animal cell. and reported "the cell is basic unit of structure and function of all life.
- K. Negeli (1817-1891) said the plant cell rise form pre-existing cells.
- Virchow (1858) established the concept of cell division in production of organism according to his theory;

✓ All living organisms (plant, animal and microorganisms) are composed of one or more cells and this cell produce further cells.

✓ All cells arise from pre-existing cells.

✓ Metabolic activity takes place in the cells.

✓ The cell is clearly defined unit of life.

Modern cell biology:

Recently number of theory has been proposed to understand the life of living organisms. In this regards Gregor Mendel discovered the fundamental laws of heredity (1865), Miescher (1871) isolated nucleon, what is now called DNA, from white blood cells. The structure of DNA given by Watson and Crick (1953). The Theory of Evolution by natural selection and the central dogma of molecular biology are the twin pillars supporting modern biology.

2.4. Classification of cells

We are not going to discuss the classification of living organisms in detail but instead would learn more about cells. We know that all organisms' body are cell organization and made up cellular organization and may contain one or many cells. Organisms that have only one cell in their body are called unicellular organism eg. Archaea, bacteria, blue green algae and protozoa etc, and organism with more than one cells in their body are called multi-cellular organism eg. most of plants and animals. Any organism, only contain one types of cell such as either Prokaryotic cell or Eukaryotic cell. In both prokaryotic and eukaryotic cells, a semi-fluid matrix called cytoplasm occupies the volume of the cell. The terms prokaryotic and eukaryotic were suggested by Hans Ris in the 1960's. This classification is based on their complexity.

2.4.1. Prokaryotic cells

Prokaryote means before nucleus in Greek. They include all cells which lack nucleus and other membrane bound organelles. Mycoplasma, virus, bacteria and cyanobacteria or blue-green algae are prokaryotes. The organisation of the prokaryotic cell is fundamentally similar range

between 1 µm to 10 µm even vary in size from 0.2 µm to 750 µm. Most prokaryotes are unicellular, exceptions being myxobacteria which have multicellular stages in their life cycles. They are membrane bound mostly unicellular organisms lacking any internal membrane bound organelles. Prokaryotes cell organalles harbor few internal structures, such as the cytoskeletons, ribosomes, which translate mRNA to proteins. The prokaryotic cell especially in bacterial cell have chemically complex envelop which consists of tightly bound three layered structure. On the basis of differences in the cell envelopes bacterial can be classified in two groups such as Gram positive and the Gram negative bacteria.

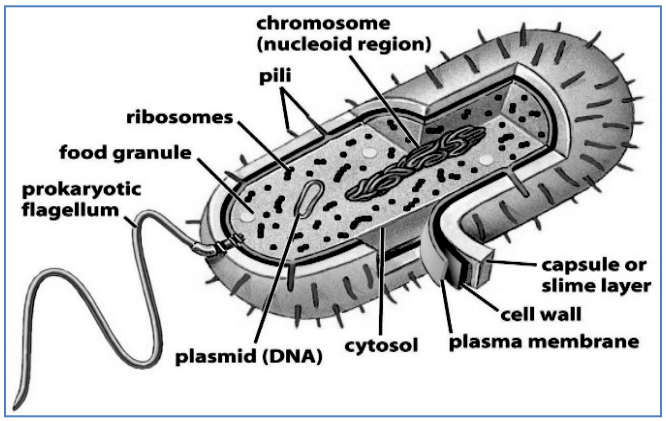

Fig. 2.1 : Schematic diagram of a prokaryotic

Flagella : It is a long, whip-like protrusion found in most prokaryotes that aids in cellular locomotion.

Capsule : The capsule is found in some bacterial cells, this additional outer covering protects the cell when it is engulfed by phagocytes and by viruses, assists in retaining moisture, and helps the cell adhere to surfaces and nutrients

Cell wall : Cell wall is the outermost layer of most cells that protects the bacterial cell and gives it shape. One exception is Mycoplasma which lacks cell wall. Bacterial cell walls are made of peptidoglycan

Cell membrane: Cell membrane surrounds the cell's cytoplasm and regulates the flow of substances in and out of the cell.

Cytoplasm : The cytoplasm of a cell is a fluid in nature that fills the cell and is composed mainly of 80% water that also contains enzymes, salts, cell organelles, and various organic molecules.

Ribosomes : Ribosomes are the organelles of the cell responsible for protein synthesis.

Nucleiod Region : The nucleoid region is possessed by a prokaryotic bacterial cell.

Plasmids: They are double-stranded and circular. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms.

Morphology of prokaryotic cells

Prokaryotic cells have various shapes; the four basic shapes are (Figure 2.2)

- Cocci - spherical
- Bacilli - rod-shaped
- Spirochaete - spiral-shaped
- Vibrio - comma-shaped

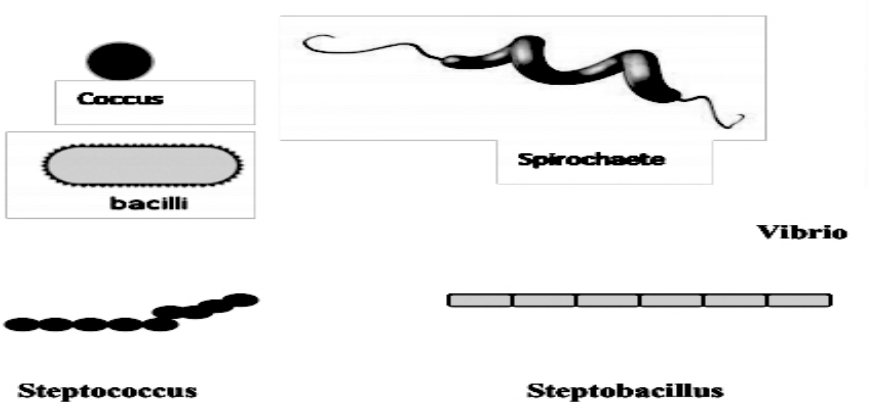

Fig. 2.2: Different morphology of prokaryotic cell

The prokaryotic cells have following characteristic which made differ from eukaryotic cells are following:

- The absence of membrane around the nuclear materials
- The absence of membrane limited the cell organelles means in prokaryotic cell the organelles such as Endoplasmic reticulum, mitochondria, Golgi body, Chloroplast and Lysosome etc are not existed.
- Due to absence of nucleolus membrane the genetic material is present in single chromosomes and represented as circle in double stranded DNA.
- In prokaryotic cell, the basic protein such as histidine is not present as compare to eukaryotic cells.
- The prokaryotic cells do not contain nuclei, cytoskeleton, centrioles, and basal body.

- The cell of prokaryotes is non cellulosic, it formed by carbohydrate and amino acids.
- Plasma membrane carries respiratory enzymes that are found in mitochondria.
- Prokaryotic cells contain 70S type's ribosomes.
- In prokaryotes ribosomes are associated with the plasma membrane of the cell.

2.4.2. Eukaryotic cells

A eukaryotic cell consists of envelop system and they are very much large than prokaryotic cells. Eukaryotic cell is large as compared to prokaryotic cell ranging from 10 to 100 micrometers. . They have a variety of internal membrane bound structures, called organelles. All species of large complex organisms are eukaryotes, including animals, plants and fungi and most species of protist microorganisms. Eukaryotes appear to be monophyletic (organisms that form a clade) and make up one of the three domains of life. Eukaryotes typically composed of plasma membrane, cytoplasm and its organelles. In addition, eukaryotic also have a variety of complex locomotory and cytoskeletal structures. Their genetic material is organized into chromosomes. Eukaryotes represent a tiny minority of all living things; even in a human body there are 10 times more microbes than human cells. Plant and animal cells are different as the former possess cell walls, plastids and a large central vacuole which are not found animal cells. On the other hand, animal cells have centrioles which are absent in almost all plant cells. The nucleolus is surrounded by the nuclear membrane in eukaryotic cell. Cell division involves separating of the genome which is in the form of tightly packed condensed structure known as the chromosomes, through movements directed by the cytoskeleton (Fig 2.3).

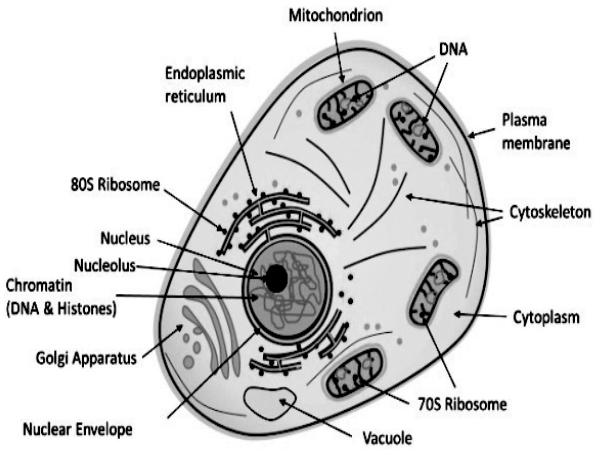

Fig.2.3: Schematic diagram of a eukaryotic cell

2.5. **Plant cells**

Plant cells are eukaryotic cells that differ in several key aspects from the cells of other eukaryotic organisms. Their distinctive features include the following organelles

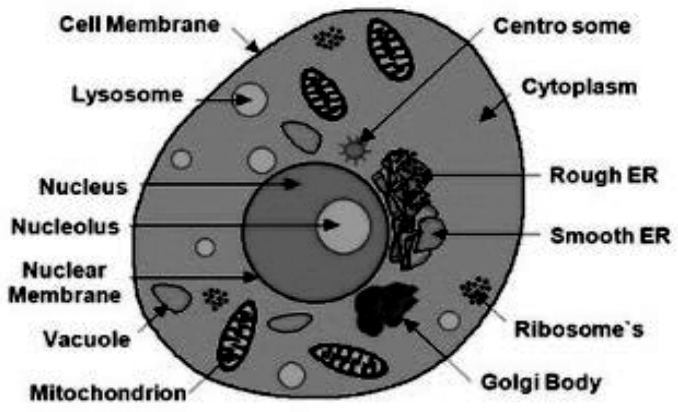

Fig.2.4. : Schematic diagram of plant cell

Vacuole: Vacuole is found in centre of plant cell. The vacuole is bound by a single membrane called tonoplast. In plant cells the vacuoles can occupy up to 90 per cent of the volume of the cell. The number of ions and other materials transported against concentration gradients into the vacuole facilitated by tonoplast in plants cell. The function of vacuole is to control movement of molecules between the cytosol and sap and also stores useful material and digests waste proteins and organelles.

Cell Wall: The cell wall is composed of cellulose, hemicellulose, and pectin and in many cases lignin, is secreted by the protoplast on the outside of the cell membrane. The cell wall is surrounded by plasma membrane. The cell wall composition varies depended on the organism. The plant cell wall is multi-layered and consists of up to three sections. From the outermost layer of the cell wall, these layers are identified as the middle lamella, primary cell wall, and secondary cell wall. The functions of cell wall are to mechanical support, withstand turgor pressure, growth regulation, regulate diffusion, communication, protection and storage etc.

Plasmodesmata: Pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

Plastids: Plastids are double membrane-bound organelles found in all eukaryotic cells such as inside plants and some algae. The primarily responsibility of plastids are related to making and storing food through photosynthesis. A typical plant cell (e.g., in the palisade layer of a leaf) might contain as many as 50 chloroplasts. Chloroplasts are the most known plastids which are responsible for photosynthesis. These are covered with thylakoids where the process of photosynthesis occurs. The other plastids are amyloplasts specialized for starch storage, etiolates specialized for fat storage, and chromoplasts specialized for synthesis and storage of pigments.

2.6. Animal Cell

Animal cell is distinct form of eukaryotic cell that makes up many tissues in animals. The animal cell differs from plant cell because notably plant cells have, as they lack cell walls and chloroplasts, and they have smaller vacuoles. Due to the lack of a rigid cell wall, animal cells can adopt a variety of shapes, and a phagocytic cell can even engulf other structures.

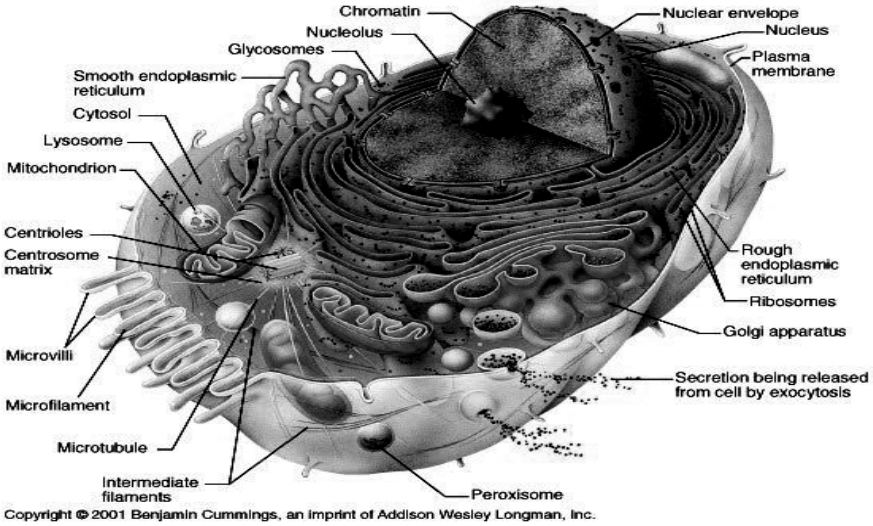

Fig.2.5. : Schematic diagram of animal cell

Cell membrane: Plasma membrane is the thin layer of protein and fat that surrounds the cell, but is inside the cell wall. The cell membrane is semi permeable, allowing selective substances to pass into the cell and blocking others.

Nucleus: They are spherical body containing many

organelles, including the nucleolus. Animal cell nucleus consists of a nuclear membrane, nucleoplasm, nucleolus and chromosomes. The nucleus is surrounded by the nuclear membrane and possesses the nucleolus, which is an organelle within the nucleus - it is where ribosomal RNA is produced.

Golgi apparatus: It is a flattened, layered, sac-like organelle involved in packaging proteins and carbohydrates into membrane-bound vesicles for export from the cell.

Ribosome and Endoplasmic reticulum: Ribosomes are small organelles composed of RNA-rich cytoplasmic granules that are sites of protein synthesis and Endoplasmic reticulum are the sites of protein maturation

Mitochondria: These are spherical to rod-shaped organelles with a double membrane. The inner membrane is infolded many times, forming a series of projections (called cristae). The mitochondrion converts the energy stored in glucose into ATP (adenosine triphosphate) for the cell and called power house.

Lysosome: Lysosomes are cellular organelles that contain the hydrolase enzymes which break down waste materials and cellular debris. They can be described as the stomach of the cell. They are found in animal cells, while in yeast and plants the same roles are performed by lytic vacuoles.

Centrosome: They are small body located near the nucleus and has a dense center and radiating tubules. The centrosomes are the destination where microtubules are made. During mitosis, the centrosome divides and the two parts move to opposite sides of the dividing cell.

Peroxisomes: Peroxisomes are found in animal cells that contain oxidative enzymes, such as D-amino acid oxidase, ureate oxidase, and catalase. Peroxisomes function to rid the body of toxic substances like hydrogen peroxide, or other metabolites.

Vacuoles and vesicles: Vacuoles are single-membrane organelles that are essentiall part of the outside that is located within the cell.

Table.2.1: Basic difference between plant and animals cells.

Characteristics	Plant cell	Animal cell
Cell shape	Have distinct edges, usually square or rectangular in shape.	Is irregular and round in shape.
Cell wall, central vacuole	Present	Absent
Plasma membrane	Present	Present
Endoplasmic reticulum	Present	Present
Nucleus	Present and lies on one side of the cell	Present and lies in the centre of the cell
Golgi apparatus, Cytoplasm, Ribosome,	Present	Present
Plastids	Present	Absent
Lysosomes,	Present but are very rare	Present
Mitochondria	Present, but fewer in number	Are present and are numerous

2.7. Units of measurement

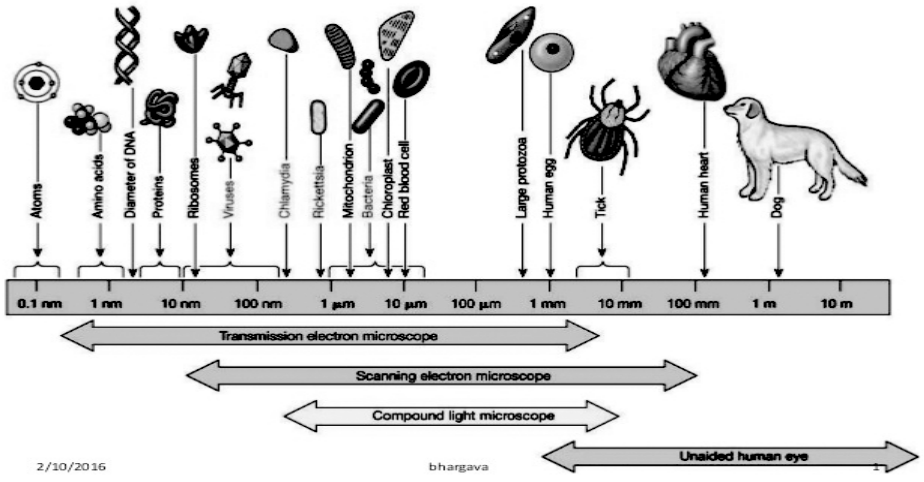

Fig.2.6. : Size of living organism

A unit of measurement is a definite magnitude of a quantity. The value of a quantity is generally expressed as the product of a number and a unit. The unit is simply a particular example of the quantity concerned which is used as a reference, and the number is the ratio of the value of the quantity to the unit. For a particular quantity, many different units may be used. For example, the speed v of a particle may be expressed in the form v = 25 m/s = 90 km/h, where meter per second and kilometer per hour are alternative units for expressing the same value of the quantity speed.

SI units

The French System International d' unit's (the SI system) is the accepted convention for all units of measurement. Table 2.2 lists basic and derived SI units. Table 2.2 lists numerical values for some physical constants in SI units. Table 2.3 lists the commonly used prefixes associated with quantitative terms. Table 2.4 gives the interconversions of non-SI units of volume.

Table: 2.2 SI units – basic and derived units

Quantity	**SI unit**	**SI unit**	**Definition of SI unit**
Basic units			
Length	metre	m	
mass	kilogram	kg	
Time	second	s	
Electric current	ampere	A	
Temperature	kelvin	K	
Luminous intensity	candela	cd	
Amount of substance	mole	mole	
Derived units			
Force	newton	N	$Kgms^{-2}$
Energy, work, heat	jule	J	$kgm^2 s^{-2}$
Power, radiant flux	watt	W	$kgm^2 s^{-3}$
Electric charge, quantity	coulomb	C	As
Electric potential difference	volt	V	$kgm^2 s^{-3}A^{-1}$
Pressure	pascal	Pa	$Kgm^{-1} s^{-2}$

Frequency	hertz	Hz	s^{-1}
Magnetic flux density	tesla	T	$kg\ s^{-2}A^{-1}$
Other units based on SI			
Area	square metre m	m^2	
Volume	cubic metre	m^3	
Density	kilogram per cubic metre	Kgm^{-3}	
Concentration	mole per cubic metre	$molm^{-3}$	

Table : 2.3 Interconversions of non-SI and SI units of volume

Non-SI unit	Non-SI subunit	SI subunit	SI unit
1 litre (l)	10^3 ml	= 1 dm^3	10^{-3} m^3
1 millilitre (ml)	1ml	1 cm^3	10^{-6} m^3
1 microlitre (ml)	10^{-3} ml	1 mm^3	10^{-9} m^3
1 nanolitre (nl)	10^{-6} ml	1 nm^3	10^{-12} m^3

Table : 2.4 Interconversions of mol, mmol and mmol in different volumes to give different concentrations

Molar (M)	Millimolar (mM)	Micromolar (mM)
1 mol dm-^{3}	1 mmol dm^{-3}	1 mmol dm^{-3}
1 mmol cm^{-3}	1 mmol cm^{-3}	1 nmol cm^{-3}
1 mmol mm^{-3}	1 nmol mm^{-3}	1 pmol mm^{-3}

2.8. Light microscope

Light microcopy some time refers to call optical microscope. The optical microscope is consists of lens or combination of lenses. The light microscope is very useful in study of microorganism and biomolecules. The light microscope has lens that tend to bend and focus light rays to produce magnified image of small object. Light microscopy is the simplest form of microscopy. It includes all forms of microscopic methods that use electromagnetic radiation to achieve magnification. The light microscope consist three lens systems such as **Condense lens system;** it occurs in beneath the specimen and it collect and focus the light on object. It is either dry or immersion lens. It magnify 40 times of specimen, **Objective**

lens system: it occurs above the specimen and it produces and magnifies the image of specimen and **Eye pieces lens system**: it occurs near the eye of observer and magnified and form the image (secondary) of the (primary) image previously produce by the objective.

In light microscope the light is transmitted and focus by mirror and condenser. The focused light illuminate the object or specimen and the reflected light is collected by an objective where primary image of object is formed, it may be real, inverted enlarged image of object. The eyepiece further magnifies this primary image into virtual, erect enlarged image, this final image that lies above the stage. Most light microscopes have three or four objectives lenses on a rotating turret. These lenses magnify the image by 4x to 100x. The light then passes up the body tube to an ocular lens that magnifies the image another 10x to 15x. One basis on contrast image, the light microscope may bright field, dark field, phase contrast ad florescence microscope are categories.

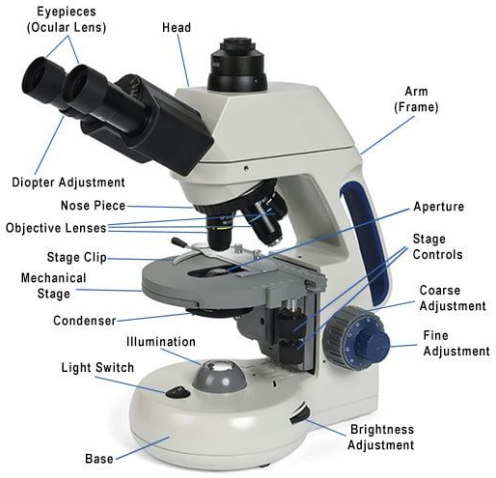

Fig. 5.7: Image of compound microscope

Bright field microscope:	In this type of microscope deflected and undeflected lights are collected by the objectives lens. The bright field microscope is useful in detection of color specimen itself. Thus the bright-field microscopy is used for pigment, tissues, or microbiological section or tissues culture cell that are strain with color dyes. Staining is increased contrast, which prevents use on live cells in many situations. The bright field microscope is very useful in the field of life science. It is due to detect the specimens or live cells.
Phase contrast	Phase contrast microscopy is a form of microscopy used to view unstained cells

microscope:	growing in tissue culture and for testing cells or cell organelles. It is also useful to study of living cells that are proliferate through cell division. The light passing through one material and another material are have slightly different reflective index that cause change in phase of image. This change creates variation in the brightness of structure. The unstained bacteria have constituents of different refractive index that reflect the light. The phase contrast microscope is useful in observing the unstained, colorless specimen, internal structure and cell organelles of the cell.
Dark field microscopy:	The dark field microscope, the dark field illumination produce image to brightly illuminated object on a black background of specimen. The dark field spectroscope used dark field condenser instead of the normal condenser. In dark field microscope, the specimen illuminated by inclined rays and the field of vision is rendered dark by preventing the axial rays with use of circular stopper in the light path. The light that enters the specimen, most is directly transmitted while some is scattered from the sample. These scattered light, along inter the objective lens and produce image. Thus the dark field spectroscope is useful to determine the outline of specimen in liquid media such as living spermatozoa, microorganism etc.
Florescence microscope:	A fluorescence microscope is basically conventional light microscope with added features and components that extend its capabilities. Here the light source comes from above the sample and the objective leans act as both condense and objective lens. Florescence microscopes also produce a magnified image of sample, but the image base on second light source. The image produces by fluorescence microscope of contrast of distinct region of fluorescence (white) and over large region of no fluorescence (black). fluorescence microscope used in determination of imaging structural components of small specimens

2.9. Electron microcopy

Electron microscopes reveal the ultra structure of cell, cell organelles and disease affected cell. There are fundamentally two different types of electron microscope: Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM). In transmission electron microscope, the electron pass through the specimen and produce image while in scanning electron microscope, the electron reflected back from the specimen (secondary electron of same frequency) collected on florescence screen and the image is produced.

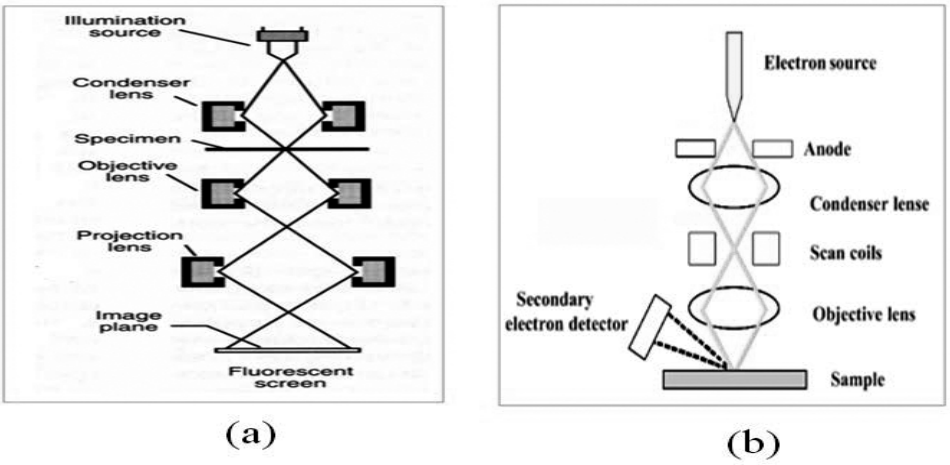

Fig.2.8: Image of (a) Transmission electron microscope (b) scanning electron microscope

Transmission electron microscope (TEM)	
Working Principle:	Transmission electron microscopy (TEM) works on principle of transmission of electron in the specimen to produce image during this process beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen and finally produce image. The electron gun is the source of electron beam, that produce at when high voltage about 400 and 100000 is pass between the anode and cathode . Since electrons cannot pass through a glass lens, magnetic lenses are used to focus the beam. The beam of electron illuminates entire specimen. Some of electrons absorb or scattered by atom of metallic

	strain, because the certain metal are used in specimen for contrast in transmission electron microscope. The object magnifies the image of specimen and the image received by the third magnetic lens which works as projection lens.
Instrumentation:	▪ **Electron Gun and anode:** electron gun is a source of light in the TEM. Electron gun consists of electrically heated tungsten or cathode, when high voltage about 400 and 100000 passes between the anode and cathode and the tungsten filament emit electron. ▪ **Microscope columns:** electron can travel in straight line in vacuum only because in the air collides with oxygen or nitrogen. ▪ **Condense lens:** It is magnetic coil, which focus or condense the electron beam in the plan of object. ▪ **Objectives lens:** it the magnetic coil which produce first magnified image of the object. It also focuses the electron which is reflected by the object and form first image. ▪ **Project lens:** It is also electromagnetic coil which magnify the first image formed by objective lens it produce first image. ▪ **Photographic plates:** Florescence screen is used for observing image of object. Final image can be captured on the photographic film. Such photographs are known as electron micrograph.
Application:	TEM is most commonly used microscope compare to other microscope. It highly useful in observation and detection of internal structure of cell and it has great

	resolving power. TEM offer very powerful magnification and resolution. TEM has a wide range of applications and can be utilized in a variety of different scientific, educational and industrial fields. TEM provide information on element and compound structure. Images are high qualified and detailed.
Scanning microscope (SEM)	
Working principle:	Scanning electron microscopy (SEM) is non-destructive techniques to surface analysis and that use in determination of morphology of solid particles. In specimen the electron does not pass but it focuses on the surface on specimen. The beam is moved rapidly back and forth by beam deflector scan the surface of specimen. When the beam is hits to the surface of specimen it induces the molecules of specimen to higher level. Due to this, the secondary electrons are emitted from metabolic surface. These secondary electrons are collected by the positively charged grid. The signal form the gird is transmitted to the television tube, which scan and form the image on the screen. The resolving power of SEM is found less than TEM and it has maximum magnification upto 20,000 times.
Instrumentation:	▪ **Electron gun:** Tungsten electron gum generally used in SEM to produce electron beam. The election beam used as source of energy energy ranging from 0.2 keV to 40 keV. It is focused by one or two condenser lenses to a spot about 0.4 nm to 5 nm in diameter. ▪ **Electron Lenses:** SEM generally used magnetic lens, which focus or condense the electron beam in

	the plan of object. SEM uses the electron lens to magnify and demagnify the electron beam. ▪ **Sample Stage:** the sample stage for the SEM can perform the following movements like horizontal movement(X, Y), vertical movement (X,Y), specimen tilting (T) and rotation (R). The X and Y movement are use for the selection of a field of view. ▪ **Detectors for all signals of interest:** The detector is used for the detection of primary and secondary detector signals emitted from the specimen. ▪ **Display/Data output devices:** The output signal of secondary electron detector is amplified and then transfer to the display unit.
Applications:	The very precise measurement of very small material about 50 nm in size is obtained through SEM. The high resolution image is produced by scanning of electron on the surface of specimen. It gives various information to recognize chemical composition of materials such as a cquiring elemental maps or spot chemical analyses using EDS and discrimination of phases based on mean atomic number using BSE.

2.1. Centrifugation for sub cellular fractionation

In biochemistry analysis, the individual cell organelles would be separated by using various techniques keeping their individual functionalities. Thus the cell organelles can be separated into its living constituent, so the cell can be separated into its functioning organelles and macromolecules. Cell fractionation is the process used to separate cellular components while preserving individual functions of each component. The various chemical processes are performed using these sub cellular functions. The cellular fraction also used in the separation of protein for further purification (Fig. 2.9). Cells can be broken up in various ways: they can be subjected to osmotic shock or ultrasonic vibration, forced

through a small orifice, or ground up in a blender. After broken the sample the disruption procedures leave organelles such as nuclei, mitochondria, the Golgi apparatus, lysosomes, and peroxisomes etc.

