COURSE INTRODUCTION

The objective of this course is to provide knowledge of cell biology and biomolecules. Cell biology is refers to the study of the formation, structure, components and function of cells and cell organelles. However, this course covers the structures and basic components of prokaryotic and eukaryotic cells, especially organelles and macromolecules. The chemical nature of biological macromolecules is briefly in this course. The course is organized into following blocks:

Block 1 covers the cell and cell organelles. Here you will learn structure and basic comments of cell and its organelles.

Block 2 deals the biomolecules Part I, this block covers the structure, nomenclature and functions of carbohydrate, and proteins.

Block 3 describes in brief of biomolecules part II, which deals the structure and functions of lipids, and nucleic acids.

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Rajarshi Tandon Open University, Prayagraj

Cell Biology and Bio-molecules

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PGBCH-101

Cell Biology and Bio-molecules

Block- I

Cell and Cell Organelles

UNIT -1

Introduction to Cell

UNIT-2

Cell Organelles

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Introduction

This first block of Cell Biology and Bio-molecules consists of following two units:

Unit-1: Introduction to cell-It's meaning, definition and types of cells and their structure. The history of biochemistry and biochemical organization will also be covered in this unit. Architecture of cells namely cell wall and cell membrane is briefly described.

Unit-2: The Cell organelles-This unit cover the structure and function of different cell organelles likes- mitochondria, nucleus, endoplasmic reticulum, chloroplast, Golgi apparatus, ribosomes, lysosomes, centrioles, cytoskeleton.

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UNIT 01: Introduction to Cell

Structure

1.1. Introduction

Objectives

1.2. Overview about biochemistry

1.3. History of biochemistry

1.4. Biochemical organization

1.5. An overview of cell

1.5.1. Prokaryotic cell

1.5.2. Eukaryotic cell

1.6. Summary

1.7. Terminal questions

1.8. Further readings

1.1. Introduction

This unit introduces about the cell. The body of organism consists of cells, which is considered to be the basic unit of biological world. The organization of cell is called tissue, the association of tissues form organ and finally organ gives the shape of an organism. Cellular macromolecules are the part of interaction and reactions taking place inside the cell and are fundamental to all living being. Cells are mainly categorized as Prokaryotic cell or Eukaryotic cell. The organisms having many cells in their body are called multi-cellular organism for example most of plants and animals are multi-cellular organisms.

Objectives:

- to learn about the basic concept of biochemistry
- to learn how the biochemistry is associated with different field of science and technology
- to get an insight into the basics of cell and cell organelles
- to define and understand the chemical organization of prokaryotic and eukaryotic cells

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1.2. Overview about Biochemistry

Biochemistry is defined as chemical process of living organisms. The biochemistry focuses on structure, function and process of biological molecules present in a living cell. The biochemistry also deals with the communication of cells during the growth, respiration, locomotion and fighting illness. In biochemistry, we study in details of all life process, which deals with chemicals that are present in the living body. It involves the study of those chemicals that regulates the growth, nutrition, respiration and metabolic activity.

Biochemistry deals with chemical process related to living organism. It is a laboratory based science which depends on the principles and analytical methods of chemistry. By using chemical knowledge and techniques, biochemist can understand and solve biological problems.

Biological molecules are most intriguing macromolecules and are the integral part of any living species. Biological macromolecules include protein, carbohydrates, vitamin, lipids and nucleic acids. We will look the structure of macromolecules and how the nature has designed then to work in the most efficient manner.

Following salient features of biochemistry is discussed in the following points

- All the biological processes are performed by macromolecules present in cells which are the subject matter of biochemistry.
- Biochemistry may be used to study the properties of biomolecules, for a variety of purposes. For example, a biochemist may study the characteristics of the keratin protein in hair so that a shampoo may be developed that enhances curliness or softness. Similarly biochemists may use recommend certain lipids as food additives. Alternatively, a biochemist might find a substitute for a usual biomolecules commonly found in the living system. Artificial sweeteners are one excellent example. Gene therapy is within the realm of biochemistry. Biochemistry is used to prepare molecular models by using computational chemistry and graphical techniques to get an insight into structural and functional attributes of biomolecules especially proteins.

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In biochemistry we mainly study the principle types of macromolecules with special reference of their structure, function and metabolic pathways. The principle types of macromolecules or biological molecules are carbohydrates, proteins, lipids and nucleic acids.

1.3. History of biochemistry

Biochemistry research has been done for around the past 400 years, although the term biochemistry itself was only coined in 1903 by the German chemist Carl Neuberg. The study of biochemistry is mainly depended on the elucidation of the cell of a organisms where the biomolecules are found. The invention of the microscope in 1665 by Robert Hooke was an important landmark for first observing the cell of an organism so that biomolecules could be studied. In 1660s, the Anton von Leeuwenhoek was first who used simple microscope or magnifies glass to see the single-celled animals and even some larger bacteria with a simple microscope. The microscopy instrument has two important role of magnification and resolution of images and provides an opportunity to see the cells and cell organelles not visiblethroughthe naked eyes.

In the 18th Century, the French scientist Antoine Lavoisier proposed a reaction mechanism for photosynthesis, which is the process by which plants make their own food using carbon dioxide, water, and sunlight and releases oxygen in the process. 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.

In the 19th Century, a prevailing belief was that protoplasm, the jelly-like inside of the cell, carried out all the processes involved with breaking down food molecules. Eduard Buchner extractedzymase from the yeast, although zymase did not contain any living cells, it could still ferment glucose to produce carbon dioxide and ethanol. .

In the 20th century, a German scientist Hens Krebs observed a series of chemical reactions during cellular respiration where glucose and oxygen are converted into ATP, carbon dioxide and water. In the same century the DNA was confirmed as the genetic material of the cell and its famous double helical structure was elucidated by James Watson and Francis Crick using previous research done by Rosalind Franklin. New

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technologies such as recombinant DNA, gene splicing and radio isotopic labeling etc. are opening new avenues in biochemistry.

1.4. Chemical organization

The body of living organism has well organized cell structure. The cells which are the basic unit structure of body, consists of complex biomolecules. The four major classes of biomolecules includes carbohydrates, lipids, proteins, and nucleic acids. Each of these biomolecules represents an important cell component and performs a wide array of functions. These biomolecules make up the majority of a cell's dry mass (recall that water makes up the majority of its complete mass). Biological macromolecules are organic, meaning they contain carbon. In addition, they may contain hydrogen, oxygen, nitrogen, and additional minor elements.

Each components of living organism have specific purpose or functions and are made up of different kinds of cells. The cells makes internal intricate structure as well as external structural components, however macromolecules that present in cell e.g. lipids, protein and nucleic acid also have specific function.

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 used in maintenance and repairing of own intricate structure of cells but also utilized in other purposeful work like locomotion. Self replication or self-assembly means is the most extra ordinary attributes of living organism as a result of which billions of daughter cells can be formed and each can carry a faithful copy of the genetic material of their parental cells. When we see the nature of living organism several questions arises in our mind like i) how the living matter is different from the non living matter, which also consists of intrinsically inanimate molecules? ii) Why the living organism does appear to be more than the sum of its inanimate parts? The basic concept of Biochemistry of cell provides an insight into living world and tries to answer these questions.

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The basic concepts of organization in biology can be understood by studying the the structure and function of different biomolecules, which are integral part of the cell and cell organelles. The biological system is an integral system comprising of biologically active structures and are characterized by a definite arrangement of their components, these components interact themselves for specific biological functions.

Another important feature of the biological system is hierarchical organization of biomolecules like low molecular weight compounds to high molecular biomolecules to cells- tissues-organ system-organism-populations- and biosphere. Therefore biological organization reflects the unity of structure, function and regulation of biological system.

1.5. An overview of cells

The German botanist Mathias Jacab Schleiden(1838) for the first time observed the cell and considered as basic unit structure of plant. Later, British biologist Theodore Schwann (1839) also studied cell and applied the theory of Schwann in different types of animal cell and reported that the cell is basic unit of structure and function of all life. Except virus, body of all living organism has cellular organization.

K. Negeli (1817-1891) said the plant cell arise form preexisting cell; Virchow (1858) established the concept of cell division in production of organism. Louis Pasture (1822-1895) has supported Virchow and extended the cell theory. Postulates of cell theory are

1. The cell is the fundamental unit of structure and function in living things.
2. All organisms are made up of one or more cells.
3. Cells arise from other cells through cellular division.

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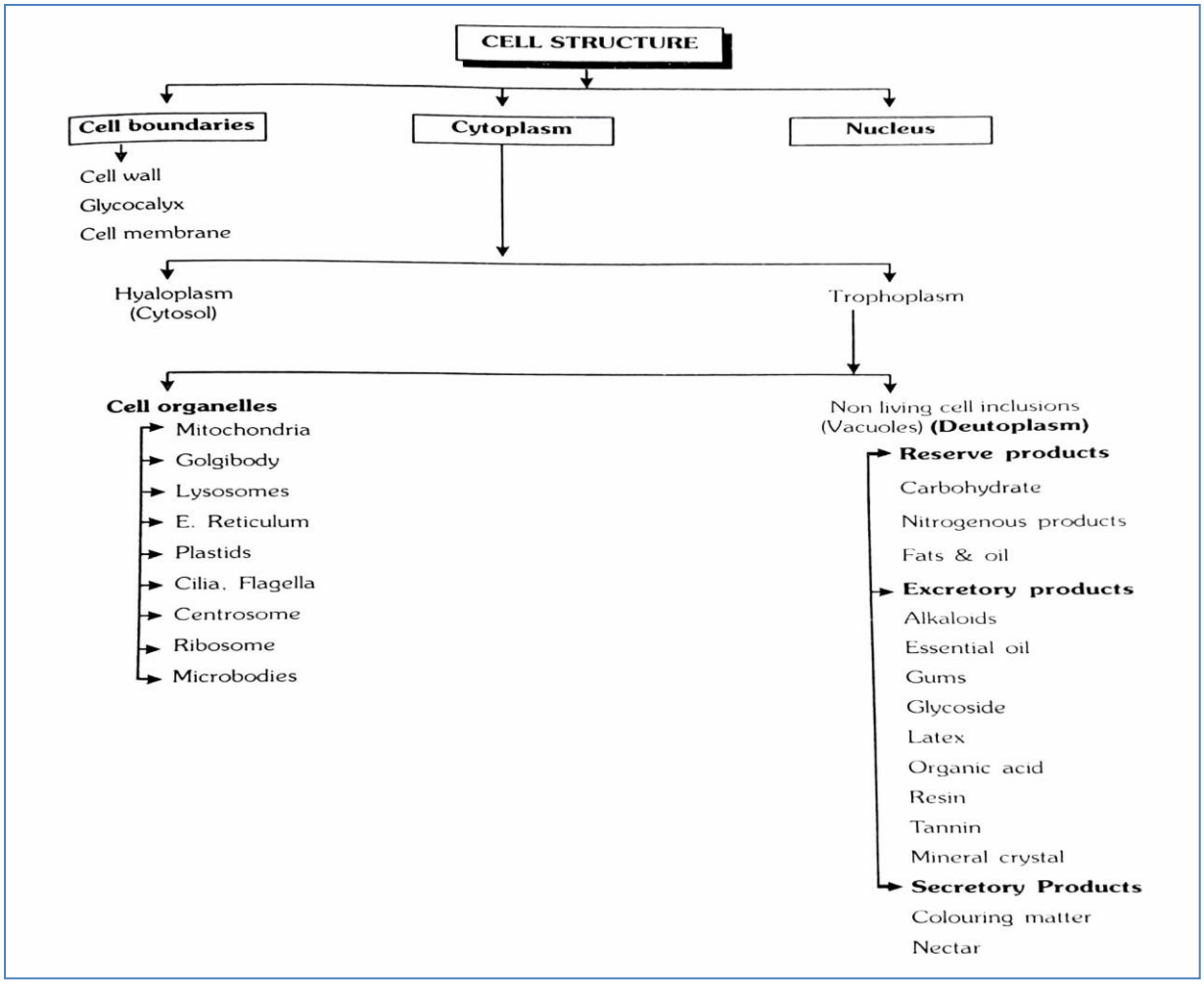

According to scientist A.G. Loewy and P. Siekvitz (1963),a cell is unit of biological activity delimited by a semi permeable membrane and capable of self- reproduction resulting in the formation of more of its copies.. Inside each cell is a dense membrane bound structure called nucleus. The organism with only one cell is called unicellular organism for example Archaea, bacteria, blue green algae and protozoa etc. The organisms having many cell in their body are called multi-cellular organism for example most of plants and animals. Based on the organization of the nucleus cells are either prokaryotic or eukaryotic cells and both prokaryotic and eukaryotic cells, a semi-fluid matrix called cytoplasm occupies most of the volume of the cell. The cytoplasm is the main centre of cellular activities in both the plant and animal cells. Various chemical reactions occur in the cell to keep the cell in the 'living state'.

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

- Cell membrane
- Nucleus
- Cytoplasm along with its organelles

Cells are categorized into two types such as prokaryotic and eukaryotic. The brief descriptions about both cells are mentioned below.

1.5.1. Prokaryotic cells:

Prokaryotic cells are smallest and most primitive. It was recognized about more than 3.5 billion years ago. For example the stromatolitesof Western Australia is known to be least 3.5 billion year old. Prokaryotic cell is the most primitive cell mostly found in bacteria (Mycoplasm, cynobacteria, and blue green algae) and archea. The organization of the prokaryotic cell is fundamentally similar even though prokaryotes exhibit a wide variety of shapes and functions. All prokaryotes have a cell wall surrounding the cell membrane enclosing the fluid matrix filled with cytoplasm. Well-defined membrane boundnucleus is absent. The genetic material is basically naked, not enveloped by a nuclear membrane. The prokaryotic cells have following characteristic which makes it different from eukaryotic cells: Examples of prokaryotes are blue-green algae, bacteria and mycoplasma. Among prokaryotes, bacteria are the most common and multiply very fast. The salient features of prokaryotic cell are:

- The absence of membrane around the nuclear material
- Due to absence of nucleolus and nuclear membrane, the genetic material is present in a single chromosome and represents as circular double stranded DNA.
- In prokaryotic cell, the basic protein such as histone is not present as compared to eukaryotic cells.
- The prokaryotic cells do not contain nuclei, cytoskeleton, centrioles, and basal body.

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

Prokaryotes have something unique in the form of cell inclusions. Reserve materials in the prokaryotic cells are stored in the cytoplasm in the form of inclusion bodies. A specialized differentiated form of cell membrane called embosoms is the characteristic of prokaryotes. The prokaryotic cell, especially bacterial cell have chemically complex envelope which consists of tightly bound three layers. Although each layer of the envelope performs distinct function, they act together as a single protective unit. 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.

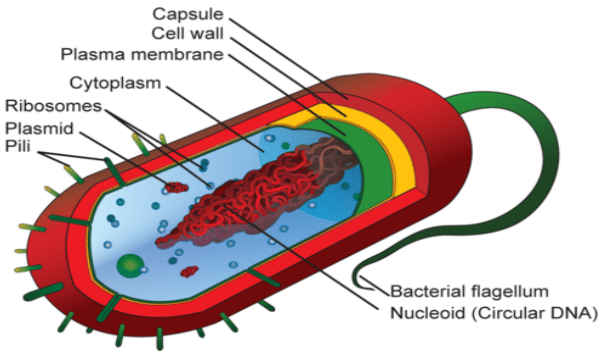

Fig.1.1: Diagram of prokaryotic cell

1.5.2. Basic difference of Gram positive and the Gram negative bacteria

As Gram positive bacteria lack an outer lipid membrane, when correctly referring to their structure rather than staining properties, are termed monoderms. The outer lipid membrane possessed by Gram negative bacteria means that, when referring to their physical structure, they are termed diderms. The gram-positive bacteria retain the crystal

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violet colour and stains purple whereas the gram-negative bacteria lose crystal violet and stain red. Thus, the two types of bacteria are distinguished by gram staining. Gram-negative bacteria are more resistant against antibodies because their cell wall is impenetrable. Gram-positive and gram-negative bacteria are categorized on the basis of the ability to hold the gram stain. The gram-negative bacteria are stained by a counter stain such as safran in and they are destained because of the alcohol wash.

1.5.3. Eukaryotic cells:

The eukaryotic cells are10 to 100 times large rthan prokaryotic cells. The nucleolus is a round body located inside the nucleus of a eukaryotic cell. It is not surrounded by a membrane but sits in the nucleus. The nucleolus and other cell organelles make the secondary membrane envelop. The eukaryotic cells are considered as true cells which are found in the protista (from protozoa to protophyta), fungi, plants (from Algae to Angiosperm) and animals (from priferans to mammals). Each eukaryotic cell has different shape, size and physiology. All cells are typically composed of plasma membrane and cytoplasm. In addition, eukaryotic cells have a variety of complex locomotory (cilia or flagella) and cytoskeletal structures. Their genetic material is well organized into chromosomes. All eukaryotic cells are not identical. 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 (Fig. 1.2).

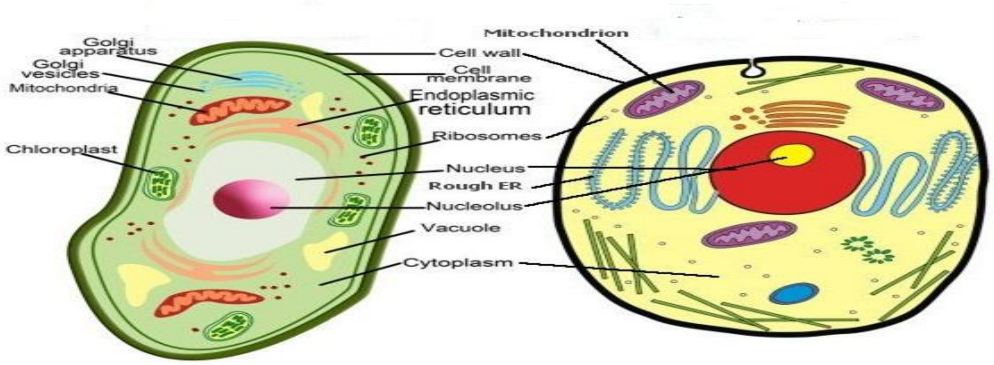

Fig.1.2: Diagram showing: (a) Plant cell (b) Animal cell

Source: https://www.pinterest.com/pin/278449189437091310/

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Table.1.1: 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 centreof 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

The major cell organelles includes nucleus, endoplasmic reticulum, mitochondria, Golgi apparatus, peroxisomes, lysosomes found in both plant and animal cells. The plant cells are comparatively larger in size (10-100 nm) than animal cells (10-30 nm).

1.6. Summary

Biochemistry is a fundamental science which aims to understand the life processes by understanding the biomolecules commonly found in all the living organisms. The structure, function and organization of basic biomolecules namely proteins, carbohydrates, lipids are important to understand the life. Further the cell is considered to be the basic fundamental unit of living organism. Understanding cell was possible by the advent of tools of microscopy which helped to observed cells in details which was not possible by naked eyes. Cell are categorized as prokaryotic and eukaryotic cell based on the organization of the genetic material in the nucleus. Cells are further organized by the presence of different types of cell organelles like miotchondria, golgi apparatus, lysosomes etc. The biochemistry aims to understand all biochemical reaction and metabolic process which occurs in body of living organism. Except virus, body of all living organism has cellular organization. Thus, cell is considered the basic unit of organization of structure of all living matter.

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1.7. Terminal questions

Q.1. What is the biochemistry? How study of biochemistry is useful to mankind?

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

Q.2. Is biochemistry and interdisciplinary subject? Discuss in your word?

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

Q.3. Define the biochemical organization of cells. How do you define the hierarchy in cells?

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

Q.4. How you can say the biochemistry is associated with biology?