Centrifugation is the first step in most fractionations, but it separates only components that differ greatly in size while the ultracentrifuge is also used to separate cellular components on the basis of their buoyant density, independently of their size and shape. Generally the obtained fractions are impure, but many of the contaminants can be removed by resuspending the pellet and repeating the centrifugation procedure several times. A finer degree of separation can be achieved by layering the homogenate in a thin band on top of a dilute salt solution that fills a centrifuge tube. When centrifuged, the various components in the mixture move as a series of distinct bands through the salt solution, each at a different rate, in a process called velocity sedimentation.

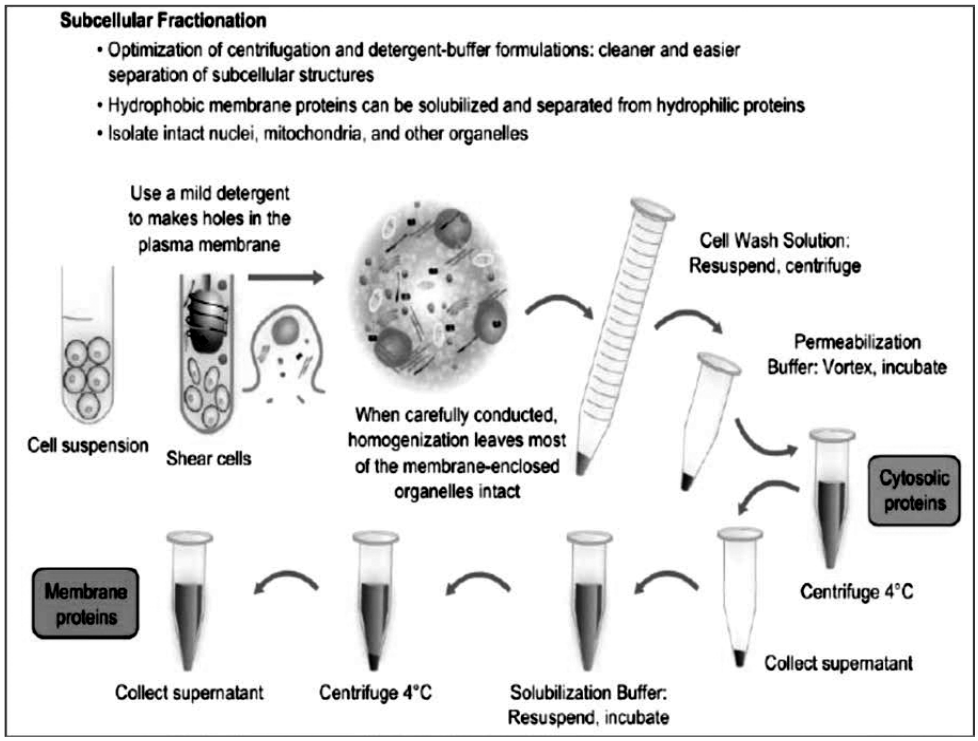

Fig.2.9: Steps involved in membrane protein extraction.

All centrifugations should be done at 4°C. Samples should be kept on ice throughout the procedure.

- Transfer cells from 10 cm plates into 500 µL fractionation buffer, eg by scraping. Incubate 15 min on ice.
- Using 1 mL syringe pass cell suspension through a 27 gauge needle 10 times (or until all cells are lysed).
- Leave on ice for 20 min.

- Centrifuge sample at 720 xg (3,000 rpm) for 5 min. The pellet will contain nuclei and the supernatant will contain cytoplasm, membrane and mitochondria.
- Transfer supernatant into a fresh tube and keep on ice. This will be dealt with in Steps 8–11.
- Wash nuclear pellet from Step 4 with 500 μL fractionation buffer. Disperse the pellet with a pipette and pass through a 25 gauge needle 10 times. Centrifuge again at 3,000 rpm for 10 min. Discard the supernatant and keep the pellet that contains nuclei.
- Resuspend the pellet from Step 6 in TBS with 0.1% SDS. Sonicate the suspension briefly to shear genomic DNA and homogenize the lysate (3 s on ice at a power setting of 2-continuous).
- Centrifuge the supernatant recovered in Step 5 at 8,000 rpm (10,000 x g) for 5 min. The pellet contains mitochondria. Transfer the supernatant into a fresh tube and keep on ice: this is the cytoplasm and membrane fraction.
- Process the mitochondrial pellet from Step 8, as described for the nuclear pellet in Step 7, to obtain mitochondrial lysate in TBS/0.1% SDS.
- For a membrane fraction, centrifuge the supernatant from Step 8 in an ultracentrifuge at 40,000 rpm (100,000 x g) for 1 h. Wash pellet by adding 400 μL of fractionation buffer. Resuspend by pipetting and pass through a 25 gauge needle. Re-centrifuge for 45 min. resuspend the membrane pellet in the same buffer as used for the nuclei.
- Optional: concentrate the supernatant by centrifuging through the filter unit. This concentrates the cytosol down to approximately 50–75 μL.

2.10. Summary

In this unit you have learn that-

The living organism is composed of single cell or multiple cells. The non living rigid structure is cells wall that gives shape to the cell and protects the cell from mechanical damage and infection, it also helps in cell-to-cell interaction and provides barrier to undesirable macromolecules. In this study you learned about the cell membrane that is composed of lipids and in a bilayer. Cell membrane is selectively permeable to some molecules present on either side of it. It provides passage for many molecules without any requirement of energy and this is called the passive transport. It also provides passage for few ions

according to their concentration gradient. Electron microscopes reveal the ultra structure of cell, cell organelles and disease affected cell. There are fundamentally two different types of electron microscope- Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM). This unit is simply a particular example of the quantity concerned which is used as a reference, and the number is the ratio of the value of the quantity to the unit. Cell fractionation is the process used to separate cellular components while preserving individual functions of each component. The various chemical processes are performed using these sub cellular functions.

2.11. Terminal question

Q.1. What is cell? write about prokaryotic and eukaryotic cells

Answer:----------

Q.2. What is the role of cell in living body? Discuss the structure of plant cell.

Answer:----------

Q.3. Discuss about animal cell? How it differ from plant cell.

Answer:----------

Q.4. What is microscope? How it useful in cell biology?

Answer: ----------

Q.5. What is transmission electron microscope? discuss it principle and functions.

Answer:----------

Q.6. Write short notes on

a) Elecromagnnnetic radiation

b) Phase contrast microscope

c) Scanning electron microscope

d) Sub cellular fractionation

Answer:----------

2.12. Further readings

1. Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Publishers and Distributors, Delhi.

2. Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company, seven editim.

3. Cell Biology (Cytology, Biomolecules and Molecular Biology): P S Verma and V K Agarwal.

4. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

UNIT-3

Cell organelles

Structure

3.1. Introduction

This unit covers structure and functions of cell with their organelles. We knew that the living organism is complicated and highly organized. The cells of which they composed and possessing intricate internal structures containing many kinds of organelles and complex molecules. In this unit we would study in details about some cell organelles which play important role in metabolic process in living organism. The cells organelles have specific purpose and function. The role of organelles is highly specific in the metabolic process and plays important role in energy production, transfer and synthesis of different kinds of metabolites. The cell organelles such as cell nucleolus, Endoplasmic reticulum, Golgi complex, Lysosome, mitochondria, chloroplasts and peroxisomes discuss briefly.

Objectives

- To understand the role of different cell organelles.
- To study the basic structure and function of cell organelles
- Describes the different part of cell organelles and their role in the metabolic or growth process.

3.2. Cell organelle overview

Cell organelle is a tiny cellular structure embedded in both prokaryotic and eukaryotic cells. In the more complex eukaryotic cells, the organelles are often enclosed by their own membrane. Analogous to the body's internal organs, organelles are specialized and perform valuable functions necessary for normal cellular operation. Organelles have a wide range of responsibilities that include everything from generating energy for a cell to controlling the cell's growth and reproduction. The name organelle comes from the idea that these structures are to cells what an organ is to the body. There are many types of organelles in eukaryotic cells. Prokaryotes were once thought not to have double membrane bound cell organelles. Single membrane bound organelles are found in prokaryotes organelles are not organized like eukaryote and they are also called the micro compartments. The eukaryotic cells contain number of cell organelles includes; mitochondria, nucleus, ribosome, vesicle, endoplasmic reticulum, golgi apparatus, cytoskeleton, vacuole, lysosome, centriole etc. These cell organelles are organized and bounded with plasma membrane.

3.3. Cell nucleus

Nucleus is prominent organelles as compare to other component of the cells which account for about 10% of cell volume. First of all, Leeuwenhoek observed nucleus in Red Blood Cells (RBCs) of fish. Nucleus play important role in controlling and regulating the activities of the cells such as growth and metabolism and carriers the genes. Nucleoli are the small bodies that found within the nucleus acts for the synthesis of ribonucleic acid (RNA) and protein. The study of nucleus is known as *Karyology*.

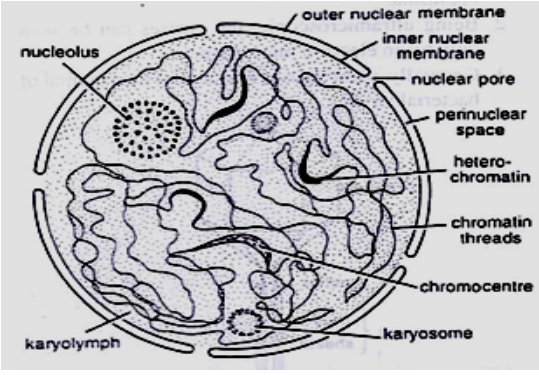

Fig.3.1. Structure of nucleus

Structure	The cell nucleus consists of a nuclear membrane, nucleoplasm, nucleolus and chromosomes. Let's discuss in brief about the several parts of a cell nucleus. **Nuclear membrane:** Nucleus is surrounded by two unit membranes, thus nucleus is double membrane structure. The space between two layers of membranes is known as perinuclear space. Outer membrane of nucleus is connected to the endoplasmic reticulum at several places and ribosome also may found on it. The nucleus communicates with the remaining of the cell or the cytoplasm through several openings called nuclear pores (300-1000A in diameter). Each nuclear pore is guarded by an octagonal discoid structure of nucleoplasm protein and structure is called as annulus (Annulus +pore=Nuclear pore complex). The inner side of inner nuclear membrane is called nuclear lamina. This structure is formed by filaments of lamina protein. **Nucleoplasm:** Nucleoplasm is the gelatinous substance within the nuclear envelope. It is a ground substances of nucleus which is a complex colloidal formed of a number of molecules like nucleotides , nucleosides, ATPs, proteins and enzymes (RNA and DNA polymerases; and nucleases and minerals), (Ca^{++},Mg^{++}) etc. **Nucleolus:** The nucleolus (plural nucleoli) is a dense, spherical-shape structure present inside the nucleus. Some of the eukaryotic organisms have nucleus that contains up to four nucleoli. The nucleolus plays an indirect role in protein synthesis by producing ribosomes. These ribosomes are cell organelles made up of RNA and proteins; They are transported to the cytoplasm, which are then attached to the endoplasmic reticulum. **Chromosomes:** Chromosomes are present in the form of strings of DNA and histones (protein molecules) called *chromatin*. The chromatin is further classified into heterochromatin and euchromatin based on this functions. The former type is a highly condensed, transcriptionally inactive form, mostly present adjacent to the nuclear membrane. On the other hand, euchromatin is a delicate, active form, less condensed organization of chromatin, which is found abundantly in a transcribing cell.
Functions	It controls the hereditary characteristics of an organism. This organelle is also responsible for the protein synthesis, cell division, growth and differentiation. Storage of hereditary material, the genes in the form of long

	and thin DNA (deoxyribonucleic acid) strands, referred to as chromatin. Storage of proteins and RNA (ribonucleic acid) in the nucleolus. Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for protein synthesis.

3.4. Endoplasmic Reticulum (ER)

The ER is most important organelle in eukaryotic cells**,** it is a network of membranes found throughout the cell and connected to the nucleus. ER was first observed by Garnier (1897) and their name of proposed by porter (1961). The credit for discovery of ER goes to Porter. ER produces transmembrane proteins and lipids for its membrane and for many other cell components. ER contains number of components which are shown in Fig. 3.2 as discussed below;

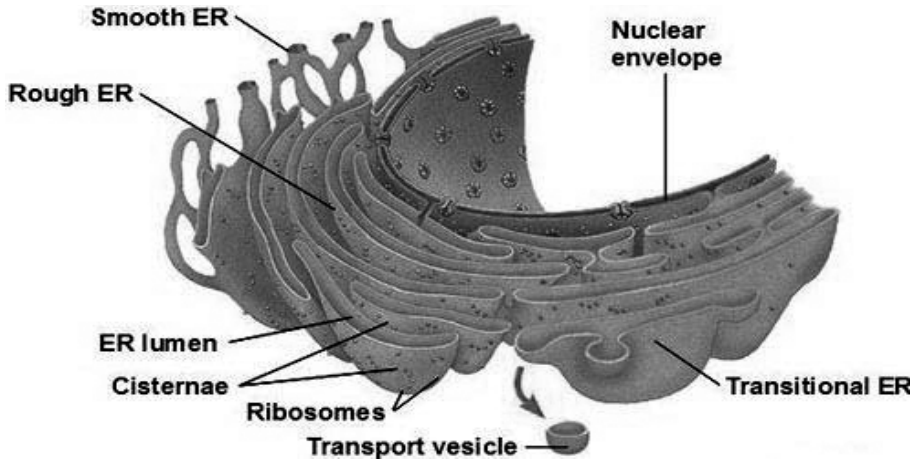

Fig.3.2. Diagram shows the different components of Endoplasmic reticulum

Source: http://botanystudies.com/endoplasmic-reticulum/

Cisternae – These are long flattened and unbranched units arranged in stacks.

Vesicles – These are oval membrane bounded structures.

Tubules – These are irregular, often branched tubes, bounded by membrane. Tubules may free or associated with cisternae.

• **Structure:**	ER has two major regions: smooth endoplasmic reticulum and rough endoplasmic reticulum. Rough ER contains attached ribosomes while smooth ER does not. The double membranes of smooth and rough ER form

	sacs called cisternae. Cisternae form a three-dimensional polygonal network. Rough ER on the other hand, is membrane-enclosed, two-dimensional flattened sacs that extend across the cytoplasm. The surface of the rough endoplasmic reticulum is studded with the protein manufacturing ribosome, which gives it a rough appearance. Hence it is referred as a rough endoplasmic reticulum. Rough ER is very important for the synthesis and packaging of proteins.
• **Functions:**	➢ Endoplasmic reticulum is mainly responsible for the transportation of proteins and other carbohydrates ➢ ER provides the increased surface area for cellular reactions such as formation of nuclear membrane during cell division. ➢ E.R. plays a vital role in the synthesis of proteins, lipids, glycogen and other steroids like cholesterol, progesterone, testosterone, etc. ➢ E.R. forms intracellular conduction system, transport of materials in cytoplasm from one place to another may occurs through the E.R. ➢ Rough ER provides site for the protein synthesis, ➢ Smooth ER concerned with detoxification of drugs, pollutants and steroids.

3.5. Golgi complex

It is one of organelle of eukaryotic cells which was discovered by C. Golgi (1898) in the nerve cells of owl. The Golgi complex also known as several names such as Dolton complex, Golgi complex, Baker's body, Dictyosome (plant Golgi body) etc. Golgi bodies are pleomorphic structures because component of Golgi body are differ in structure & shape in different cells. The Golgi body is made up of a series of flattered, stacked pounces called cisternae. The Golgi apparatus has three primary compartments, known generally as "cis" (cisternae nearest the endoplasmic reticulum), "medial" (central layers of cisternae), and "trans" (cisternae farthest from the endoplasmic reticulum).The proteins and lipids received at the cis face arrive in clusters of fused vesicles.

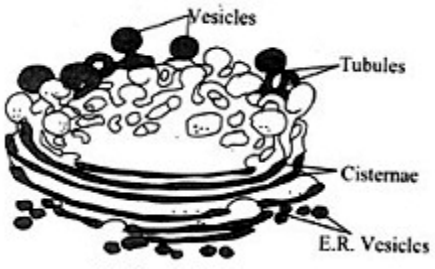

Fig.3.3: Golgi apparatus in section

• **Structure:**	It contains cisternae, tubules, vacuoles and vesicles in their structure. The cisternae are arranged in a stack and are unbranched saccules like smooth endoplasmic reticulum. Convex surface of Cisternae which is toward the nucleus is called cis-face or forming face. Other component of golgi body tubules are branched and irregular tubs like structure associated with cisternae. Vacuoles which are large spherical structures it also associated to the tubules. Whereas vesicles are spherical structure arise by budding from tubules. Golgi body is single membrane bound cell organelle Fig(3.3). About 60% proteins and 40% phospholipids occur in Golgi body.
• **Functions:**	➢ The chief function of Golgi body is secretion of macromolecules. ➢ Golgi complex involves in secretion of zymogen granules from pancreas and lactoprotein from mammany glands. ➢ Products from the Golgi apparatus goes to three main destinations: (1) inside the cell to lysosomes (2) on the plasma membrane (3) outside the cell. It receives biochemicals in a bulk flow from the rough endoplasmic reticulum.

3.6. Lysosome

The Lysosome found in all types of eukaryotic cell and responsible of digestion. The lysozyme was discovered by Christian De Duve (1955). Lysosome generally found in the cytoplasm of animal cell and exists in polymorphism. Each lysosome is surrounded by a membrane that maintained an acidic environment within the interior via a proton pump. In plant cells large central vacuole functions as lysosome. So in higher plant lysosome is less frequent but number of lysosome is high in fungi.

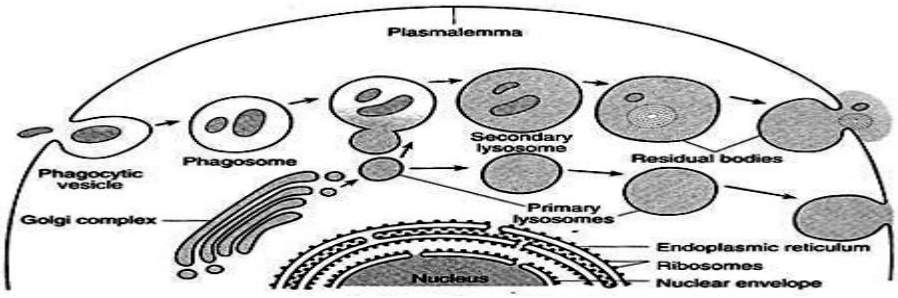

Fig.3.4: Diagram showing the origin and different phase of Lysosome

• **Structure:**	Lysozyme is spherical bag like structures (0.1-0.8υm) which is covered by single unite membrane. They are large in Phagocytes (WBC) (0.8 to2υm). Lysozyme are filled with 50 different type of digestive enzymes termed as acid hydrolyses. These acid hydrolyses function in acidic medium (pH=5). Membrane of lysosome has an active H^+ lumen pumps of lysosome. Lysosome is highly polymorphic cell organelle (Fig.3.4). The Lysozymes are basically categories in four types.
• **Functions:**	➢ It functions as the digestive system of the cell, serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself. ➢ it acts as autophagy in which it digested old or dead cell organelles. Autophagy takes place during starvation of cell.

3.7. Mitochondria

Mitochondria is called "powerhouse of the cell" because it produce about 90% of chemical energy that cell need to survive or used in metabolic reactions. The term mitochondrion was given by C. Bendra and F. Meves in year 1904. They first observed mitochondria in plants *Nymphea*. The mitochondrion is present in large numbers i.e.1000-1600 per cell. The size of mitochondria is often between 0.75 and 3 micrometers and is not visible under the microscope unless they stained. Mitochondria are split into different compartments or regions, each of which carries out distinct roles. Usually plant cells have fewer mitochondria as compared to animal cell. In higher animals maximimum mitochondria are found in flight muscles of birds. Mitochondria can make its shape as ellipsoidal, oval, spherical or spiral.

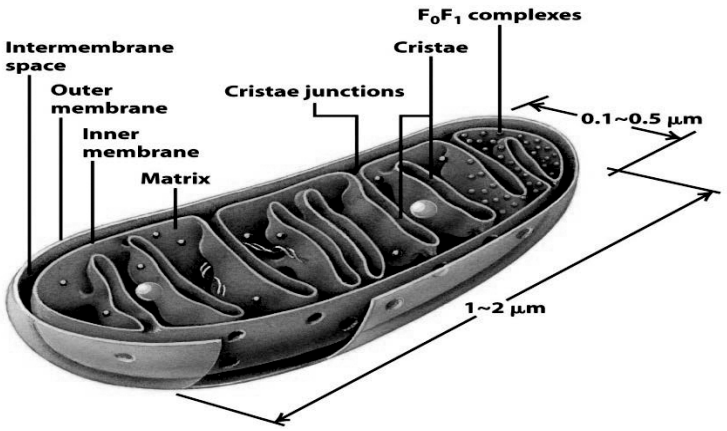

Fig.3.5: structure of mitochondria

Source: Molecular Cell Biology, Sixth Edition@ 2008; W. H.Freeman and Company

• **Structure:**	Mitochondria have two membranes, a one outer and an inner one. Each membrane has different function. The basic difference in cells structure of mitochondria is due to present of more phospholipids and cholesterol in outer membrane as compared to inner membrane. Each membrane of 60-75 A˚ thick along with mitochondrial spaces. Some finger likes structure is found in mitochondria is known as cristae shown in Fig.3.5. The cristae are the folds of the inner membrane. The inner membranes of mitochondria have cytochromes which act as carrier for electron transfer. Mitochondria matrix have enzyme for kreb's cycle.
• **Functions:**	➢ Mitochondria produce energy for oxidative metabolism, where organic compounds are broken down to release & store metabolic energy in the form of ATP molecules. ➢ The main job of mitochondria is to perform cellular respiration. This means it takes in nutrients from the cell, breaks it down, and turns it into energy. This energy is then in turn used by the cell to carry out various functions. ➢ Mitochondria help in vitelloenesis in oocytes. ➢ Mitochondrial kinase makes the yolks viscous and insoluble longer duration storage.

3.8. Chloroplasts:

Chloroplasts are the most known plastids which are responsible for photosynthesis. These are covered with thylakoids where the process

of photosynthesis occurs. Chloroplasts are typically found in the fleshy fruits, flowers as well as various other pigmented parts of the plant such as leaves. These are plastids which contain different types of pigment (carotenes, Xanthophylls etc.), chloroplast either absent or occur in very less amount.

Structure	Chloroplasts can be found in the cells of the mesophyll in plant leaves. There are usually 30-40 per enlonoplasts mesophyll cell. The chloroplast has an inner and outer membrane with an empty intermediate space in between. Inside the chloroplast are stacks of thylakoids, called grana, as well as stroma, the dense fluid inside of the chloroplast. These thylakoids contain the chlorophyll that is necessary for the plant to go through photosynthesis. The space which the chlorophyll fills is called the thylakoid space.
Functions	➢ They provide colour to fruits and flowers. ➢ They help in storage of proteins, starch and oil. ➢ They trap solar energy to manufacture food through the process of photosynthesis. ➢ They help in maintaining balance between carbon dioxide and oxygen during photosynthesis. Chloroplasts are the centres of synthesis and metabolism of carbohydrates. ➢ During the dark reaction, glucose is synthesized through Calvin cycle in stroma.

3.9. Ribosome

Ribosomes are the granular structures first observed under the electron microscope as dense particles by George Palade (1953). They are composed of ribonucleic acid (RNA) and proteins and are not surrounded by any membrane. The eukaryotic ribosomes are 80S while the prokaryotic ribosomes are 70S. Ribosomes are located inside the animal, human cell, and plant cells. They are situated in the cytosol, some bound and free-floating to the membrane of the coarse endoplasmic reticulum.

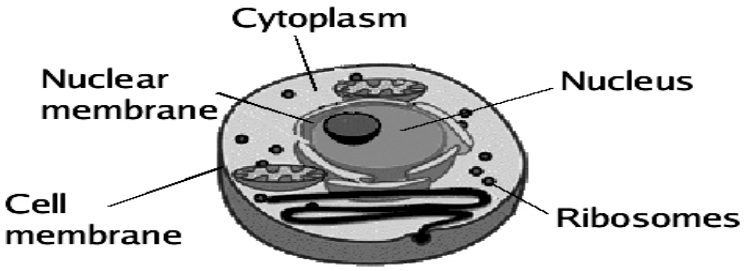

Fig. 3.6: Structure of ribosomes in cell

Structure:	Ribosomes are made of proteins and ribonucleic acid found in almost equal amounts. It comprises of two parts, known as subunits. The smaller subunit is the place where the mRNA binds and it decodes, whereas the bigger subunit is the place the amino acids are included. Ribosomes found in cytoplasm and also connected to the endoplasmic reticulum. So that it called the rough endoplasmic reticulum. Around 37 to 62% of ribosome is comprised of RNA and the rest is proteins.

3.10. Peroxisomes

Peroxisomes are cell organelles found in eukaryotic cells. The peroxisome derives its name from the fact that many metabolic enzymes that generate hydrogen peroxide as a by-product are sequestered here because peroxides are toxic to cells. Peroxisomes are bound by a single membrane that separates their contents from the cytosol and contain membrane proteins critical for various functions, such as importing proteins into the organelles and aiding in proliferation.

Structure	Peroxisomes are created by taking in proteins and lipids from the cytoplasm of the cell. The influx of proteins and lipids makes the peroxisome grow in size. Once the peroxisome is large enough, it divides through fission to create two daughter peroxisomes. Peroxisomes are created in this manner because they don't have their own DNA to give instructions on making the proteins that they need to function.
Functions	➢ The principal function of peroxisome is to house many metabolic pathways that are involved in various aspects of lipid metabolism. ➢ The enzymes involved in the derivative oxidation. ➢ It involve the early steps in the synthesis of ether glycerolipids or plasmalogens; ➢ It involve in formation of bile acids, dolichol, and cholesterol.

	➢ It involves in catabolism of purines, polyamines, and amino acids, and the detoxification of reactive oxygen species such as hydrogen peroxide, superoxide anions.

3.11. Summary

After study this unit you have learn that-

The cell consists of cell organelles which are the structural and functional part of cells. These cell organelles have specific purpose and function. The roles of organelles are highly specific in the metabolic process and plays important role in energy production, transfer and synthesis of different kinds of metabolites and glucose.

- The cell nucleus consists of a nuclear membrane, nucleoplasm, nucleolus and chromosomes. Its control and regulates the activities of the cells such as growth and metabolism and carriers the genes. The study of nucleus is known as Karyology.
- Endoplasmic reticulum is the important part of cell responsible for the transportation of proteins and other carbohydrates. Endoplasmic reticulum has two major types: smooth endoplasmic reticulum and rough endoplasmic reticulum. Rough ER has attached ribosomes while smooth ER does not.
- Golgi bodies are pleomorphic structures because component of Golgi body are differ in structure & shape in different cells is secretion of macromolecules.
- Mitochondria is called “powerhouse of the cell” because it produce about 90% of chemical in form ATP energy, that cell need to survive or used in metabolic reactions. Mitochondrial kinase makes the yolks viscous and insoluble for longer duration storage.
- Chloroplasts are responsible for photosynthesis. These has number of thylakoids where the process of light phase of photosynthesis occurs. Chloroplasts are typically found in the fleshy fruits, flowers as well as various other pigmented parts of the plant such as leaves
- Ribosomes are composed of ribonucleic acid (RNA) and proteins. The eukaryotic ribosomes are 80S while the prokaryotic ribosomes are 70S.
- Peroxisomes are bound by a single membrane that separates their contents from the cytosol and contain membrane proteins critical for various functions.

3.12. Terminal questions

Q.1. Define the roll of cell organelles in cell briefly.

Answer:----------

Q.2. What is Endoplasmic reticulum? Discuss the structure and function of ER.

Answer:----------

Q.3. How you can say that the mitochondria are power house of the cell?

Answer:----------

Q.4. Discuss the structure and function of mitochondria.

Answer:----------

Q.5. What is Golgi body's complex? discuss its functions?

Answer:----------

Q.6. Write short notes on

(e) Ribosomes

(f) Lysosome

(g) Peroxisomes

(h) Nucleoplasm

Answer:----------

3.13. Further readings

1. Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Publishers and Distributors, Delhi.

2. Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company, seven editim.

3. Cell Biology (Cytology, Biomolecules and Molecular Biology): P S Verma and V K Agarwal

4. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

5. Wikipedia and internet sources

Uttar Pradesh Rajarshi Tandon
Open University

Bachelor in Science

UGBCH-101

Introduction
To
Biochemistry

Block

2

Amino acids, Proteins and carbohydrates

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Introduction

This is the second block on Amino acids, proteins and carbohydrates. It consists of the following three units:

Unit-4: In this unit we know about the amino acids and its functions, understand the structure and physical and chemical properties of amino acid. We also study the different classifications (based on structure, chemical nature, nutritional requirement and metabolic fate) of amino acid.

Unit-5: It deals about characteristics of proteins, understand the different structures (primary, secondary, tertiary and quaternary) of proteins, to know about different classifications (based on function, chemical nature, solubility and nutritional importance) of proteins. In this unit classification of peptides and its conformational structure have also been discussed.

Unit-6: In this unit we discussed about characteristics of carbohydrates and their classifications. We can also understand about structures of monosaccharides, disaccharides and polysaccharides. Study about the ring structure of sugars. We can also discuss about structure of aldose and ketoses.