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

Q.5. Discuss about the typical structure of plant and animal cell with suitable diagram.

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

Q.6. Discuss about different types of cells and write the characteristic feature of prokaryotic cells.

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

1.8. Further readings

1. General biochemistry: J.H. Weil, New Age International (P) Limited, 5th edition, 2013,
2. Principles of Biochemistry: Lehninger, Nelson and Cox. Student edition, CBS 1439 Publishers and Distributors, Delhi.

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3. Biochemistry: T.A. Brown, Viva book publication. First edition, 2018.

4. Elements of biochemistry: J.L. Jain, S. Chand publication. **Seventh Edition**

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

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Unit 2: Cell organelles

2.1. Introduction

Objectives

2.2. Structure of cell wall

2.3. Cell membrane

2.4. Cell organelles

2.4.1. Mitochondria

2.4.2. Golgi complex

2.4.3. Lysosome

2.4.3.1. Primary Lysosome

2.4.3.2. Digestive vacuoles

2.4.4. Endoplasmic reticulum (ER)

2.4.5. Plastids

2.4.5.1. Chloroplasts

2.4.5.2. Leucoplasts

2.4.5.3. Chromatoplasts

2.4.5.4. Etioplasts

2.4.6. Nucleus

2.4.7. Ribosome

2.4.8. Vesicle

2.4.9. Cytoskeletal

2.4.10. Vacuole

2.4.11. Cytosol

2.4.12. Lysosome

2.4.13. Centrioles

2.4.14. Summary

2.4.15. Terminal questions

2.4.16. Further readings

2.1. Introduction

This unit covers structure and functions of cell and their organelles. We knew that the living organism is complicated and highly organized. Their cell possesses intricate internal structures consisting of many kinds of organelles and complex molecules. The detail study of cell reveals the basic structure and living phenomena, i.e., physiological and behavioral processes of an organism. All organisms are composed of cells. Some are composed of single cells which are called as unicellular organisms while others are composed of more than one cells, are called multicellular organisms. Cell membrane and cell wall are the specific feature in cell providing an outer barrier and play important role in cell growth, formation of intercellular junctions, , secretion, endocytosis and cell division etc. All cell 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.

Objectives

- To learn about basic component of cell, their structure and functions.
- To learn cell and cell membrane
- To discuss in brief the various types of cell organelles, their structure, types and functions.

2.2. Structure of cell wall

As you may recall, a non-living rigid structure called the cell wall forms an outer covering for fungi and plants. Cell wall not only 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. Algae have cell wall, made of cellulose, galactans, mannans and minerals like calcium carbonate, while in other plants it consists of cellulose, hemicellulose, pectins and proteins.

The cell wall of a young plant cell, the primary wall has capacity of growth, which gradually diminishes as the cell matures and the secondary wall is formed on the inner (towards membrane) side of the cell. The middle lamella is a layer mainly of calcium

pectate which holds or glues the different neighboring cells together. The cell wall and middle lamellae may be traversed by plasmodesmata which connect the cytoplasm of neighboring cells.

2.3. Cell membrane

After invention of electron microscopy in 1950, first time cell membrane was studied. This study reveals that all the membrane consists of double layer of lipids molecules in which proteins are embedded. These proteins account about 50% of mass of membrane. It has been found that lipids are arranged within the membrane with the polar head towards the outer sides and the hydrophobic tails towards the inner part. This ensures that the nonpolar tail of saturated hydrocarbons is protected from the aqueous environment. The cell membrane contains two types of proteins

- Lipoproteins- it contains lipids. It works as enzymes and ions regulation.
- Glycoproteins- it contains carbohydrate, which works as receptors.

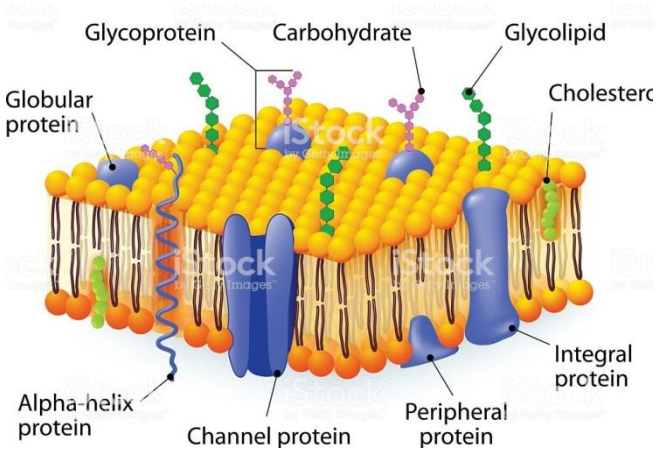

Fig.2.1: Cell membrane

Source: https://www.istockphoto.com/in/vector/cell-membrane-gm509862902-86007545#/close

Some proteins are located in the inner surface of membrane which are called as intrinsic proteins. Some are found in outer surface of membrane known as extrinsic proteins, whereas those proteins which extend through the membrane are called as trans membrane proteins.

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The lipid component of the membrane not only contains phosphoglycerides but also possess protein and carbohydrate. The ratio of protein and lipid varies considerably in different cell types. In 1972, Singer and Nicolson have proposed a model for cell membrane popularly known as fluid mosaic model. This model is considered as advanced or improved model for cell membranes. According to this model the quasi-fluid nature of lipid enables lateral movement of proteins within the overall lipid bilayer. These moments of proteins is measured as its fluidity. The fluid nature of the membrane is also important from the point of view of functions like cell growth, formation of intercellular junctions, secretion, endocytosis, cell division etc. Water may also move across this membrane from higher to lower concentration. Some ions such as Na+/K+ maintain homeostatic conditions in cell membrane. They pass through it against concentration gradients by utilizing energy produced form ATP by osmosis.

- **Function of cell membrane:**
  - Protection: it form outermost boundary of cell organelles to protect them
  - Transport properties: Active transport is the movement of molecules across a membrane from a region of their lower concentration to a region of their higher concentration i.e. against the concentration gradient. It transfers macromolecules across the cell membrane. It also provides movement of molecules across the membrane without requirement of energy by means of passive transport.
  - Properties of selective permeability: it allows passage of selective molecules/ions across the membrane which is important to maintain the homeostatic conditions of the cell.
  -
  - Link adjacent cells together for cell to cell communication and also for the formation of tissues by combining different cells.

2.4. Cell organelles

An organelle is a tiny cellular structure embedded in both prokaryotic and eukaryotic cells with specific functions. 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. The eukaryotic cell contain number of cell organelles, mitochondria, nucleus, ribosome, vesicle, endoplasmic reticulum, Golgi apparatus, cytoskeleton, vacuole, cytosol lysosome, centriole etc. these cell organelles are organized and bounded with plasma membrane.

2.4.1. Mitochondria:

Mitochondria are organelles found in every cell of complex organism. It is also called "powerhouse of the cell" because it produce about 90% of chemical energy that cell need to survive or use in metabolic reactions. Kolliker (1880) first observed mitochondria as cytoplasmic granules in striped muscles of insect. Flemming and Altman were credited for the discovery of mitochondria, however the term mitochondria was given by C. Bendra and F. Meves in year 1904. They first observed mitochondria in plants *Nymphea*. Seikevitz called them power house. The mitochondria is present in several 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 are stained. Mitochondria are split into different compartments or regions, each of which carries out distinct roles. Mitochondria also have a special role in programmed cell death (apoptosis). This may sound strange, but it is vital for the processes of growth and development. Sometimes cells don't die when they should, and start to grow uncontrollably. All the mitochondria present in a cell are collectively called *chondriome*. Usually plant cells have fewer mitochondria as compared to animal cell. In higher animals maximum mitochondria are found in flight muscles of birds. Mitochondria may vary in shape as ellipsoidal, oval, spherical or spiral.

- **Structure:**

Mitochondria have two membranes, an 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 is of 60-75 A˚ thick and separated by a space (80-100 A˚) called peri-mitochondrial spaces. The space has enzymes require for oxidation of fats and pyruvic acid. Some finger likes structure is found in mitochondria known as cristae as shown in Fig.2.2. The cristae are the folds of the inner membrane. They increase the surface area of the membrane, therefore increasing the space available for chemical reaction. The inner membranes of mitochondria have cytochromes which act as carrier for electron transfer. Inner membrane is studded with pin head particles called oxysomes or elementary particles or F_1-F_0 particles (10_4 to 10^6 in number).

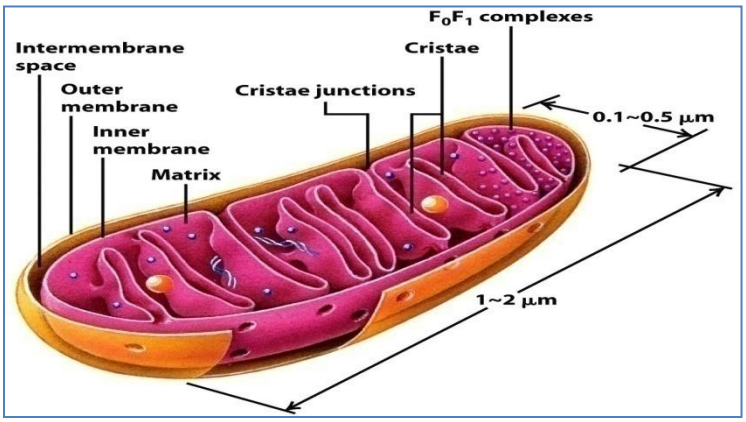

Fig.2.2: structure of mitochondria

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

Mitochondrial matrix have enzyme for Kreb's cycle. Besides these enzymes, matrix have a complete protein synthesis apparatus (Ribosome 70S, DNA, few RNAs & few enzymes) so mitochondria are called as semiautonomous cell organelles. One or many (6 kb to 36 kb long) double stranded mainly circular naked DNA is present in mitochondrial matrix. Mitochondrial DNA can code the synthesis of 10 to 37 different types of proteins. Enzymes for replication and transcription of DNA like DNA-Polymerase and RNA polymerase are found in mitochondrial matrix. Mitochondria of mammals have 55s ribosomes.

- **Functions:**

The mitochondria actsto produce energy for oxidative metabolism and ATP production, where by organic compounds are broken down to release & store metabolic

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energy in the form of ATP molecules. This energy is then in turn used by the cell to carry out various functions. Mitochondria help in vitellogenesis (yolk deposition) in oocytes. Mitochondrial protein kinases make the yolks viscous and insoluble for longer duration storage.

2.4.2. Golgi complex:

Golgi complex is also called Golgi apparatus or Golgi body. 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 by several names such as Dolton complex, Golgi complex, Baker's body, Dictyosome (plant Golgi body) etc. The cytoplasm surrounding Golgi body has fewer or no other organelles. It is called Golgi ground substance or zone of exclusion. Golgi bodies are pleomorphic structures because component of Golgi body differ in structure & shape in different cells. The Golgi body is made up of a series of flattened, stacked pounces called cisternae. Cisternae are flat sacs that are stacked in a semicircular, bent formation. Each formation has a membrane to separate it from the cytoplasm of the cell. 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.

- **Structure:**

Golgi complex is made up of four parts such as cisternae, tubules, vacuoles and vesicles. 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. Convex surface of Cisternae which is membrane is called trans-face or maturing face. Other component of golgi body tubules are branched and irregular tubs like structure associated with cisternae. Vacuoles which is large spherical structures is also associated to the tubules. Whereas vesicles are spherical structure arise by budding from tubules. Golgi body is single membrane bound cell organelle(Fig 2.3). About 60% proteins and 40% phospholipids occur in Golgi body.

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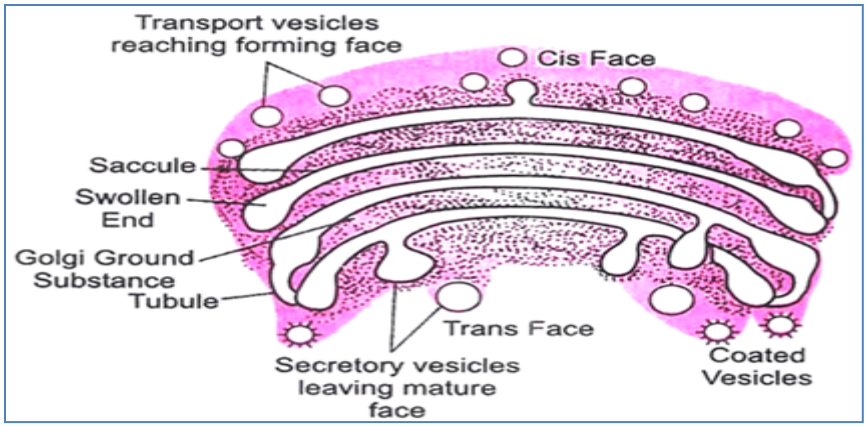

Fig.2.3: Golgi apparatus in section

Source:https://www.aplustopper.com/structure-function-golgi-apparatus/

- **Functions:**

The chief function of Golgi body is secretion of macromolecules. Secretion involves three steps: Golgi body receives the materials from E.R. through its cis- face. These materials are chemically modified by Golgi body. Glycosidation of proteins and lipids takes place in Golgi body. After chemical modification materials are packed in vesicles. In addition, the golgi complex is involved in secretion of zymogen granules from pancreas and lacto protein from mammany glands. Different types of macromolecules are to be sent outside the cell move through the Golgi body. Products from the Golgi apparatus go to three main destinations: (1) inside the cell to lysosomes (2) the plasma membrane (3) outside the cell. Thus Golgi body termed as "director of macromolecular traffic in cell" or middle men of cell. Golgi apparatus also receives biochemicals in a bulk flow from the rough endoplasmic reticulum. This is only organelle in the cell that receives, sorts, modifies, concentrates, packs and dispatches biochemicals for use inside and outside the cell.

2.4.3. Lysosome

Lysosomes are generally found in the cytoplasm of animal cell and exhibits polymorphism. The Lysosome was discovered by Christian De Duve (1955) and named it as Lysosome. The lysosomes are found in all types of eukaryotic cell and are responsible for digestion because it contain array of enzymes capable of breaking down all types macromolecules such as protein, nucleic acids, carbohydrate and lipids. Each lysosome is surrounded by membranes that are maintained in 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.

- **Structure:**

Lysosome is spherical bag like structures (0.1-0.8μm) which is covered by single unit membrane. They are large in Phagocytes (WBC) (0.8 to2μm). Lysosome are filled with 50 different type of digestive enzymes termed as acid hydrolyses. These acid hydrolyses function in acidic medium (pH=5).

- **Primary Lysosome:**

A primary lysosome is a membrane bounded sac that buds from the golgi apparatus. A primary lysosome contains many enzymes (collectively called acid hydrolases) that are synthesized on the RER and sorted in the Golgi. The newly formed primary lysosome stores enzyme acid hydrolases in the inactive from (Enzymes synthesized on ribosomes in cytoplasm) these are newly formed lysosome.

- **Digestive vacuoles:**

These lysosome is formed by the fusion of primary lysosome and phagosomes. It is also called as secondary lysosomes. Lysosome containing undigested material is called residual bodies. These are also called as telolysosomes. Lysosome containing cell organelles to be digested are known as autophagosome. In normal cells the autophagosome contains cellular components targeted for degradation. The contents of the autophagosome are degraded when it fuses to a lysosome.

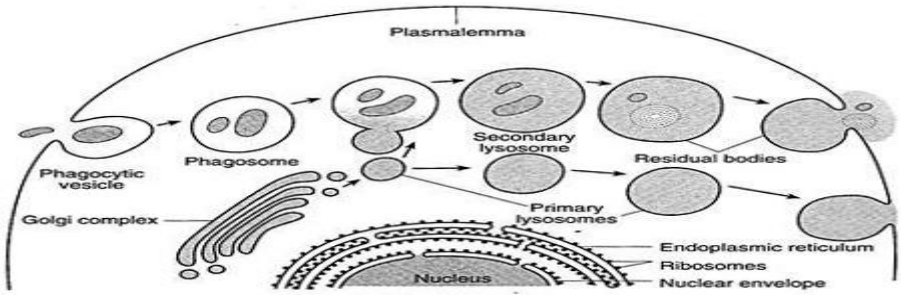

Fig.2.4: Diagram showing the origin and different phase of Lysosome

Source:http://www.biologydiscussion.com/lysosomes/lysosomes-meaning-structure-and-function-with-diagram/38571

- **Functions:**

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Lysosomes function 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. Autophagy takes place during starvation of cell. Excessive secretary granules of hormone in endocrine gland may be used for digestion by lysosome. Sometimes all lysosome of a cell burst to dissolve the cell completely. Lysosome is helpful in digestion of egg membrane to assist fertilization and also trigger the cell division.

2.4.4. Endoplasmic Reticulum (ER):

Structurally, the endoplasmic reticulum is a network of membranes found throughout the cell and connected to the nucleus. The membranes are slightly different from cell to cell and a cell's function determines the size and structure of the endoplasmic reticulum (ER). The ER is most important organelle in eukaryotic cells. ER was first observed by Garnier (1897) and their name was proposed by Porter (1961) thus credit for discovery of ER goes to Porter. ER produces transmembrane proteins and lipids for its membrane and for many other cell components including lysosomes, secretory vesicles, the Golgi apparatus, the cell membrane, and plant cell vacuoles. ER contains number of components which are shown in Fig. 2.4 as discussed below;

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

Vesicles- These are oval membrane bound structures.

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

- **Modification of E.R**.

Sarcoplasmic Reticulum: This smooth E.R. occurs in skeletal and cardiac muscles. S.R. Stores Ca^{+2} and energy rich compounds required for muscle contraction.

T-Tubules: These are transversely arranged tubules in skeletal and cardiac muscle cells. These transmits stimulus for contraction of muscles.

Ergastoplasm: When the ribosomes are accumulated on the small parallel cisternae of E.R. they are called as Ergastoplasm.

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Myeloid Bodies: Myeloid bodies are the specialized smooth E.R. which found in pigmented epithelial cells of the retina. Myeloid body is light sensitive structure and may be involved in pigment migration.

Microsome: These are pieces of E.R. with associated ribosomal particles (Claude 1951). These can be obtained by fragmentation and high speed centrifugation of cell. They do not exist as such in the living cell.

- **Structure:**

The structure of ER contains a network of tubules and flattened sacs. 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 (Fig.2.5). Protein molecules are synthesized and collected in the cisternal space/lumen. Smooth ER (SER) acts as a storage organelle. It is important in the creation and storage of lipids and steroids. 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 in the synthesis and packaging of proteins. In certain cell types, smooth ER plays an important role in the synthesis of steroid hormones from cholesterol. In cells of the liver, it contributes to the detoxification of drugs and harmful chemicals. The sarcoplasmic reticulum is a specialized type of smooth ER that regulates the calcium ion concentration in the cytoplasm of striated muscle cells.