UNIT-4

Amino Acids

Structure

4.1. Introduction

The amino acid is regarded as building blocks of protein. It is a nitrogenous compound having both an acidic carboxyl (−COOH) and a basic amino group ($-NH_2$). They are classifying in different ways based on the structure, polarity, nutritional importance and their metabolic fate. The amino acids are termed as α-amino acids, if both the carboxyl and amino groups are attached to the same carbon atom. They mostly exist in the ionized form in the biological system. The α-carbon atom binds to a side chain represented by R which is different for each of the 20 amino acids found in proteins. lf a carbon atom is attached to four different groups, it is asymmetric and therefore exhibits optical isomerism. The amino acid exist in two active forms: those having $-NH_2$ group to the right are designated as D-forms while those having $-NH_2$ group to the left are designated as L-forms.

Objectives :

After going through the course of this unit student will be able to:

- Understand the structure and functions of amino acids
- Know about the types of amino acids and its importance.

- Understand the different types of classification of amino acids.
- To know about the chemical and physical properties of amino acids.

4.2. What are amino acids?

Amino acids are a group of organic compounds containing two functional groups amino ($-NH_2$) and carboxyl group ($-COOH$) along with a side chain (R group) specific to each amino acid. The key elements of an amino acid are carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), although other elements are found in the side chains of certain amino acids. The amino acids are essential components of peptides and proteins. 20 amino acids known as standard are repeatedly found in the structure of proteins. Besides the 20 standard amino acid there are several non standard amino acids which are biologically important.

4.3. Classification of amino acids

There are different ways of classifying amino acids:

A. Based on the structure of amino acid:

Each amino acid is assigned a three letter or one letter symbol which are used to represent the amino acids in protein structure.

i. Amino acids with aliphatic side chains :

These are monoamino monocarboxylic acids. This group consists of the most simple amino acids- Glycine (Gly), Alanine (Ala), Valine (Val), Leucine (Leu) and Isoleucine (Ile). The last three amino acids (Leu, lle, Val) contain branched aliphatic side chains, hence thev are referred to as branched chain amino acids.

ii. Hydroxyl group containing amino acids :

Serine, threonine and tyrosine are hydroxyl group containing amino acids. Tyrosine-being aromatic in nature.

iii. Sulfur containing amino acids : Cysteine with sulfhydryl group and methionine with thioether group are the two amino acids incorporated during the course of protein synthesis.

iv. Acidic amino acids and their amides :

Aspartic acid and glutamic acids are dicarboxylic monoamino acids while asparagine and glutamine are their respective amide derivatives. All these four amino acids possess distinct codons for their incorporation into proteins.

v. Basic amino acids :

The three amino acids lysine, arginine (with guanidino group) and histidine (with imidazole ring) are dibasic monocarboxylic acids. They are highly basic in character.

vi. Aromatic amino acids :

Phenylalanine, tyrosine and tryptophan (with indole ring) are aromatic amino acids. Besides these, histidine may also be considered under this category.

vii. lmino acids: Proline containing pyrrolidine ring is a unique amino acid. lt has an imino group (=NH), instead of an amino group (−NH2) found in other amino acids. Therefore proline is an α-imino acid.

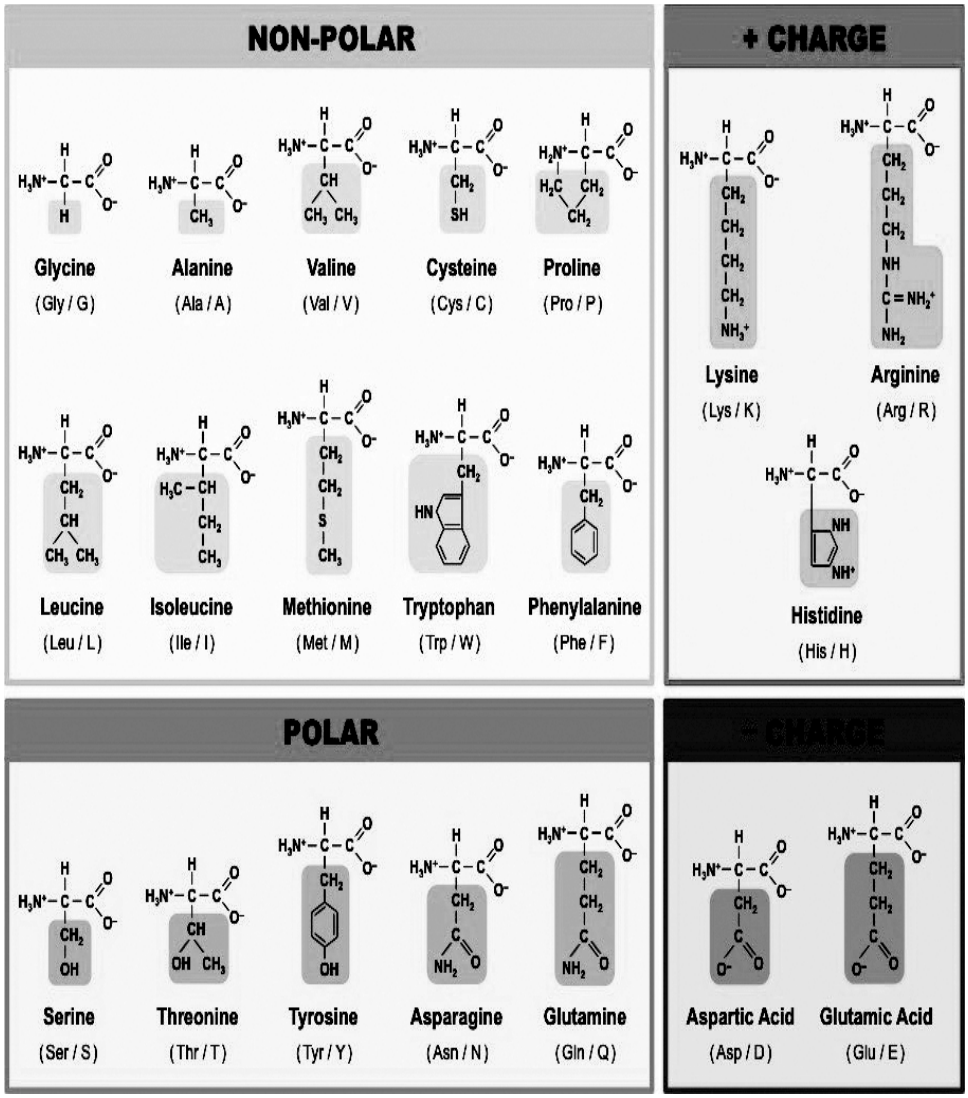

Fig. 4.1: Twenty Amino acids

B. Classification Based on polarity of amino acids:

The polarity in turn reflects the functional role of amino acids in protein structure. They are divided into on 4 groups

i. Non-polar amino acids with no charge on 'R' group:

These amino acids are also known as hydrophobic. They have no charge on the 'R' group. The amino acids included in this group are − alanine, leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and proline.

ii. Polar amino acids with no charge on 'R' group:

They have no charge on the 'R' group. They have groups such as hydroxyl, sulfhydryl and amide. They participate in hydrogen bonding of protein structure. The amino acids in this group are- glycine, serine, threonine, cysteine, glutamine, asparagines and tyrosine.

iii. Polar amino acids with positive 'R' group:

Lysine, arginine and histidine are included in this group.

iv. Polar amino acids with negative'R' group:

The dicarboxylic monoamino acids - aspartic acid and glutamic acid are included in this group.

v. Nutritional classification of amino acids:

Based on the nutritional requirements divided into two classes:

a) Essential or indispensable amino acids:

They cannot be synthesized by the body and therefore need to be supplied through the diet are called essential amino acids. They are required for proper growth and maintenance of the individual.

The ten essential amino acids are – Arginine, Valine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine and Threonine. Among all these two amino acids Arginine and Histidine are considered as semi-essential.

b) Non-essential or dispensable amino acids:

They can synthesize by body to meet the biological needs, hence they need not be consumed in the diet. These are – glycine, alanine,serine, cysteine, aspartate, asparagines, glutamate, glutamine, tyrosine and proline.

C. Based on metabolic fate of amino acid:

Divided into three groups –

i. **Glycogenic amino acids:** These can serve as precursors for the formation of glucose or glycogen. Eg. alanine, aspartate, glycine, methionine etc.

ii. **Ketogenic amino acids:** Fat can be synthesized from these amino acids such as lysine and leucine.

iii. **Glycogenic and Ketogenic amino acids:** The four amino acids isoleucine, phenylalanine, tryptophan, tyrosine are precursors for synthesis of glucose as well as fat.

4.4. Structure of amino acid

Amino acids are containing two functional groups amino and carboxyl. The amino group ($-NH_2$) is basic while the carboxyl group ($-COOH$) is acidic in nature. The amino acids are termed as α-amino acids, if both the carboxyl and amino groups are attached to the same carbon atom. The amino acids mostly exist in the ionized form in the biological system. The α-carbon atom binds to a side chain represented by R which is different for each of the 20 amino acids found in proteins.

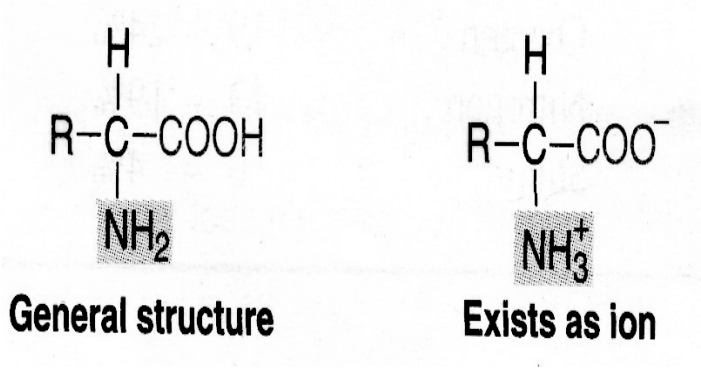

Fig.4.2: conjugated structure of amino acid

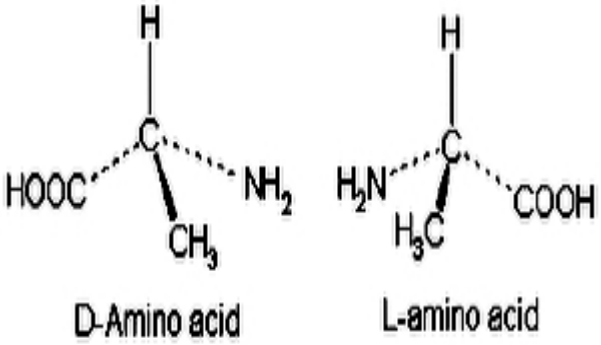

Fig: 4.3: Optical isomers of amino acids

If a carbon atom is attached to four different groups, it is asymmetric and therefore exhibits optical isomerism. The amino acids (except glycine) possess four distinct groups (R, H,COO^-, NH_3^+) held by α-carbon. Thus all the amino acids (except glycine where R = H) have optical isomers. The amino acid exist in two active forms: those having $-NH_2$ group to the right are designated as D-forms while those having $-NH_2$ group to the left are designated as L-forms. The proteins are composed of L-α-amino acids.

4.5. Functions of amino acid

- **Precursors for protein synthesis:** Of more than 10.000 protein molecules as enzymes, hormones, serum, albumin, immuno-globulins, clotting factors, etc.
- **Act as Buffers:** Since they are amphoteric molecules. Because of weak positively charged amino group & weak negatively charged carboxylic group.
- **In Detoxification of drugs & metabolic by-products :** For e.g. Benzoic acid (toxic) + glycine → Hippuric acid (non-toxic) water soluble → excreted in urine .
- **In Neurotransmitters:** Some amino acid derivatives are used as direct neurotransmitters : For e.g.-
- Tyrosine → Dopamine, Epinephrine, NE.
- Tryptophan → Serotonin , Melatonin
- Precursors for some biologically active oligopeptides: For e.g. oxytocin, vasopressin & GSH and also for biological molecules like: Histamine, NAD^+ etc.

4.6. Basic properties of amino acids

A. Physical Properties

i. Solubility:

Amino acids are soluble in water and insoluble in organic solvents.

ii. Melting points:

They Melt at higher temperatures, often above 200 °C.

iii. Taste:

iv. They may be Sweet (Glycine, alanine, valine), tasteless (Leucine) or bitter (Arginine, Isoleucine). Monosodium glutamate (MSG) is a salt of glutamic acid which employed as a flavoring agent in food industry to increase taste and flavor.

v. Optical properties:

All amino acids except glycine have optical isomers due to the presence of asymmetric carbon atom. Isoleucine and threonine also have a second asymmetric carbon.

vi. Amino acids as ampholytes:

Amino acids contain both acidic (−COOH) and basic ($-NH_2$) groups. They can donate a proton or accept protons hence are regarded as ampholytes. Zwitterion or dipolar ion: Zwitter ion is a hybrid molecule containing positive and negative ionic groups. Each amino acid has a characteristic pH (eg. Leucine, pH 6.0) at which it carries both positive and negative charges and exists as zwitterions.

B. Chemical properties:

Reaction due to presence of –COOH group:

i. Formation of salts: They form salts (−COONa) with bases and esters (−COOR') with alcohols.

ii. Decarboxylation: Amino acids undergo decarboxylation to produce corresponding amines. This reaction significant in the formation of many biologically important amines in the living cells.

iii. Reaction with ammonia: The carboxyl group of dicarboxylic amino acids reacts with NH_3 to form amide-

iv. Aspartic acid + $NH_3 \rightarrow$ Asparagine

v. Reaction due to presence of –NH_2 group:

vi. Formation of salts: They behave as bases and combine with acids to form salts (NH_3Cl).

vii. Reaction with ninhydrin: The α-amino acids react with ninhydrin to form a purple, blue or pink colour complex (Ruhemann's purple).

viii. Colour reactions of amino acids: Amino acids can be identified by specific colour reactions.

ix. For e.g. Biuret reaction for two peptide linkages; Ninhydrin reaction for α-amino acids and Xanthoproteic reaction for Benzene ring of aromatic amino acids.

x. Transamination: an amino group transfer from an amino acid to a keto acid to form a new amino acid known as Transamination which is important for redistribution of amino groups and production of non essential amino acids.as per the requirement of cells. This reaction diverts the excess amino acids towards energy generation.

xi. **Oxidative deamination:** They undergo oxidative deamination to liberate free ammonia from the amino group of amino acids coupled with oxidation. This reaction provides NH_3 for urea synthesis and α-keto acids for a variety of reactions.

4.7. Summary

Amino acids are a group of organic compounds, building blocks of proteins (polymer of amino acids). Amino acids possess two functional groups namely carboxyl (-COOH) and amino (-NH_2). In the physiological system, they exist as dipolar ions commonly referred to as zwitterions. They are 20 in number and classified into different groups based on their structure, chemical nature, nutritional requirement and metabolic fate. Amino acids are containing two functional groups, amino and carboxyl. The amino group (−NH_2) is basic while the carboxyl group (−COOH) is acidic in nature. They are classified in different ways based on the structure, chemical nature, nutritional requirement and metabolic fate. The important functions of amino acids are precursors for protein synthesis; act as buffer, in detoxification of drugs & metabolic by-products and neurotransmitters.

4.8. Self assessment questions

Q.1. Describe the classification of amino acids.

Answer:----------

Q.2. Write about the essential and non-essential amino acids.

Answer:----------

Q.3. Describe the physical and chemical properties of amino acids.

Answer:----------

Q.4. Write about the important functions of amino acid.

Answer:----------

Q.5. Describe the structure of amino acid.

Answer:----------

Q.6. Write shot notes on

(a) Ampholytes:

(b) Non polar amino acids

(c) Sulfur containing amino acids

(d) Basic amino aicds

Answer:----------

4.9. Further Readings

1. Jain, J.L, Jain, S. & Jain, N. Fundamentals of Biochemistry. S Chand and Company limited, New Delhi.
2. Biochemistry. Books and Allied: Satyanarayan, & Chakrapani, Kolkata.
3. Concise Medical Biochemistry: Dandekar, S.P. Elsevier.
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UNIT-5

Proteins

Structure

5.1. Introduction:

Protein is a macronutrient. It is one of the three essential nutrients found in food that the body needs in large amounts. Proteins are predominantly constituted by five major elements Carbon, Hydrogen, Oxygen, Nitrogen and Sulfur. It is essential for the maintenance and building of body tissues and muscles. Proteins are classified on the basis of their function, chemical nature and solubility properties and nutritional importance. The primary structure represents the linear sequence of amino acids. The twisting and spatial arrangement of polypeptide chain is the secondary structure. Tertiary structure constitutes the three dimensional structure of a functional protein. The assembly of similar or dissimilar polypeptide subunits comprises quaternary structure. Proteins are classified into three major groups. Simple proteins contain only amino acid residues (e.g. albumin). conjugated proteins contain a non-protein

moiety known as prosthetic group, besides the amino acids (e.g. glycoproteins). and Drived proteins are obtained by degradation of simple or conjugated proteins

Objectives:

After going through the course of this unit student will be able to:

- Understand the nature of proteins.
- To study the different types of classifications of proteins.
- Know about the different types of protein structure (Primary, secondary, tertiary and quaternary).
- Understand the conformational structure of peptides.
- Study of classification of peptides.

5.2. What is protein?

Proteins are the polymers of L-α-amino acids. The term protein is derived from a Greek word proteios, meaning holding the first place. Mulder (Dutch chemist) in 1838 used the term proteins for the high molecular weight nitrogen rich and most abundant macromolecules present in animals and plants. Proteins perform various specialized functions. Certain proteins perform brick and mortar roles and are primarily responsible for structure and strength of body. These include collagen and elastin found in bone matrix, vascular system and other organs and α-keratin present in epidermal tissues. Proteins acting as enzymes, hormones,blood clotting factors, immunoglobulins, membrane receptors, storage proteins, besides their function in genetic control, muscle contraction, respiration etc.

5.3. Nature of proteins

1. **Solubility :** Proteins form colloidal solutions instead of true solutions in water.

2. **Molecular weight :** vary in their molecular weights, dependent on the number of amino acid residues. Majority of proteins / peptides may be composed of 40 to 4,000 amino acids with a molecular weight ranging from 4,000 to 440,000.

3. **Shape :** variation in the shape, may be globular (insulin), oval (albumin) fibrous or elongated (fibrinogen).

4. **lsoelectric pH :** The nature of the amino acids (particularly their ionizable groups) determines the lsoelectric pH (pl) of a protein. The acidic amino acids (Asp, Glu) and basic amino acids (His, Lys, Arg) strongly influence the pl. At isoelectric pH, the proteins exist as zwitterions or dipolar ions. They are electrically neutral (do not migrate in

the electric field) with minimum solubility, maximum precipitability and least buffering capacity. The isoelectric pH (pl) for some proteins are given here:

5. Pepsin- 1.1; Casein-4.6; Human albumin-4.7; Urease-5.0; Hemoglobin-6.7; Lysozyme-1l.0.

6. Acidic and basic proteins: Proteins in which the ratio (ε Lys + ε Arg/(ε Glu + ε Asp) is greater than 1 are referred to as basic proteins. For acidic proteins, the ratio is less than 1.

7. Precipitation of proteins: Proteins exist in colloidal solution due to hydration of polar groups (-COO- , $-NH_3^+$,-OH). Proteins can be precipitated by dehydration or neutralization of polar groups.

- The proteins in general are least soluble at isoelectric pH.
- The process of protein precipitation by the additional of neutral salts such as ammonium sulfate or sodium sulfate is known as salting out.
- Heavy metal ions like $Pb^{2+,}$ Hg^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+} cause precipitation of proteins.
- Proteins can be precipitated by trichloroacetic acid, sulphosalicylic acid, phosphotungstic acid, picric acid, tannic acid, phosphomolybdic acid etc.
- Organic solvents such as alcohol are good protein precipitating agents

8. Colour reactions of proteins : The proteins give several colour reactions which are often useful to identify the nature of the amino acids present in them.

9. Denaturation : The phenomenon of disorganization of native protein structure is known as denaturation. The results show loss of secondary, tertiary and quaternary structure of proteins. This involves a change in physical, chemical and biological properties of protein molecules.

a) Agents of denaturation

b) Physical agents: Heat, violent shaking, X-rays, UV radiation.

c) Chemical agents: Acids, alkalies, organic solvents (ether, alcohol), salts of heavy metals (Pb, Hg), urea, salicylate.

5.4. Classification of proteins

Proteins are classified on the basis of their function, chemical nature and solubility properties and nutritional importance.

A. Functional classification of Protein:

Based on the functions they perform, proteins are classified into the following groups:

1. **Structural proteins:** Protein that serve as supporting framework of cells, e.g. Keratin of hair and nails, collagen of bone.
2. Enzymes or catalytic proteins: these acts as biocatalyst, e,.g. Hexokinase, pepsin.
3. Transport proteins: Proteins involved in the process of transportation, e.g. Hemoglobin, serum albumin.
4. Hormonal proteins: Involved in communication between tissues, e.g. Insulin, growth hormone.
5. Contractile proteins : Protein of skeletal muscle involved in muscle contractions and relaxation, e.g. Actin, myosin.
6. Storage proteins: Ovalbumin, glutelin
7. Genetic proteins: Nucleoproteins.
8. Defense proteins: involved in defence mechanisms, e.g. Snake venoms, lmmunoglobulins.
9. Receptor proteins for hormones, viruses.

B. Classification based on chemical nature and solubility:

lt is based on the amino acid composition, structure, shape and solubility properties. Proteins are broadly classified in to 3 major groups:

1. Simple proteins:

They are composed of only amino acid residues.

i. **Globular proteins:** These are spherical or oval in shape, soluble in water or other solvents and digestible.

ii. **Albumins:** Soluble in water and dilute salt solutions and coagulated by heat. e.g. serum albumin, ovalbumin (egg), lactalbumin (milk).

iii. **Globulins:** Soluble in neutral and dilute salt solutions e.g. serum globulins, vitelline (egg yolk).

iv. **Glutelins** : Soluble in dilute acids and alkalies and mostly found in plants e.g. glutelin (wheat), oryzenin (rice).

v. **Prolamines:** Soluble in 70 % alcohol e.g. gliadin (wheat), zein (maize).

vi. **Histones:** Strongly basic proteins, soluble in water and dilute acids but insoluble in dilute ammonium hydroxide e .g. thymus histones of codfish sperm.

vii. **Globins:** These are generally considered along with histones. However, globins are not basic proteins and are not precipitated by NH_4OH.

viii. Protamines: They are strongly basic and resemble histones but smaller in size and soluble in NH_4OH. They are also found in association with nucleic acids e.g. sperm proteins.

2. Fibrous proteins:

These are fiber like in shape, insoluble in water and resistant to digestion, Albuminoids or scleroproteins constitute the most predominant group of fibrous proteins.

i. **Collagens:** connective tissue proteins lacking tryptophan. On boiling with water or dilute acids, yield gelatin which is soluble and digestible.

ii. **Elastins:** These proteins are found in elastic tissues such as tendons and arteries.

iii. **Keratins:** These are present in exoskeletal structures e.g. hair, nails, horns. Human hair keratin contains as much as 14% cysteine.

3. Conjugated proteins:

Besides the amino acids, these proteins contain a non-protein moiety known as prosthetic group or conjugating group.

a. **Nucleoproteins:** Nucleic acid (DNA or RNA) is the prosthetic group e.g. nucleohistones, and nucleoprotamines.

b. **Glycoproteins:** The prosthetic group is carbohydrate, which is less than 4% of protein. The term mucoprotein is used if the carbohydrate content is more than 4% e.g. mucin (saliva) ovomucoid (egg white).

c. **Lipoproteins:** Protein found in combination with lipids as the prosthetic group e.g. serum lipoproteins, membrane lipoproteins.

d. **Phosphoprotein**: Phosphoric acid is the prosthetic group e.g. casein (milk), vitelline (egg yolk).

e. **Chromoproteins:** The prosthetic group is coloured in nature e.g. hemoglobins, cytochromes.

f. **Metalloproteins**: These proteins contain metal ions such as Fe, Co, Zn, Cu, Mg etc., e.g. ceruloplasm (Cu), carbonic anhydrase (Zn).

4. Derived proteins:

These are the denatured or degraded products of simple and conjugated proteins. They are two types –

a) Primary derived proteins: Denatured or coagulated or first hydrolysed product of proteins.

i. **Coagulated proteins:** These are the denatured proteins produced by agents such as heat, acids, alkalies etc. .e.g. cooked proteins, coagulated albumin (egg white).

ii. **Proteans:** These are the earliest products of protein hydrolysis by enzymes, dilute acids, alkalies etc. which are insoluble in water e.g. fibrin formed from fibrinogen.

iii. **Metaproteins:** These are the second stage products of protein hydrolysis obtained by treatment with slightly stronger acids and alkalies, e .g. acid and alkali metaproteins.

b) Secondary derived proteins:

These are the progressive hydrolytic products of protein hydrolysis. These include proteoses, peptones, polypeptides and peptides.

c) Nutritional classification of proteins

The nutritive value of proteins is determined by the composition of essential amino acids. From t he nutritional point of view, proteins are classified in to 3 categories.

1. **Complete proteins**: These proteins have all the ten essential amino acids in the required
2. Proportion by the human body to promote good growth. e.g. egg albumin, milk casein.
3. **Partially incomplete proteins**: These proteins are partially lacking one or more essential amino acids and hence can promote moderate growth. e.g. wheat and rice proteins.
4. **Incomplete proteins:** These proteins completely lack one or more essential amino acids. Hence they do not promote growth at all e.g. gelatin, zein,

5.5. Structure of protein

The structure of proteins is rather complex which can be divided into 4 levels of organization.

1. **Primary structure**: The linear sequence of amino acids forming the backbone of proteins (polypeptides).
2. **Secondary structure:** The spatial arrangement of protein by twisting of the polypeptide chain.
3. **Tertiary structure:** The three dimensional structure of a functional protein.
4. **Quaternary structure:** Some of the proteins are composed of two or more polypeptide chains referred to as subunits. The spatial arrangement of these subunits is known as quaternary structure.

5.5.1. Primary structure of protein

Each protein has a unique sequence of amino acids which is determined by the genes contained in DNA. The primary structure of a protein is largely responsible for its function. The amino acid composition of a protein determines its physical and chemical properties.

The amino acids are held together in a protein by covalent peptide bonds or linkages. These bonds are rather strong and serve as the cementing material between the individual amino acids (considered as bricks). When the amino group of an amino acid combines with the carboxyl group of another amino acid, a peptide bond is formed. A dipeptide will have two amino acids and one peptide bond. Peptides containing more than 10 amino acids (decapeptide) are referred to as polypeptides.

The peptide bond is rigid and planar with partial double bond in character. lt generally exists in trans configuration. Both -C=O and –NH groups of peptide bonds are polar and are involved in hydrogen bond formation. The amino acids in a Peptide or Protein are represented by the 3-letter or one letter abbreviation. This is the chemical shorthand to write proteins. For naming peptides, the amino acid suffixes-ine (glycine), -an (tryptophan), -ate (Glutamate) are changed to -yl withthe exception of C-terminal amino acid. Thus a tripeptide composed of an N-terminal glutamate, a cysteine and a C-terminal glycine is called glutamyl-cysteinyl-glycine.

The primary structure comprises the identification of constituent amino acids with regard to their quality, quantity and sequence in a protein structure. A pure sample of a protein or a polypeptide is essential for the determination of primary structure which involves 3 stages:

i. **Determination of amino acid composition in a protein:** The protein or polypeptide is completely hydrolysed to liberate the amino acids which are quantitatively estimated'. The hydrolysis may be carried out either by acid or alkali treatment or by enzyme hydrolysis. Treatment with enzymes, however results in smaller peptides rather than amino acids.

Pronase is a mixture of non-specific proteolytic enzymes that causes complete hydrolysis of protein. The mixture of amino acids liberated by hydrolysis can be determined by chromatographic techniques.

ii. **Degradation of protein into smaller fragments:** Protein is a large molecule which is sometimes composed of individual polypeptide chains. Separation of polypeptides is essential before degradation.

(a) **Liberation of polypeptides:** Treatment with urea or guanidine hydrochloride disrupts the non-covalent bonds and dissociates the protein into polypeptide units. For cleaving the disulfide linkages between the polypeptide units, treatment with performic acid is necessary.

(b) **Number of polypeptides:** The number of polypeptide chains can be identified by treatment of protein with dansyl chloride. It specifically binds with N-terminal amino acids to form dansyl polypeptides which on hydrolysis yield N-terminal dansyl amino acid. The number of dansyl amino acids produced is equal to the number of polypeptide chains in a protein.

(c) **Breakdown of polypeptides into fragments:** Polypeptides are degraded into smaller peptides by enzymatic or chemical methods. The proteolytic enzymes such as trypsin, chymotrypsin, pepsin and elastase exhibit specificity in cleaving the peptide bond. Cyanogen bromide (CNBr) chemical is commonly used to split polypeptides into smaller fragments.

iii. **Determination of amino acid sequence:** The polypeptides or their smaller fragments are conveniently utilized for the determination of sequence of amino acids. This is done in a step wise manner to finally build up the order of amino acids in a protein. Certain reagents are employed for sequence determination.

Sanger's reagent: Sanger used 1-fluoro 2, 4-dinitrobenzene (FDNB) to determine insulin structure.

Edman's reagent: Phenyl isothiocyanate is the Edman's reagent. lt reacts with the N-terminal amino acid of peptide to form a phenyl thiocarbamyl derivative.

5.5.2. Secondary structure of protein

The conformation of polypeptide chain by twisting or folding is referred to as secondary structure. The amino acids are located close to each other in their sequence. Two types of secondary structures, α-helix and β-sheet, are mainly identified.

Conformational structure:

α- Helix

It is the most common spiral structure of protein. lt has a rigid arrangement of polypeptide chain. α-Helical structure was proposed by

Pauling and Corey (1951) The salient features of α-helix are given below –

- It is a tightly packed coiled structure with amino acid side chains extending outward from the central axis.
- It is stabilized by extensive hydrogen bonding.lt is formed between H atom attached to peptide N and O atom attached to peptide C. The hydrogen bonds are individually weak but collectively they are strong enough to stabilize the helix.
- All the peptide bonds, except the first and last in a polypeptide chain, participate in hydrogen bonding.
- Each turn of α-helix contains 3.6 amino acids and travels a distance of 0.54 nm. The spacing of each amino acid is 0.15 nm.
- α-Helix is a stable conformation formed spontaneously with the lowest energy.
- The right handed α-helix is more stable than left handed helix.
- Certain amino acids (particularly proline) disrupt the α-helix. Large number of acidic (Asp, Glu) or basic (Lys, ,Arg, His) amino acids also interfere with α-helix structure.