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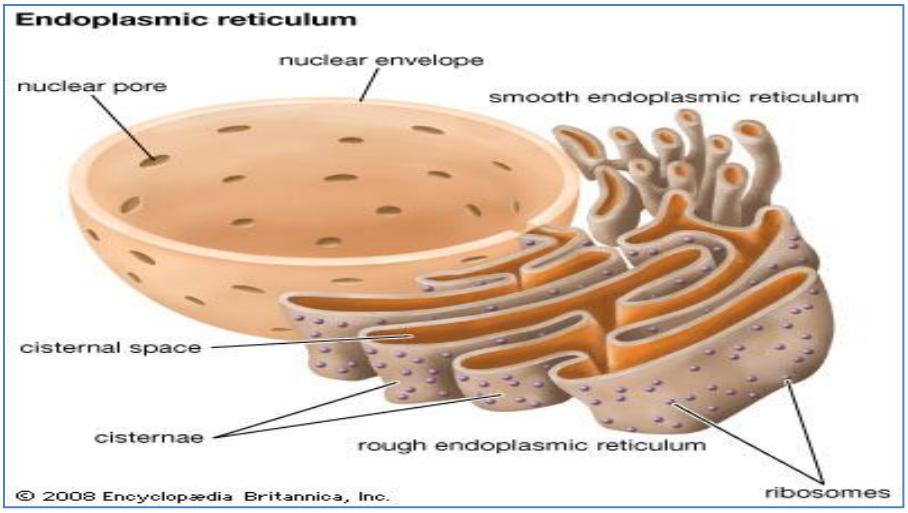

Fig.2.5: Diagram shows the different components of Endoplasmic reticulum

Source:https://alevelbiology.co.uk/notes/endoplasmic-reticulum-structure-and-function/

- **Functions:**

Endoplasmic reticulum is mainly responsible for the transportation of proteins and other carbohydrates to another organelle, which includes lysosomes, Golgi apparatus, plasma membrane, etc. 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, because rough E.R. has ribosome on its surface. Endoplasmic reticulum seems to play a role in breakdown of glycogen (glycogenolysis). Smooth ER are concerned with detoxification of drugs, pollutants and steroids.

2.4.5. Plastids

Plastids are double membrane-bound organelles found in all eukaryotic cells such as inside plants and some algae. The primary responsibility of plastids are related to making and storing food. Many plastids are photosynthetic, but some are not. The term "Plastid'' was first used by Haeckel and Choloroplast name was proposed by Erera (Fig. 2.5a, b).Some of common plastids are chloroplasts, chromoplasts, gerontoplasts and leucoplasts:

- **Chloroplasts:**

Chloroplasts are organelles specializing in the conversion of radiant energy to chemical energy. These organelles are only found in plant cells and some protists such as algae. Animal cells do not have chloroplasts. Chloroplasts work to convert solar energy into sugars that can be used by cells. The entire process is called photosynthesis and it all depends on the little green chlorophyll molecules in each chloroplast. Two membranes contain and protect the inner parts of the chloroplast. They are appropriately named as the outer and inner membranes. The inner membrane surrounds the stroma and the grana (stacks of thylakoids). Single thylakoid stack is called a granum.

- **Chrormoplasts**

Chrormoplasts are brightly colored plastids that act as the site of pigment accumulation. Chromoplasts are found in flowers, leaves, roots and ripe fruits, they contain carotenoids (lipid-soluble pigments ranging from yellow to red in color), which lend color to the plant tissues containing them. Chrormoplasts occur mainly in pericarp and petals. Red color of chillies and red tomatoes is due to the red pigment "Lycopene'' of chrormoplasts. Lycopene is a type of carotene. Yellowish-orange colors of fruits are due to α- cartone β, -carotene, and ϒ-carotene. β- Carotene is a precursor of vitamin- A. Richest source of β-carotenes are carrot roots.

- **Leucoplasts:**

Leucoplasts are the non-pigmented organelles which are colorless. They are usually found in most of the non-photosynthetic parts of the plant like roots depending on what the plant needs. They act as storage sheds for starches, lipids, and proteins depending on the needs of plant. Generally occur in non green and underground plant cells. They are more readily used for synthesizing amino acids and fatty acids. Leucoplasts are of three types namely amyloplasts, proteinoplasts and elaioplasts. Amyloplasts are larger than proteinoplasts and elaioplasts and are concerned with storage of starch while proteinoplasts helps in protein storage. The elaioplasts are specialized for the storage of lipids in plants.

- **Etioplasts:**

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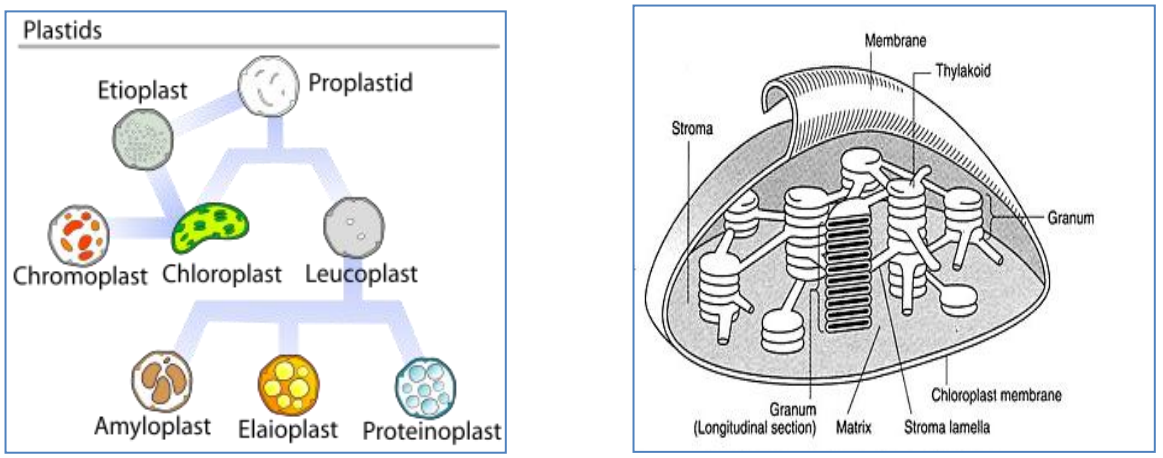
These are plastids without pigments, stored food and lamellar structures. These plastids occurs in etiolated plants due to the absence of light.



(a) **(b)**

Fig.2.6: a) Structure of different types of plastids, **b)** three dimensional structure of chloroplast.

Source: https://en.wikipedia.org/wiki/Plastid

- **Function**

- They provide color 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 centre of synthesis and metabolism of carbohydrates.
- During the dark reaction, glucose is synthesized through Calvin cycle in stroma.

2.4.6. Nucleus:

Nucleus is prominent organelles as compared to other component of the cells organelles which account for about 10% of cell volume. First of all Leeuwenhoek observed nucleus in Red Blood Cells (RBCs) of fish. In 1831 scientist Robert Brown again observed nucleus in orchid root cells and he performeddetailed study about this organelle. Thus the

credit of discovery of nucleus and nucleolus goes to Robert Brown. Nucleus is double membrane bound dense protoplasmic body, which control all cellular metabolisms and encloses the genetic information of cell. Nucleus play important role in controlling and regulating the activities of the cells such as growth and metabolism and contains all the genes. Nucleoli are the small bodies found within the nucleus, acts a site for the synthesis of ribonucleic acid (RNA) and protein. The gel-like matrix in which the nuclear components are suspended is called *nucleoplasm*. Enclosing the nucleoplasm is the nuclear envelope (nuclear membrane), which is made up of two layers of membrane: an outer membrane and an inner membrane.

Thus nucleus is very important and largest component of cell. However, some eukaryotic cells are without nucleus for example, red blood cells, whereas, some are multinucleated (consists of two or more nuclei), for example, slime molds. Nucleus is separated from the rest of the cell or the cytoplasm by double layer, the nuclear membrane. The study of nucleus is known as *Karyology*.

- **Structure:**

The cell nucleus consists of a nuclear membrane, nucleoplasm, nucleolus and chromosomes as shown in Fig.2.7. Nucleoplasm, also known as *karyoplasm*, is the matrix present inside the nucleus. Let's discuss in brief about the several parts of a cell nucleus.

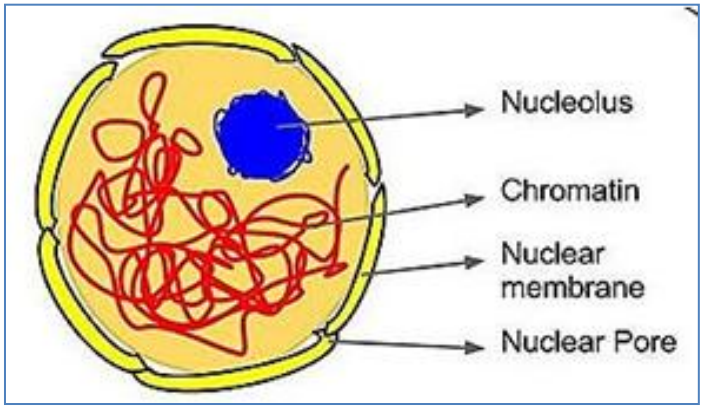


Fig.2.7. Structure of nucleus

Source: https://alevelbiology.co.uk/notes/nucleus-structure-and-functions/

- **Nuclear membrane:**

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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 be 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 nuclear membrane is also called by 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 form made up of a number of chemical like nucleotides, nucleosides, ATPs, proteins and RNA and DNA polymerases, end nucleases, minerals (Ca^{++},Mg^{++}) etc.

- **Nucleolus:**

The nucleolus (plural nucleoli) is a dense, spherical-shaped 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 basic proteins histones (protein molecules) called *chromatin*. The chromatin is further classified into heterochromatin and euchromatin based on the 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, less condensed organization of chromatin, which is found abundantly in a transcribing cell.In human beings, there are two types of chromosomes: autosomes and allosomes (also known as sex chromosomes). Each human cell normally contains 23 pairs of chromosomes. Twenty-two of these pairs, called

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autosomes, look the same in both males and females. The 23rd pair, the sex chromosomes, differ between males and females. Chromosomes are especially important in the process of cell division. If chromosomes don't replicate, new cells cannot be formed, and this will result in no growth and ultimately death. Chromosomes help keep DNA stable in its physical make-up as well as its biological make-up. They help to ensure that DNA is distributed and remade properly.

- **Functions:**

Speaking about the functions of a cell nucleus, it controls the hereditary characteristics of an organism. This organelle is also responsible for the protein synthesis, cell division, growth and differentiation. Here is a list of the important functions carried out by a cell nucleus.

Storage of hereditary material.

Nucleolus stores protein and RNA.

Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for protein synthesis.

During the cell division, chromatins are arranged into chromosomes in the nucleus. Production of ribosomes (protein factories) occurs in the nucleolus.

2.4.7. 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. Here 'S' stands for the sedimentation coefficient expressed in Svedgberg unit; it is indirectly a measure of density and size. Both 70S and 80S ribosomes are composed of two subunits. The 70S ribosome is made up of a 50S and 30S subunits while 80S ribosomes comprises of 60S and 40S subunits.

- **Structure:**

Ribosomes are made of proteins and ribonucleic acid found in almost equal amounts. It has small and big units that are called subunits. Ribosomes are found in

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cytoplasm and are also connected to the endoplasmic reticulum. Such endoplasmic reticulum having ribosomes are referred as rough endoplasmic reticulum. Around 37 to 62% of ribosomes are comprised of RNA and the rest is proteins. Prokaryotes have 70S (30S and 50S) and Eukaryotes have 80S (40S and 60S) ribosomes subunits.

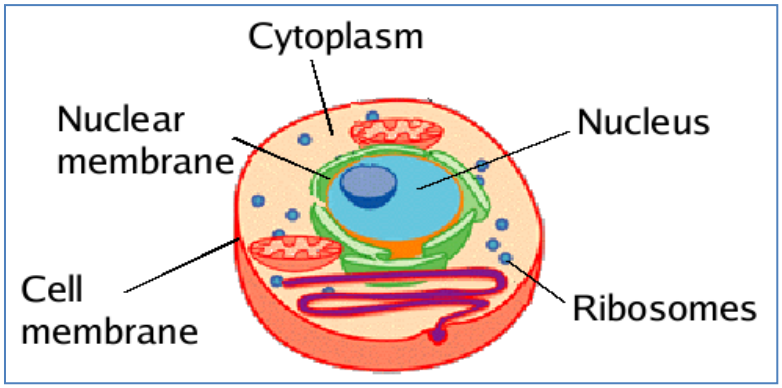

Fig. 2.8.: Structure of ribosomes in cell
Source:https://www.microscopemaster.com/ribosomes.html

- **Functions:**

- The procedure of creation of proteins, the deoxyribonucleic acid makes mRNA by the DNA transcription.
- The arrangements of protein assembly amid protein synthesis are indicated in the mRNA.
- The mRNA is arranged in the nucleus and is moved to the cytoplasm for an additional operation of protein synthesis.

2.4.8. Vesicle

Vesicles are composed of lipids which used to transport materials from one place to another. It is specific cell organelles used for storage as well as performs metabolic and enzymatic process. Fig 2.9 shows the overall structure of a simple vesicle. As it is composed of a lipids layer, it has a completely self-contained environment that is different from the inside of the cell. There are essentially four types of vesicles such as

Lysosomes

, transport vesicles,

and secretory vesicles which used in cell.

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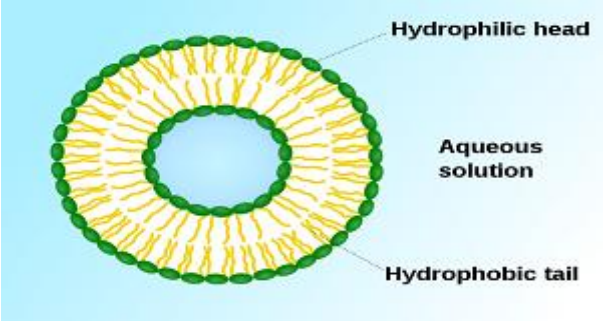

Fig. 2.9 : Diagram of simple types of vesicles

Source: https://study.com/academy/lesson/vesicles-definition-function-quiz.html

2.4.9. Cytoskeleton

An elaborate network of filamentous proteinaceous structures present in the cytoplasm is collectively referred to as the cytoskeleton. The cytoskeletons in a cell are involved in many functions such as mechanical support, motility and maintenance of the shape of the cell. The use of high-voltage electron microscopy on whole cells has helped to demonstrate that there is a highly structured, three-dimensional lattice in the ground cytoplasm. Figure 2.10 gives an overview of the cytoskeletal system. They are classified based on their size, function and distribution within the cell. The primary types of fibers comprising the cytoskeleton are:

Microfilaments: it forms part of the cytoskeleton and are primarily composed of polymers of actin, but in cells. Microfilaments also provide some rigidity and shape to the cell. They can depolymerize (disassemble) and reform quickly, thus enabling a cell to change its shape and move.

Intermediate filaments: Intermediate filaments are found in cells of vertebrates, and many invertebrates. Intermediate filaments have a diameter of about 10 nm are composed of a family of related proteins sharing common structural and sequence features. Intermediate filaments contribute to cellular structural elements and are often crucial in holding together tissues like skin.

Microtubules:it is microscopic hollow tubes made of the proteins alpha and beta tubulin that are part of a cells.

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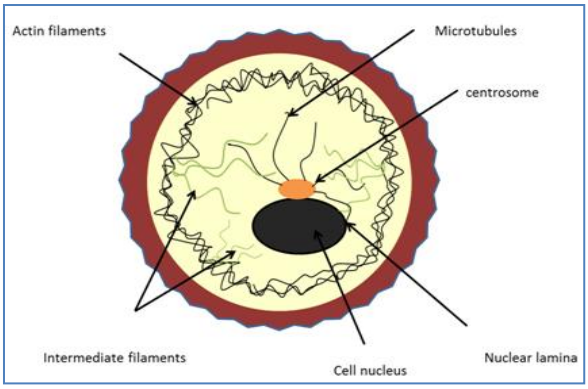

Fig.2.10 : The cytoskeletal system

Source: https://nptel.ac.in/courses/102103012/4

2.4.10. Vacuole

The vacuole is the special type of structure in cytoplasm which contains water, sap and excretory substances which are not useful for the 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 is facilitated by tonoplast in plants cell. In Amoeba the contractile vacuole is important for excretion. In many cells, as in protists, food vacuoles are formed by engulfing the food particles.

Function:

Vacuoles can serve a wide variety of functions in a cell, and their importance depends on what role they play within the cell. Typically, their job includes isolating harmful materials, storing waste products, storing valuable water in a plant cell, helping maintain the pressure within a cell, balancing the pH of a cell, exporting products out of the cell, and storing proteins for seed germination.

2.4.11. Cytosol:

Cytosol is a water based fluids in which organelles, proteins and other structures of the cell are embedded. The cytosol of any cell is a complex solution, whose properties allow the functions of life to take place (Fig.2.11). Cytosol contains different macromolecules. In cytosol about 70 % water is present which cover large part of cells. In addition to water,

cytosol also consists of small molecules, dissolved ions and large water-soluble water molecules (e.g., proteins).

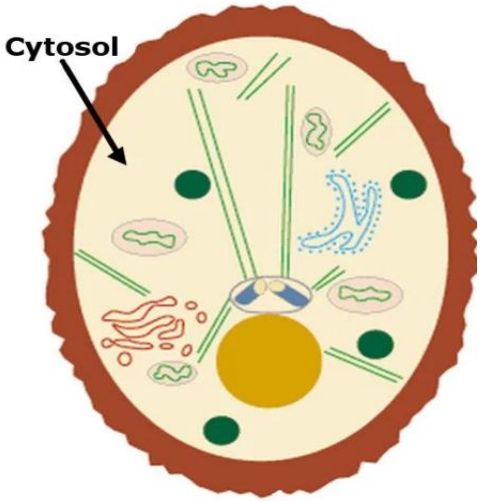

Fig.2.11 : Cytosol consists of dissolved ions and water-soluble molecules.

Source:https://www.scienceabc.com/pure-sciences/what-is-cytosol-how-is-it-different-from-cytoplasm.html

- **Functions:**

Cytosol serves as medium for transfer of proteins, ions and other ingredients inside the cell. The enzymatic and signal transduction activities takes place in cytosol because it regulates the pH levels, salt concentration and other environmental condition during enzymatic activity. Messenger molecules may diffuse through the cytosol to change the function of enzymes, organelles, or even DNA transcription.

2.4.12. Lysosome:

Lysosomes are cell organelles first discovered by Novikoff. It is spherical bag like structure which cover single unit membrane as shown in Fig. 2.12. Lysosomes are highly polymorphic cell organelles showing different morphological status. There are several types of lysosomes such as primary or storages granules lysosomes, digestive vacuoles, residual bodies and autophagic lysosomes. The isolated lysosomal vesicles have been found to be very rich in almost all types of hydrolytic enzymes optimally active at the acidic pH. These enzymes are capable of digestion of different macromolecules.

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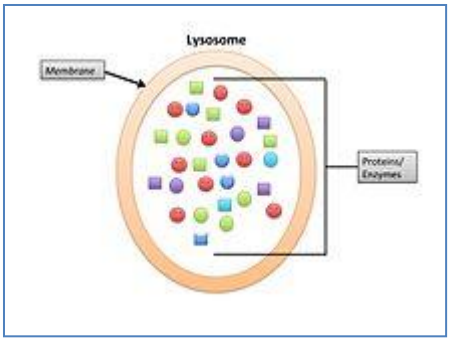

Fig. 2.12: Diagram of Lysosome.

- **Functions:**

Heterophagy lysosomes are associated with digestion of foreign materials in cells and autophagy is useful in digestion of old or dead organelles of the cell. Excessive secretory granules of hormones in endocrine gland may be digested e by lysosomes. Lysosomes are helpful in digestion of egg membrane to assist fertilization and are also known to trigger the cell divisions.

2.4.13. Centriole:

There are two short cylindrical structures called centriole found in the cell especially during cell divisions (Fig.2.13). They are located near the nucleus and are arranged in such a way that they are right angle to each other tubules in group of three run longitudinally in the wall of centriole. Centrioles might be found in animal cells, lower plants and in base of cilia and flagella (as basal bodies). With a diameter of about 250nm and a length ranging from 150-500nm in vertebrates, centrioles are some of the largest protein-based structures. The nine triplets microtubules are some of the most recognizable features of this organelle. In human beings, however, among other higher animals, they exist as complex triplets that make up the scaffold of the microtubules arranged in a circle (at an angle) around the central core.