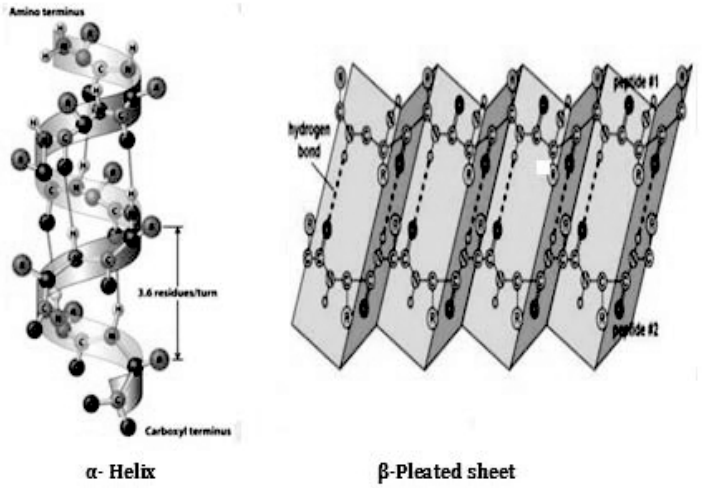

Fig. 5.1: Secondary structure of protein

β-Pleated sheet

This is the second type of structure proposed by Pauling and Corey. β-Pleated sheets (or simply β-sheets) are composed of two or more segments of fully extended peptide chains. ln the β-sheets, the hydrogen bonds are formed between the neighbouring segments of polypeptide chains.

The polypeptide chains in the β-sheets may be arranged either in parallel (the same direction) or anti-parallel (opposite direction). β-Pleated sheet

may be formed either by separate polypeptide chains (H-bonds are interchain) or a single polypeptide chain folding back on to itself (H-bonds are intrachain).

Ramachandran Plot:

The conformation of the main polypeptide chain can be completely determined if the values ϕ and ψ for each amino residue in the chain are known. In a fully stretched polypeptide chain, ϕ=ψ= 180°. Ramachandran (1963) recognized that an amino acid residue in a polypeptide chain cannot have just any pair of values of ϕ and ψ. By assuming that atoms behave as hard spheres allowe ranges of ϕ and ψ can be predicted and visualized in steric contour diagram called **Ramachandran Plot.** Such a plot for poly-L-alanine (or any amino acid except glycine and proline) shows three separate allowed ranges. One of them contains ϕ−ψ values that generate the antiparallel β sheet, the parallel β sheet and the collagen helix. A second region has ϕ−ψ values that produce the right handed α helix: a third, the left handed αhelix.

For glycine these three allowed regions are larger and a fourth appears because a hydrogen atom causes less steric hindrance than a methyl group. Glycine anables the polypeptide backbone to make turns that would not be possible with another residue.

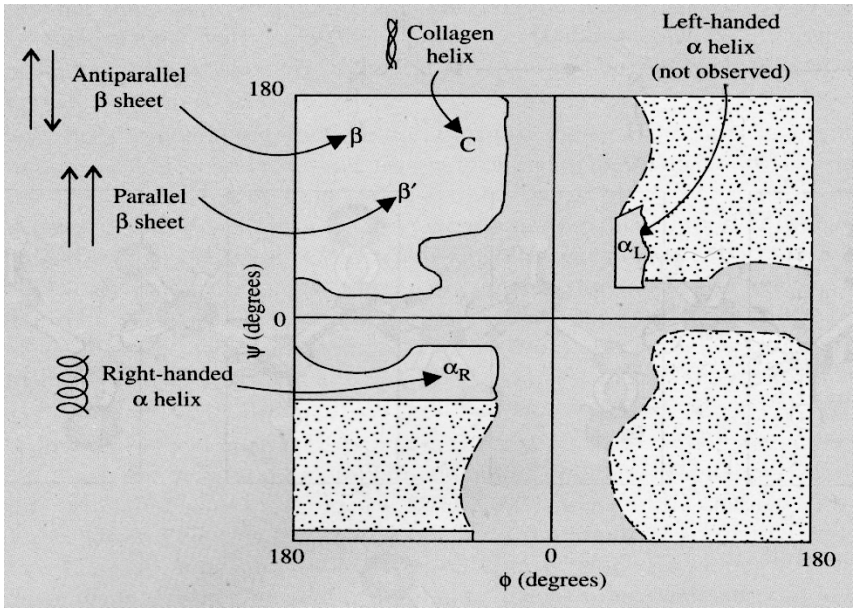

Fig.5.2: Ramachandran Plot

Proline too is special. The 5-membered ring of proline prevents rotation about the N−C_α bond which fixes ϕ at about - 65°. Hence a proline residue has a markedly restricted range of allowed conformations. The residue on the N-terminal side of a proline is also constrained because of steric hindrance imposed by the 5-membered ring. Proline also disfavours α-helix formation because it lacks an amide H atom for hydrogen bonding.

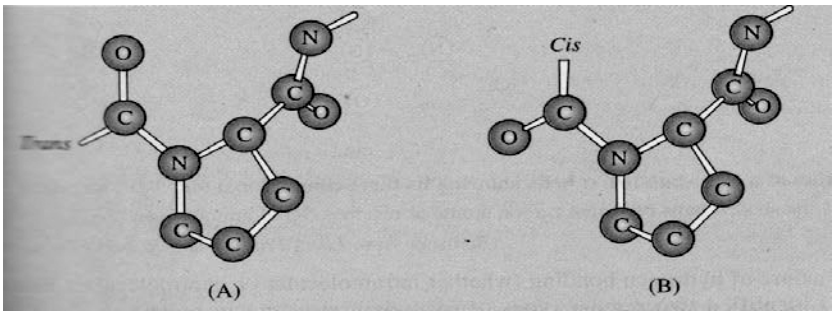

Fig.5.3: Formation of (A) Trans and (B) cis

5.5.3. Tertiary structure of protein

The three-dimensional arrangement of protein structure is referred to as tertiary structure. lt is a compact structure with hydrophobic side chains held interior while the hydrophilic groups are on the surface of the protein molecule. This type of arrangement ensures stability of the molecule.

Besides the hydrogen bonds, disulfide bonds (-S-S), ionic interactions (electrostatic bonds) and hydrophobic interaction also contribute to the tertiary structure of proteins.

5.5.4. Quaternary structure of protein

Some of the proteins consist of two or more polypeptides which may be identical or unrelated. Such proteins are termed as oligomers and possess quaternary structure. The individual polypeptide chains are known as monomers, protomers or subunits. A dimer consist of two polypeptides while a tetramer has four. The monomeric subunits are held together by nonconvalent bonds namely hydrogen bonds, hydrophobic interactions and ionic bonds.

These proteins play a significant role in the regulation of metabolism and cellular function.

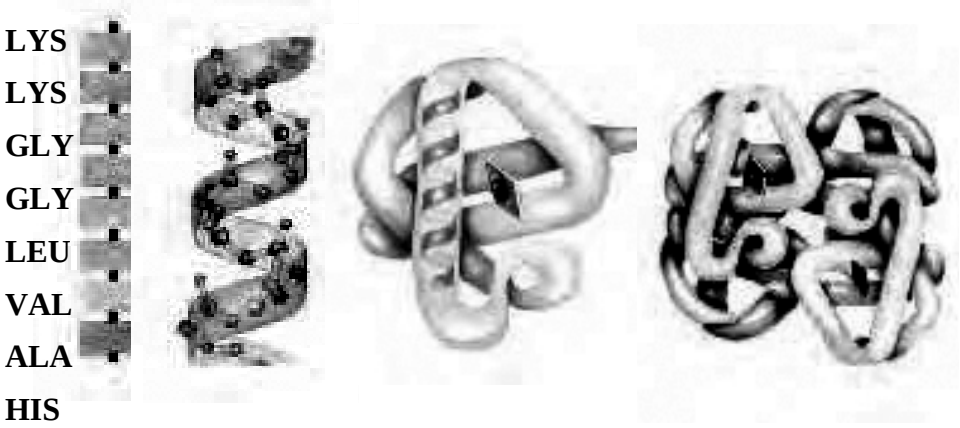

Fig.5.4: Levels of structure in proteins.

The primary structure consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be coiled into units of secondary structure, such as an α-helix. The helix is a part of the tertiary structure of the folded polypeptide, which is itself one of the subunits that make up the quaternary structure of the multisubunit protein, in this case hemoglobin.

5.6. Peptides

Peptides are chains of amino acids. Two amino acid molecules can be covalently joined through a substituted amide linkage, termed a **peptide bond,** to yield a dipeptide. Such a linkage is formed by removal of the elements of water (dehydration) from the α-carboxyl group of one amino acid and the α-amino group of another. Peptide bond formation is an example of a condensation reaction, a common class of reactions in living cells. Three amino acids can be joined by two peptide bonds to form a tripeptide; similarly, amino acids can be linked to form tetrapeptides, pentapeptides, and so forth. When a few amino acids are joined in this fashion, the structure is called an **oligopeptide.** When many amino acids are joined, the product is called a **polypeptide.** Proteins may have thousands of amino acid residues. Although the terms "protein" and "polypeptide" are sometimes used interchangeably, molecules referred to as polypeptides generally have molecular weights below 10,000, and those called proteins have higher molecular weights. A polypeptide is a long, continuous, and unbranched peptide chain. Proteins consist of one or more polypeptides arranged in a biologically functional way and are often bound to cofactors, or other proteins. Long peptides such as amyloid beta can be considered proteins, whereas small proteins such as insulin can be considered peptides. Peptide bonds are strong with partial double bond character. They are not broken by usual denaturing agents like heating or high salt concentration. They can be broken by Prolonged exposure to strong acid or base at elevated temperatures and by specific enzymes such as digestive enzymes. Peptide bonds are rigid and planner resisting free rotation, therefore they stabilize protein structure. Peptide bonds are uncharged but polar: they contain polar hydrogen atoms of amino groups (with a partial positive charge) and polar oxygen atoms of carboxyl groups (with a partial negative charge). This allows hydrogen bonds to form between peptide bonds in different parts of the chain.

Classiffication of Peptide:

Peptides are divided into several classes, depending on how they are produced:

1. Milk peptides: They are formed from milk proteins by enzymatic breakdown by digestive enzymes or by the proteinases formed by lactobacilli during the fermentation of milk.

2. Ribosomal peptides: They are synthesized by translation of mRNA. They are often subjected to proteolysis to generate the mature

form. These function, typically in higher organisms, as hormones and signaling molecules. Some organisms produce peptides as antibiotics, such as microcins.

3. Nonribosomal peptides: These peptides are assembled by enzymes that are specific to each peptide, rather than by the ribosome. The most common non-ribosomal peptide is glutathione, which is a component of the antioxidant defenses of most aerobic organisms. -Other non-ribosomal peptides are most common in plants, and fungi and are synthesized by enzyme complexes called nonribosomal peptide synthetases.These peptides are often cyclic and can have highly-complex cyclic structures, although linear non-ribosomal peptides are also common.

4. Peptones: They are derived from animal milk or meat digested by proteolytic digestion. In addition to containing small peptides, the resulting spray-dried material includes fats, metals, salts, vitamins and many other biological compounds. It is used in nutrient media for growing bacteria and fungi.

5. Peptide fragments: It refer to fragments of proteins that are used to identify or quantify the source protein. Often these are the products of enzymatic degradation performed in the laboratory on a controlled sample, but can also be samples that have been degraded by natural effect

5.7. SUMMARY

Proteins are nitrogen containing, most abundant organic macromolecules, widely distributed in animals and plants. They perform structural and dynamic functions in the organisms. Proteins are polymers composed L-α-amino acids. Besides the 20 standard amino acids present in proteins, there are several non-standard amino acids. These include the amino acid derivatives found in proteins (e.g. hydroxyproline, hydroxylysine) and, non-protein amino acids (e.g. ornithine, citrulline). The structure of protein is divided into four levels of organization. The primary structure represents the linear sequence of amino acids. The twisting and spatial arrangement of polypeptide chain is the secondary structure. Tertiary structure constitutes the three dimensional structure of a functional protein. The assembly of similar or dissimilar polypeptide subunits comprises quaternary structure. The determination of primary structure of a protein involves the knowledge of quality, quantity and the sequence of amino acids in the polypeptide. Chemical and enzymatic methods are employed for the determination of primary structure. The secondary structure of protein mainly consists of α-helix and or β-sheet, α-Helix is stabilized by extensive hydrogen bonding. β-pleated sheet is composed of two or more segments of fully extended polypeptide chains. The tertiary and quaternary structures of protein are stabilized by non-

covalent bonds such as hydrogen bonds, hydrophobic interactions, ionic bonds etc. Proteins are classified into three major groups. Simple proteins contain only amino acid residues (e.g. albumin). Conjugated proteins contain a non-protein moiety known as prosthetic group, besides the amino acids (e.g. glycoproteins). Derived proteins are obtained by degradation of simple or conjugated proteins. In addition to proteins, several peptides perform biologically important functions.

5.8. Terminal questions

Q.1. Describe the classification of proteins with suitable examples.

Answer:----------

Q.2. Describe the quaternary structure of protein.

Answer:----------

Q.3. Write about the conformational structure of peptide.

Answer:----------

Q.4. Discuss the important biologically active peptides.

Answer:----------

Q.5. Write about the α-Helix and β-pleated sheet.

Answer:----------

Q.6. Comments on Secondary structure of protein.

Answer:----------

Q.7. Discuss about the properties of protein.

Answer:----------

5.9. Further readings

1. Jain, J.L, Jain, S. & Jain, N. Fundamentals of Biochemistry. S Chand and Company limited, New Delhi, seven editim.
2. Biochemistry. Books and Allied: Satyanarayan, & Chakrapani, Kolkata.
3. Concise Medical Biochemistry: Dandekar, S.P. Elsevier.
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UNIT-6

Carbohydrates

Structure

6.1. Introduction

Carbohydrates are the most abundant organic molecules in nature. They are primarily composed of the elements carbon, hydrogen and oxygen. The name carbohydrate till literally means 'hydrates of carbon'. Carbohydrates are the major source of energy for the living cells. They are broadly classified into three major groups – monosaccharides (glucose, fructose), oligosaccharides (lactose, sucrose) and polysaccharides (starch, glycogen). This categorization is based on the number of sugar units. The monosaccharides are also divided into different categories (Aldoses & Ketoses) based on the functional group and the number of carbon atoms. Stereoisomerism is an important characters of monosaccharides. Manosaccharides participate in several metabolic reactions. These include oxidation, reduction, dehydration and osazone formation etc. Formation of esters and glycosides by monosaccharides is of special significance in biochemical reactions. Among the oligosaccharides, disaccharides are the most common (lactose, maltose and sucrose). Polysaccharides are the polymers of monosaccharides or their derivatives, held together by glycosidic bonds.

Objectives

After going through the course of this unit, student will be able to:

- To know about the carbohydrates and its important functions.
- Understand the structure of all classes of carbohydrates.
- To study the classification of carbohydrates
- To know about the formation of disaccharides and polysaccharides.

6.2. Carbohydrate overviews

Carbohydrates are the major source of energy for the living cells. As such, carbohydrates are the first cellular constituent synthesized by green plants during photosynthesis from carbon dioxide and water, on absorption of light. Carbohydrates may be defined as polyhydroxyaldehydes or ketones or compounds which produce them on hydrolysis. The principal dietary carbohydrates are polysaccharides (starch, glycogen), disaccharides (lactose, sucrose) and, to a minor extent, monosaccharides (glucose, fructose). The important functions of carbohydrates are:

- They are the most abundant dietary source of energy (4 Cal/g) for all organisms.
- Carbohydrates syntheses by photosynthesis are precursors for the organic compounds (fats, amino acids).
- Carbohydrate (as glycoproteins and glycolipids) participates in the structure of cell membrane and cellular functions such as cell growth, adhesion and fertilization.
- They are structural components of many organisms. These include the fiber (cellulose) of plants, exoskeleton of some insects and the cell wall of microorganisms.
- Carbohydrates also serve as the storage form of energy (glycogen) to meet the immediate energy demands of the body

6.3. Classification of Carbohydrates

Carbohydrates are often referred to as saccharine (Greek: sakcharon-sugar). They are broadly classified into three major groups monosaccharides, oligosaccharides and polysaccharides. This categorization is based on the number of sugar units. Mono- and oligosaccharides are sweet to taste, crystalline and soluble in water, hence thev are commonly known as sugars.

6.4. Monosaccharides:

- Monosaccharides (Greek: mono-one) are the simplest group of carbohydrates and are often referred to as simple sugars.
- They have the general formula $Cn(H_2O)n$.
- They cannot be hydrolysed further.
- They are subdivided into, trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) and heptoses (7C), based on the number of carbon atoms.
- Based on the functional group and the number of carbon atoms The monosaccharides are also divided into different categories –

Aldoses : When the functional group in monosaccharides is an aldehyde are known as aldoses e.g. glyceraldehyde, glucose.

Ketoses: When the functional group is a keto group, they are referred to as ketoses e.g. dihydroxyacetone, fructose.

6.4.1. Structure of Monosaccharides

Stereoisomerism is an important character of monosaccharides. Stereoisomers are the compounds that have the same structural formulae but differ in their spatial configuration. A carbon is said to be asymmetric when it is attached to four different atoms or groups. The number of asymmetric carbon atoms (n) determines the possible isomers of a given compound which is equal to 2^n. Glucose contains 4 asymmetric carbons, and thus has 16 isomers.

Optical isomers (D-and L-isomers): The D and L isomers are mirror images of each other. The spatial orientation of -H and -OH groups on the carbon atom (C_5 for glucose) that is adjacent to the terminal primary alcohol carbon determines whether the sugar is D- or L-isomer. If the -OH group is on the right side, the sugar is of D-series, and if on the left side, it belongs to L-series. The structures of D- and L-glucose based on the reference monosaccharide, D- and L-glyceraldehyde (glycerose).

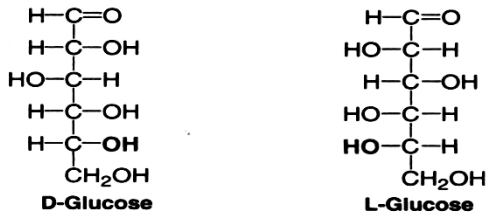


Fig. 6.1: D- and L- forms of Glucose

Optical activity of sugars:

Optical activity is a characteristic feature of compounds with asymmetric carbon atom. When a beam of polarized light is passed through a solution of an optical isomer, it will be rotated either to words the right or left. The term

dextrorotatory (+) and levorotatory (-) are used to compounds that respectively rotate the plane of polarized light to the right or to the left. An optical isomer may be designated as D (+), D(-), L(+) and L(-) based on its structural relation with glyceraldehyde. D- and L-configurations of sugars are primarily based on the structure of glyceraldehyde, the optical activities however, may be different.

Racemic mixture: lf D- and L-isomers are present in equal concentration, it is known as racemic mixture or DL mixture. It does not exhibit any optical activity, since the dextro- and levorotatorv activities cancel each other.

Configuration of D-aldoses

The configuration of possible D-aldoses starting from D-glyceraldehyde is shown in Fig. 6.2. This is a representation of Killiani-Fischer synthesis by increasing the chain length of an aldose, by one carbon at a time. Thus, starting with an aldotriose(3C),aldotetroses (4 C), aldopentoses (5C) and aldohexoses (6C) are formed. Of the 8 aldohexoses glucose, mannose and galactose are the most familiar. Among these, D-glucose is the only aldose monosaccharide that predominantly occurs in nature.

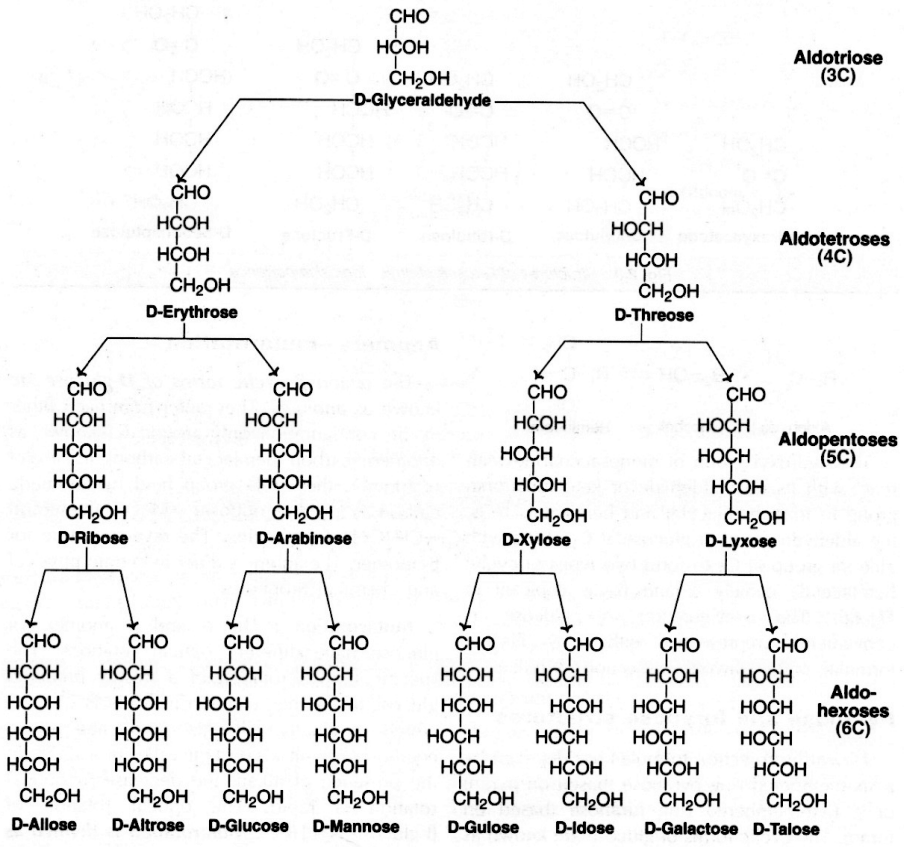


Fig.6.2: The structural relationship between D-aldoses shown in Fischer projection

Configuration of D-ketoses

Starting from dihydroxyacetone (triose), there are five keto-sugars which are physiologically important.

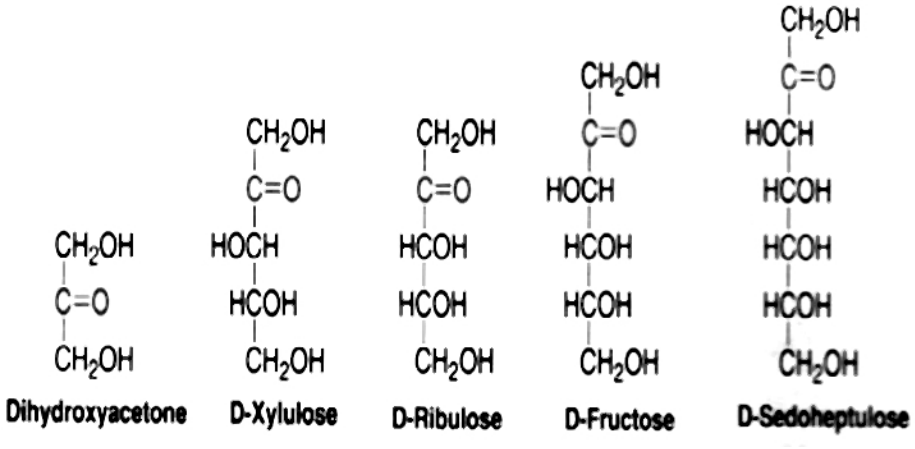


Fig. 6.3. Structures of Ketoses

Epimers:

If two monosaccharides differ from each other in their configuration around a single specific carbon (other than anomeric) atom, they are referred to as epimers to each other. For example glucose and galactose are epimers with regard to carbon 4 (C_4-epimers). They differ in the arrangement of -OH group at C_4. Glucose and mannose are epimers with regard to carbon 2 (C_2-epimers). The interconversion of epimers (e.g. glucose to galactose and vice versa) is known as epimerization, and a group of enzymes namely-epimerases catalyse this reaction.

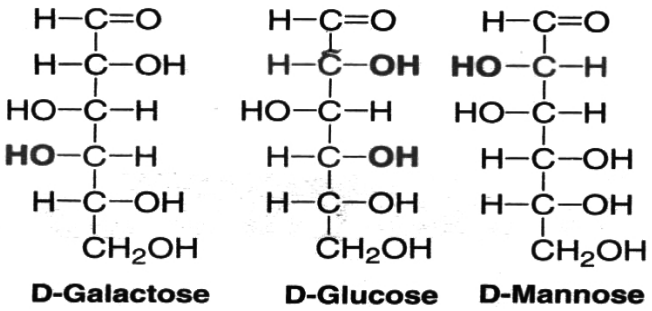


Fig.6.4. Structures of epimers

Enantiomers:

Enantiomers are a special type of stereoisomers that are mirror images of each other. The two members are designated as D- and L-sugars. Majority of the sugars in the higher animals (including man) are of D-type. The term diastereomers is used to represent the stereoisomerism that are not mirror images of one another.

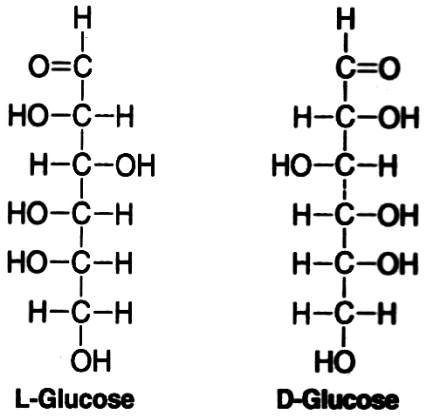


Fig. 6.5. Enantiomers of Glucose

6.4.2. Structure of Glucose

The hydroxyl group of monosaccharides can react with its own aldehyde or keto functional group to form hemiacetal and hemiketal. The aldehyde group of glucose at C_1 reacts with alcohol group at C_5 to form two types of cyclic hemiacetals namely α and β. The configuration of glucose is conveniently represented either by Fischer formulae or by Haworth projection formulae. Haworth projection formulae are depicted by a six-membered ring pyranose (based on pyran) or a five-membered ring furanose (based on furan). The cyclic forms of glucose are known as α-D-glucopyranose and α-D-glucofuranose.

Anomers-mutarotation:

The α and β cyclic forms of D-glucose are known as anomers. They differ from each other in the configuration only around C_1 known as anomeric carbon (hemiacetal carbon). In case of α- anomer, the -OH group held by anomeric carbon is on the opposite side of the group $-CH_2OH$ of sugar ring. The reverse is true for β-anomer. The anomers differ in certain physical and chemical properties.

Mutarotation : The α and β anomers of glucose have different optical rotations. The specific optical rotation of a freshly prepared glucose (α-anomer) solution in water is $+112.2^o$ which gradually changes and attains an equilibrium with a constant value of $+52.7^o$. ln the presence of alkali, the decrease in optical rotation is rapid. The optical rotation of β-glucose is $+18.7^o$. Mutarotation is defined as the change in the specific optical rotation representing the interconversion of α and β forms of D-glucose to an equilibrium mixture. The equilibrium mixture contains 63% β-anomer and 36% α-anomer of glucose with

1 % open chain form. ln aqueous solution, the β form is more predominant due to its stable conformation. The α and β forms of glucose are interconvertible which occurs through a linear form. The latter, as such, is present in an insignificant quantity in aqueous solution.

Fructose also exhibits mutarotation. The pyranose ring (six-membered) is converted to furanose (five-membered) ring till an equilibrium is attained. Fructose has a specific optical rotation of -92 ^{o} at equilibrium.