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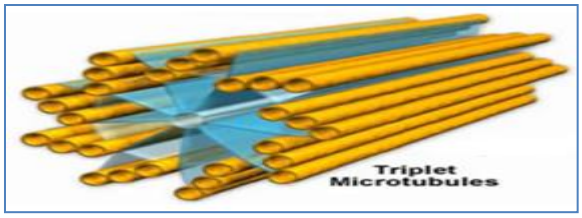

Fig.2.13. Diagram of centriole

- **Functions:** They are concerned with the moments of chromosomes during cell division.

2.4.14. 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 protect the cell from mechanical damage and infection, it also helps in cell-to-cell interaction and provides barrier to undesirable macromolecules. In this chapter you have learned about the cell membrane that is composed of lipids and proteins. Cell membrane is selectively permeable in nature as it allows passage of specific molecules/ions across the membrane.. It provides passage for many molecules without any requirement of energy by the means passive transport. It also provides passage for few ions according to their concentration gradient. The structural and functional attributes of different cell organelles like mitochondria, plastids, ribosomes, endoplasmic reticulum, nucleus, golgi apparatus, lysosomes, centrioles, vacuoles etc.. has been described in this chapter..

2.4.15. Terminal questions

Q.1. Discuss structure and function of cell wall.

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

Q.2. Write difference between prokaryotic and eukaryotic cell ?

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

Q.3. What are different cell organelles? Write functions of different cell organelles.

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

Q.4. Discuss structure and function of mitochondria.

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

Q.5. What are plastids? Discuss types and role of plastids in plant cell.

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

Q.6. Discuss structure and function of cell membrane.

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

2.4.16. Further readings

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Rajarshi Tandon Open University, Prayagraj

PGBCH-101

Cell Biology and Bio-molecules

Block- II

Biomolecules part -I

UNIT -3

Carbohydrates

UNIT-4

Proteins

Introduction

The second block comprises Bio-molecules. It consists of following two units:

Unit-3: In this unit we deal with the Carbohydrate, its basic definition, structure, types and function. Properties of monosaccharides, disaccharides, and polysaccharides have been discussed. Derivatives of monosaccharides with their stereoisomerism are also discussed briefly.

Unit-4: This unit covers the basic structure, function and acid base reaction of amino acids. The chemical composition, primary, secondary, tertiary and quaternary structures of protein are also defined. The protein-protein and protein- nucleic acids interactions are discussed briefly. The synthesis and structure of hemoglobin function and malfunction of protein has also been discussed.

Unit-3: Carbohydrates

Structure

3.1. Introduction

Objectives

3.2. About Carbohydrate

3.3. Monosaccharides

3.3.1. Aldose and Ketoses

3.3.2. Structure of monosaccharides

3.3.3. Classification of monosaccharides

3.3.4. Stereoisomerism of monosaccharides

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3.1. Introduction

This unit covers the introduction, structure and classifications of carbohydrates. Carbohydrates are important macromolecules because they are the main source of energy in living organism. At chemical level carbohydrate consists of carbon, hydrogen, and oxygen element. The carbohydrates are mains molecules that are daily require for human body. According to National Institute of Health, the recommended daily amount (RDA) of carbohydrates for adults is 135 grams. Generally carbohydrates are classified as monosaccharides, disaccharides, oligosaccharides and polysaccharides. Here in this unit you will study the structure of aldose and ketoses form of carbohydrates. The derivatives of monosaccharides have specific features due to their structural combinations. The monomers, of carbohydrates are linked together by glycosidic bond which determines the shape and size of carbons chains which is also present in structure. In addition, brief study of the general features and acid base reactions of carbohydrates is also discussed.

Objectives

- To study the carbohydrate macromolecules.
- To study the structure and classification of carbohydrates.
- To discuss structure and features of various derivatives of monosaccharides.
- To illustrate family of Aldose and Ketoses in carbohydrates.
- To know the physical and chemical properties of various carbohydrates.

3.2. Overview on carbohydrate

Carbohydrates are polyhydroxy aldehydes or ketones. Carbohydrates are the body's main source of energy. They are called carbohydrates because, at the chemical level, they contain carbon, hydrogen and oxygen. They are primarily produced by plants and form a very large group of naturally occurring organic substances. The recommended daily amount (RDA) of carbohydrates for adults is 135 grams, according to the National Institutes of Health (NIH). However, people with diabetes should not eat more than 200 grams of carbohydrates per day, while pregnant women need to take at least 175 grams. It is the fuel for the central nervous system and energy for working muscles. Carbohydrates

represent the most important source of energy for the body, and are vital for a varied and balanced diet and accounts for one of the four major bimolecular classes including proteins, lipids, and nucleic acids. The name "carbohydrate" is derived from 'hydrates of carbon', and they arise from photosynthesis, where they exist as products from carbon dioxide and water.

$$nCO_2 + nH_2O + (\text{Solar energy}) \rightarrow ADP + C_nH_{2n}O_n + nO_2$$

Where, the general empirical structure for carbohydrates is $(CH_2O)_n$ means ratio of 1 Carbon: 2 Hydrogens: 1 Oxygen and ADP (Adenosine diphosphate) is a product that can be synthesized to form ATP (Adenosine-5'-triphosphate) –ATP is the form of chemical energy used in cells which acts as a fuel of metabolism in plants and animals, through aerobic cellular respiration. Digestive system changes carbohydrate into glucose (blood sugar). Your body uses this sugar for energy for your cells, tissues and organs. It stores any extra sugar in your liver and muscles for when it is needed.

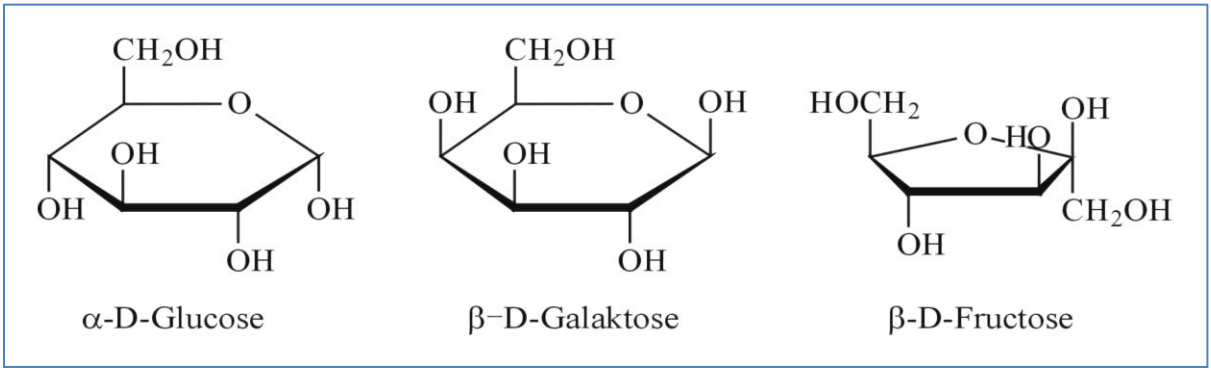
Carbohydrate is one out of the three macronutrients in our diet. All macronutrients are obtained through diet; the body cannot produce macronutrients on its own. Protein is the one of them, responsible for the structure, function and regulation of the body tissues and organs. And third one is fat which is required for healthy bones and skin.

All carbohydrates are made of building blocks called sugars; it can be further classified on the basis of number of sugar units. They are monosaccharides, disaccharides, oligosaccharides and polysaccharides. Sugars have the ability to form cyclic compounds, typically five or six member heterocyclic rings. Simple carbohydrates include sugars found naturally in fruits, vegetables, milk, and milk products. Complex carbohydrates found in whole grain, breads, cereals, starchy, vegetables and legumes are good sources of fiber.

3.3. Monosaccharides

Monosaccharides, meaning one sugar (mono = one, saccharide = sugar), consists of a single polyhydroxy aldehyde or ketone unit. The monosaccharides are simple sugars that serve as fuel molecules as well as fundamental constituents of living organisms, and also simplest carbohydrates. Glucose, fructose (found in fruits) and galactose (found in milk products) are prominent examples among this single unit sugars i.e. monosaccharides.

$(CH_2O)_x$ where *x* is any number between three and eight. The D-glucose is considered as parental monosaccharide from which the other are derived. The D- glucose is basic building block of the most abundant polysaccharides, such as starch and cellulose. The monosaccharides is found in fruits, berries, vegetables, honey and glucose-fructose syrups. Most of monosaccharides have a sweet taste. The monosaccharides are white crystalline in nature and soluble in water but non soluble in nonpolar solvents.



3.3.1. Aldose and ketoses

We know that carbohydrate consists of carbon, hydrogen and oxygen in their carbon chain. The simplest carbohydrate is monosaccharides which represents by simple formula CH_2O. Where n represent the number of carbon present in chain of carbohydrate molecules. The carbon chain when consists of number of hydroxyl (OH) groups and either one aldehyde group (CHO) is known to be aldose. Beside this, when carbon chain consists number of hydroxyl (OH) group and either one ketone (=C=O) group is known to be ketose. The smallest, carbohydrate is called trioses. Thus glyceraldehyde is triose that has an aldehyde group and so as an aldose. Thus it can also be called as aldotriose, similarily dihydroxyacetone is a ketotriose.

Fig.3.1: Structure of glyceraldehyde and dihydroxyacetone

3.3.2. Structure of monosaccharides:

Although the chemical structure of each sugar differs, but the chemical formula is the same as $C_6H_{12}O_6$. This designates a central carbon molecule bonded to two hydrogen and one oxygen. The oxygen here is bonded to hydrogen creating a hydroxyl group. One of the carbon in the chain form double bond with an oxygen is called carbonyl group. If this carbonyl group occurs at the end of the chain, the monosaccharide is in the *aldose* family. If the carboxyl group is in the middle of the chain, the monosaccharide is in the *ketose* family. Monosaccharides may be subcategorized as aldoses or ketoses depending on whether it consists of aldehyde or ketone functional groups respectively. The aldoses or ketoses are found as either L or D enantiomer. Dihydroxyacetone is the simplest ketose sugar and glyceraldehyde is the simplest aldoses. In both classes, monosaccharides (aldoses or ketoses) hexoses are by far the more abundant. Glucose is most common monosaccharides found in nature (Fig.3.2) and it is used by nearly every form of life.

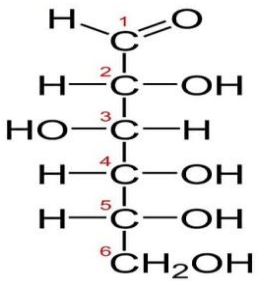


Fig.3.2: Structure of Glucose

3.3.3. Classification of monosaccharides

The monosaccharides are the simplest form of the carbohydrates, since they contain only one polyhydroxy aldehyde or ketone unit. Monosaccharides may be further classified, which is based on the number of carbon atoms as triose (n=3), tetrose (n=4), pentose (n=5), hexose (n=6), heptose (n=7), etc. For example, n=5 carbon aldopentose is ribose; n=6 carbon aldose is glucose, etc. the simple monosaccharides having the three carbon i.e. triose, like glyceraldehyde and dihydroxyacetone. The presence of an aldehyde is indicated by the prefix aldo- and a ketone by the prefix keto-. It is important to note that sugars can be reducing or non-reducing depending on whether a sugar has a carbonyl moiety in its linear form. This means that the sugar in cyclic form is either in hemiacetal or hemiketal

form which represented by the Fischer Projection, while in cyclic form, shown by using Haworth projections (Fig.3.3).

Table 3.1: Name of some carbohydrates

#Carbon	Category name	Examples of monosaccharides
3	Triose	Glyceraldehyde, Dihydroxyacetone
4	Tetrose	Erythrose
5	Pentose	Ribose, Ribulose, Xylulose
6	Hexose	Glucose, Galactose, Mannose, Fructose
7	Heptose	Sedoheptulose
9	Nonose	Neuraminic acid, also called sialic acid

Fig.3.3: Fisher and Haworth projection in monosaccharides

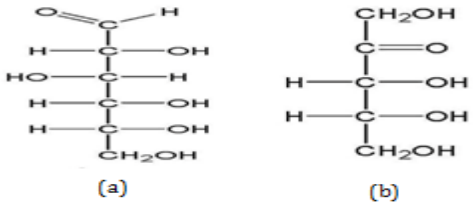
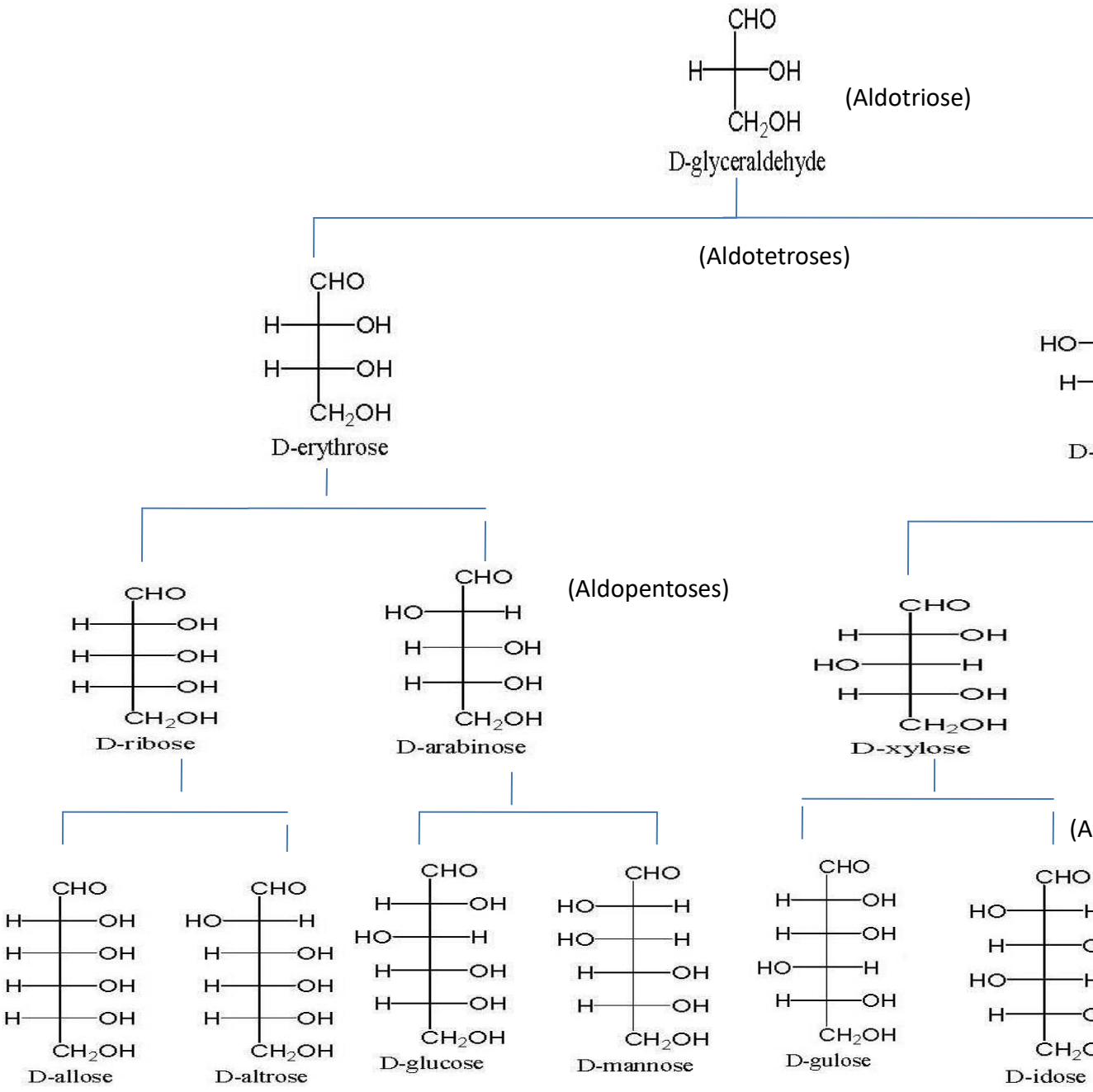
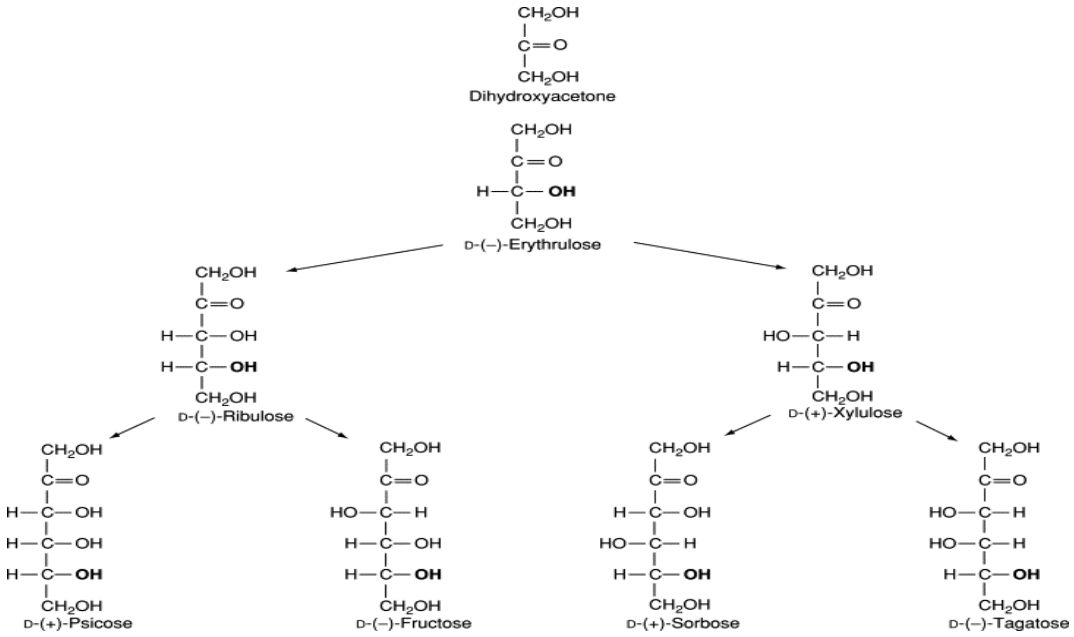


Fig.3.4: The monosaccharides: a) glucose an aldohexose having six carbons, b) ribose a ketopentose having five carbons.


(Aldotriose)
D-glyceraldehyde
(Aldotetroses)
D-erythrose
(Aldopentoses)
D-ribose
D-arabinose
D-xylose
D-allose
D-altrose
D-glucose
D-mannose
D-gulose
D-idose

(a)



(b)

Fig.3.5 : The family of (a) aldose and (b) ketoses having form the three to six (C3-C6) carbon atoms shown in open chain structural formulas.

3.3.4. Stereoisomerism of monosaccharides:

In monosaccharides, each carbon atom bonded to a hydroxyl group except for the first and last one is a stereo center of R or S, potentially forming many isomers for the same chemical formula of saccharide. Generally all the monosaccharide contains one or more asymmetric carbon atoms and thus form chiral carbon. It is known that the chiral carbons are those carbons which connect to four different function groups and it gives non-super imposable mirror images, this carbon is also called chiral carbon or a center of chirality. In addition, if any of the groups on the carbon are the same, the carbon atom cannot be chiral carbon. There are some examples of chiral carbon as shown in Fig 3.6.

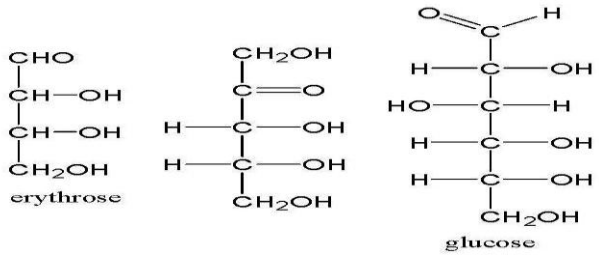


Fig.3.6: Chiral carbon atom in carbohydrate.