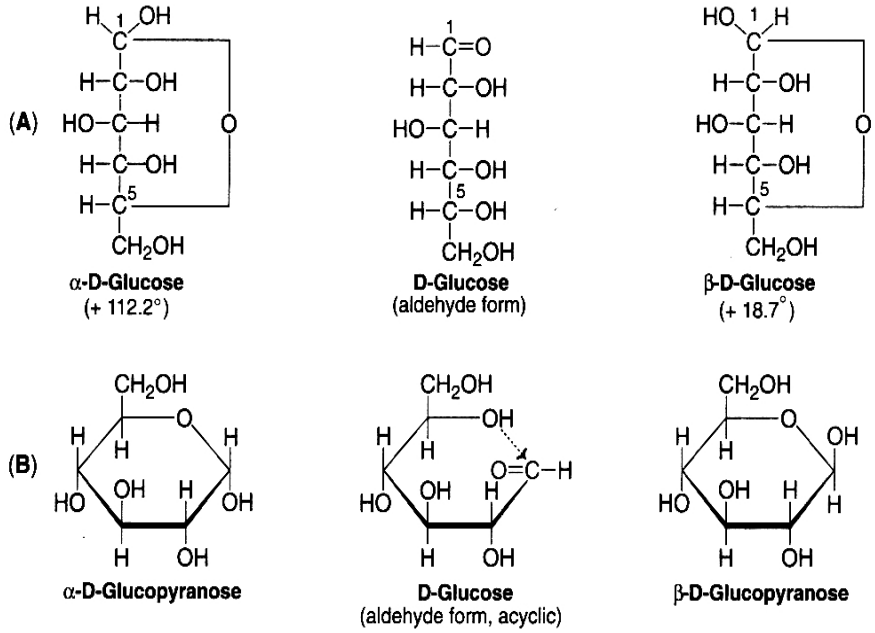


Fig.6.6: Mutarotation of Glucose representing α and β anomers

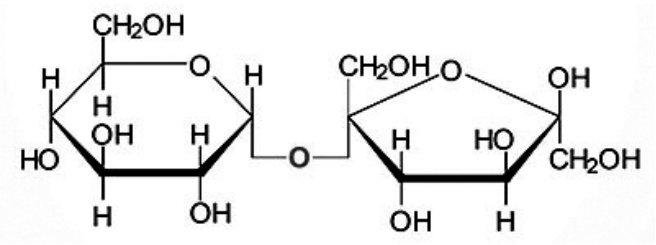
(A) Fisher projections (B) Haworth projections

6.5. Disaccharides

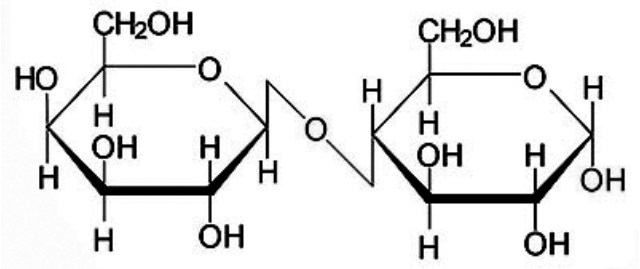
Double units of monosaccharides are called disaccharides in which both units joined together by glycosidic linkage. The most common disaccharides are lactose (milk sugar), maltose and sucrose shown in Fig (6.7). Sucrose is a common sweetener, which is formed by D-fructose and α-D-glucose linked through a double glycosidic bond between C1 of α-glucose to C2 of β-fructose and occurs naturally in sugar beet, sugar cane and fruits. Sucrose does not undergo mutation and does not react with phenyl hydrazine to form osazones. It also does not act as a reducing sugar. The sucrose gets readily hydrolyzed than other disaccharides. Another example is Lactose, a disaccharide consisting of glucose and galactose, is the main sugar in milk and dairy products but does not occurs in nature. It yields D- galactose and D-glucose on hydrolysis. Maltose is an disaccharide, occurs in malt and starch derived syrups and found in beer and some vegetables being the most widely known. Maltose, composed of two D-glucoses linked by a α-glycosidic bond from the C1 of one to the OH at C4 of the other glucose (α-1→4 glycosidic bond). It is acetal of the anomeric carbon atom 1 of D-glucose; one hydroxyl group is furnished intermolecular by carbon atom 5 and the other by carbon atom 4 of a second D–glucose molecule.

Table 6.1: Some example of disaccharides.

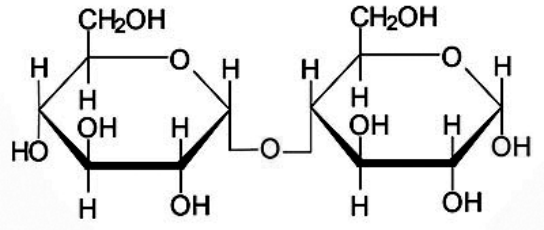
Name	Components of sugar	Description
Sucrose	Glucose + Fructose	From sugar cane and sugar beet
Lactose	Glucose + Galactose	Milk sugar
Maltose	Glucose + Glucose	Malt sugar, from germinating cereals
Trehalose	Glucose + Glucose	Made by plant and fungi
cellobiose	Glucose + Glucose	Breakdown product of cellulose



(a) Sucrose



(b). Lactose



(c) Maltose

Fig. 6.7: Chemical structure of some disaccharides: (a) Sucrose (b**).** Lactose (c) Maltose

6.6. Trisaccharides

The trisaccharides occur naturally. Trisaccharides are oligosaccharides composed of three monosaccharides with two glycosidic bonds connecting them. The trisaccharides raffinose (α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside) is the first member of a series of homologous oligosaccharides named "raffinose family oligosaccharides. Raffinose found in abundant in sugar beets and many other higher plants. Fig.(6.8) The tetrasaccharides (degree of polymerization (*DP*=4)) is known as stachyose, and the pentasaccharide and hexasaccharide, are named verbascose (*DP*=5) and ajugose (*DP*=6), respectively. Oligosaccharides are typically classified as carbohydrates comprising 3-10 monosaccharide units.

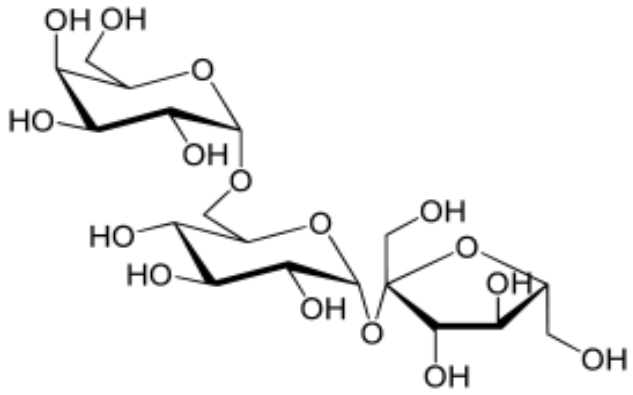


Fig.6.8: Chemical structure of trisaccharides – raffinose

6.7. Oligosaccharides:

Oligosaccharides (Greek: oligo-few) contain 2-10 monosaccharide molecules which are liberated on hydrolysis. Based on the number of monosaccharide units present, the oligosaccharides are further subdivided to disaccharides, trisaccharides etc.

Disaccharides:

Among the oligosaccharides, disaccharides are the most common. It consists of two monosaccharides units (similar or dissimilar), held together by a glycosidic bond. They are crystalline, water-soluble and sweet to taste. The disaccharides are of two types –

1. Reducing disaccharides with free aldehyde or keto group e.g. maltose, lactose.
2. Non-reducing disaccharides with no free aldehyde or keto group e.g. sucrose, trehalose.

Maltose:

It is composed of two α-D-glucose units held together by α (1→ 4) glycosidic bond. The free aldehyde group present on C_1 of second glucose answers the reducing reactions, besides the osazone formations (sunflower-shaped). Maltose can be hydrolysed by dilute acid or the enzyme maltase to liberate two molecules of α-D-glucose. ln isomaltose, the glucose units are held together by α (1→6) glycosidic linkage. Cellobiose is another disaccharide, identical in structure with maltose, except that the former has β (1→4) glycosidic linkage. Cellobiose is formed during the hydrolysis of cellulose.

Sucrose:

Sucrose (cane sugar) is the sugar of commercial importances mostly produced by sugar cane and sugar beet. Sucrose is made up of α-D-glucose and β- D-fructose. The two monosaccharides are held together by a glycosidic bond ($\alpha_1 \rightarrow \beta_2$), between C_1 of α-glucose and C_2 of β-fructose. The reducing groups of glucose and fructose are involved in glycosidic bond, hence sucrose is a non-reducing sugar, and it cannot form osazones.

Sucrose is the major carbohydrate produced in photosynthesis. lt is transported into the storage organs of plants (such as roots, tubers and seeds). Sucrose is the most abundant among the naturally occurring sugars. lt has distinct advantages over other sugars as a storage and transport form. This is due to the fact that in sucrose, both the functional groups (aldehyde and keto) are held together and protected from oxidative attacks.

Sucrose, as such is dextrorotatory ($+66.5^o$). But, when hydrolysed, sucrose becomes levorotatory (-28.2^o). The process of change in optical rotation from dextrorotatory (+) to levorotatory (-) is referred to as inversion. The hydrolysed mixture of sucrose, containing glucose and fructose, is known as invert sugar.

Lactose:

Lactose is more commonly known as milk sugar since it is the diasccharide found in milk. Lactose is composed of β-D-galactose and β-D-glucose held together by β (1→4) glycosidic bond. The anomeric carbon of C_1 glucose is free, hence lactose exhibits reducing properties and forms osazones (powder-puff or hedgehog shape). Lactose of milk is the most important carbohydrate in the nutrition of young mammals. It is hydrolysed by the intestinal enzyme lactase to glucose and galactose.

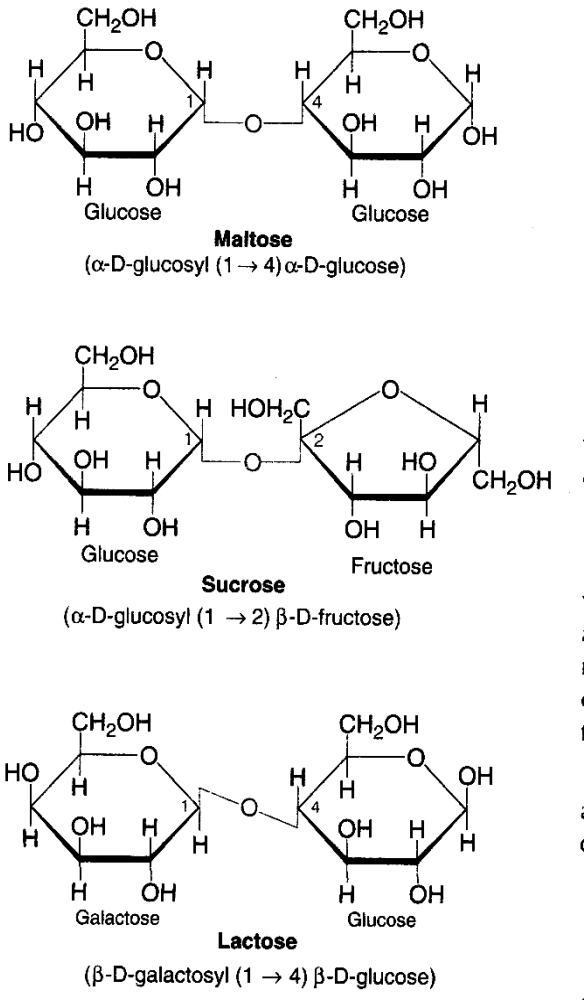


Fig.6.9: Structures of disaccharides

6.8. Polysaccharides:

Polysaccharides (Greek: poly-many) are polymers of monosaccharide units with high molecular weight (up to a million), held together by glycosidic bonds. They are usually tasteless (non-sugars) and form colloids with water. Polysaccharides are linear as well as branched polymers.They are primarily concerned with two important functions-structural and storage of energy.

The polysaccharides are of two types homopolysaccharides and heteropolysaccharides.

i. Homopolysaccharides: which on hydrolysis yield only a single type of monosaccharide. They are named based on the nature of the monosaccharide unit. Thus, glucans are polymers of glucose whereas fructosans are polymers of fructose.

ii. Heteropolysaccharides**:** on hydrolysis yield a mixture of more than one types of monosaccharides or their derivatives.

i. Homopolysaccharides:

Starch:

Starch is a homopolymer composed of D-glucose units held by α-glycosidic bonds. lt is known as glucosan or glucan. It is the carbohydrate reserve of plants which is the most important dietary source for higher animals, including man. High content of starch is found in cereals, roots, tubers, vegetables etc. Starch consists of two polysaccharide components-water soluble amylose (1 5-20%) and a water insoluble amylopectin (80-85%). Chemically, amylose is a long unbranched chain with 200-1,000 D-glucose units held by α (1→4) glycosidicl linkage. Amylopectin, on the other hand, is a branched chain with α (1→6) glycosidic bonds at the branching points and α (1→4) linkages everywhere else. Amylopectin molecule containing a few thousand glucose units looks like a branched tree (20-30 glucose units per branch). Starches are hydrolysed by amylase (pancreatic or salivary) to liberate dextrins, and finally maltose and glucose units. Amylase acts specifically on α (1→4) glycosidic bonds.

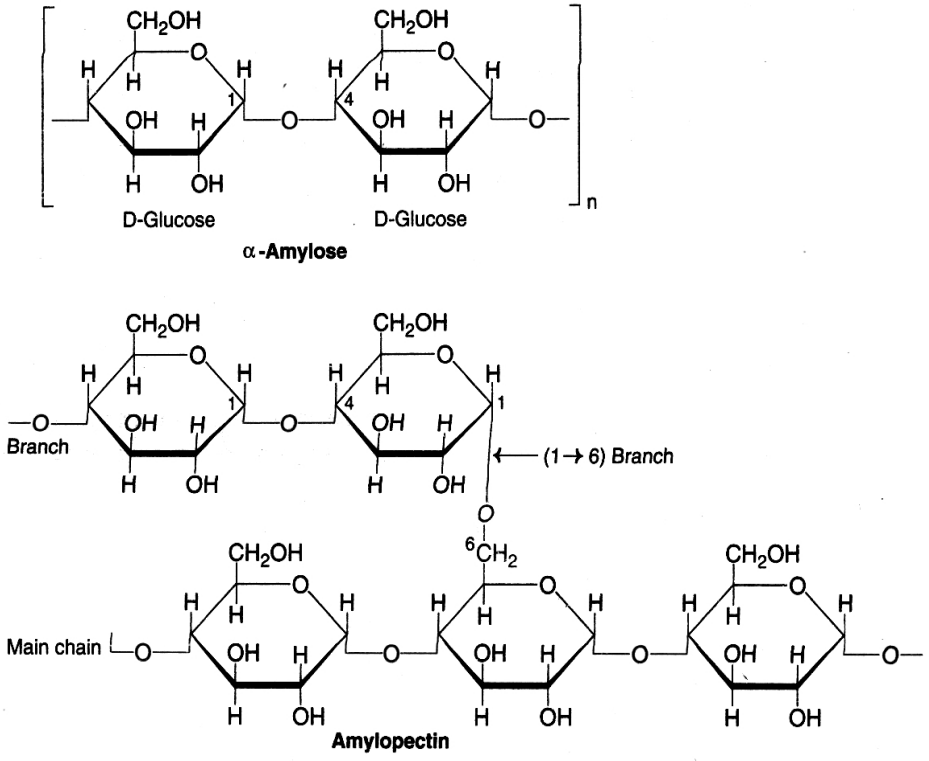


Fig. 6.10: Structure of Starch

Dextrin's:

Dextrin is the breakdown products of starch by the enzyme amylase or dilutes acids. Starch is sequentially hydrolysed to different dextrins and,

finally, to maltose and glucose.The various intermediates (identified by iodine colouration) are soluble starch (blue), amylodextrin (violet), erythrodextrin (red) and achrodextrin (no colour).

Inulin:

Inulin is a polymer of fructose i.e., fructosan. It occurs in dahlia bulbs, garlic, onion etc. lt is a low molecular weight (around 5,000) polysaccharide easily soluble in water. It is not utilized by the body. lt is used for assessing kidney function through measurement of glomerular filtration rate (GFR).

Glycogen:

Glycogen is the carbohydrate reserve in animals, hence often referred to as animal starch. It is present in high concentration in liver, followed by muscle, brain etc. It is also found in plants that do not possess chlorophyll (e.g. yeast, fungi). The structure of glycogen is similar to that of amylopectin with more number of branches.

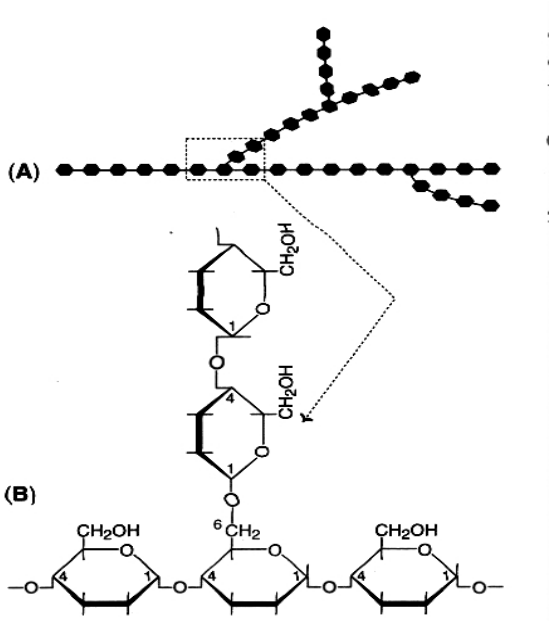


Fig. 6.11: Structure of Glycogen (A) General structure (B) Enlarged at a branch point

Glucose is the repeating unit in glycogen joined together by α (1 → 4) glycosidic bonds and α (1 → 6) glycosidic bonds at branching points. The molecular weight (up to 1 x 10^8) and the number of glucose units (up to 25,000) vary in glycogen depending on the source from which glycogen is obtained.

Cellulose:

lt is a predominant constituent of plant cell wall. Cellulose is totally absent in animal body. Cellulose is composed of β-D-glucose units linked by β (1→4) glycosidic bonds. It cannot be digested by mammals including man-due to lack of the enzyme that cleaves β-glycosidic bonds. Certain

ruminants and herbivorous animals contain microorganisms in the gut which produce enzymes that can cleave β-glycosidic bonds. Hydrolysis of cellulose yields a disaccharide cellobiose, followed by β-glucose. lt is a major constituent of fiber, the non-digestable carbohydrate. The functions of dietary fiber in human nutrition include decreasing the absorption of glucose and cholesterol from the intestine, besides increasing the bulk of feces.

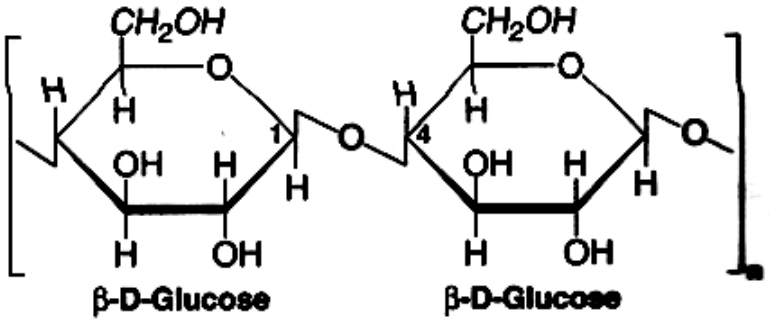


Fig. 6.12: Structure of cellulose

Chitin:

Chitin is composed of N-acetyl D-glucosamine units held together by β (1→4) glycosidic bonds. lt is a structural polysaccharide found in the exoskeleton of some invertebrates e.g. insects, crustaceans.

ii. Heteropolysaccharides:

When the polysaccharides are composed of different types of sugars or their derivatives, they are referred to as Heteropolysaccharides or heteroglycans.

Mucopolysaccharides

Mucopolysaccharides are heteroglycans made up of repeating units of sugar derivatives, namely amino sugars and uronic acids. These are more commonly known as glycosaminoglycans (GAG). Acetylated amino groups, besides sulfate and carboxyl groups are generally present in GAG structure. The presence of sulfate and carboxyl groups contributes to acidity of the molecules, making them acid mucopolysaccharides. Some of the mucopolysaccharides are found in combination with proteins to form mucoproteins or mucoids or proteoglycans. Mucoproteins may contain up to 95%, carbohydrate and 5% protein.

Mucopolysaccharides are essential components of tissue structure. The extracellular spaces of tissue (particularly connective tissue-cartilage, skin, blood vessels, tendons) consist of collagen and elastin fibers embedded in a matrix or ground substance. The ground substance is predominantly composed of GAG. The important mucopolysaccharides are hyaluronic acid, chondroitin 4 -sulfate, heparin, dermatan sulfate and keratan sulfate.

6.9. Summary

Carbohydrates are the polyhydroxyaldehydes or ketones, or compounds which produce them on hydrolysis. The term sugar is applied to carbohydrates soluble in water and sweet to taste. Carbohydrates are the major dietary energy sources, besides their involvement in cell structure and various other functions. Carbohydrates are broadly classified into 3 groups-monosaccharides, oligosaccharides and polysaccharides. The monosacchsrides are further divided into different categories based on the presence of functional groups (aldoses or ketoses) and the number of carbon atoms (trioses, tetroses, pentoses, hexoses and heptoses).

Glyceraldehyde (triose) is the simplest carbohydrate and is chosen as a reference to write the configuration of all other monasaccharides (D- and L- forms). If two monosaccharides differ in their structure around a single carbon atom, they are known as epimers. Glucose and galactose are C4-epimers. Monosaccharides participate in several reactions. These include oxidation, reduction, dehydration, osazone formation etc. Formation of esters and glycosides by monosaccharides is of special significance in biochemical reactions.

Among the oligosaccharides, disaccharides are the most common. These include the reducing disaccharides namely lactose (milk sugar) and maltose (malt sugar) and the non-reducing sucrose (cane sugar). Polysaccharides are the polymers of monosaccharides or their derivatives, held together by glycosidic bonds. Homopolysaccharides are composed of a single monosaccharide (e.g., starch, glycogen, cellulose and inulin). Heteropolysaccharides contain a mixture of few monosaccharides or their derivations (e.g., mucapolysacaharides). Starch and glycogen are the carbohydrate reserves of plants and animals respectively. Cellulose, exclusively found in plants, is the structural constituent.

6.10. Terminal questions

Q.1. Define and classify above carbohydrates.

Answer:----------

Q.2. Describe the structure of monosaccharides.

Answer:----------

Q.3. Write about the Oligosaccharides with examples.

Answer:----------

Q.4. Describe mucopolysaccharides.

Answer:----------

Q.5. Discuss the structure and functions of 3 important disaccharides.

Answer:----------

Q.6. Define trisachharides in brief.

Answer:----------

6.11. Further Readings

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Uttar Pradesh Rajarshi Tandon
Open University

Bachelor in Science

UGBCH-101

Introduction
To
Biochemistry

Block

3

Lipids, Nucleic acids and Vitamins

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Introduction

This the third block of introduction to biochemistry. It consists of following three units:

Unit-7: In this unit we cover the introduction lipids. This unit discuss about fundaments of lipids and their biochemistry. The structure and types of lipids are describes. The fatty acids and their derivatives that is the building blokes of lipids. The role of saturated and unsaturated fatty acids in the lipids mentioned here briefly. Students will learn about nomenclature and significant of lipids.

Unit-8: In this unit we cover nucleotides and nucleosides-structure and properties. The components of nucleotides also discuss briefly. Roles of nitrogenous basis in nucleic acid reveals in the unit. The structure of DNA and RNA also mentioned here.

Unit-9: This unit covers the vitamins. Vitamins are natural components of foods; usually present in very small amounts. They are essential for normal physiologic function (eg. growth, reproduction, etc). Types of vitamin such as water soluble and fat soluble vitamins briefly discuss. Deficiency diseases and their symptoms.

UNIT-7

Lipids

Structure

7.1. Introduction

Lipid is a general term employed to all those substances which are extracted from plant and animal, tissues by dissolving them in 'Fat Solvents' such as chloroform, carbon tetrachloride, benzene, acetone, ether etc. Generally lipids are defined as esters of fatty acids with glycerol. The fatty acids may be defined as those monocarboxylic long chain acids which have more than four carbons and are relatively insoluble in water. Lipids, largely stored as reserve food material, play a very important role in metabolism. Along with carbohydrates, they act as biological fuels in the body. Although lipids form a large proportion of the mass of all cells.

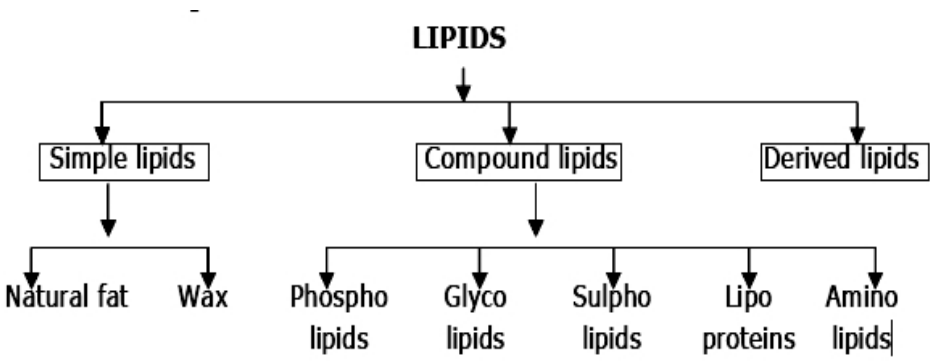
Objectives:

- structures and functions of different lipids molecules

- describes fatty acids structure and their derivatives
- Different physical and chemical properties of lipids

7.2. Classification of lipids

Lipids are classified into three classes on the basis of their chemical structure as in Fig. (7.1).



Fig, 7.1: Classification of lipids

1. Simple lipids: Triglycerides (Fats and oils) and waxes are the simple lipids. They contain only carbon, hydrogen and oxygen. Simple lipids are esters of one or more fatty acids and an alcohol, mostly glycerol

2. Compound lipids : Compound lipids contain other elements such as phospholipids containing in addition to fatty acids and an alcohol, a phosphoric acid residue, glycolipids compounds of fatty acids with carbohydrate, containing nitrogen but no phosphoric acids.

3. Derived lipids : This group is generally termed as the "Catch all" group. These are derivatives of simple or compounds lipids but do not have any ester linkage. Most of these are complex compounds having an active hydroxyl group e.g. steroids, sterols, certain fat soluble vitamins (A, D, E and K).

7.3. Building blocks of lipids

In this section, we shall get to know about building blocks of lipids. These are one glycerol molecule and at least one fatty acid with a maximum of three fatty acids.

7.3.1. Fatty acids

Fatty acids are compounds consisting of a long hydrocarbon chain with a carboxylate group at one end. The general formula is $CH_3(CH_2)_nCOOH$. The fatty acids have the configuration shown here in their free form,

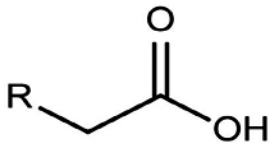


Fig. 7.2 : Fatty acid

Fatty acids are divided into two classes depending upon whether or not the carbon chain carries the maximum possible number of attached hydrogens. If all carbon atoms are fully saturated with hydrogen, the fatty acid is called saturated, when a fatty acid contains one or more double bonds, it is said to be unsaturated.

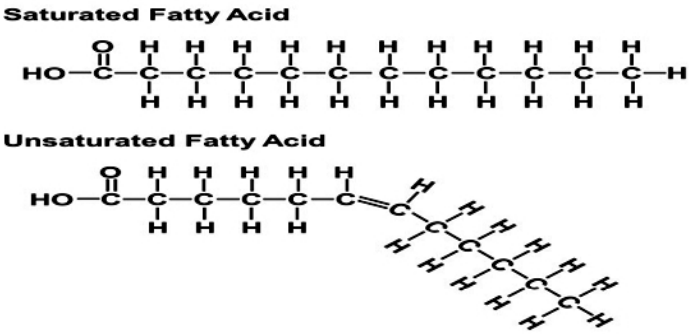


Fig: 7.3 : Saturated and unsaturated fatty acid

7.3.1.1. Saturated Fatty Acid

The most abundant saturated fatty acids are palmate acid, and stearic acid . These are some other acids along with glycerol form the bulk of the body fat in most organisms. It has a polar end with a free -COOH group. In Figure 7.4 some naturally occurring saturated fatty acids are illustrated. In fatty acids solubility decreases in water, with an increase in carbon atoms. Melting point also increases with increasing number of carbon atom.

Table 7.1 : Common Saturated Fatty Acids of Food Fats

Common Name	Molecular formula	Occurrence
Butyric acid	$C_3H_7.COOH$	Butter
Caproic acid	$C_5H_{11}.COOH$	Butter
Caprylic acid	$C_7H_{15}.COOH$	Fats of plant origin
Capric acid	$C_9H_{19}.COOH$	Fats of plant origin
Lauric acid	$C_{11}H_{23}.COOH$	Palm Kernel, Cinnamon, coconut oil
Myristic acid	$C_{13}H_{27}.COOH$	Nutmeg, Palm Kernel, coconut oil
Palmitic acid	$C_{15}H_{31}.COOH$	Palm oil, animal and plant fat
Stearic acid	$C_{17}H_{35}.COOH$	Animal fat and plant oil
Arachidic acid	$C_{19}H_{29}.COOH$	Groundnut oil
Lignoceric acid	$C_{23}H_{47}.COOH$	Groundnut oil

7.3.1.2. Unsaturated Fatty Acids

Typical unsaturated fatty acids are Oleic acid and linoleic acid. The melting point of a fatty acid depends on its nature of kind. The greater the degree of unsaturation the lower the melting point. For example, the melting point of the stearic acid (saturated) is 70ºC and that of Oleic acid (one double bond) and linoleic acid (2 double bonds) is 4ºC and -5ºC respectively. For this reason most animal fats are solids and most vegetable oils are liquids at room temperature. The oils are more unsaturated than the fats because they are composed of the glycerides of unsaturated fatty acids. The hydrogenation of the double bonds of the fatty acid presenting oil (converting them in to saturated acids) raises the melting point of the glycerids. The "hardening" of vegetable oils such as the cotton seed or groundnut oil is an important commercial process by which the cooking fats like "Dalda", etc. are produced. Fatty acids having single double bond are called as mono unsaturated fatty acids (MUFA) while those with two or more double bonds are called poly unsaturated fatty acid (PUFA). Unsaturated fatty acids show different types of isomerism.

Fatty acids with same molecular formula and same number of double bonds may differ in the location of double bonds and exhibit positional isomerism. Another type of isomerism like geometric isomerism in which orientation of two hydrogen atoms attached to the two carbon atoms joined by the double bonds may differ. If both hydrogen atoms are placed on the same side of the double bond, cis-isomer results but if these are placed on either side of double bond, it is a trans-isomer as shown in Figure. 7.4.

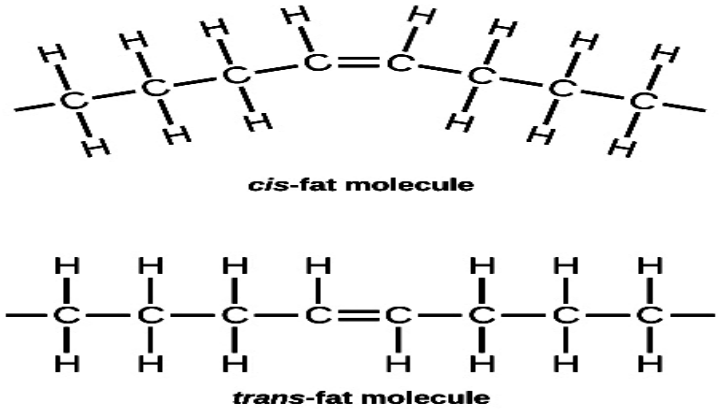


Fig. 7.4: Cis and trans-isomers

7.4. Nomenclature of fatty acid

The most frequently used systematic nomenclature names the fatty acid after the hydrocarbon with the same number and arrangement of carbon atoms, with oil being substituted for the final of fatty acid. This, saturated acids end in -anoic, e.g., octanoic acid and unsaturated acids with double bonds end in -enoic, e.g., octadecenoic acid (Oleic acid).