Glyceraldehyde contains only one asymmetric carbon atom and therefore can exists as two different stereoisomers as shown in Fig (3.6). Other molecules have different stereoisomers such as Aldotetroses have two (2^n, 2^2 = 4,), Aldopentoses three (2^n, 2^3 = 6,), and Aldohexoses have four (2^n, 2^4 = 8,), asymmetric carbon atom, respectively. The monosaccharides with asymmetric carbon atom are expected to be optically active molecules. For example, the usual form of glucose found in nature is dextrorotatory and the usual form of fructose is levorotatory but molecules are D-series, since their absolute configuration are related to D-glyceraldehyde.

Fig.3.7: Glyceraldehyde exists in two isomeric forms that are stereoisomers of each other.

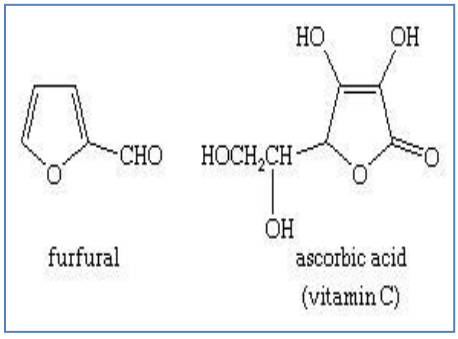
Fig.3.8: D- glucose and L-fructose configuration

Monosaccharides are classified as the or β anomers in their cyclic forms where the carbon atom of the carbonyl oxygen is called the anomeric carbon atom. Because the carbonyl carbon is sp^2 hybridized in the open chain form, when the sugar cyclizes, the carbonyl carbon forms a new chiral center; the anomer of glucose has the hydroxyl group attached to anomeric carbon in a Trans position relative to heteroatom oxygen. The β-anomer occurs when the hydroxyl group is on the same side or in cis position as the heteroatom oxygen. Both anomers are in equilibrium and are constantly changing between the ring and straight-chain conformations.

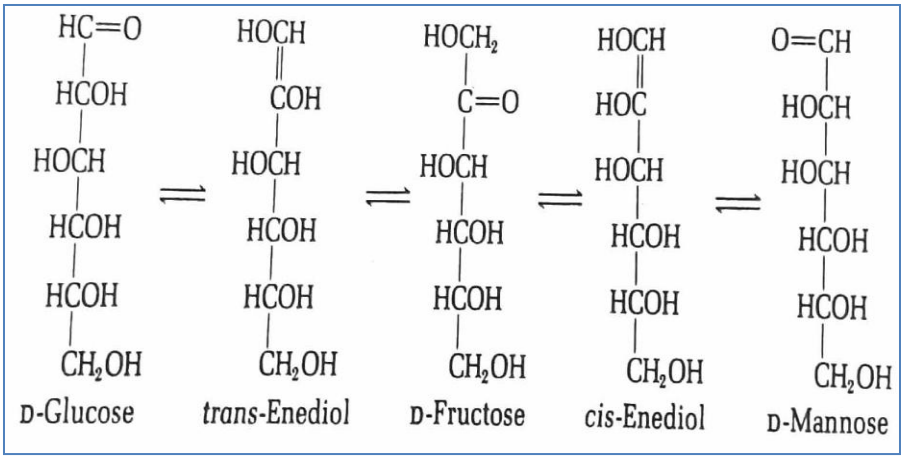
Anomeric carbon is defined as hemiacetal or hemiketal carbon which results in different forms of stereoisomer for the saccharide. The anomeric position is easily oxidized and can form glycosidic linkages readily.

3.3.5. Effect of acid and base on monosaccharides:

The monosaccharides are mostly found to be stable at hot dilute mineral acids. But at concentrated acid the sugar molecules give furfurals and aldehyde derivatives of furan. When D-glucose is heated with strong HCl, it gives 5-hydroxymethylfurfural as shown in Fig (3.9a). However in the presence of phenols furfurals get condensed and give orcinolie. color compound used in colorimetric analysis of sugar. For example when D-glucose is treated with dilute alkali it gives equilibrium mixture of D-glucose, D-fructose and D-manose as shown in Fig. (3.9b). However, it has been found that high temperature or high alkali concentrations causes free monosaccharides which undergoes further rearrangement and give fragmentation or polymerization.



(a)



(b)

Fig.3.9: (a) Furfural formation form glucose at conc. acid. (b) Isomerization of D-glucose by dilute base.

3.3.6. Derivatives of monosaccharides:

Due to the presence of functional groups, the monosaccharides have several derivatives. Some of which have physiological importance such as sugar phosphate, , deoxy and amino sugars; sugar alcohol and sugar acids. Some of the derivatives are discussed as follows;

3.3.6.1. Sugar acids:

Sugar acid is an oxidized form in which aldehyde or alcohol functional groups are oxidized into carboxylic acids. Thus, the sugar acids are obtained from aldose in which the function group (aldehyde carbon or primary alcohol) present at carbon C1 is oxidized into carboxylic acids. Sugar acids are important component of many polysaccharides. For example, Gluconic acid is produced from glucose by oxidation of aldehyde (C1 group) whereas glucuronic acid is formed when primary alcohol group (C6) is oxidised as shown in Fig (3.10). There are three important types of sugar acids namely aldonic, aldaric and uranic acids. When the aldose oxidized by weak oxidizing agents the alonic acid is obtained. The strong oxidizing agents when applied, both the aldehydic carbon and the carbon atom bearing primary alcohol are oxidized to carboxylic group and yields aldaric acid. The aldaric acid is also called saccharic acid. The uranic acids are obtained when only the carbon atom bearing primary alcohol is oxidized to carboxylic group. The uranic acid

derived from D-glucose is D-glucuranic acids. Vitamins are also most important sugar acids that have biological importance.

Fig.3.10: Sugar acids: gluconic and glucuronic acid.

3.3.6.2. Sugar alcohol:

The sugar alcohol is produced by reduction of aldoses or ketoses. Here the carbonyl oxygen of the parent monosaccharides is reduced and produces a polydroxy alcohol. For example, glycerol and myo-inositol are the important components of lipids. Ribitol is a component of FMN and FAD. Sugar alcohol D- Glucitol is also formed by the reduction of L-sorbose and often called L-sorbitol whereas the D- Mannitol is obtained from the reduction of D-Mannose. Glycerol and inositol are important alcohol sugar found abundantly in nature. The structure of some sugar alcohol is shown in Fig.3.11.

Fig. 3.11:Sugar alcohol, sarbito and mannitol.

3.3.6.3. Amino sugars

The amino sugars are obtained by replacement of one or more hydroxyl group of the monosaccharides by amino groups. The wide distribution of two amino sugars such as D-glucosamine and N-acetylneuraminic acid shows in which the hydroxyl group at carbon atom at 2position is replaced by the amino group which is shown in Fig. (3.12). The D-

glucosamine is a major component of chitin and occurs in many polysaccharides of vertebrate tissues. The amino groups of amino sugars are sometimes acetylated eg. N-acetyl-D-glucosamine. N-acetylneuraminic acid (NANA) is derivates of N- acetylmannose and pyruvic acid. It is important constituent of glycoprotein's and glycolipids.

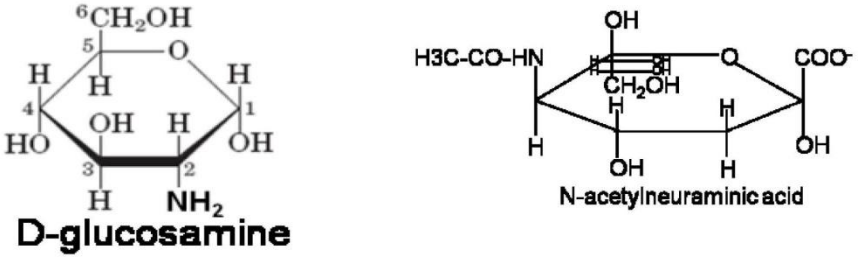


Fig. 3.12: Amino sugars; D-glucosamine and N-acetylneuraminic acid.

3.3.6.4. Sugar phosphates:

Phosphate derivatives of monosaccharides are found in all living cells, in which they serve as important intermediates in carbohydrates metabolism. Representative sugar phosphates are shown in Fig (3.13)

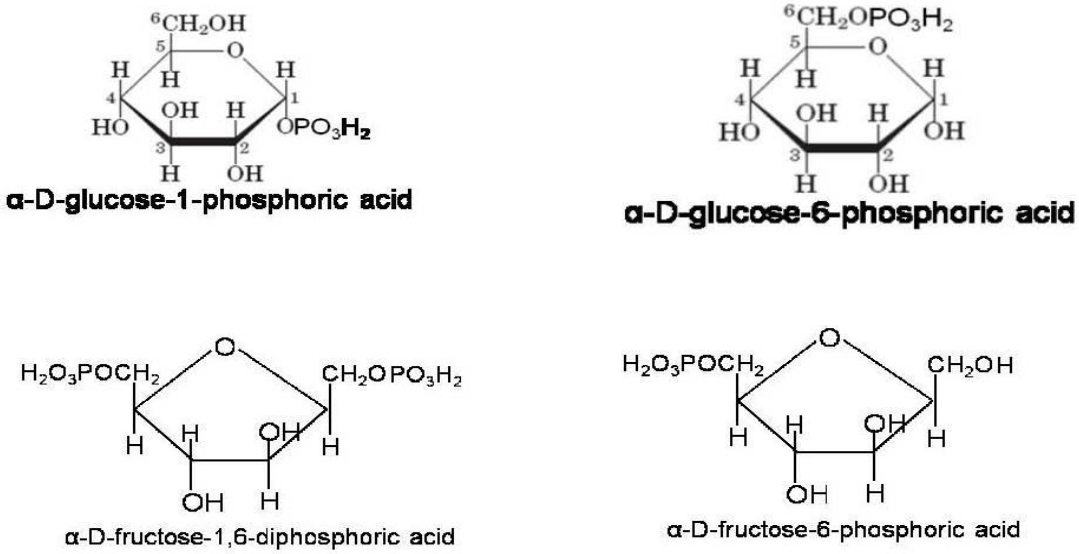


Fig.3.13: Sugar phosphates–α-D-glucose-1phosphoric acid, α-D-glucose-6-phosphoric acid, α-D-fructose-1-6-diphosphoric acid, and α-D-fructose-6-phosphoric acid.

3.3.6.5. Deoxy sugars:

These are the sugar which contains one oxygen atom less than that present in the parental molecule. The groups -CHO and CH_2OH become CH_2 and $-CH_3$ due to absence of oxygen. D-2-deoxyribose is the most important deoxy sugar since it is a structural constituent of DNA shown in Fig.3.14.

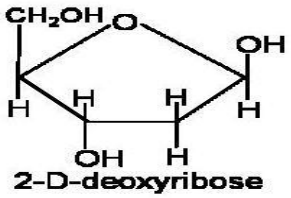


Fig.3.14: Deoxy sugar- 2-D- deoxyribose.

3.3.6.6. Glycosides:

Glycosides are formed when the anomeric hydroxyl group of a monosaccharide undergoes condensation with the hydroxyl group of a second molecule, with the elimination of water. Aldopyranoses readily react with alcohol in the presence of a mineral acid to form anomeric α and β-glycosides. The glycosides are asymmetric mixed acetals formed by the reaction of the anomeric carbon atom of the intermolecular hemiacetal or pyranose of the aldohexose with a hydroxyl group furnished by an alcohol. The linkage resulting from such a reaction is known as a *glycosidic bond*. Glycosides are named for the sugar that provides the hemiacetal group. Thus, if glucose provides the hemiacetal group, the resultant molecule is a glucoside; if galactose provides the hemiacetal group, the result is a galactoside (Fig.3.15). Classification of glycosides on the basis of the linkage between glycone and aglycone part are:

O-glycosides : in these glycosides the sugar part is linked with alcoholic or phenolic hydroxyl or carboxyl group.

S-glycosides : in these glycosides the sugar attached to a Sulfur atom of aglycone such as in sinigrin.

N-glycosides : in these glycosides the sugar linked with Nitrogen atom of (-NH2,-NH-) amino group of aglycone like in nucleosides DNA,RNA.

C-glycosides : in these glycosides the sugar linked (condensed) directly to Carbon atom of aglycone like in aloin.

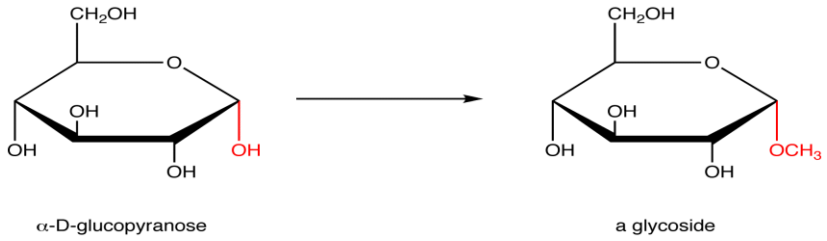


Fig.3.15: Glycosides structure

3.3.6. Physical and chemical properties of monosaccharides:

Monosaccharides are solid at room temperature and extremely soluble in water. The presence of OH groups makes it much more water soluble despite of higher molecular weight than other similar molecular weight compounds. For example the glucose can be dissolved in minute amounts of water to make syrup (1g/ml H_2O). Most of monosaccharides are called sugar because they have sweet test. Monosaccharides do not usually exist in solution in their "open-chain" forms: an alcohol group can add into the carbonyl group in the same molecule to form a pyranose ring containing a stable cyclic hemiacetal or hemiketal.

In the pyranose form of glucose, carbon-1 is chiral, and thus two stereoisomers are possible: one in which the OH group points down (α-hydroxy group) and one in which the OH group points up (β-hydroxy group). These forms are anomers of each other, and carbon-1 is called the anomeric carbon.

Table3.2: Relative sweetness of different carbohydrates.

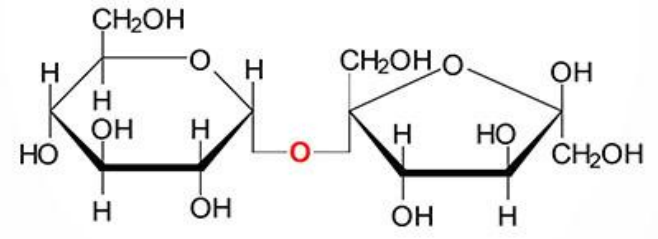
Sugar	Relative sweetness	Type
Lactose	0.16	Disaccharide
Galactose	0.22	Monosaccharide
Maltose	0.32	Disaccharide
Xylose	0.40	Monosaccharide
Glucose	0.74	Monosaccharide
Sucrose	1.00	Disaccharide
Invert sugar	1.30	Mixture of glucose and fructose
Fructose	1.73	Monosaccharide

3.4.Diasachharides

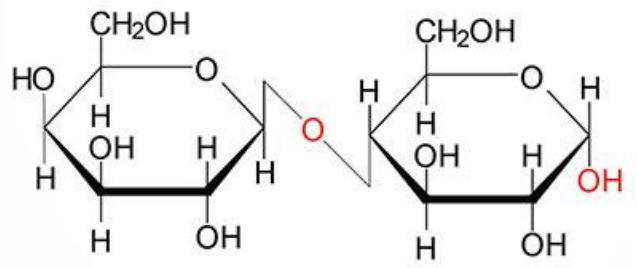
Double units of monosaccharides are called disaccharides in which both units are joined together by glycosidic linkage. The most common disaccharides are lactose (milk sugar), maltose and sucrose shown in Fig (3.16). Sucrose is common sweetener, which are formed by D-fructose and α-D-glucose linked through 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 maturation 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 another 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 mixed 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 –glucose molecule.

Table 3.3: Some example of disaccharides.

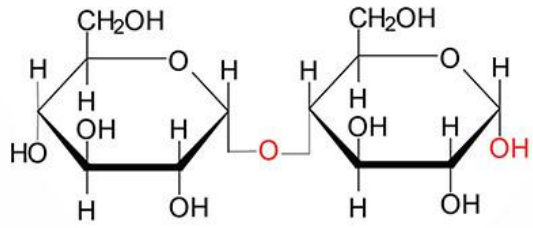
Name	Components of sugar	Description
Sucrose	Glucose + Fructose	From sugar cane and sugar beet
Lactose	Glucose + Galactose	Milk sugar
Maltose	Glucose + Glucose	Malt sugar, from germinating cereals
Trehalose	Glucose + Glucose	Made by plant and fungi
cellobiose	Glucose + Glucose	Breakdown product of cellulose



(a) Sucrose



(b). Lactose



(c) Maltose

Fig. 3.16: Chemical structure of some disaccharides:(a) Sucrose (b**).**Lactose (c) Maltose

3.5.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(3.17) 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.

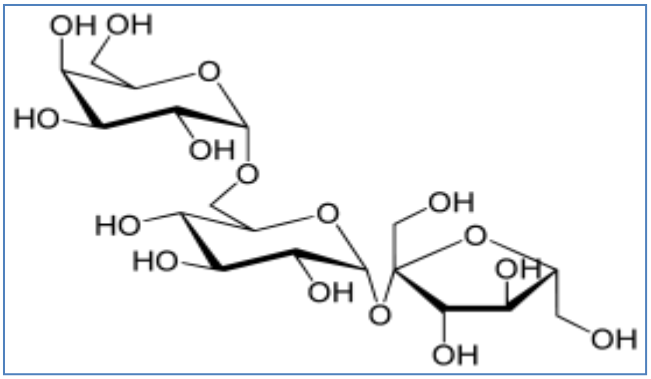


Fig.3.17: Chemical structure of trisaccharides – raffinose

3.6.Polysaccharides or glycans

Double units of monosaccharides are called disaccharides in which both units joined together by glycosidic linkage. The most common disaccharides are lactose (milk)

Complex carbohydrates are also known as polysaccharides or glycans. They are polymeric macromolecules i.e. have three or more sugars or Polysaccharides contains more than 10 monosaccharide units and can be quite large. Mostly carbohydrates found in nature are polysaccharides with high molecular weight. Due to hydrolysis with acid or specific enzymes these polysaccharides yield monosaccharides or simple monosaccharides derivatives. The predominant monosaccharide found in polysaccharides is D-glucose, but polysaccharides of D-mannose, D-fructose, D- galactose, and D-xylose are also common. They are often referred to as starchy foods and include beans, peas, lentils, peanuts, potatoes, corn, parsnips, whole-grain breads and cereals. Polysaccharides which are also called glycans differ in the nature of their recurring monosaccharides units in the length of their chain and in the degree of their branching.

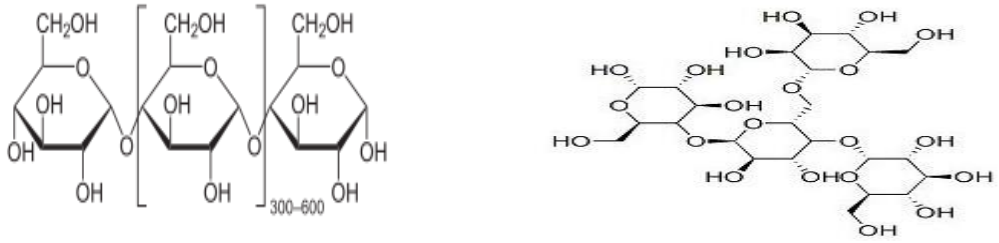
Depending upon the type of monomeric units, they are divided into homopolysaccharides and heteropolysaccharides. Polysaccharides composed of a single type of monosaccharide as a building block, are termed as homopolysaccharides while those composed of more than one type of monosaccharide are termed as heteropolysaccharides. Starch which contains only D- glucose units is a homopolysaccharides whereas Hyaluronic acid consists of alternating residues of D-glucuronic acid and N- acetyl-D-glucosamine is an example of heteropolysaccharides.