Carbon atoms are numbered from the carboxyl carbon (carbon No. 1). The carbon atoms adjacent to the carboxyl carbon (Nos. 2, 3 and 4) are also known as the α and β carbon, respectively.

Various conventions use Δ^9 indicates a double bond between carbons 9 and 10 of the fatty acid; w9 indicates a double bond on the ninth carbon counting from the w-carbon. Table 7.2 presents the common unsaturated fatty acids found in nature.

Table 7.2: Naturally occurring common unsaturated fatty acids

Common Name	Chemical Structure	Occurrence
Palmitoleic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	All fats
Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	All fats, abundant in olive
Linoleic acid (LA)	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	Mainly vegetable oils, also in some animal fats
Elaidic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	Hydrogenated fat, margarine
Linolenic acid (LNA)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH-(CH_2)_7COOH$	Mainly vegetable oils, particularly linseed oil
Arachidonic acid	$CH_3(CH_2)4CH=CHCH_2CH=CHCH_2-CH=CHCH_2CH=CH(CH_2)_3COOH$	Peanut oil, groundnut oil, traces in some animal fats

7.5. Glycerol

Glycerol is common to all fats and oils and their properties (fats and oils) are dependent only on the fatty acid component. Most of the fatty acids forming oils and fats have the following properties:-

a. They are mono carboxylic with a variable number of carbon atoms.

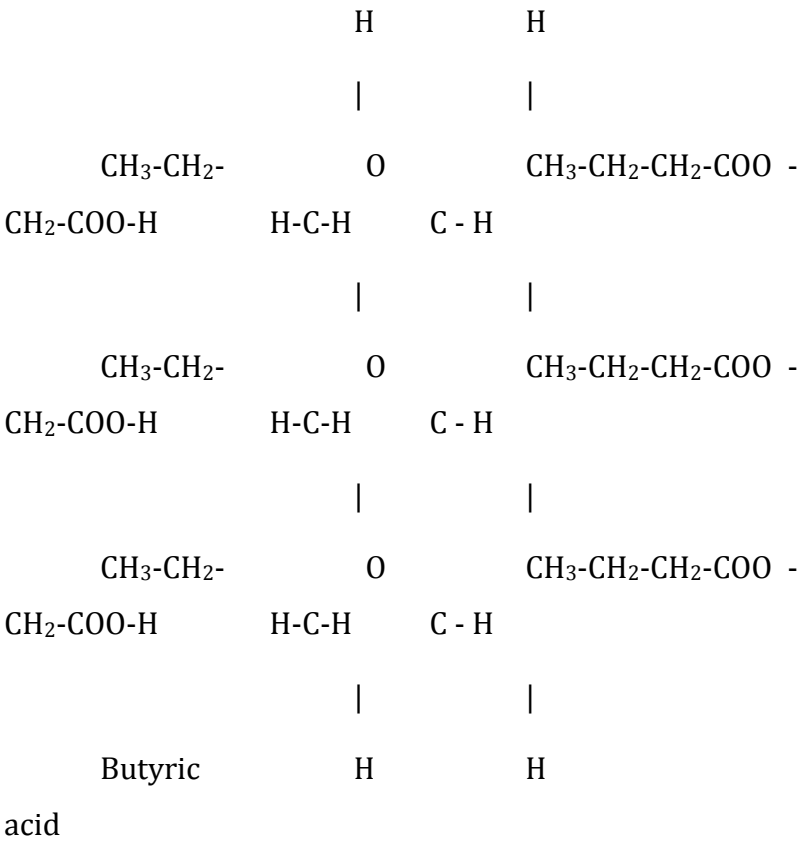
b. The numbers of carbon atoms are nearly always even ranging from 4-30.

c. The carbon atoms form a side chain which is usually unbranched.

d. The side chains may be saturated or unsaturated. Unsaturated fatty acids have a variable number of double bonds

depending on the degree of saturation.

Fats and oils are esters of fatty acid and glycerol. Three molecules of fatty acid combine with one molecule of glycerol to form a triglyceride. In this process three molecules of water are lost (Shown in figure 7.5).



Tributyrin (a simple lipid found in butter) + $3H_2O$

Fig. 7.5: Chemical formulae of Tributyrin

Triacylglycerols (Fig. 7.6) are the main storage water forms of fatty acids. These are esters of the trihydric alcohol glycerol and fatty acids. Mono and dia-cylglycerols, wherein one or two fatty acids are esterified with glycerol, are also found in the tissues.

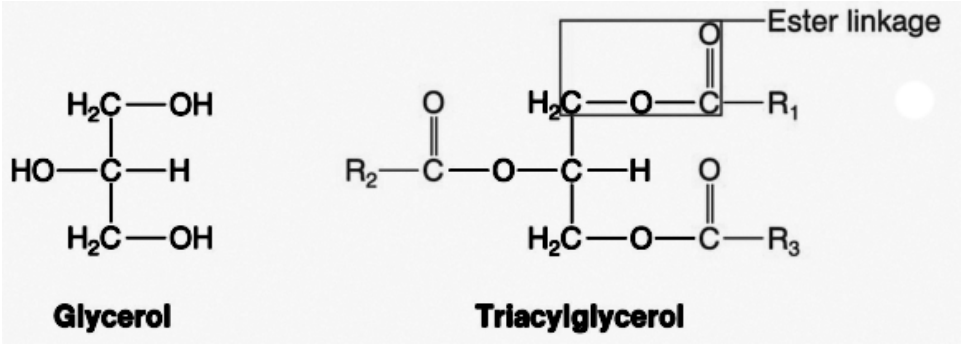


Fig. 7.6: Triacylglycerol

To number the carbon atoms of glycerol unambiguously, the -sn (stereo chemical numbering) system is used. It is important to realize that carbons 1 and 3 of glycerol are not identical when viewed in three dimensions. (Shown as a projection formula in Fig. 7.7).

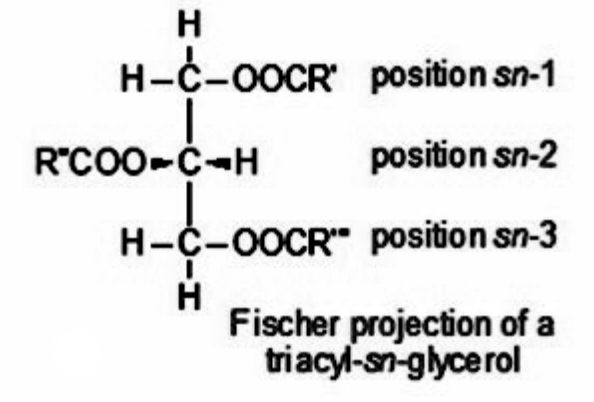
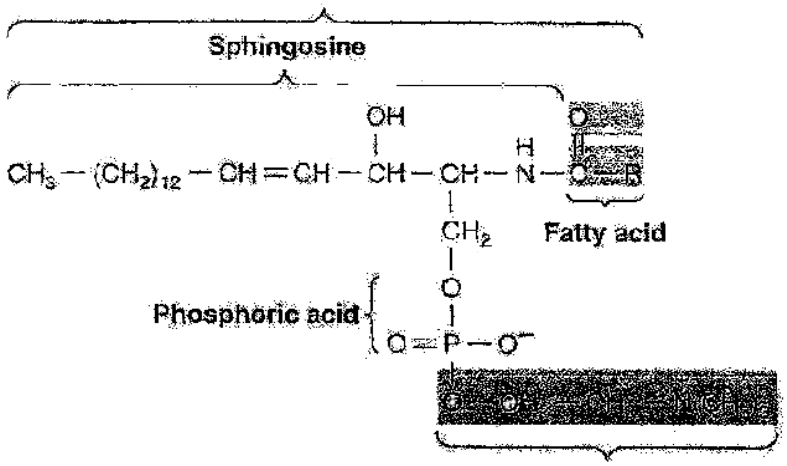


Fig.7.7: Triacyl-sn-glycerol

7.6. Ceramide

Sphingomyelins are found in large quantities in brain and nerve tissue. On hydrolysis, the sphingomyelins yield a fatty acid, phosphoric acid, choline and a complex amino alcohol, Sphingosine (Figure 7.8). No glycrerol is present. The combination of sphingosine plus fatty acid is known as ceramide, a structure also found in the glycosphingolipids.

Galactosyl ceramide is a major glyco sphingolipid of brain and other nervous tissue. It contains a number of characteristic C_{24} fatty acids, e.g., cerebronic acid. Galactosylceramide (Figure 7.8) can be converted to sulfogalacto sylceramide (Sulfatide), present in high amounts in myelin.



Choline

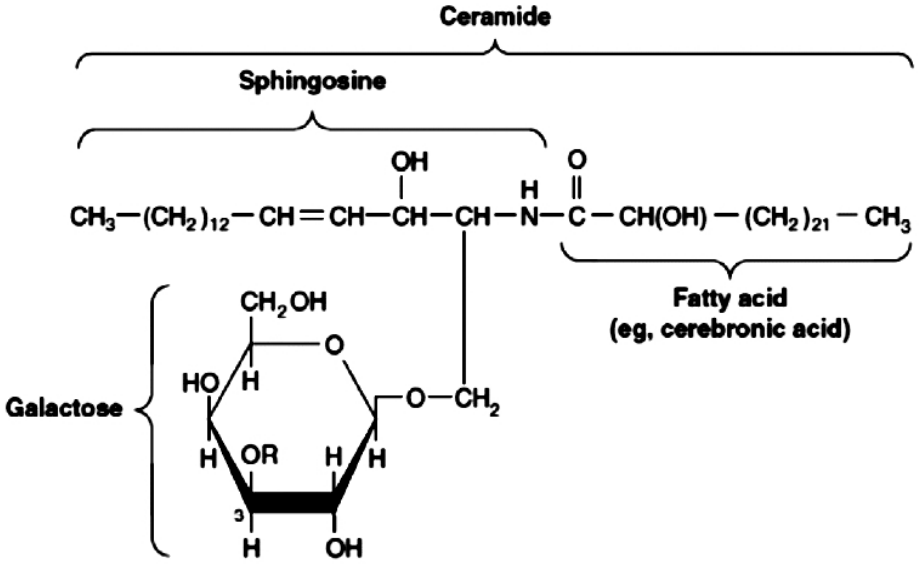


Fig. 7.8: Galactosylocerami

7.7. Structure of fatty acids and their derivatives

Compound lipids contain other elements such as sulphur, phosphorus, or nitrogen, in addition to the carbon, hydrogen, and oxygen of the simple lipids. Some compound lipids are described as follows :

Phospholipids : These are compound lipids, containing two fatty acids (diglycerides) attached to the glycerol molecule, Instead of the third fatty acid, they have a phosphate molecule, usually with an additional water-soluble molecule attached to it. Hence, they are also called glycerophosphatides. This chmical constitution makes the phospholipid molecule amphipathic, with a non-polar, water- insoluble part (the fatty acids) and a highly polar, water- soluble part (the rest of the molecule) as shown below :

glycerol + two fatty acids + phosphate + water soluble molecule

Thus, phospholipids are all phosphate esters of glycerol, with the following general formula :

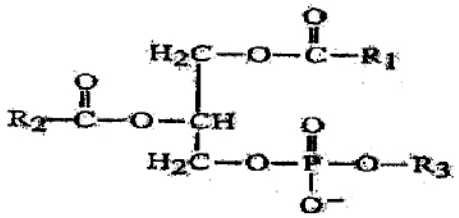


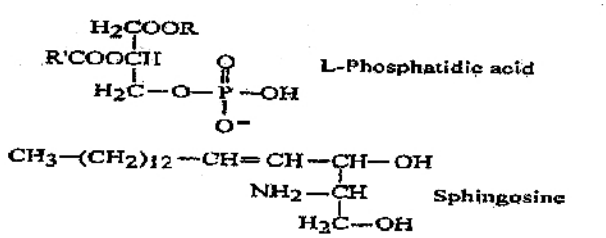
Fig.7.9: Phospholipids

R_1 and R_2 are fatty acids; R_3 represent a variety of residues attached to phosphoric acid. The phosphorus-containing portion of the phospholipid molecule carries one or more electric charges, so this portion

is hydrophilic (water-loving). The two fatty acid regions, however are hydrophobic (water- fearing). Thus, in a biological membrane, phospholipids line up in such a way that the nonpolar hydrophobic "tails" pack tightly together to form the interior of the membrane, and the phosphorus containing "heads" face outward where they interact with water, which is excluded from the interior of the membrane. The phospholipids form a bilayer, i.e., a sheet of two molecules thick.

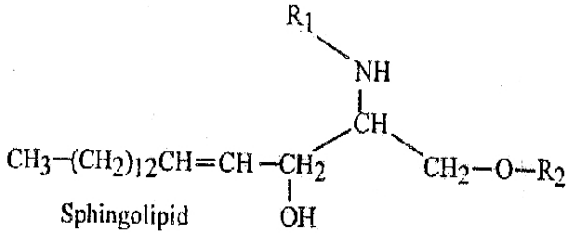
In cell membranes, there are five main phospholipids which differ according to the type of water-soluble molecule attached to the phosphate. The most abundant form is lecithin in which this additional molecule is choline, a vitamin

Therefore, lecithin is called choline phosphoglyceride. The phospholipids are bound into the structure of the cell membrane.



When the phosphate group has no attached molecules, the phospholipid is slightly less polar. This group of phospholipids includes the phosphoglycerides derived from phosphatidic acid.

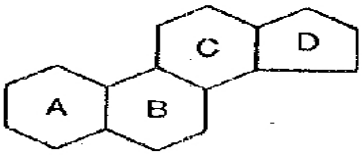
It has been suggested that it functions as a movable molecule within the cell membrane, acting as a carrier for ions.



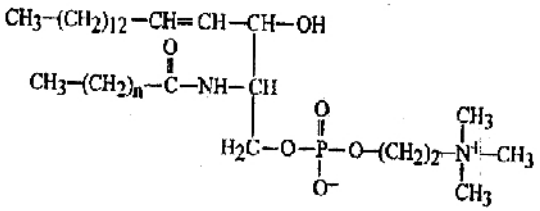
Sphingolipids: These are derivatives of sphingosine. an amino alcohol possessing a long unsaturated hydrocarbon chain.

Thus, the structure common to all sphingolipids is a sphingosine residue on which two other residues (R_1 and R_2) are substituted as shown here. (R_1 may be a fatty acid while R_2 can vary widely). The myelin sheath surrounding many nerve cells is particularly rich in the sphingolipid and sphingomyelin.

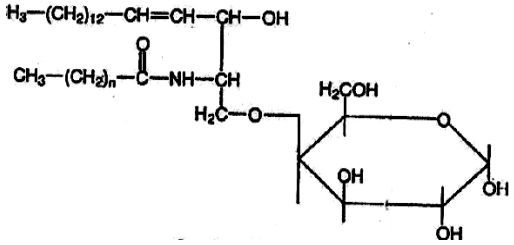
In sphingomyelin, the amino group of the sphingosine skeleton is linked to a fatty acid and the hydroxyl group is esterified to phosphorylcholine.



Basic unit of all steroids



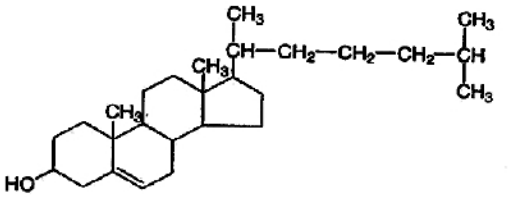
Sphingomyelin



Glycolipids: Glycolipids (and glycoproteins) occur in the plasma membrane. The former play vital roles in immunity, blood group specificity and cell-cell recognition. The lipid portion of a glycolipid is similar to sphingosine with the amino group of the sphingosine skeleton acylated by a fatty acid (as in sphingomyelin) and the hydroxyl group associated with the carbohydrate.

Cerebroside (a glycolipid): The simplest glycolipids are the cerebrosides, which, as the name suggests, are abundant ii the brain tissue where they occur in the myelin sheaths and may account for as much as 20% of the sheath's dry weight. The sugar of a cerebroside is either glucose or galactose. In the gangliosides of the nerve tissue, the carbohydrate portion of the molecule consists of a chain of sugar molecules usually including glucose, galactose, and neuraminic acid.

Steroids: These are fat-soluble derivatives of cyclopentanoperhydro-phenanthrene and play important roles in metabolic activities of the organism. They include cholesterol, vitamin D, cholic acid and a number of sex hormones:



Cholesterol

Cholesterol: The most common animal steroid is cholesterol, found in all cell membranes, in the myelin sheath of nerves and in many lipids. From the Greek hole and stereos followed by the chemical suffix 01 for an alcohol, is an organic molecule. A complete cholesterol test finds the sums of "good" and "bad" cholesterol. A complete Cholesterol test is also called "lipid profile". Cholesterol levels are measured in milligrams (mg) of cholesterol per deciliter (dL) of blood.

Ideal results in most adults are :

i. **LDL:** 70-130 md/dL (the lower number the better)

ii. **HDL:** more than 40-60 mg/dL (the higher the number the better)

iii. **Total Cholesterol** : less than 200 mg/dL (the lower the number, the better)

iv. **Triglycerides** : 10-150 mg/dL (the lower the number the better)

If your cholesterol numbers are outsides the normal range, you may be at higher risk of heart disease and atherosclerosis. It is also the metabolic precursor of most steroid hormones. In its structure, there is phenanthrene nucleus (rings A, B and C) and the attached cyclopentane ring (D) characteristic of all steroids. Cholesterol gives rise to three basic types of steroid hormones:

(a) Androgens (C19 compounds)

(b) Estrogens (C18 compounds)

(c) Progesterones and corticosterones (C21 compounds).

The blood contains cholesterol (215 mg/liter) as free cholesterol and cholesterides (esters of cholesterol with fatty acids). Cholesterol is also present in bile (0.6 to 1.7 g/liter). The concentration of the bile which occurs in the gall bladder may lead to a further rise in the cholesterol concentration and crystallization. Cholesterol crystals in turn form nuclei for the crystallization of bile pigments and calcium salts (bile stones).

A diet high in saturated fats and cholesterol can lead to reduced blood flow caused by the deposit of fatty materials on the linings of blood vessels. Cholesterol is ferried in the blood by two types of plasma proteins

- LDL and HDL. LDL (called bad" or low density lipoprotein) transports cholesterol to the tissues from the liver, and HDL (called "good" or high-density lipoprotein) transports cholesterol out of the tissues to the liver. When LDL level in blood is abnormally high or the HDL level is abnormally low, cholesterol accumulates in the cells. When cholesterol-laden cells line the arteries, plaque develops; it bulges out into the lumen of an artery, obstructing blood flow. Individuals with a high level (240 mg/100 ml) should always be further tested to determine what their LDL blood cholesterol is. If the LDL level is 160 mg/100 ml or higher, and/or if the total cholesterol-to-HDL cholesterol is higher than 4.5, the person is considered at risk.

7.8. Physiological role of lipids

Fats and fatty acids are important food storage compounds in most organisms. The fat is stored in the adipose tissue. Like carbohydrates and proteins, fats also play significant roles as structural components of cells. For example, certain phospholipids form an important component of the cell membranes and of enzyme systems in the mitochondria. Phosphatides have also been considered essential for the formation of one of the blood clotting factors. Phospholipid, cephalin helps in the formation of prothrombinase which converts prothrombin into thrombin during blood coagulation. Some complex lipids are also found in the brain and nerve tissue and in the heat and skeletal muscles. Besides, the oxidation of fats provides a large amount of energy to the body cells. The oxidation of 1 gm fat yields about 9300 calories whereas 1 gm of protein or carbohydrate releases 4100 calories.

The animal fats are the most important sources of some of the vitamins, i.e., A and D. The steroids are also of great physiological importance and the cholesterol is the main precursor of steroid hormones, (e.g., estrogen progesterone, corticosterone) which affect cellular activities by influencing gene expression. Some steroids are vitamins (e.g., vitamin D_2) and influence the activities of certain cellular enzymes. Other steroids are regular constituents of membranes, where they influence membrane structure, permeability and transport.

7.9. Summary

The fatty acids may be defined as those monocarboxylic long chain acids which have more than four carbons and are relatively insoluble in water. Compound lipids contain other elements such as sulphur, phosphorus, or nitrogen, in addition to the carbon, hydrogen, and oxygen of the simple lipids. This, saturated acids end in -anoic, e.g., octanoic acid and unsaturated acids with double bonds end in -enoic, e.g., octadecenoic acid (Oleic acid). The most common animal steroid is cholesterol, found in all cell membranes, in the myelin sheath of nerves and in many lipids. These characteristics distinguish the living from the non-living

and are the criteria by which we measure the dynamic aspects of the protoplasmic system.

7.10. Terminal questions

Q.1. What is an amino acid? How amino acids are arranged in a protein?

Answer:----------

Q.2. What is a protein? Describe the levels of structure of protein molecules.

Answer:----------

Q.3. Write about saturated and unsaturated fatty acids.

Answer:----------

Q.4. Write biological properties of protoplasm.

Answer:----------

Q.5. Write a short note on the following:

(a) Cholesterol

(b) Hormones

(c) Protoplasm

(d) Phospholipids

Answer:----------

Q.6. Complete the following sentences :

(a) Glycogen is often called _____ ____.

(b) _____ ____is a monosaccharide.

(c) _____ ____the amino acid sequence is one, aspect of protein.

(d) Structure _____ _____ of the molecule is another.

Answer:----------

7.11. Further readings

1. Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Publishers and Distributors, Delhi.

2. Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company.

3. Cell Biology (Cytology, Biomolecules and Molecular Biology): P S Verma and V K Agarwal.

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5. Principle of Biochemistry: Nelson, D.L. & Cox, M.M. Lehninger. University of New Mexico and Karen Ocorr, University of California, San Diego.

UNIT - 8

Nucleic acids

Structure

8.1. Introduction

In unit 7, we described lipids, which are a class of biomolecules grouped together mainly on the basis of common solubility properties. In Unit 8, we shall study another type of vital biomolecules, the nucleic acids. These biomolecules are important component of living organisms. In this unit you will learn about the structure and biological importance of nucleic acids. You shall also learn about building blocks of nucleic acids, types of nucleic acids, helical structure of DNA and about RNA.

Objectives

After studying this unit you will be able to:

- Explain the structure of nucleic acid.

- Describe the properties of nucleic acid.
- Explain the functions of nucleic acid.
- Differentiate between DNA and RNA.
- Describe the function of various type of RNA.
- Explain the double helical model of DNA.

8.2. Nucleotides

Nucleotides are the building block of (monomer units) the nucleic acids. Nucleotide is united formed by the association of three components.

A Nitrogenous base (Nitrogen containing base)

A Pentose Sugar

A phosphate group

The molecule without the phosphate group is called a *nucleoside.* Presence of sugar and phosphate in nucleotides make the backbone of double helix DNA and bases are present in centre. This backbone is held together by chemical bonds (hydrogen bond) that are formed between the phosphate component of one nucleotide and the sugar component of the other nucleotide.

8.3. Nucleosides

The nucleosides are the compound in which nitrogenous bases conjugated to the pentose sugar (ribose or deoxyribose) by β glycosidic. The β glycosidic linkage involves the C-1' of sugar and hydrogen atom of N-9 (In the case of Purines) or N-1 (In case of pyrimidines), thus eliminating the water molecules. Therefore, in purnes, nucleosides are N-9 glycosides and in the pyrimidine, nucleosides are N-1 glycosides. Nucleosides are usually obtained by chemical or enzymatic decomposition of nucleic acids.

The nucleosides are generally named as the particular purine or pyrimidine present in it. Nucleosides containing ribose are called Ribonucleoside while those having deoxyribose as Deoxyribonucleoside.

8.4. Nucleic acid

Nucleic acid enzymes Aqueous NH_3 Nuclease

Sugar + Purines + Pyrimidines + Phosphoric acid

The nucleotides are the building blocks of the following three types of compounds:

1. **High-energy compounds:** The most important high energy molecule is the ATP, which is synthesized in the mitochondria of living cells mainly.

2. **Coenzymes:** Coenzymes are also chemical derivatives of nucleotides; A coenzyme is a carrier molecule that functions in conjunction with a particular enzyme. Important electron and hydrogen carrying cellular coenzymes are NAD^+, $NADP^+$, FMN and FAD.

3. **Nucleic acids**: Nucleic acids (DNA and RNA) are polymers of nucleotides. They are an especially significant group of compounds found in the nucleus (DNA) and in the cytoplasm (RNA). In the nucleus, they are found in the chromosome and are important in transmitting information from the nucleus to the cytoplasm. In the cytoplasm, they are most closely concerned with the synthesis of proteins. The DNA is the material that forms the genes, the cellular components that have long been recognized as the carriers of heredity, but now they are known to be the ultimate controllers of all metabolism, or the 'secrets of life'.

Other Functions of Nucleotides

In addition to their role as the sub units of nucleic acids, nucleotides have a variety of other functions, some of which are as follows:

1. **As energy carriers**: Nucleotides carry chemical energy in cells. The energy released by the hydrolysis of ATP and the other nucleotide triphosphate is accounted for the structure of triphosphate group.

2. **Components of enzyme co-factors**: They are unrelated structurally except for the presence of adenosine.

3. **As chemical messengers**: They serve as regulatory molecules. For example, C-Amp, formed from ATP in a reaction catalyed by adenylcyclase.

8.5. Structure of nucleic acids

The nucleic acids are long chain polymers built by a large number of units called nucleotides. These nucleotides joined by phosphodiester bonds spanning from the 5' position of one nucleotide to the 3' position of

the adjacent nucleotide. Every nucleotide is made up of an organic nitrogenous base, a pentose sugar and phosphate, as shown in Figure 8.1(a) within the nucleotide the combination of a pentose with a nitrogen base constitutes a *nucleoside*.

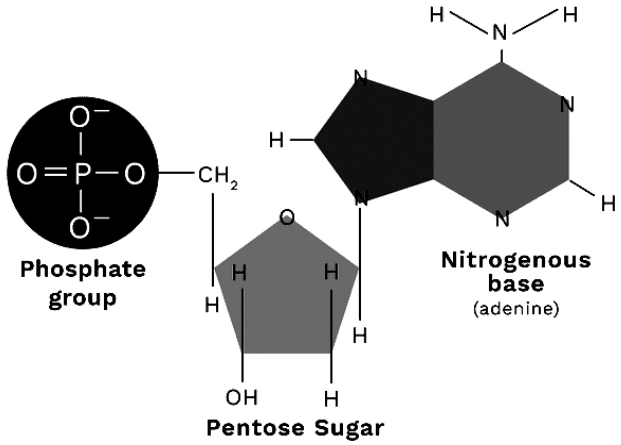


Fig 8.1: Three components of nucleotides

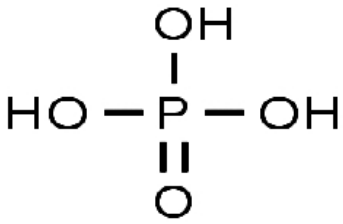
There are two kinds of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Both types of nucleic acids are present in all plants and animals. Viruses also contain nucleic acids; however, unlike a plant or animal, a virus has either RNA or DNA, but not both upon hydrolysis, the nucleic acids yield 3 components: Phosphoric acid, a pentose sugar and nitrogenous base.

Table 8.1: summarizes the structural components of nucleic acids.

Components	Ribonucleic acid	Deoxyribonucleic acid
Acid	Phosphoric acid	Phosphoric acid
Pentose Sugar	ribose	deoxyribose
Nitrogenous bases		
Purines	Adenine, Guanine	Adenine, Guanine
Pyrimidines	Cytosine, Uracil	Cytosine, Thymine

- **Phosphoric Acid**

The molecular formula of phosphoric acid is H_3PO_4. It contains 3 mono valent hydroxyl group and a divalent oxygen atom, all linked to the pentavalent phosphorus atom.



Phosphoric acid

- **Pentose Sugar**

The two types of nucleic acids are distinguished primarily on the basis of the pentose (5-carbon) sugar which they possess. One possesses deoxyribose, hence the name deoxyribose nucleic acid or deoxy ribo nucleic acid, while the other contains ribose, hence the name ribose nucleic acid or ribonucleic acid.

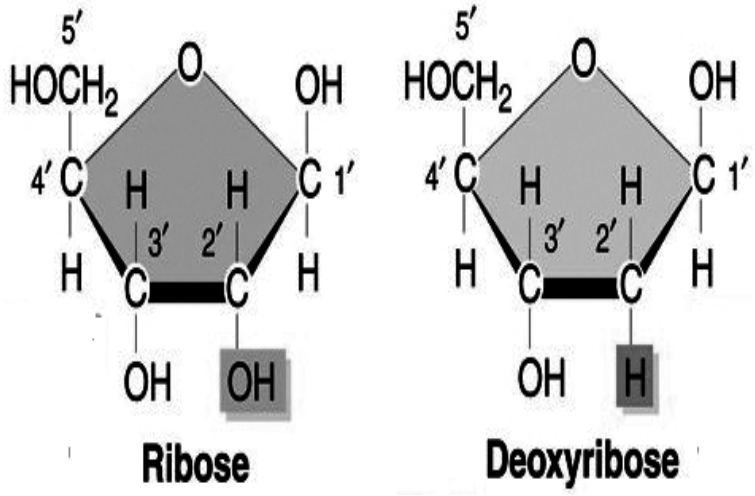


Fig 8.2: The two Pentose Sugars present in the RNA and DNA

- **Nitrogenous Bases**

Two types of nitrogenous bases are found in all nucleic acids.