3.6.1. Starch

Starch is a *Homopolysaccharide* shown in Fig. 3.18. It is found in plants as a food reserve. Its structure is identical to glycogen, except for a much lower degree of branching (about every 20–30 residues). Unbranched starch is called **amylase**; branched starch is called **amylopectin**. In amylose, 1000–5000 D-glucose units are joined linearly by α-1→4 glycosidic bonds while in amylopectin, polymer of over 600,000 glucose units are inserted on the main chain by α-1→6 bonds. Amylase is not truly soluble in water but forms hydrated micelles which give a blue color with iodine. The amylopectins yieldcolloidal or miceller solution giving a red –violet color with iodine. The major components of starch can be enzymatically hydrolyzed in two different ways. Amylase can be hydrolyzed by α-amylase which is present in saliva and pancreatic juice and participate in digestion of starch in gastrointestinal track. It hydrolyzes α-[1→4] linkage at random to yield a mixture of glucose and free maltose.

3.6.2. Glycogen

Glycogen is the major form of stored carbohydrate in animals. Glycogen is also known as a Glucon i.e. it is made up exclusively of D-glucose units. It is also a polysaccharide. This crucial molecule is a homopolymer of glucose in α–(1, 4) linkage; it is also highly branched, with α–(1,6) branch linkages occurring every 8-10 residues. Glycogen has a very compact structure that results from the coiling of the polymer chains. This compactness allows large amounts of carbon energy to be stored in a small volume, with little effect on cellular osmolarity. It is structurally similar to amylopectin, but with more branches.



(a) **(b)**

Fig.3.18: Structure of polysaccharides, a) starch b) glycogen

Dextrins are end products of partial hydrolysis of amylopectin by amylase. Dextrans, are branched d-glucose polymers, like amylopeptin and glycogen, with different glycosidic bonds. Insulin is a polymer of fructose molecules bound via α-2→1.

3.6.3. Cellulose

Cellulose have important structural role in plants; it is a linear polymer of glucose with β-1→4 bonds. The connection though is different from starch and glycogen, it is a beta linkage. So the linkage is β-glucosidic linkage (Fig 3.19). The structure is not helical since the beta linkage confines the polysaccharide to a straight-chain form. In the structure of cellulose -OH groups point outside the chain structure. Whenever two chains come close to each other they tend to form a stack on each other due to hydrogen bonding between these hydroxyl groups. As a result, we get a fibrous insoluble structure which is suitable for the functions of cellulose in the cell walls (Fig. 3.20).

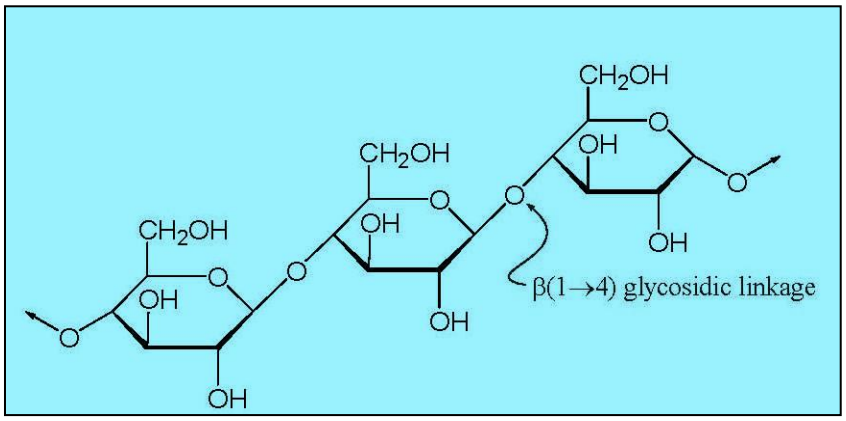


Fig.3.19: Structure of glycosidic linkage.

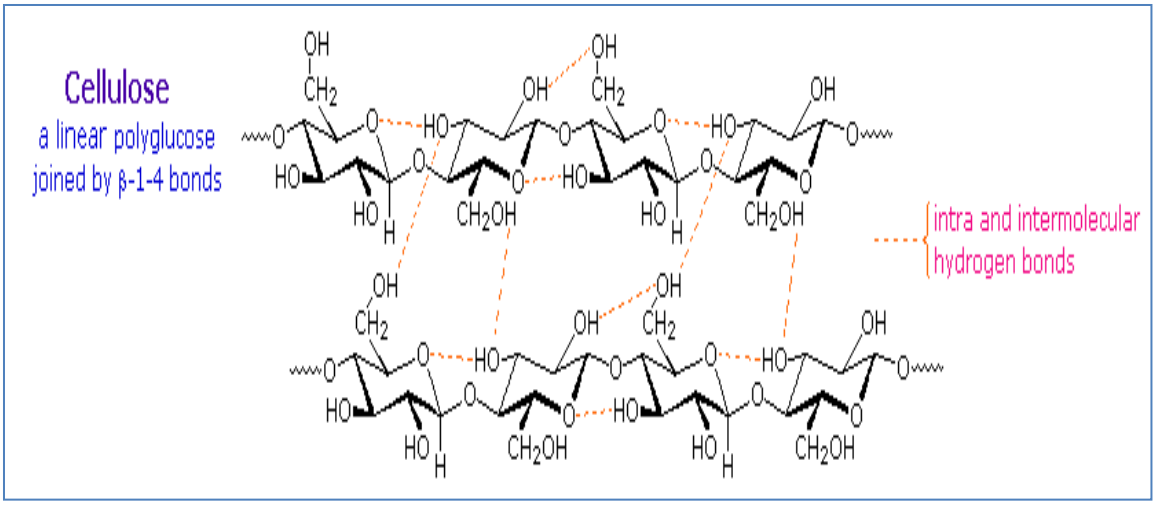


Fig.3.20: Structure of cellulose.

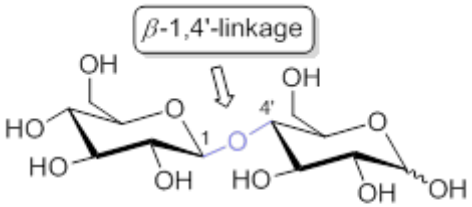
Chitin constitutes the exoskeleton of insects and crustaceans and it is a polymer of *N*-acetyl-d-glucosamine units, linked by β-1→4 bonds.

Heteropolysaccharides include glycosaminoglycans (hialuronic acid, chondroitin sulfate, dermatan, heparan and keratan sulfates, and heparin). Heteropolysaccharides bound to other kind of molecules constitute proteoglycans, peptidoglycans, glycolipids (gangliosides), and glycoproteins. Proteoglycans result from the association of glycan chains (chondroitin sulfate, dermatan sulfate, keratan), bound via glycosidic bonds to the hydroxyl of serine or threonine residues (O-glycosidic bond), or to the N of asparagine residues (N-glycosidic bond) of proteins. Peptidoglycans are the main component of bacterial cell walls. They consist of *N*-acetyl-d-glucosamine and *N*-acetyl-muramic acid. Glycoproteins are carbohydrates conjugated to proteins by O- or *N*-glycosidic linkages. Gangliosides and glycoproteins differ from proteoglycans because they have shorter carbohydrate chains. They play important roles in antigen/antibody recognition on the surface of cells.

3.7. Glycosidic bond:

A glycosidic bond is a covalent bond that joins a carbohydrate to another functional group or molecule. A substance containing a glycosidic bond is termed a glycoside. Glycosides are formed when the anomeric (hemiac-etal or hemiketal) hydroxyl group of a monosaccharide undergoes condensation with the hydroxyl group of a second molecule,

with the elimination of water. Glycosides are named afterthe sugar that provides the hemiacetal group. Thus, if glucose provides the hemiacetal group, the resultant molecule is a glucoside; if galactose provides the hemiacetal group, the result is a galactoside. We can see this type of bond in adenine and ribose in the molecule adenosine. *Cellobiose* also contains two D-glucose subunits. The only difference from maltose is that the two glucose subunits are joined through a β -1,4'-glycosidic linkage.



cellobiose

4-*O*-(β-D-glucopyranosyl)-D-glucopyranose

Glycosidic bonds are labeled according to the identity of the atom on the second carbohydrate or the functional group. There are also N-, S-, and C-glycosidic bonds. Covalent bonds between the hemiacetal or hemiketal to -SR form thioglycosides.

3.8. Summary

In this unit you have learned that

The carbohydrates are the important macromolecules for energy production in living organism. The carbohydrates are classified as mono, di, tri, and polysaccharides. The name "carbohydrate" is derived from 'hydrates of carbon', and they arise from photosynthesis, where they exist as products from carbon dioxide and water. It is one out of the three macronutrients in our diet. Complex carbohydrates found in whole grain breads cereals; starchy vegetables and legumes are good sources of fiber. The Monosaccharides are simple sugars that serve as fuel molecules as well as fundamental constituents of living organisms. Glucose, fructose and galactose are monosaccharides. Sugar acids, sugar alcohol, amino sugars, sugar phosphates, deoxy sugars and glycosides

are important derivatives of monosaccharides. Complex carbohydrates are also known as polysaccharides or glycan. They consists more than three molecules of sugars. Mostly carbohydrates are found the in nature as polysaccharides with high molecular weight.

3.9.Terminal questions

Q.1: What is the carbohydrate? Discuss the basic unit of carbohydrates?

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

Q.2: What are disaccharides? Discuss with suitable examples.

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

Q.3: What are monosaccharides? Discuss the family of aldose and ketose based carbohydrates.

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

Q.4: Discuss the structure and different types of polysaccharides.

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

Q.5: Write the effect of acid and base on monosaccharides.

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

Q.6: Define the sources of carbohydrates and its physical and chemical properties.

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

3.10. Further readings

1. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.
2. Principles of Biochemistry: Lehninger, Nelson and Cox. Student edition, CBS 1439 Publishers and Distributors, Delhi.
3. General biochemistry: J.H. Weil, New Age International (P) Limited, 5th edition, 2013,
4. Biochemistry: T.A. Brown, Viva book publication. First edition, 2018.
5. Elements of biochemistry: J.L. Jain, S. Chand publication. Seventh edition.

Unit-4: Proteins

Structure

4.1. Introduction

Objectives

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4.2.1. Classification of amino acids

4.2.1.1. Non-polar (Hydrophobic) R group

4.2.1.2. Uncharged Polar R-group

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4.2.2. Some basic point on amino acids

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4.6.1. Synthesis of hemoglobin

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4.7.2. Methods of protein-protein interaction

4.7.2.1. Yeast 2 Hybrid Method (Y2H)

4.7.2.2. Co-Immunoprecipitation (Co-IP)
4.7.2.3. Protein affinity chromatography
4.8. Protein–Nucleic Acid Interaction
4.8.1. Interaction types
4.8.1.1. Specific types
4.8.1.2. Non specific types
4.9. DNA - Protein Interactions
4.10. Protein–RNA interactions
4.11. Functions of protein
4.12. Summary
4.13. Terminal questions
4.14. Further readings

4.1. Introduction

This unit covers the basic unit of proteins means amino acids structure and types of proteins. The primary structure of proteins defined as covalent backbone structure of polypeptide chains. In this unit describes the primary, secondary, tertiary and quaternary structure of proteins. The chemical composition of proteins reveals that carbon; hydrogen, nitrogen, oxygen and rare sulfur are the chief elements while others such as phosphorous, iron, zinc and copper also may be part of proteins. Some protein contains only one peptide chain and some two or more. There are 20 amino acids which constitutes the proteins structure while arranged in special sequences. The amino acids differ from each other in the structure of their distinct side chains, called R groups. One the basis of R group amino acids can be classified in four types such as Non-polar, polar, positive charge polar and negative charge polar. The straight chain amino acids are combined which gives primary structure of proteins. The spatial arrangement of two or more poly peptides or tertiary structures unites by different types of bond to form quarterly structure of protein

Objectives:

- To study the different types of amino acids and its classifications
- To discuss the types of proteins.
- To briefly discuss proteins structures.
- To understand the structure and function of hemoglobin as an example of protein.

4.2. Amino Acids

There are 20 amino acids commonly found in proteins in which about 10 amino acids are not synthesized in the body of animals so they must be taken in diet. These 20 amino acids shown in Table 4.1 is also known as standard amino acid. The essential amino acids includes Theonine, Valine, Leucine, Lysine, Isoleucine, Methionine, Phenylalanine, Tryptophan, Arginine, Histidine. Arginine and Histidine are semi essential. Other 10 amino acid are synthesized in animal body so these are called non-essential amino acids such as Glycine, Analine, Serine, Cystine, Aspartic acid, Glutamic acid, Asparagine, Glutamine, tyrosine, and Proline. The amino acids differ from each other in the structure of their distinct side chains, called R groups. On the basis of R group present in amino acids molecules, the amino acids can be classified.

4.2.1. Classification of amino acids:

On the basis of R group the amino acids are classified in four types:

4.2.1.1. Non-polar (Hydrophobic) R group:

In this group, eight amino acids having non polar group are present. In this family, five amino groups such alaine, valine, leucine, proline, isoleucine have aliphatic hydrocarbon R group where as remaining two amino acid such as phenylalanine and tryptophan have aromatic hydro carbon R group and one amino acid Methionine contain sulphur group. Due to presence of sulphur group this amino acids is less water soluble than the amino acid having R group.

4.2.1.2. Uncharged Polar R-group:

The amino belong to this group are found less water soluble than those with nonpolar because R group contains natural (uncharged) polar functional groups which is actively participate in hydrogen bonding with water molecules. In these groups includes serine, cystine, tyrosine, asparagine, glutamine. Here the polarity of some amino is contributed by their hydroxyl group and some have amide group. The amino acids such as serine, threoine and tyrosine contain hydroxyl groups whereas amino acid such as asparagine and glutamine contains amide group. Cystine is contributed by its (-SH) group. Glycine, the member of this group shows border line between both nonpolar and unchanged polar groups but most of the time it is considered in non polar group.

4.2.1.3. Positive charge polar R-group:

In these types of amino acids, the R groups have a net positive charge at pH 7.0. The amino acids for example lysine, arginine and histidine have six carbon atoms in their structure. The lysine contains positively charge amino group at the asylum position whereas arginine contain positively charged quanindium group. But histidine has weakly basic imidazolium function and present at borderline in its properties.

4.2.1.4. Negatively charge polar R-group:

In this category aspartic acid and glutamic acid is considered, each have second carboxylic group that is fully ionized thus it also called acidic amino acid.

Except glycine, each amino acid has two enantiomer isomers

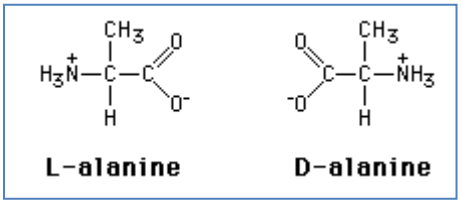


Fig.4.1: Enantiomer isomers of amino acids

Eukaryotic proteins have L-amino acid while D-amino acid occurs in bacteria and antibodies.Amino acids are joint with peptide bond to form protein. Peptidyl transfer ease enzyme catalyses the peptide bonds formation.

4.2.2. Some basic points on amino acids:

- Glycine is the simplest and Tryptophan is complex amino acid
- Cysteine, Cystine, Methionine are the sulpher containing amino acids.
- Phenylalaine, Tyrosine, Tryptophan amino acid are aromatic amino acids.
- Serine and Theonine are alcoholic amino acid.
- Histidine, Proline and Hydroxy proline are heterocyclic amino acid.
- All the amino acid is laevo-rotatory, except Glycine which is non- rotator

Table 4.1 : Amino acids and their functional groups and pK_a values

Amino acid	**Three-letter**	**Other letter**	**pK_a**		
			Carboxylic group	Amino group	Side-chain

Alanine	Ala	A	2.34	9.69	
Arginine	Arg	R	2.01	9.04	12.48
Asparagine	Asn	N	2.02	8.80	
Aspartic Acid	Asp	D	2.10	9.82	3.86
Cystine	Cys	C	2.05	10.25	8.00
Glutamic Acid	Glu	E	2.10	9.47	4.07
Glutamine	Gln	Q	2.17	9.13	
Glycine	Gly	G	2.35	9.78	
Histidine	His	H	1.82	9.17	6.00
Isoleucine	Lie	I	2.32	9.76	
Leucine	Leu	L	2.33	9.74	
Lysine	Lys	K	2.18	8.95	10.53
Methionine	Met	M	2.28	9.21	
Phenylalanine	Phe	F	2.58	9.24	
Proline	Pro	P	2.00	10.60	
Serine	Ser	S	2.21	9.15	
Theonine	Thr	T	2.09	9.10	
Tryptophan	Trp	W	2.38	9.39	
Tyrosine	Tyr	Y	2.20	9.11	10.07
Valine	Val	V	2.29	9.72	

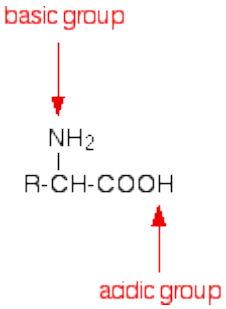
4.2.3. Non protein amino acids

There are several rare amino acids found in nature in addition to the 20 amino acids. In which most are the derivaties of α amino acids found in proteins. These rare amino acids are mostly obtained as intermediates metabolites. Some rare amino acids such as β-analine is considered as building blocks of vitamin pantothenic acid; homocysteine and homoserine are intermediate in amino acid metabolism; and citrulline is intermediate in synthesis of arginine. Some nonprotein amino acids also have the D configuration for example D- Glutamic acid, analine and D-serine etc. The non protein amino acid found in some fungi and higher plants vary in structure.

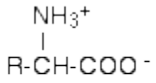
4.2.4. Acid base reaction of amino acids

The acid base properties of amino acids are useful to understand the properties of protein. Crystalline amino acids have high melting point and decomposition point and are much more soluble in water. This property is because of crystalline nature. It is stabilized by electrostatic forces of attraction between opposite charge and groups. In addition, the non ionic crystalline form of amino acid is stabilized by weaker Van der Waals forces and would have low melting points. This evidences lead to conclusion that the amino acid occur in crystalline from, and in

neutral aqueous solution as dipolar ions or zwitterions. The amino acid is dissociated in water and it can acts as either as acid or base water represented in equations.

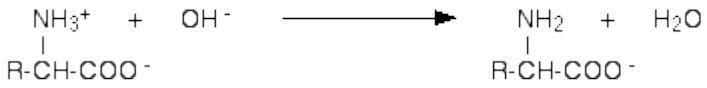


In this reaction the internal transfer of hydrogen ion occurs from –COOH group to NH_2 group to leave and with both a negative charge and a positive charge. This is called a zwitterion.



a zwitterion

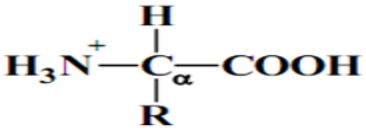
The crystalline form of amino acid is soluble in water that it present in zwitterions form. A zwitterion is a compound with no overall electrical charge, but which contains separate parts which are positively and negatively charged. It increases the pH of a solution of an amino acid by adding hydroxide ions; the hydrogen ion is removed, which form the $-NH_3^+$ group. And the amino acid is converted into negative charge ion.



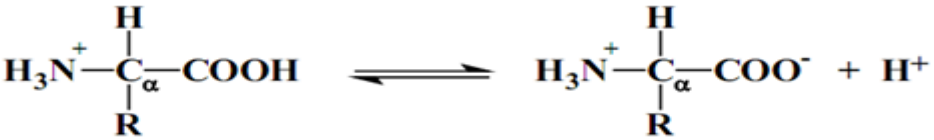
If you decrease the pH by adding an acid to a solution of an amino acid, the -COO- part of the zwitterion picks up a hydrogen ion.

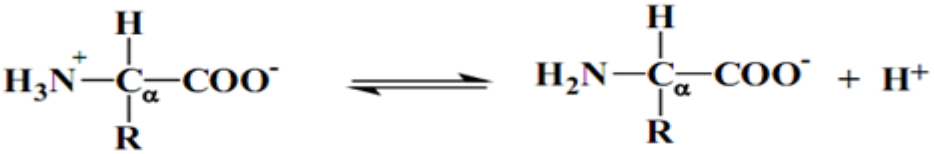
If we start the reaction under acidic condition and slowly add alkali to it, we get back to the zwitterion.

When we add just the right amount of alkali, the amino acid no longer has a net positive or negative charge. The pH at which there no ion moves towards either the cathode or anode during electrophoresis is known as the isoelectric point of the amino acid and symbolized as pH_I. The isoelectric point (isoelectric pH; pH_I) is the pH at which the amino acid has a net zero charge. The isoelectric pH is the arithmetic of pK_1 and pK_2; that is $pH_I = \frac{1}{2} pK'_1 + pK'_2$. All mono amino acids and monocarboxylic acid shows essentially the same behavior. The pK' values of some amino acids shows in Table 4.1. For example the titration curves of the amino acid glycine shown in Fig. 4.2.



Glycine is a diprotic amino acid which means it has two dissociable protons, one on the α amino group and the other on the carboxyl group. In the case of Glycine, the R group does not contribute a dissociable proton.





The dissociation of proton proceeds in a certain order which depends on the acidity of the proton: the one which is most acidic and having a lower pK_a will dissociate first. So, the H^+ on the α-COOH group (pK_{a1}) will dissociate before that on the α-NH_3 group (pK_{a2}).

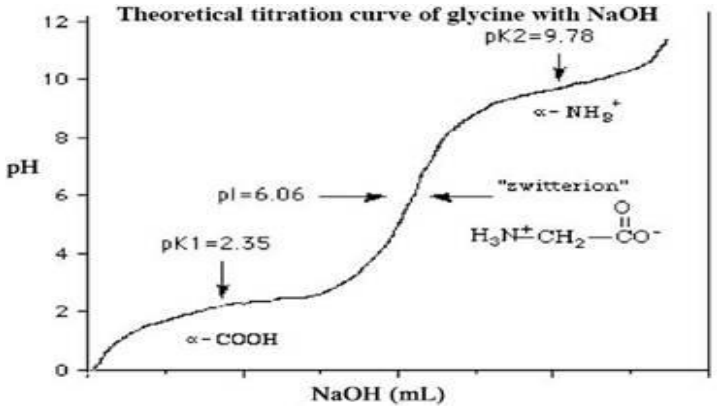


Fig.4.2: Acid-base titration curve of Glycine amino acids.

Source: https://www.chem.fsu.edu/chemlab/bch4053l/character/titration/index.html

The ionic form of the amino acid present in the aqueous solution is dependent upon the solution's pH. The acid base titration is very useful for the identification of amino acids as shown the titration curve of glycine in Fig 4.2. The glycine have two association steps, in their first step they lose the H^+ from the acidic group from carboxylic acid at low pH and in their second steps it lose H^+ from amino group at higher pH. The pKa value for each dissociable group of an amino acid can be determined from such a titration curve by extrapolating the midpoint of each buffering region (the plateau) within the curve.

4.2.5. Functions of amino acids

- Formation of proteins
- Formation of antibiotics
- Formation of enkephalins
- Amino acids acts as a biological buffer
- Synthesis of histamine

4.3. Overviews on proteins:

Protein is a biopolymer constituted by amino acids. Protein is quite abundant and naturally occurring organic molecules found in every part of every cell. It is a fundamental constituent of cell structure and functions. Different kind of protein is specialized for various biological functions. Moreover, most of the genetic information is expressed by protein. The

amino acids are the monomeric unit of proteins. The amino acids linked together to formed unbranched chains called polypeptide. In protein molecules the two amino acids are linked by peptide bond. Each polypeptide bond forms between the carboxylic acid and amino groups of adjacent amino acids by condensation of reaction that releases the water molecules. Thus protein is also called polypeptide. The polypeptide or proteins folds into a defined three dimensional structure responsible for specific function. Due to various folds in protein shows complex structure. The folding in protein occurs simultaneously. Polypeptide contains mixture of 20 different amino acids. The difference between a peptide and polypepetide is that a peptide is usually a short chain of amino acids while a polypeptide is a longer chain of a set of amino acids.

In general we can say that all the proteins are polypeptides but all polypeptides are not proteins because polypeptides may not fold into a definite three dimensional structure as occurred in true proteins. The chemical bonding between portions of polypeptides gives special properties to hold a protein which provides shape to protein molecules. There are two free terminuses i.e. amino terminus NH_2- or N terminus and a carboxylic terminus COO- or C terminus are exposed to formation of peptide bond. Each amino acid has the same general structure shown in Fig. (4.3). Polypeptides therefore have a chemical direction that can be express as a N→C (left to right) or C → N (right to left). Protein synthesis occurs in the N→C directionv which means each new amino acid is added to the free carboxylic group of the growing polypeptides. These comprises a central carbon atom, called the chiral carbon, to which four different chemical groups are attached. These group are a hydrogen atom, (-H), a carboxylic group (-OH), an amino group (-NH_2), and the R group or side chain. There are several R groups which are going to behave different based on the different properties that is has. For example, in glycine, the R group is simply a hydrogen atom, whereas for phenylalanine, tryptophan and tyrosines they have large organic structure.

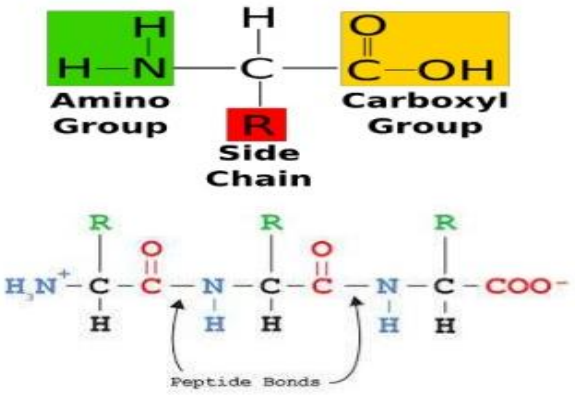


Fig.4.3: Primary structure of protein and peptide bond

The sequence of amino acid residues of this protein is that it starts with a protonated amino terminus and end with a carboxylic acid terminus which does not have the proton attached to it.

The shape of a protein is very important to its function. To understand how a protein gets its final shape or conformation, we need to understand the four levels of protein structure namely primary, secondary, tertiary, and quaternary.

4.3.1. Chemical composition of proteins:

We know that twenty different α-amino acids commonly present as the building blocks of proteins. There are several proteins that have been isolated and all are having carbon, hydrogen, nitrogen, and oxygen; nearly all contain sulfur in their structure. In addition some other elements particularly phosphorous, iron, zinc and copper are also found in proteins. The α-amino acids linked together and form peptide chain. Some protein contains only one peptide chain and some two or more. The polypeptide chain contains hundreds of amino acids and each peptide chain has a definite molecular weight, chemical composition, sequential order of its amino acids and three dimensional shapes. On the basis of composition of protein, proteins are classified as simple, conjugated and derived proteins.

- **Simple proteins:** On hydrolysis they yield only the amino acids and occasionally small carbohydrate compounds. The chemical composition of simple protein is C 50%, H 7%, O 23% N 16% and S 0-3%. Examples are: albumins, globulins, glutelins, albuminoids, and histones.

- **Conjugated proteins**: These are simple proteins combined with some non-protein component. The non amino acid portion of a conjugated protein is called prosthetic group. It is also classified on the basis of chemical composition of prosthetic group. Nucleoproteins, glycoproteins, phosphoproteins, haemoglobins and lecithoproteins are all the example of conjugated proteins.
- **Derived proteins**: These are proteins derived from simple or conjugated proteins by physical or chemical means.

4.3.1.1. Protein structure:

We have earlier discussed that the protein are the biopolymers and comprises of amino acid. So, the amino acids are called the building blocks of proteins. However, the proteins are long chain-like structure with amino acids being the main ingredients. These amino acids are connected together with peptide bond and make the polypeptide chain. Now one or more of these polypeptide chains twist or fold spontaneously and thus form a protein.

Simple peptide contains two, three, four or more amino acid residues and form dipeptides, tripeptides, tetrapeptides, respectively. These are joined covalently through peptide bonds, are formed on partial hydrolysis of much longer polypeptide chains of proteins. The size of the proteins varies greatly. It actually depends on the number of polypeptide molecules it contains. One of the smallest protein molecules is insulin, and the largest being titin which consist of 34,350 amino acids. Let us now look at the four types of protein structure that make up a protein molecule.

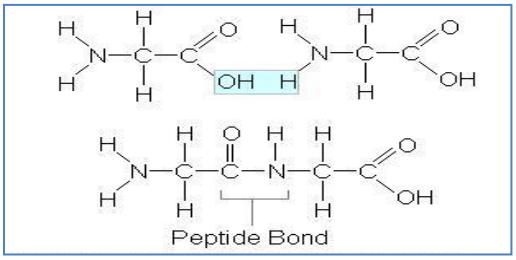


Fig. 4.4: A molecule of water is removed from two glycine amino acids to form a peptide bond.

4.3.1.2. Primary Protein Structure:

A straight chain of amino acids linked by peptide bond forms the primary structure of protein (Fig.4.5). The primary structure of protein is unique in structure in which straight chain

amino acids are combined in such a way which reflects the specific sequence of amino acids. However, this sequence of amino acids in protein determines its properties. These protein molecules are most unstable in nature. When the two amino acids are in such a position that the carboxylic groups of each amino acid are adjacent to each other, they can be combined by undergoing a dehydration reaction, which result in the formation of a peptide bond. Our body contains all twenty amino acids; all of these have a carboxylic group and an amino group. But each has a different variable group known as the "R" group. It is this R group that lends a particular protein its unique structure.

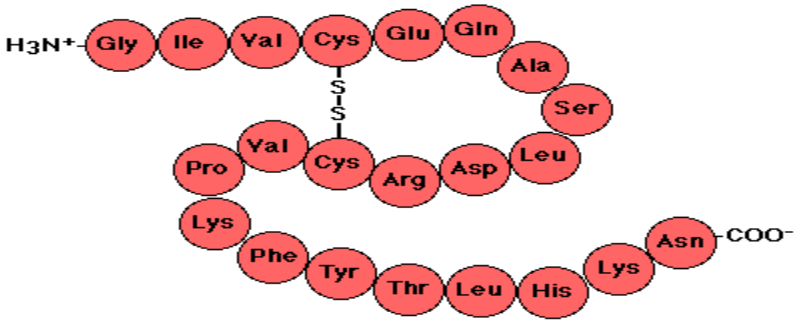


Fig.4.5: Simple primary structure of protein

The protein is determined by the sequencing of the amino acids (Fig. 4.5). The formation and ordering of these amino acids in proteins are extremely specific. If we alter even one amino acid in the chain it results in a non-functional protein and this may result from a gene mutation. The minimum size of a protein is defined as about 50 amino acids residue and this chain referred to simply chain of peptides. Thus we can say the primary structure of a small protein would consist of a sequence of 50 or so residues. Even such small proteins contain hundreds of atoms and have molecular weights of over 5000 Daltons (Da).

4.3.1.3. Secondary structure of proteins:

The protein molecules of secondary structure are spirally coiled and here the peptide backbone of protein structure fold onto itself to give protein their unique structure. This folding of the polypeptide chains happens due to the interaction between the carboxyl groups along with the amine groups of the peptide chains In addition to peptide bond, amino acids are linked by hydrogen bond between oxygen of one amide group and hydrogen of another amide group. The secondary structure is two types:

α-helix :- The backbone follows a helical structure. In this case, right handed rotation of spirally coiled chain with approximately $3\frac{1}{2}$ amino acids in each turn occurs. This structure has intermolecular hydrogen bonding i.e. between two amino acids of same chain. The hydrogen bonds with the oxygen between the different layers of the helix, gives helical structure (Fig.4.6a). Example of protein α-helix structure is Keratin, Myosin and Tropomyosin.

β pleated sheet structure:-In β-helix the polypeptide chains are stacked next to each other and their outer hydrogen molecules form intramolecular bonds to give it sheet-like structure. Generally the primary structure folds back on itself in either a parallel or antiparallel arrangement, producing a parallel or antiparallel β sheet. In this arrangement, side chains project alternately upward and downward from the sheet (Fig.4.6b). Protein molecule has zig-zag structure. Two or more protein molecules are held together by intermolecular hydrogen bonding for example Fibroin. The secondary structure of protein is insoluble in water and fibrous in appearance.

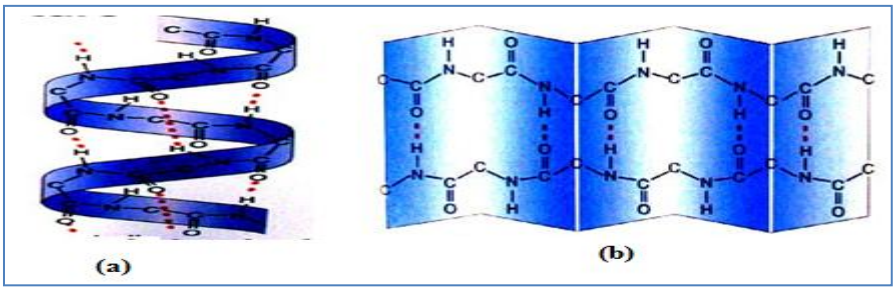


Fig.4.6: Secondary structure: (a) α-helix structure, (b) pleated sheath structure

Source:https://schoolworkhelper.net/protein-structures-primary-secondary-tertiary-quaternary/

4.3.1.4. Tertiary structures:

This is the structure that gives protein 3-D shape and formation. After the amino acids form bonds (secondary structure) and shapes like helices and sheets, the structure can coil or fold at random. This is what we call the tertiary structure of proteins. The proteins of tertiary structure are highly folded to give a globular appearance (Fig.4.7). Majority of the proteins and enzymes exhibits tertiary structure. They are water soluble. The structure of protein has following bonds:

Peptide bond= strongest bond in protein

Hydrogen bond

Disulphide bond- these bond form between–SH group of amino acid. This bond is second largest bond that stabilize tertiary structure of protein.

Hydrophobic bond- this bond formed between amino acid which has hydrophobic side chains for e.g. Aromatic amino acid.

Ionic bond- formation of ionic bond occurs between two opposite ends of protein molecule due to electrostatic attraction.

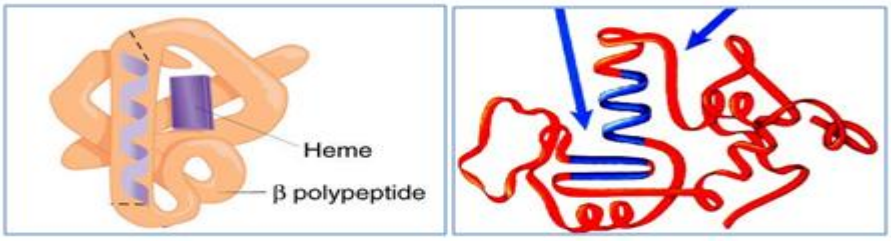


Fig4.7: Tertiary structure of proteins molecules

Source:https://schoolworkhelper.net/protein-structures-primary-secondary-tertiary-quaternary/

4.3.1.5. Quaternary Structure:

The spatial arrangement of two or more polypeptide or tertiary structures unite by different types of bond to form quarterly structure of protein. Quaternary structure is most stable structure of the protein (Fig.4.8). The quaternary structure also refers to the number and arrangement of the individual polypeptide chains. Each polypeptide is referred to as a subunit of the protein. The same forces and bonds that create tertiary structure also hold subunits together in a stable complex to form the complete protein. Primary, secondary and tertiary structures are present in all natural proteins, but the same is not true for quaternary structure.

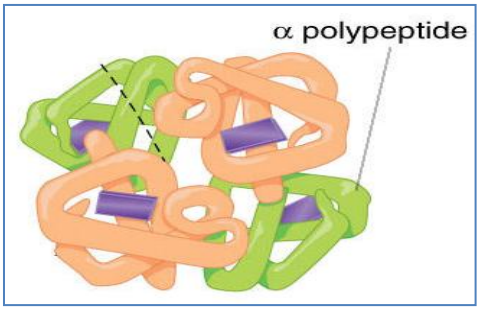


Fig.4.8: Quaternary structure of protein.

Source:https://www.mun.ca/biology/scarr/iGen3_06-04.html

4.3.2. Some biologically important proteins:

- Some protein acts as enzymes such as flavorproteins, metalloprotiens, etc.
- Some protein acts as hormones- somatic hormones, growth hormones, insulin etc.
- Nucleoproteins- protamine, histones, non-histones chromosomal protein
- Proteins and antibiotics- tyrocidine penicillins, gramicidin.
- Blood plasma proteins- albumin, α globulin, β globulin etc.
- Proteins and transport- albumin, lipoprotein, hemoglobin, myoglobin and transferrin.

4.4. Enzyme mechanisms

The enzymes catalyze the reaction without change themselves in which they create chemical transformation. These chemical transformations through enzymes govern by a set of mechanism. In the enzymatic mechanism the enzymes act on the substrate and the substrate convert into complex that is called ***active centre*** or site. The enzymes and substrate form active complex at active site. In the formation of active complexes reduction in activation entropy occurs due to the loss of translation and rotational entropy. The different types of chemical and physic chemical bond (ionic, hydrogen, Van der Waal forces) would be responsible for binding between enzymes and subtract. The active sites on enzymes have different functional group in their surface that activity involves in chemical binding and form transition (intermediate) compound called enzymes subtract complex (ES). The enzymes catalysis reaction gives exergonic reaction and release energy during chemical bonding, which raises energy level of substrate molecules. This raises energy level of substrate is known as activation energy or energy of activation. In other word activation energy is least possible amount of energy of system which is required to start a reaction a reaction.

$$\underset{\text{(Enzymes)}}{E} + \underset{\text{(Substrate)}}{S} \longrightarrow \text{ES complex}$$

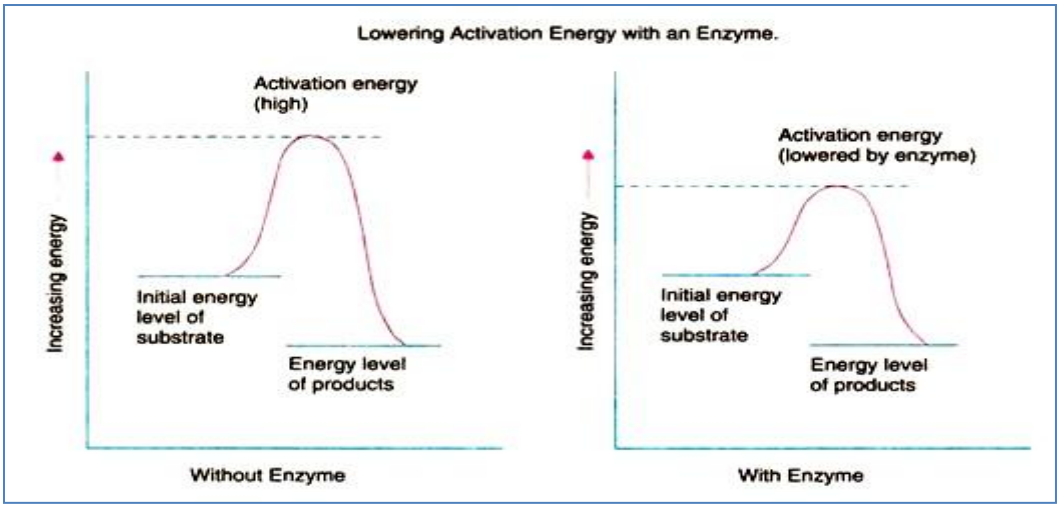


Fig. 4.9 : Mode of enzymes actions

4.4.1. Types of mechanism of enzymes:

Generally it was realized that enzymes catalysis is similar in principle to other types of chemical catalysis, for instance by involving a combination of several different types of analysis. To understand the enzymatic mechanism for substrate-enzymes complex formation there are two theory, Lock and key theory (template model) and induced-fit theory has been proposed.