- **Pyrimidine**

These are all derived from their parent heterocyclic compound Pyrimidine, which contains a six membered ring with two nitrogen atoms and three double bonds. It has a melting point of 22ºC and a boiling point of 123.50ºC. The common pyrimidine derivatives found in nucleic acids are uracil, thymine and cytosine (**Fig.** 8.3).

- **Uracil ($C_4H_4O_2N_2$) :** Found in RNA molecules only MW = 112.10 Daltons and melting point 338ºC.
- **Thymine ($C_5H_6O_2N_2$)** : Found in DNA molecules only MW = 126.13 Daltos. It was first isolated from thymus, hence called as Thymine.
- **Cytosine ($C_4H_5ON_3$)** : Found in both RNA and DNA MW = 111.12 Daltons.

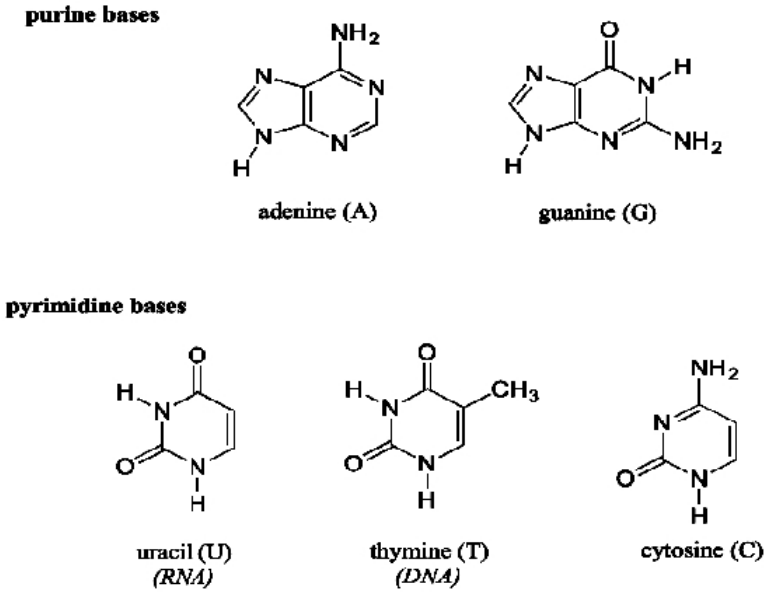


Fig 8.3: Nitrogenous Bases (purine and pyrimidine base)

- **Purine Derivatives**

These are all derived from their parent compound pyrine, which contains a six-membered pyrimidine ring fused to the five membered imidazole ring and is related to uric acid. It has melting point of 216ºC. The purine derivatives found in nucleic acids are adenine and guanine (Fig.)

- **Adenine ($C_5H_5N_5$)**: Found in RNA and DNA, MW = 135.15 Daltons and melting point 360 - 365ºC.
- **Guanine ($C_5H_5ON_5$):** Found in both RNA and DNA. it is colourless, insoluble in water, a white amorphous crystalline substance. MW = 151.15 Daltons. It was first isolated from guano (bird manure) hence named guanine.

8.6. Structure of DNA

The molecular organization of DNA was explained by Watson and Crick, a concept for which James D. Watson of the United States, Francis H.C. Crick and Maurice H.F. Wilkins of England Shared the Nobel Prize of 1962. According to Watson and Crick model, the DNA molecule consists of two anti-parallel polynucleotide strands twisted around each other like a 2-ply wool to form a double-stranded helix or spiral.

In the individual strands, the adjacent nucleotides are linked with each other by sugar-phosphate bonds. Each phosphate links the 3'-carbon atom on one sugar to the 5' carbon atom on the sugar of the next nucleotide forming 3'-5' phosphodiester bonds. The nitrogenous bases are attached through one of their nitrogens by an N-glycosidic bond to carbon 1' of the pentose sugar.

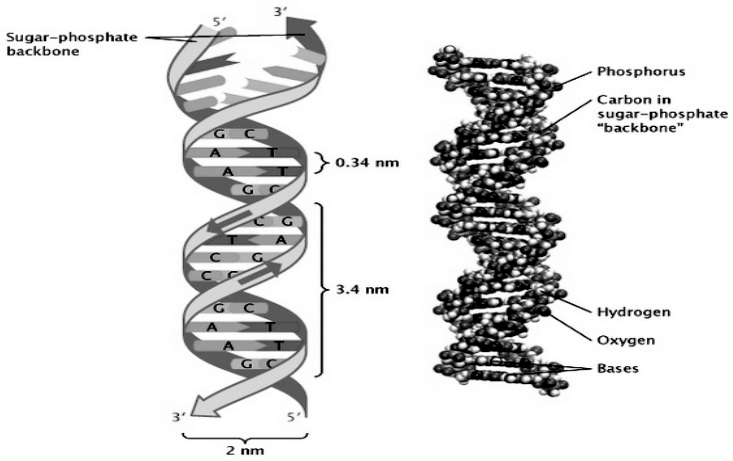


Fig.8.4: DNA model as proposed by Watson and Crick

Source:https://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397/

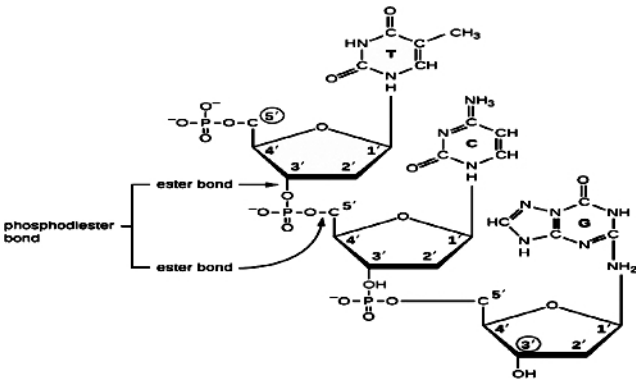


Fig 8.5: The chemical formula of a deoxyribonucleotide

The schematic diagram of nucleotide chain as shown below is frequently useful; it can be drawn either in a horizontal or vertical direction.

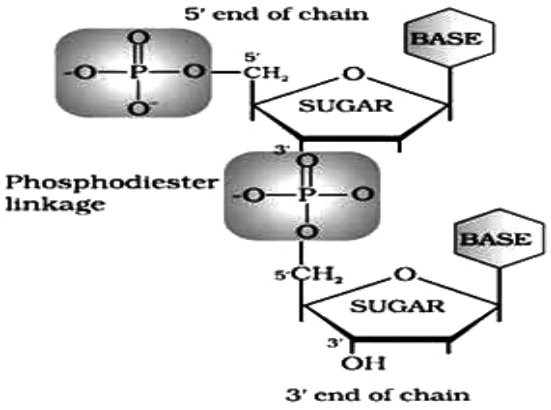


Fig.8.6: Schematic diagrams of nucleotide chain, 3' and 5' are the carbons of the pentose sugar.

The two polynucleotide strands of the DNA molecule are anti-parallel. Chemically, they are arranged in opposite directions, i.e., the structure P-5' - sugar - 3' - P opposes the structure P - 3' - sugar - 5' - P. Consequently, when the chains are traced from 5' end to 3' (5'3') the direction is up the helix on one chain and down it on the other (Fig.8.6).

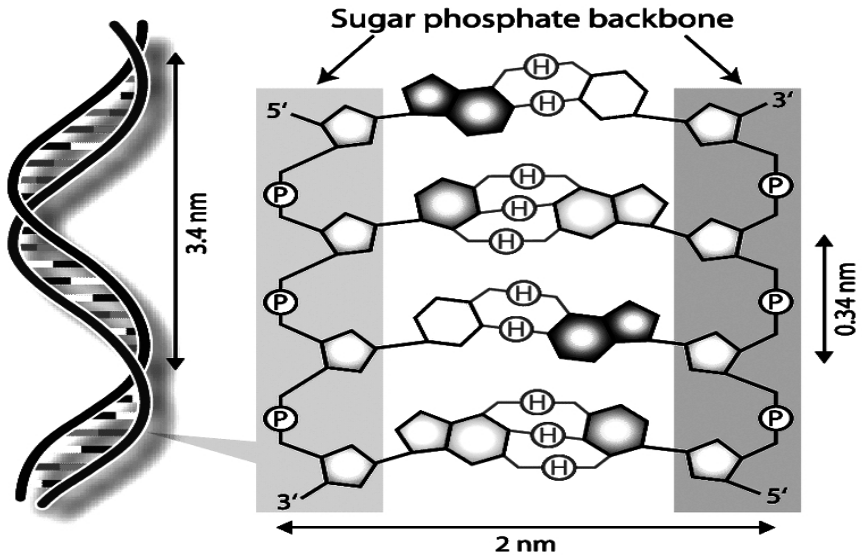


Fig. 8.7: Segment of a DNA molecule showing anti parallel orientation of the complementary chains.

The two polynucleotide strands of the DNA molecule are linked within the helix by hydrogen bonds between pairs of bases. In the formation of these hydrogen bonds, adenine (A) always pairs with thymine (T), and cytosine (C) with guanine (G). Thus, the linking is invariably A-T and C-G. This has been described as base-pairing of the Watson and Crick model. Obviously, pyrimidines join only with purines. Only these arrangements are possible because two purines will occupy too much and two pyrimidines too little space to allow the formation of a helix. Therefore, if the base order in one strand is known, the sequence of bases in the other strand can be predicted. For instance, if one strand has a region which goes thymine, guanine, cytosine, adenine, then the corresponding region in the complementary strand will go adenine, cytosine, guanine, and thymine.

The hydrogen bonds, described above, are formed by the sharing of hydrogen atoms of the two members of a base. In case of A-T linkage there are two hydrogen bonds, whereas in the C-G linkage there are three such bonds. The hydrogen bonds holding the purines and pyrimidines are weaker than the bonds holding these to the sugar-phosphate groups. Therefore, the DNA molecule easily splits into its two strands.

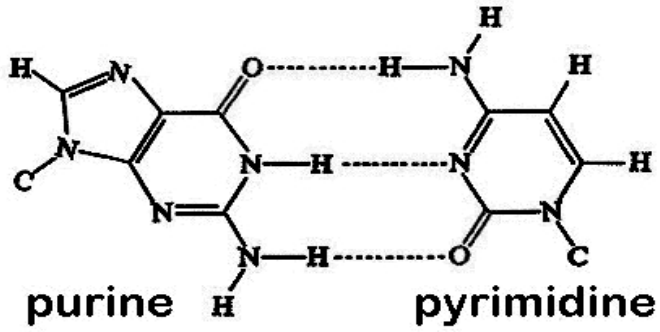


Fig.8.6: Possible hydrogen bonding between the bases of DNA

In short, the full structure of DNA resembles a winding stair-case, with the sugar and phosphate molecules of the nucleotides forming the railings and the linked nitrogen bases (either adenine thymine or guanine-cytosine) forming the steps. Deoxyribonucleic acid molecules may be very large. For example, in the bacterium *Escherichia* coli, DNA is about 1000 m (1 mm) in length, and contains about 4,000,000 base pairs. Plant and animal cells may possess DNA molecules which are even longer.

From the base-pairing rule it follows that the A : T (A/T) and G : C (G/C) ratio should always be 1 : 1 on any DNA molecule. But the (A+T) : (G+C) is not always one. Higher plants and animals have an excess of A+T over (G+C) in their DNA content. For example, the (A+T) : (G+C) in wheat is 122, in man it is 1.40. But among the viruses, bacteria, and lower plants there is much variation; both (A+T) - rich and (G+C) - rich DNAs have been reported. For instance, in *Pseudomonas aeruginosa* and *Bacillus megaterium* the (A+T) : (G+C) are 0.51 and 1.66, respectively.

The sequence of nucleotides on the DNA strand determines the nature of the offspring. Therefore, the exact sequence of nucleotide base in DNA of an organism is transmitted unchanged from one generation to the next. To ensure this, DNA must be copied very exactly during the life cycle of a cell before division. This process is known as replication.

DNA Exists in Relaxed & Super coiled Forms

In some organisms such as bacteria, bacteriophages, many DNA-containing animal viruses, as well as organelles such as mitochondria, the ends of the DNA molecules are joined to create a closed circle with no covalently free ends This of course does not destroy the polarity of the molecules, but it eliminates all free 3’ and 5’ hydroxyl and phosphoryl groups. Closed circles exist in relaxed or super coiled forms. Supercoils are introduced when a closed circle is twisted around its own axis or when a linear piece of duplex DNA, whose ends are fixed, is twisted. This energy-requiring process puts the molecule under tensional stress, and the greater the number of super- coils, the greater the stress or torsion (test this by twisting a rubber band). Negative supercoils are formed when the molecule is twisted in the direction opposite from the clockwise turns of

the right-handed double helix found in B DNA. Such DNA is said to be underwound. The energy required to achieve this state is, in a sense, stored in the super coils. The transition to another form that requires energy is thereby facilitated by the under winding. One such transition is strand separation, which is a prerequisite for DNA replication and transcription. Supercoiled DNA is therefore a preferred form in biologic systems. Enzymes that catalyze topologic changes of DNA are called topoisomerases. Topoisomerases can relax or insert supercoils. The best-characterized example is bacterial gyrase, which induces negative supercoiling in DNA using ATP as energy source. Homologs of this enzyme exist in all organisms and are important targets for cancer chemotherapy.

DNA provides a template for replication and transcription

The genetic information stored in the nucleotide sequence of DNA serves two purposes. It is the source of information for the synthesis of all protein molecule of the cell and organism, and it provides the information inherited by daughter cells or offspring. Both of these functions require that the DNA molecule serve as a template - in the first case for the transcription of the information into RNA and in the second case for the replication of the information into daughter DNA molecules.

The complementarily of the Watson and Crick double-stranded model of DNA strongly suggests that replication of the DNA molecule occurs in a semi-conservative manner. Thus, when each strand of the double-stranded parental DNA molecule separates from its complement during replication, each serves as a template on which a new complementary strand is synthesized. The two newly formed double-stranded daughter DNA molecules, each containing one strand (but complementary rather than identical) from the parent double-stranded DNA molecule, are then sorted between the two daughter cells. Each daughter cell contains DNA molecules with information identical to that which the parent possessed; yet in each daughter cell the DNA molecule of the parent cell has been only semi-conserved.

Ribonucleic acid (RNA) is a polymer of purine and pyrimidine ribonucleotides linked together by 3’,5’- phosphodiester bridges analogous to those in DNA. Although sharing many features with DNA, RNA possesses several specific differences:

1. In RNA, the sugar moiety to which the phosphates and purine and pyrimidine bases are attached is ribose rather than the deoxyribose of DNA.

2. The pyrimidine components of RNA differ from those of DNA. Although RNA contains the ribonuclcotides of adenine, guanine, and cytosine, it does not possess thymine except in the rare case mentioned below. Instead of thymine, RNA contains the ribonucleotide of uracil.

3. RNA exists as a single strand, whereas DNA exists as a double-stranded helical molecule. However, given the proper complementary base

sequence with opposite polarity, the single strand of RNA is capable of folding back on itself like a hairpin and thus acquiring double-stranded characteristics.

4. Since the RNA molecule is a single strand complementary to only one of the two strands of a gene, its guanine content does not necessarily equal to its cytosine content, nor does its adenine content necessarily equal to its uracil content.

5. RNA can he hydrolyzed by alkali to 2',3' cyclic diesters of the mononucleotides, compounds that cannot be formed from alkali-treated DNA because of the absence of a 2'-hydroxyl group. The alkali liability of RNA is useful both diagnostically and analytically.

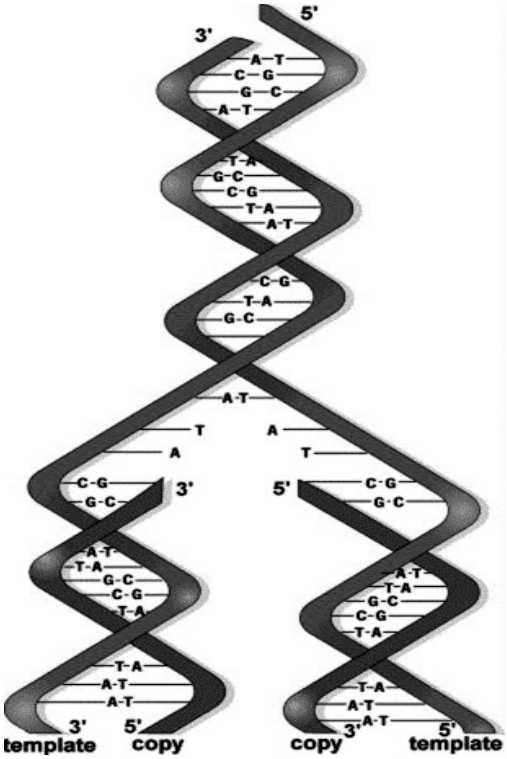


Fig 8.7: Double-stranded structure of DNA

The double-stranded structure of DNA and the template function of each old strand (dark shading) on which a new complementary strand (light shading) is synthesized.

8.7. Types of RNA

Those cytoplasmic RNA molecules that serve as tiny plates for protein synthesis (ie, that transfer genetic information from DNA to the protein-synthesizing machinery) are designated as messenger RNAs, or mRNAs. Many other cytoplasmic RNA molecules (ribosomal RNAs; rRNAs) have structural roles wherein they contribute to the formation and functions of ribosomes (the organellar machinery for protein synthesis) or serves as adapter molecules (transfer RNAs; tRNAs) for the translation of

RNA information into specific sequences of polymerized amino acids. Some RNA molecules have intrinsic catalytic activity. The activity of these ribosomes often involves the cleavage of a nucleic acid. An example is the role of RNA in catalyzing the processing of the primary transcript of a gene into mature messenger RNA.

Messenger RNA (mRNA)

The Messenger RNA (mRNA) is synthesized during the process of transcription. In which sequence of bases in one strand of chromosomal DNA is enzymatically transcript in the single strand of mRNA. In process of transcription, DNA is transcript into an RNA message during a protein synthesis that involves copying the genetic information.

Transfer RNA(t-RNA)

Transfer RNA (tRNA) plays an important role in the translation process of protein synthesis. Its job is to translate the message within the nucleotide sequences of mRNA into specific amino acid sequences. The amino acid sequences are joined together to form a protein. Transfer RNA is shaped like a clover leaf with three hairpin loops.

Ribosomal RNA (r-RNA)

Ribosomal RNA (rRNA) is a component of cell organelles called ribosomes. A ribosome consists of ribosomal proteins and rRNA. Ribosomes are typically composed of two subunits: a large subunit and a small subunit. Ribosomal subunits are synthesized in the nucleus by the nucleolus. Ribosomes contain a binding site for mRNA and two binding sites for tRNA located in the large ribosomal subunit

8.8. Summary

Nucleic acid is most important molecules that define the continuity of life. Generally nucleic acid form by the combinations of nitrogen bases, sugar and phosphate bond. DNA and RNA are two main nucleic acids carry the genetic blueprint of a cell. The two types of nucleic acids are distinguished primarily on the basis of the pentose (5-carbon) sugar which they possess. Nucleotide, the monomer comprising DNA or RNA molecules, consists of a nitrogenous heterocyclic base that can be a purines or pyrimidine, a five-carbon pentose sugar, and a phosphate group. Adenine and guanine are classified as purines. The primary structure of a purine consists of two carbon-nitrogen rings. Cytosine, thymine, and uracil are classified as pyrimidines which have a single carbon-nitrogen ring in their primary structure. DNA has a double-helix structure, with sugar and phosphate on the outside of the helix, forming the sugar-phosphate

backbone of the DNA. Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. The Messenger RNA (mRNA) is synthesized during the process of transcription Transfer RNA (tRNA) plays an important role in the translation portion of protein synthesis. Ribosomal RNA (rRNA) is a component of cell organelles called ribosomes.

8.9. Terminal questions

Q.1. What are nucleotides and nucleostides?

Answer:----------

Q.2. Role of nitrogenous base in formation of nucleotides

Answer:----------

Q.3. What do you understand by RNA? Write different types of RNA.

Answer:----------

Q.4. What do you men by nucleic acid? describe it?

Answer:----------

Q.5. Roles of phosphate in Nucleotides.

Answer:----------

Q.6. Write role of sugar in nucleotides.

Answer:----------

8.10. Further readings

1. The Fat-solubel Vitamins, Deluca, H.F. (ed.), 1978, , Plenum, New York.

2. The Fat-soluble Vitamins, Deluca, H.F. and Suttie, J.W. (eds.), 1970, University of Wisconsin Press, Madison.

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UNIT-9

Vitamins

Structure

9.1. Introduction

Vitamins are organic compounds that are essential in very small amounts for supporting normal physiologic function. We need vitamins in our diet, because our bodies can't synthesize them quickly enough to meet our daily needs. Vitamins have three characteristics: They are natural components of foods; usually present in very small amounts. They are essential for normal physiologic function (eg. growth, reproduction, etc). When absent from the diet, they will cause a specific deficiency Vitamins are generally categorized into the following types water soluble and fat soluble. This unit covers type's structure and functions of some important vitamins.

Objectives

- Determination and structure and function of vitamins.
- Define nature of water soluble and insoluble vitamins.
- Deficiency, diseases and symptoms.
- Role of vitamins in human being

9.2. Vitamins overview

Vitamins are an assorted group of chemicals essential for the maintenance of normal metabolic functions. Most of these are not synthesized in the body and must, therefore, be present in the diet. Nearly seventy years ago, it was thought that a diet consisting only of proteins, fats, carbohydrates and some inorganic substances was enough for sustaining normal life. Nevertheless when an artificial diet consisting of these materials was given to rats they died after sometime. However, when small amount of milk was added to the above diet the rats did not die. The unspecified substances provided by milk were called accessory food factors. About the same time it was also found that exclusive eating of polished rice (rice in which the pericarp or bran was removed) caused a disease known as beri-beri. Addition of polishings to the diet cured this disease. This discovery led to the concept of deficiency diseases. Funk (1911) isolated a concentrate from rice polishings which was found to contain nitrogenous bases (amines). As these substances were vital for sustaining life, they were called vitamins. Later, when it was discovered that all these essential substances, were not exclusively amines the letter 'e' was dropped and the name (vitamin was given to all the essential substances which must be present in the diet in small quantities and whose deficiency causes disease. The vitamins are divided into two major categories - (a) fat soluble, and (b) water soluble vitamins.

Table: 9.1. List of Vitamins

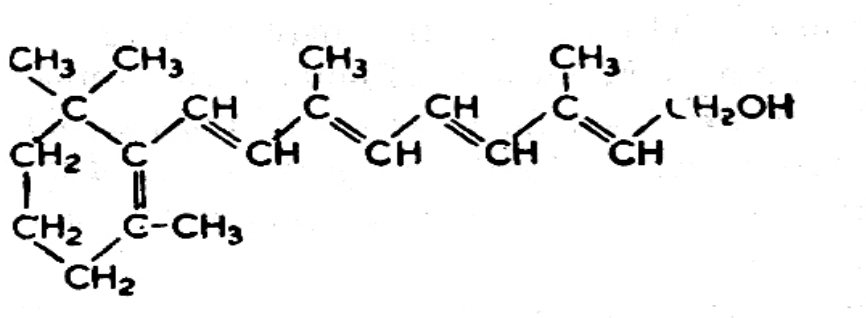
Fat soluble vitamins	Water soluble vitamins
Vitamin A	Thiamine - Vitamin B_1
Vitamin D	Riboflavin - Vitamin B_2
Vitamin E	Nicotinic acid - Niacin
Vitamin K	Pyridoxine - Vitamin B_8
Vitamin C	Pteroyl glutamic acid - Folic acid
Lipoic acid	Pantothenic acid
	Cyanocobalamin - Vitamin B_{12}
	Biotin
	Choline
	Inositol
Para amino benzoic acid	PABA (All these along with Lipoic acid form Vitamin B complex)

The knowledge of the chemical structure, physiological role, deficiency diseases and available sources of vitamin is essential in nutritional studies. A detailed description of the different vitamins follows:

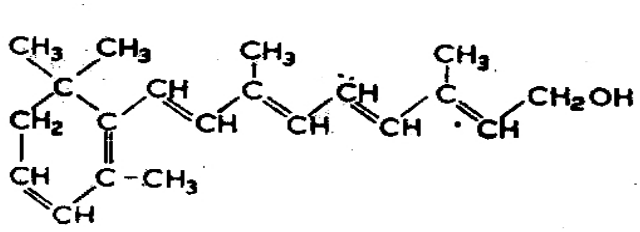
9.3. Fat soluble vitamins

9.3. 1. Vitamin - A (Antixeropthalmic vitamin)

Vitamin A is a primary unsaturated alcohol which exists in nature in two forms, vitamin A_1 and vitamin A_2 called retinol and dehydroretinol respectively.



Retinol - Vitamin A_1



(3, Dehydroretinol)

Vitamin A exists in several cis-trans isomers which are easily interconvertible in the body. This vitamin is essential for vision and for the maintenance of the various epithelial cells. The rods of the retina contain a pigment rhodopsin or visual purple. Rhodopsin is a combination of the protein opsin and a compound called retinene. Retinene in fact is the aldehyde of cis vitamin A. Similarly the cones of the retina contain another pigment called iodopsin. This pigment is responsible for colour vision. It is made of phoropsin and retinene. When human beings are fed on diets deficient in vitamin A their ability for dark adaptation is lost which results in night blindness. Severe deficiency of vitamin A causes keratinization of cornea; cells resulting in a disease called rexopihalmia.

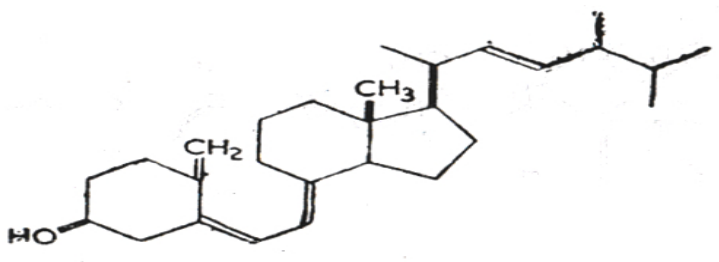
Absence of vitamin A also causes atrophy of epithelial cells. In the alimentary canal its deficiency results in the damage of intestinal mucosa resulting in diarrhoea. Defect in the dentine of the teeth or even its absence is caused by acute deficiency of vitamin A.

The daily human requirement of vitamin A is 6,000 international units I.U. = 0.30 micrograms). Very high consumption of vitamin A, on the other hand, causes a disease hypervitaminosis A. This is marked by deep swellings in the forearms, shanks and feet which are very painful.

Most vitamin A is stored in the liver. Cod liver oil, halibut liver oil etc. are commercially sold as sources of this vitamin. In addition to the above, edible fats like milk, butter, ghee, etc. also contain vitamin A. This vitamin is slowly destroyed in the fats and oils even at room temperature. Plants too, contain vitamin A in the form of pigments. Two such sources of the vitamin are β-carotene and cryptoxanthin from the yellow-green parts of plants, which are converted into vitamin A by the liver and alimentary canal.

9.3.2. Vitamin D (Calciferol)

There are at least ten different substances possessing properties of vitamin D. All these have a general structure similar to the sex hormones and cortisones, and are derivatives of cyclopentaphenanthrene. With the help of ultraviolet radiation present in the sun rays vitamin D is synthesized in the skin by molecular rearrangement in already existing sterols. The two most important sterols which are converted into the vitamin are ergosterol and 7-dehydroxycholesterol. These two on exposure to ultraviolet rays, change into vitamin D_2 (calciferol) and vitamin D_3 respectively.



Vitamin D_2 (Caleiferol)

Vitamin D_2 helps in maintaining th levels of calcium and phosphorous in the body and is involved in resorption of calcium from calcified bones by osteolytic cells. It also increases the permeability of the intestinal mucosa to calcium which helps to raise the blood calcium level of the body. Vitamin D along with parathyroid hormone and calcitonin is crucial for the metabolism of calcium and phosphorous.

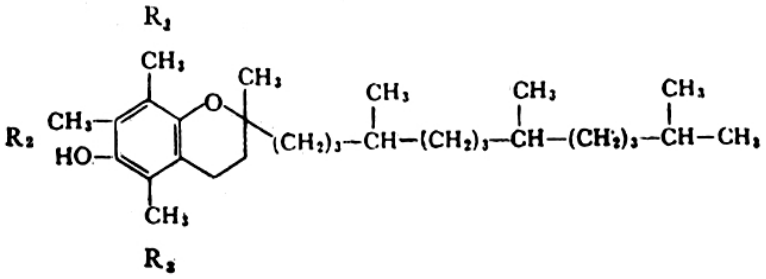
Deficiency of vitamin D in children (6 months to 3 years in age causes a disease called rickets (rachitis, hence the name antirachitic for vitamin D. This disease is characterised by deformities of the skeleton especially the limbs, spine bones and skull. Rickets can be cured rapidly by the administration of vitamin D. Since ultraviolet radiation helps in the synthesis of vitamin D in the body, exposure of rickety children to sunlight may help in the cure of the disease. Another ricket-like disease osteomalcia is found in adults in India and China.

The daily human requirement of vitamin D is 500 LU. (One I.U. is equivalent to 0.025 microgram of vitamin D_3 contained in U.S.P. vitamin D reference standard), Excess vitamin D (150,000 I.U./day or more) causes a disease called hypervitaminosis D. In this, the bones become weak (osteoporosis) due to calcium resorption and there is calcification of soft tissue especially the kidney, heart, lungs etc.

The important sources of vitamin D are the liver oils of tunny, halibut, cod and other fishes. Of these, tunny liver oil is the richest source of this vitamin. Besides these egg yolk and milk also contain vitamin D.

9.3.3. Vitamin E (Tocopherols)

There are three types of vitamin E (tocopherols). These are ∝, β and γ tocopherols. The structure of ∝ tocopherol is given below.



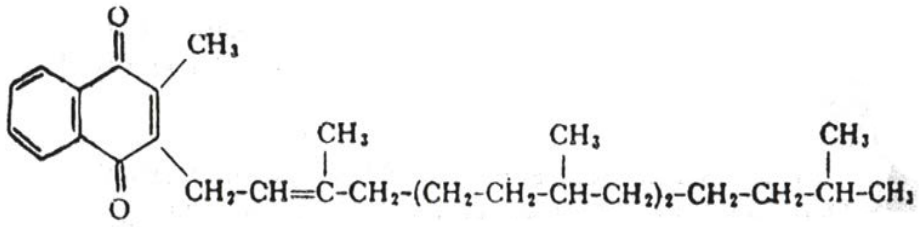
∝-tocopherol

The β and γ tocopherols differ from the ∝ compound in having two instead of three methyl groups. The tocopherol has only R_2, R_3, R_1 are H. The γ tocopherol has R_1, R_2, R_3 are H. Of these the tocopherol is biologically active. Its deficiency in experimental animal causes degeneration of the germinal epithelium of both sexes. In males it results in immobile sperms. Although vitamin E deficient females can have

fertilization, the embryo gets aborted after some time. Vitamin E, therefore, is also referred to as antisterility vitamin. Its utility in human beings has not been conclusively proved. Vitamin E is found in most of vegetables, milk and meat. It also is found abundantly in vegetable oils. Of these wheat germ oil is the richest source of vitamin E.

9.3.4. Vitamin K

Vitamin K occurs in two forms, vitamin K_1 (farnoquinone) and vitamin K_2 (phylloquinone). Both of these contain a 2 methyl 1, 4 naphthoquinone. They differ from each other in their side chain R.



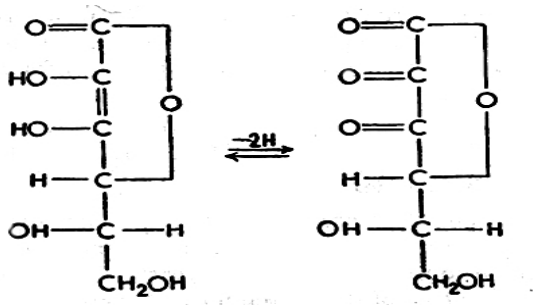
2-methyl-1, 4 naphthoquinone

Vitamin K plays an important role in blood coagulation. Deficiency of vitamin K reduces the concentration of prothrombin and factors VII, IX and X in the liver. As these factors are essential for blood clotting, deficiency of vitamin K increases the clotting time. New born infants deficient in vitamin K are in danger of bleeding to death. Administration of the vitamin in such cases may prevent the tragedy.

The amount of vitamin K required by an individual is not known. However, a normal diet provides enough of the vitamin.. Vitamin K is abundantly found in green vegetables like spinach, cauliflower, cabbage and alphalpha. Besides this, the intestinal bacteria can also synthesize this vitamin.

9.3.5. Vitamin C (Ascorbic Acid)

Ascorbic acid has an asymmetrical carbon and is, therefore, found both in L and D forms. However, only the L-isomer is found in nature and has biological activity. Ascorbic acid is a powerful reducing agent as it gives a pair of hydrogen atoms and gets oxidized to dehydroascorbic acid. Because of this property ascorbic acid is unstable and gets easily oxidised during cooking. Freshly chopped vegetables and fruits contain an enzyme ascorbic acid oxidase which is capable of oxidising this vitamin. The exact mode of action of ascorbic acid is not known. However, it is thought that vitamin C may have an important role in maintaining the SH-containing enzymes in the reduced form. In high concentration in the adrenals, it prevents epinephrine from being oxidized. Besides this, vitamin C may also have a role in the synthesis of adreno-corticotrophic hormone (ACTH). It is essential for the maintenance of collagen fibres, epithelial tissue, bone matrix and capillary lining. Vitamin C is both water and fat soluble. It has the following structural formula:



Ascorbic acid Dedydroascorbic acid

(Vitamin C)

In case of vitamin C deficiency the capillary linings develop leaks causing haemorrhage. The deficiency of this vitamin causes a disease called scurvy. This is characterized by spongy and bleeding gums, bleeding under the skin and extreme weakness. Since Vitamin C is continuously oxidised in the body, the daily requirement of the vitamin is rather high. 10 mg/day of the vitamin is enough to prevent scurvy. As a safety margin 30 mg/day is suggest. ed for a healthy adult. An intake of 70 mg/day should, therefore, make enough allowance for destruction by cooking.

Ascorbic acid is found in diffruits like strawberry, oranges and lemons. All citrus fruits are a rich source of vitamin C. Vegetables like cauliflower, cabbage, tomatoes and potatoes also contain the vitamins. Foods derived from animals, like meat, fish, egg, etc. have very little vitamin C. Only milk contains a small amount of this. Vitamin C is also synthesized by animals except for guineapigs, frogs, apes and man.

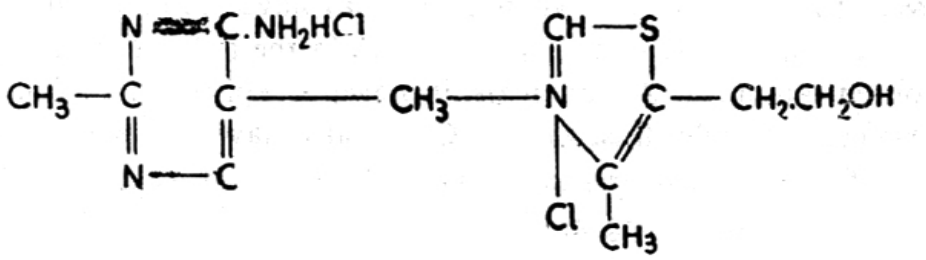
9.4. Water soluble Vitamins

9.4.1. The Vitamin B Complex

The vitamin B complex comprises of many vitamins, all grouped together because they are water soluble and can be obtained from the same source i.e. liver and yeast. However, these two are not the only sources of these vitamins. Several of these vitamins are essential parts of important enzymes. The vitamin Bs known so far are given in Table. A detailed discussion of these is given below.

9.4.2. Thiamine - Vitamin B_1 or Aneurin

Thiamine was the first vitamin B to be identified, hence the name vitamin B1. Thiamine is a complex molecule having a pyrimidine and a thiazole nucleus joined by a methyl bridge. The thiazole nucleus as far as is known is unique and appears only in thiamine.



Thiazole ring Pyrimidine ring

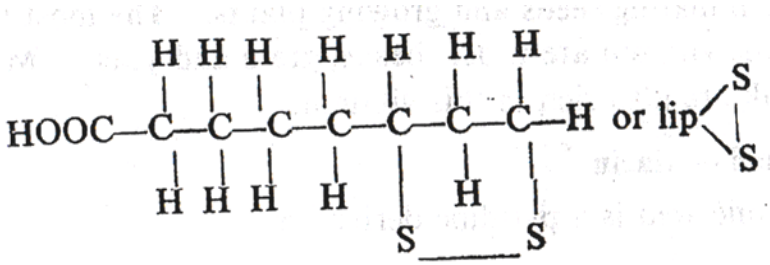
Thiamine is phosphorylated with ATP to form a pyrophosphate ester called thiamine pyrophosphate. The later in association with lipoic acid, forms the prosthetic group co-carboxylase for the enzyme carboxylate. This enzyme is needed for the decarboxylation of ∝ keto acids like pyruvic acid. Thiamine is, therefore, very important in carbohydrate metabolism.

Thiamine deficiency leads to a disease known as beri-beri (Singhalese-beri = weakness). Beri-beri is a disease of the nervous system which is characterised by partial paralysis of the extremities, emaciation and anemia. This is caused due to the degeneration of peripheral nerves (polyneuritis). Even before the concept of vitamins was developed beri-beri was described as a deficiency disease. It was found that it occurred in individuals eating polished rice. Administration of rice polishings cured the disease.

The normal person needs about 333 1.U. (1 nag thiamine hydrochloride) of the vitamin every day. Thiamine has the highest concentration in the heart followed by liver, kidney and brain. It is also present in nuts, cereals, pulses and yeast. The bran of pulses and grains is a very rich source of this vitamin ; it is because of this that it is advisable to eat whole flour and unpolished rice. Raw flesh of fish and molluscs contains an enzyme thiaminase which destroys the vitamin in the body: People eating raw fish and molluscs, therefore, are liable to suffer thiamine deficiency.

9.4.3. Lipoic acid (Thioctic acid)

This is an exception of the vitamin B series as it is fat soluble rather than water soluble.

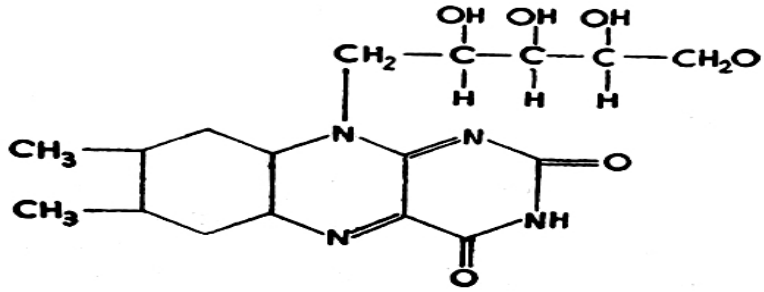


α-Lipoic acid

As said before, lipoic acid in association with thiamine forms the prosthetic group of carboxylase. Lipoic acid is synthesized by most animals, hence, it is not an essential factor of the diet. It is found in the liver of most edible animals.

9.4.4. Riboflavin - Vitamin B_2

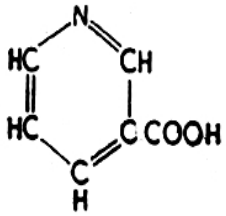
Riboflavin is a component of all oxidative enzymes called flavoproteins which consist of a coenzyme flavin-adenine dinucleotide (FAD). The flavoproteins play a very important role in the oxidation of hydrogen (electron transfer). Since all energy eventually comes from electron transfer, FAD occupies a key place in energy metabolism. Riboflavin is a yellow coloured substance having the following structure.



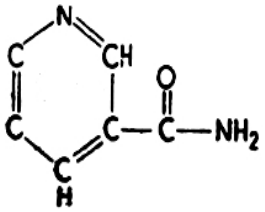
RiboflavinRiboflavin deficiency results in the growth of fine blood vessels in the cornea which give it a blood shot appearance and the eyes become photophobic. In severe deficiency the skin becomes scaly, the lips become red and cracked, and the tongue enlarged and magenta coloured. The daily human requirement is about 2 mg. Riboflavin is found in germinating seeds and growing plants. The most important source of this vitamin are peas, beans, grain and yeast. Milk, meat and egg yolk are also rich in this vitamin.

9.4.5. Nicotinic acid - Niacin

Nicotinic acid is a pyridine derivative.



Nicotinic acid



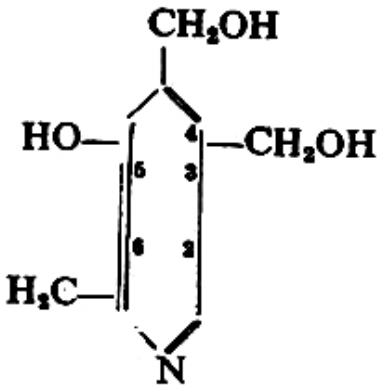
Nicotinic acid amide

The amide of nicotinic acid combines with one molecule each of adenine, a pentose and phosphate to form nicotinanide adenine dinucleotide (NAD^+). NAP^+ is a dehydrogenase of extreme importance in metabolism. Similar to NAD, nicotinamide is also a constituent of the other dehydrogenase, NADP, A description of these two coenzymes has been given. The liver can synthesize niacin from the amino acid tryptophan. The disease caused by the deficiency of niacin is called pellagra (rough skin) a condition characterized by disorders of the skin, digestive and nervous systems. In severe cases, there is burning in the alimentary tract,

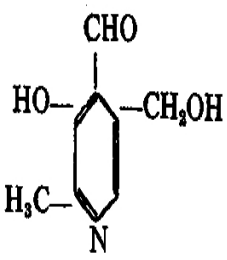
nausea and vomiting; skin becomes red and itchy and various forms of mental disturbances appear. The average human requirement of niacin is 10-15 mg/day. The richest sources for this are liver, kidney, yeast, wheat and green vegetables; milk and meat also contain this vitamin.

9.4.6. Pyridoxine - Vitamin B_6

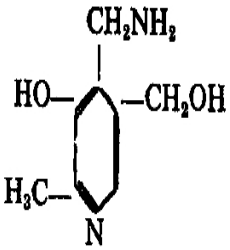
Pyrodoxine like niacin is a pyridine derivative. This vitamin exists in three forms, pyridoxine, pyridoxal and pyridoxamine. These differ from each other in the nature of the substituent on the fourth carbon.



Pyridoxine



Pyridoxal



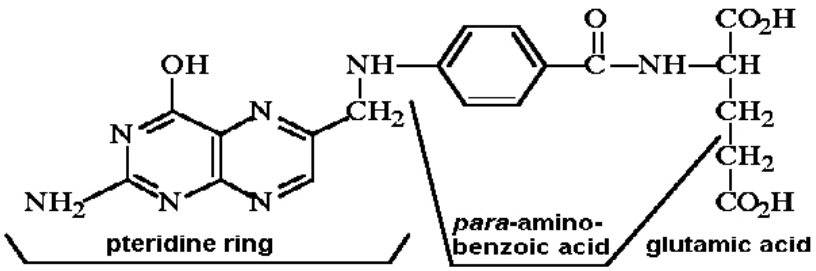
Pyridoxamine

Phosphorylated pyridoxine and its derivatives are coenzymes for gransaminases. As the intestinal bacteria are capable of synthesizing vitamin B6, its deficiency is riot normally found in human beings. In cases where deficiency does occur, the skin near the eyes, nose etc. develops seborrhea - like (oily crusts or scales on the skin) Symptoms. Nervous convulsions have also been reported in experimental animals having vitamin B_6 deficiency.

The daily human requirement ranges from 0.2 to 1 mg. It is abundantly found in foods of both plant and animal origin.

9.4.7. Pteroylglutamic acid - Folic acid

Folic acid contains a pteridine nucleus linked to p-aminobenzoic acid to form pteroic acid. The latter is attached to glutamic acid.



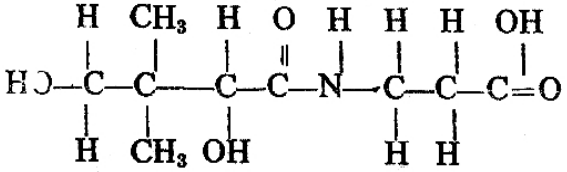
Folic acid itself is not an active vitamin in mammals. However, it takes up hydrogen to get converted to folinic acid or citrovorum factor (C.F.) which along with vitamin B_{12} acts as coenzyme F. The later is essential for the methylation of pyrimidine to form thymine and the formation of purines by introducing to 2- and B carbon atoms in the molecule. It is also important in the synthesis of serine aid choline. It is believed that its-ability to synthesize the above depends on its property of lifting one-carbon units from the so called formate pool. Pyridoxine is thus extremely important in the synthesis of pyrimidines and purines which form the nucleic acids. Deficiency of folic acid leads to disturbances arising out of incomplete nucleic acid synthesis, such as failure of mitosis and meiosis. This deficiency is most marked in tissues which have a rapid rate of cell division. The first evidence of folic acid deficiency is seen in impaired erythropoiesis (production of R.B.C.). The later causes anemia. Other complications of folio acid deficiency are diarrhoea and gingivitis.

The daily human requirement is 0.1 mg to 0.2 mg. Folic acid is synthesized by the micro-organisms of the human colon. Important sources of folic acid are liver and green vegetables, especially spinach.

9.4.8. Pantothenic acid

This vitamin is bound with adenylic acid, 2-mercapto ethylamine ($H\text{-}CH_2\ CH_2\text{-}NH_2$) and three phosphates to form coenzyme A. which is important for fat and carbohydrate metabolism. Thus five members of vitamin B Complex i.e., thiamine, lipoic acid, riboflavin, niacin and pantothenic acid are essential for cellular metabolism.

This is a complex organic acid having the following structure.



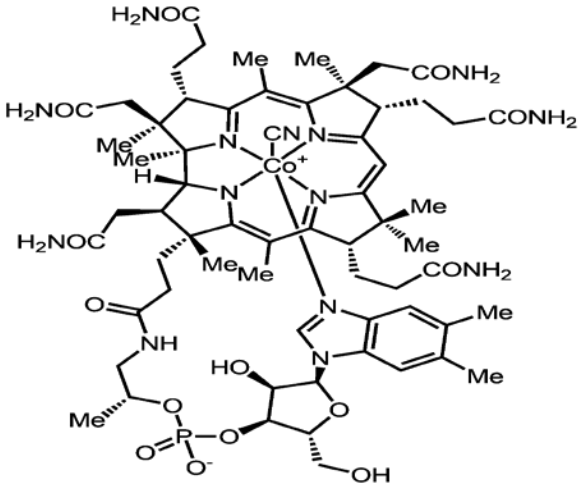
Pantothenic acid

Pantothenic acid deficiency is not known in man. However, in experimental animals it causes degenerative changes in the skin, heart, kidneys, lungs and the nervous system. The daily human requirement of

pantothenic acid is 5 mg It is synthesized in the colon of man by symbiotic bacteria. It is found in yeast, most vegetables and foods of plant sources.

9.4.9. Cyanocobalamin - Vitamin B_{12}

A number of compounds called corrinoids come under the common name of vitamin B_{12}. All corrinoids essentially have a corrin nucleus. This has four pyrrole rings linked in a manner similar to the porphyrin nucleus of haemoglobin. In this, however, porphyrin is formed around cobalt instead of iron.



Cyanocobalamin - vitamin B_{12}

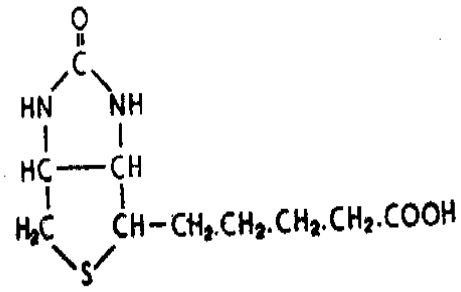
The various members of the vitamin B_{12} group are formed by the replacement of CN by other groups. The molecule which contains CN is called cyanocobalamin. Vitamin B_{12b} is hydroxycobalamiñ since the CN in this is replaced by a hydroxyl group. Similarly vitamin B_{18c} is nitrocobalamine since. CN in this is replaced by NO_2.

Vitamin B_{12} is converted into a coenzyme called coenzyme B_{12}. This coenzyme converts methyl malonyl coenzyme A to succinyl coenzyme A which is essential for the maintenance of the SH groups of enzymes in the reduced form. This form is necessary for the functioning of many enzymes. Vitamin B_{12} is essential in nutrition and growth. It is essential in highly proliferating tissues like bone marrow which produces about 200,000,000 R.B.C. per minute. Deficiency of vitamin B_{12} therefore, causes pernicious anemia. This disease is characterized by changes in the bone marrow and reduction in the number of circulating R.B.C. Vitamin B_{12} deficiency also causes destruction of the lining of the alimentary canal. In the stomach, secretions of pepsin and HCI are reduced. In severe cases of vitamin B_{12} deficiency myelinated nerve fibres are destroyed, resulting in various kinds of nervous disorders.

The average human requirement per day is only 1 μg of vitamin B_{12}. This vitamin is generally absent in most plant products. Kidney, liver and milk contain more than the body requirements of the vitamin. A normal meat diet, therefore, is sufficient for supplying the requisite amount of vitamin B_{12}.

9.10. Biotin

Biotin acts as a coenzyme in the carboxylation of pyruvate to oxaloacetic acid. Biotin deficiency is normally not found in human beings. Biotin is a complex organic acid which is optically active.

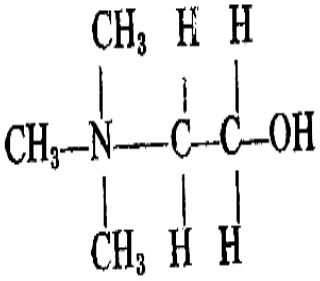


Biotin

In experimental animals its deficiency produces greasiness of the skin, drowsiness, muscular pain and heart distress. The intestinal bacteria synthesize biotin in such high quantities that the amount excreted in urine and feces exceeds the intake. This is the reason why the daily human requirement is not known. Biotin is found in yeast, liver, kidney and egg yolk.

9.4.11. Choline

The chemistry of this compound has been dealt in Chapter. It is an important lipotropic agent, and participates in the mobilization of fat from the liver; its absence, therefore, causes accumulation of fat in the hepatic tissues. Choline is a quaternary ammonium compound.

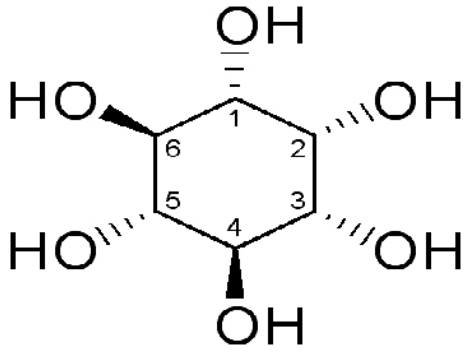


Choline

Whenever methylation is required, choline acts as an important methyl donor in intermediary metabolism. The ester of choline with acetic acid i.e. acetylcholine is an important neurotransmitter. Choline can be synthesized in the body by the methylation of ethanolamine and therefore, strictly speaking, it is not a vitamin. Its requirement ranges from 1.5 to 3 g/day. The best source for this is vegetable foods.

9.4.12. Inositol

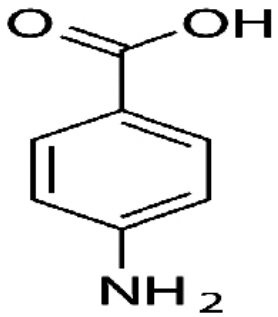
Inositol is a hexahydroxycyclohexane. There are eight possible isomers of this. Of these only i-inositol or mesa inositol is biologically active. Inositol is a component of phospholipids. Its exact physiological function is not known. Inositol deficiency causes impaired growth and insufficient lactation in experimental animals. The amount of inositol needed by man is not known.



Meso-inositol (i-inositol)

9.4.13. Para aminobenzoic acid - PABA

PABA is essential in micro-organisms for the synthesis of folic acid. In case of human beings its presence helps in the synthesis of folic acid by intestinal bacteria. There is no clearly defined deficiency disease of PABA and there is no evidence that it is a dietary essential for human beings. The physiological role of PABA is not fully understood. It has the following structure:



Para aminobenzoic acid

9.5. Deficiency and toxicity of vitamins

Hypervitaminosis is a condition of abnormally high storage levels of vitamins, which can lead to toxic symptoms. Specific medical names of the different conditions are derived from the vitamin involved: an excess of vitamin A, for example, is called hypervitaminosis A. Hypervitaminosis are primarily caused by fat-soluble vitamins (D and A), as these are stored by the body for longer than the water-soluble vitamins.

Vitamins	Deficiency	Toxicity
Vitamin B1 (Thiamine)	Symptoms include burning feet, weakness in extremities, rapid heart rate, swelling, anorexia, nausea, fatigue, and gastrointestinal problems.	None known.
Vitamin B2 (Riboflavin)	Symptoms include cracks, fissures and sores at corner of mouth and lips, dermatitis, conjunctivitis, photophobia, glossitis of tongue, anxiety, loss of appetite, and fatigue.	Excess riboflavin may increase the risk of DNA strand breaks in the presence of chromium.
Vitamin B3 (Niacin)	Symptoms include dermatitis, diarrhea, dementia, and stomatitis.	Niacin from foods is not known to cause adverse effects. Supplemental nicotinic acid may cause flushing of skin, itching, impaired glucose tolerance and gastrointestinal upset.
Vitamin B5 (Pantothenic acid)	Very unlikely. Only in severe malnutrition may one notice tingling of feet.	Nausea, heartburn and diarrhea may be noticed with high dose supplements.
Vitamin B6 (Pyridoxine)	Symptoms include chelosis, glossitis, stomatitis, dermatitis (all similar to vitamin B2 deficiency), nervous system disorders, sleeplessness, confusion, nervousness, depression, irritability, interference with nerves that supply muscles and difficulties in movement of these muscles, and anemia.	High doses of supplemental vitamin B6 may result in painful neurological symptoms.
Vitamin B7 (Biotin)	Very rare in humans. Keep in mind that consuming raw egg whites over a long period of time can cause biotin deficiency. Egg whites contain the protein avidin, which binds to biotin and prevents its absorption.	Not known to be toxic.
Vitamin B9 (Folic	One may notice anemia	vitamin B12 and

acid)	(macrocytic/megaloblastic), sprue, Leukopenia, thrombocytopenia, weakness, weight loss, cracking and redness of tongue and mouth, and diarrhea. In pregnancy there is a risk of low birth weight and preterm delivery.	folic acid deficiency can both result in megaloblastic anemia.
Vitamin B12 (Cobalamin)	Symptoms include pernicious anemia, neurological problems and sprue.	. Only a small amount is absorbed via the oral route, thus the potential for toxicity is low.
Vitamin C (Ascorbic acid)	Symptoms include bruising, gum infections, lethargy, dental cavities, tissue swelling, dry hair and skin, bleeding gums, dry eyes, hair loss, joint paint, pitting edema, anemia, delayed wound healing, and bone fragility. Long-term deficiency results in scurvy.	Possible problems with very large vitamin C doses including kidney stones, rebound scurvy.
Vitamin A (Retinoids)	One may notice difficulty seeing in dim light and rough/dry skin.	Vitamin A is rapidly absorbed and slowly cleared from the body. Nausea, headache, fatigue, loss of appetite, dizziness, and dry skin can result. Excess intake while pregnant can cause birth defects.
Vitamin D (Calciferol,	In children a vitamin D deficiency can result in rickets, deformed bones, retarded growth, and soft teeth. In adults a vitamin D deficiency can result in osteomalacia, softened bones, spontaneous fractures, and tooth decay.	Hypervitaminosis D is not a result of sun exposure but from chronic supplementation.
Vitamin E (tocopherol)	Only noticed in those with severe malnutrition. However, suboptimal intake of vitamin E is relatively common.	Minimal side effects have been noted in adults taking supplements in doses less than 2000 mg/day. There is a potential for

		impaired blood clotting. Infants are more vulnerable.
Vitamin K	Tendency to bleed or hemorrhage and anemia.	May interfere with glutathione. No known toxicity with high doses.

9.6. Summary

Vitamins are organic nutrients with essential metabolic functions, generally required in small amounts in the diet because they cannot be synthesized by the body. The lipid-soluble vitamins (A, D, E and K) are hydrophobic molecules requiring normal fat absorption for their efficient absorption and the avoidance of deficiency symptoms. Vitamin A (retinol), present in meat, and the provitamin (β-carotene), found in plants, form retinaldehyde, utilized in vision, and retinoic acid, which acts in the control of gene expression. Vitamin D is a steroid prohormone yielding the active hormone derivative calcitriol, which regulates calcium and phosphate metabolism; deficiency leads to rickets and osteomalacia. Vitamin E (tocopherol) is the most important antioxidant in the body, acting in the lipid phase of membranes protecting against the effects of free radicals. Vitamin K functions as cofactor to a carboxylase that acts on glutamate residues of precursor proteins of clotting factors and bone proteins to enable them to chelate calcium. The water soluble vitamins of the B complex act as enzyme coactors. Thiamin is a cofactor in oxidative decarboxylation of α-keto acids and transketolase in the pentose phosphate pathway. Riboflavin and niacin are important cofactors in oxidoreduction reactions, present in flavoprotein enzymes and in NAD and NADP, respectively. Pantothenic acid is present in coenzyme A and acyl carrier protein, which act as carriers for acyl groups in metabolic reactions. Pyridoxine as pyridoxal phosphate, is the coenzyme for several enzymes of amino acid metabolism, including the transaminases, and of glycogen phosphorylase. Biotin is the coenzyme for several carboxylase enzymes. Besides other functions, vitamin B12 and folic acid take part in providing one-carbon residues for DNA synthesis; deficiency results in megaloblastic anemia. Vitamin C is a water-soluble antioxidant that maintains vitamin E and many metal cofactors in the reduced state. Inorganic mineral elements that have a function in the body must be provided in the diet. When intake is insufficient, deficiency may develop, and excessive intakes may be toxic.

9.7. Terminal questions

Q.1. Classify vitamins and briefly discuss their functions and deficiency disorders.

Answer:----------

Q.2. Describe the chemistry, biochemical functions, daily requirements, sources and deficiency manifestations of vitamin A.

Answer:----------

Q.3. Write an account of folic acid involvement in one carbon metabolism.

Answer:----------

Q.4. Discuss the biochemical functions of vitamin C. Add a note on the therapeutic use of megadoses of this vitamin.

Answer:----------

Q.5. Write briefly about the coenzymes involved in oxidation-reduction reactions.

Answer:----------

Q.6. Write short notes on

(a) Vitamin D is a hormone-justify,

(b) Thiamine pyrophosphate,

(c) Coenzymes of niacin,

(d) Pyridoxal phosphate in transamination,

Answer:----------

9.8. Suggested Readings

1. Deluca, H.F. (ed.), 1978, The Fat-solubel Vitamins, Plenum, New York.

2. Deluca, H.F. and Suttie, J.W. (eds.), 1970, The Fat-soluble Vitamins, University of Wisconsin Press, Madison.

3. Dyke, S.F., 1965, The Chemistry of the Vitamins, Interscience, Yew York.

4. Munson, P.L., Diczfalusy, E., Glover, J. and Olson, R.E. (eds.), 1978, Vitamins and Hormones, Vol. 36, Academic Press, New York.

5. Sebrell, W.H. Jr. and Harris, R.S. (eds.), 1972, The Vitamins, Vols. 1-5, Academic Press, New York.

6. Wanger, A.F. and Folkers, 1964, Vitamins and Coenzymes, Interscience, New York.