4.4.1.1. Lock and key theory

The lock and key theory has been given by Email Fischer's in 1894. The basic concept of this theory to explain specificity of enzyme with a single substrate. According to this theory reaction of substrate and enzyme is analogous to lock and key. Analogous means the chemical whose chemistry is similar to substrate but fail to react to enzymes. In this theory it was assumed that the enzyme is analogous to key which fixed in socket that has similar geometrical configuration. Here in case of enzyme substrate reaction the substrate got fixed geometrical configuration like socket where key got fixed. However, according to this theory particularly one substrate can be found at active site of particular enzyme forming substrate enzymes complex. Enzyme-substrate complex remains in tight fitting and active sites of enzymes are complementary to substrate molecules. The activity of reaction sites made enzyme substrate complex, because here transformation of substrate into product occurred.

Fig.4.10: Diagram shows the mechanism of lock and key model

Source: https://www.news-medical.net/life-sciences/Enzyme-Mechanisms.aspx

4.4.1.2. Induced Fit Theory:

The so called rigid enzyme has been failed to explain for the enzymatic reaction that why it's get modification called the induced-fit theory has been proposed. According to induced fie theory ES bonding found rigid in structure but it has some flexibility when substrate attached the enzymes and gives final shape. This theory also explains why certain compound can bind to enzyme but do not react because the enzyme has been distorted too much. It induced a change in binding pocket. When a substrate molecule collides with an enzyme it structure becomes more precisely enclosed to the enzymes because enzyme has specificity. In this process the shape of the enzymes active site will change so that the substrate fits into it and an enzyme-substrate complex can form. Thus the specificity of enzymes for substrate get increased that why the substrate binding tightly but not react this is called induced fit model.

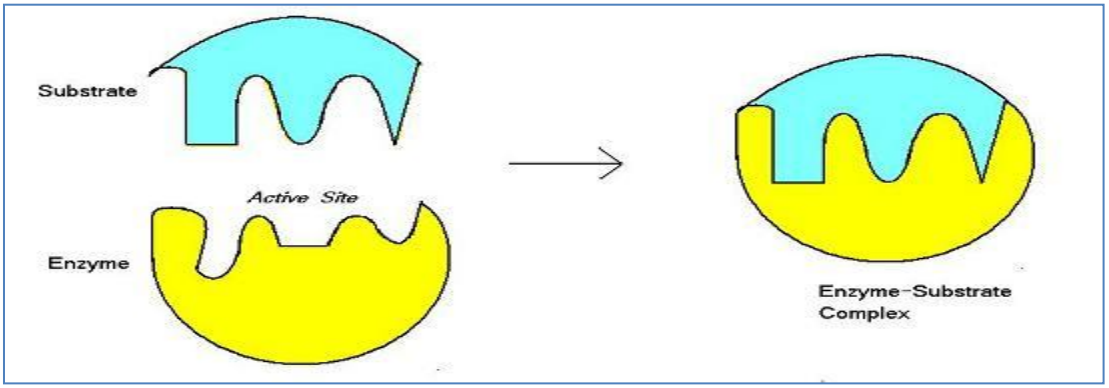


Fig.4.11: Diagram shows the mechanism of induced fit model

Source:https://alevelnotes.com/notes/biology/biological-molecules/enzymes/enzymes

4.5. Enzymes kinetics

We know enzymes are usually protein molecules that manipulate other molecules the enzymes substrates reaction. In this enzymes substrates reaction the target molecules bind to an enzymes active site and are transformed into products through a series of steps known as the enzymatic mechanism.

$$E + S \rightleftarrows ES \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P$$

Kinetic studies on enzymes substrates can we understand through Michaelis and Mound model.

According to this model when enzyme E combines with substrate (S) that form enzyme substrate (ES) complex. In reversible reaction this ES complex can be reverse and can proceed chemically to be give enzyme E and substrate S as.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

The rate constants k_1, k_2 and k_3 describe the rates association with each step of the catalytic process. This reaction shows the ES maintain their steady state because the ES remains approximately constant unit nearly all the substrate is used in backward reaction. It is know, that the initial velocity (V_o) of any enzyme at low substrate concentration is directly proportional to to [S]. While at high substrate concentration the velocity tends to shift at maximum value, that is the rate becomes independent of [S].

Shown in Fig. 6.6, this is maximum velocity known as maximum velocity ($V_{max}/2$). The maximum velocity express in unite μmol min^{-1}. To describe this observation the Michaelis and Mound derived as equation that is known as Michaelis and Mound equation:

$$V_o = \frac{V_{max}\, X\, [S]}{K_m + [S]}$$

This equation describes the hyperbolic curve as shown the experimental data in Fig. 4.12. After solving the equation Michaelis and Mound defined a new constant, K_m called as Michaelis constant.

$$K_m = \frac{k_2 + k_3}{k_1}$$

K_m is refers to the stability of the ES complexes. It is equal to equal to the sum of the rates of breakdown of ES over its rate of formation. The k_2 value for many enzymes is found much greater than k_3 in this circumstances K_m becomes a measure of the affinity of an enzyme for its substrate since its value depends on relatives values of k_1 and k_2 for ES formation and dissociation, respectively. The k_m be determined experimentally by the fact that its values is equivalent to the substrate concentration at which the velocity is equal to half of V_{max}. Inadition, the K_m is equal to the sum of the rates of breakdown of the enzyme-substrate complex over its rate of formation, and is a measure of the affinity of an enzyme for its substrate. The Km values for most of enzymes lies between 10^{-1} and 10^{-7} M.

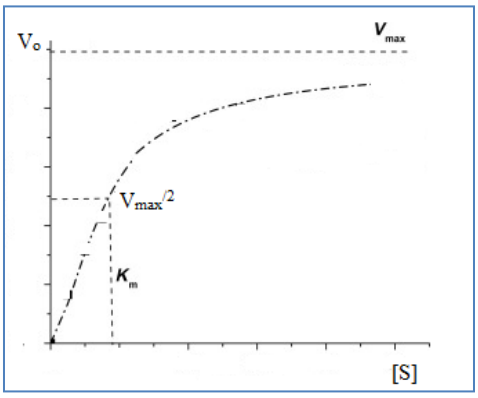


Fig.4.12: The relationship between [S] and V_o in enzyme catalytic reaction

4.6. Structure of hemoglobin

Hemoglobin is oligomeric protein; it was first shown by M.F. Perutz and his colleges in England. Hemoglobin consists of the protein goblin (polypeptides) and pigments heme (heme). Hemoglobin is red in color and an oxygen carrying pigment in red blood cells of vertebrates. Haem is an iron containing porphyrin called iron proto porphyrin IV. The porphyrin molecules is tetrapyrrole means it consists of 4 pyrrole ring, which is joined together with four methione (CH-) bridges (Fig.4.13). The pyrrole ring is numbered as I, II, II, IV, the carbon atom of the methane bridge are labeled as α, β, γ and δ. The side chain at 1, 3, 5, and 8 position are methyl ($-CH_3$); 2 and 4 are vinyl ($-CH=CH_2$), and 6 and 7 are propionic acids ($-CH_2CH_2COOH$). Globin contains two α-chain and two β-chains for peptide bond. Both the α and β chains have about 70%

α helical character, as is true for myoglobins. The hemoglobin chain is arranged by tetrahedral geometry and each chain has irregularity folded confirmation in which length of pure α-helical regions are spherical by bonds. Two α chain contains 141 amino acids and of two β chain contains 146 amino acids. Each chain has compact spherical structure with dimension 6.4 by 5.5 by 5.0.nm. Here iron (Fe^{2+}) is attached to the N of each pyrrole ring. Each iron combines loosely and reversible with one molecules of oxygen and combination of heme with oxygen is called oxygenation. Oxygenation of first hem molecules is hemoglobin, which increase the affinity of second hem for oxygenation and oxygenation of second increase the affinity of third heme and their increase affinity of five. The shifting of hem oxygenation of oxygen gives sigmoid shape of oxygen hemoglobin and increase deoxygenating. Generally all hemoglobins contain a histidine R group coordinated with the iron atom of the hemo groups.

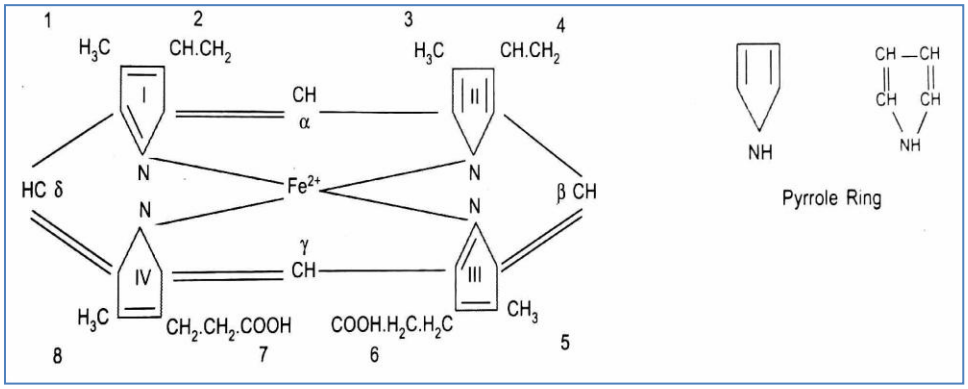


Fig.4.13: structure of hemoglobin molecules

Oxyhemoglobin: hemoglobin reacts with oxygen to form oxyhemoglobin and is represented by HbO_2. This reaction is influence by the pH, temp and concentration of 2, 3 diphosphoglycerate in RBCs which finely produce glucose.

- **Carbon hemoglobin:** When the carbon react with hemoglobin to form carbon hemoglobin.
- **Reduced (Deoxygenated) hemoglobin**: It occurs due to removal of oxygen from hemoglobin and is representing as Hb.
- **Carbon monoxyhemoglobin**: When carbon mono oxide reacts with hemoglobin is called Carbon monoxyhemoglobin.

- **Methaemoglobin:**When either reduced or oxygenated hemoglobin is exposed to various drugs and agents the Fe^{2+} (ferrous) is oxidized into Fe^{3+} (ferric) formed a compound is called methaemoglobin.

4.6.1. Synthesis of hemoglobin:

For synthesis of hemoglobin require proteins, vitamins and minerals (especially iron). It may take part in developing RBCs.

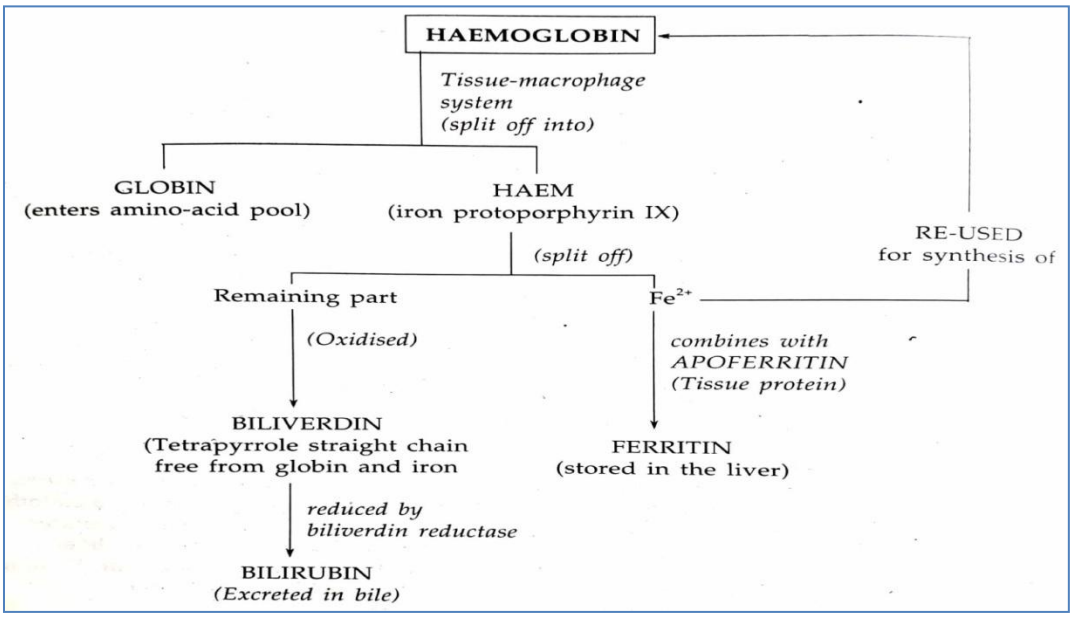


Fig.4.14: Fate of hemoglobin in the body.

4.6.2. Function of hemoglobin:

There are three mains functions of hemoglobin represented as

- Transport of oxygen from lungs to tissues
- Transport of carbon dioxide form tissues to lungs
- It acts an excellent acid base buffer.

4.7. Protein-Protein Interactions (PPIs)

The protein-protein interactions (PPIs) refer to interactional physical contacts established between two or more proteins as a result of biochemical event and or electrostatics forces. Protein-protein complex formation has been investigated for the decades, for their importance in biochemistry, molecular dynamics, chemical biology, signal transduction and other metabolic or genetic/epigenetic pathways. It play important role in predicting the protein function of target protein and drug ability of molecules. The wide range of biological process like cell to cell interaction, metabolic and development control are influenced by protein- protein interactions. . The significance properties of PPIs marked by Phizicky and Fields are as follow:

- It can modify the kinetic properties of enzymes
- It act as a general mechanism to allow for substrate channeling
- It contrast a new bonding site for small effectors molecules
- It activate or suppress a proteins
- Change the specificity of proteins
- It provides e upstream and downstream regulation.

The PPIs imply physical contact between proteins does not mean that such contacts are static or permanent. There are several limitations that cover the protein-protein interaction. The function and activity of a protein are often influenced by the presence of other proteins with which it interacts. However, interaction depends on followings:

- Cell types
- Cell cycle
- Phase state
- Development stage
- Environmental condition
- Protein modification
- Presence of cofactor
- Presence of other binding parameters

The main example of PPIs is such as

- Signals transduction
- Transport across membranes
- Cell metabolism

- Muscles contraction.

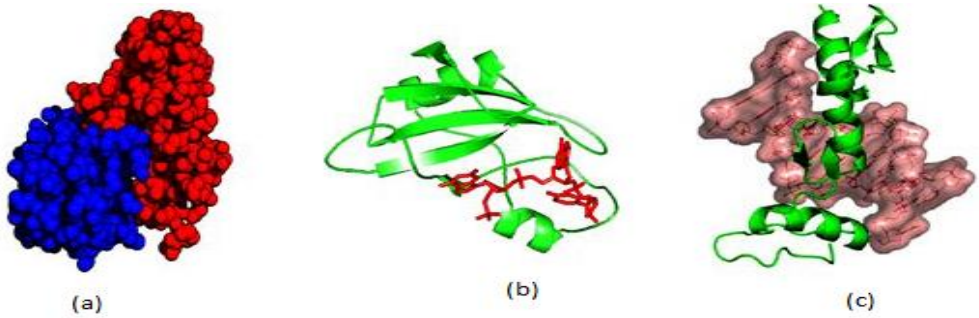


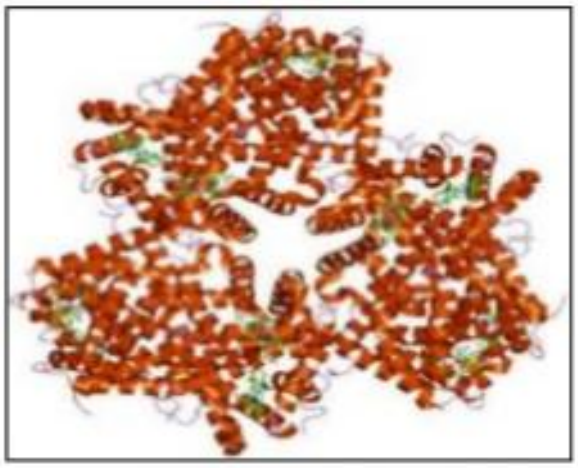
Fig 4.15: A protein can interact with (a) other protein, (b) small ligand molecule and (c) nucleic acid.

4.7.1. Types of protein interactions

The protein interaction is different types that depend on several factors. However the PPIs can be understand by

A. On the basis of their composition

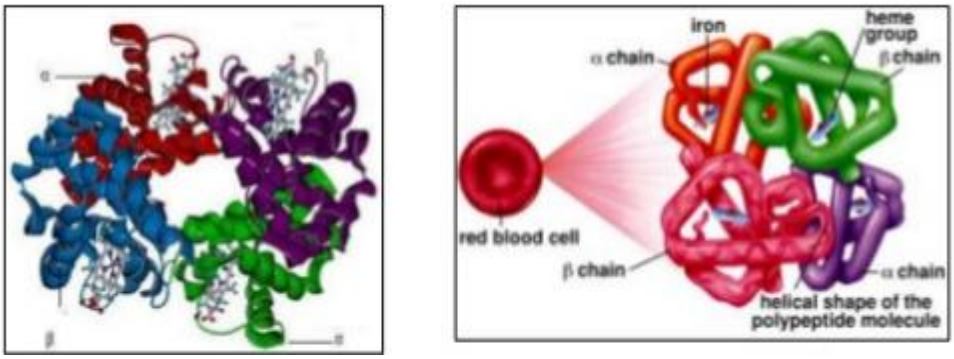
i. **Homo-oligomers:** It is macromolecular complex constituted by only one types of protein subunit called homo-oligomers or homomer (Fig.4.16) by the process of oligomerization. Oligomerization is a chemical process in which monomers convert into macromolecular complex through defined degree of polymerization. Protein subunit assembly is guided by establishment of non convent interaction in the quaternary structure of the protein. Eg. PPIs are observed in muscles contraction. Several enzymes, carrier protein and transcription regulatory factors carry out their function as homo-oligomers.



Source: https://www.slideshare.net/shrikantyankanchi/protein-protein-interactions-73707285

Fig.4.16: Homo-oligomers interaction in same proteins

ii. **Hetero-oligomers:** In hetero oligomers distinct protein subunit interact in hetero-oligomers which are essential to control several cellular functions. For example interaction in hemoglobin (**Fig.4.17)**, PPIs between cytochrome oxidase and $TRPC_3$ (transient receptor potential cat in channels)



Source: https://www.slideshare.net/shrikantyankanchi/protein-protein-interactions-73707285

Fig 4.17: Hetero-oligomers in hemoglobin Hb or Hgb

B. On the basis of their binding:

i. **Covalent binding:** The protein is strongly associated by covalent bonding such as disulphide bond or electron sharing. Thus the post translation modification is carried out. Example: Ubiquition and sumoylation.

ii. **Non-covalent**: These types of bond are established during interaction by combination of weaker bonds. Such as hydrogen bonding, ionic interaction, van-der waals forces and hydrophobic bonds.

C. On the basis of their duration of interaction:

i. Transient interactions: Interaction that that is for a short period of time and in reversible manner. For example**:** Protein coupled, receptor only transiently binds to Gi/O protein when they are activated by extracellular ligands**.**

ii. **Stable interaction:** Protein interacts for long time taking part of permanent complex subunits. In which structural and functional roles is carried out for example cytochrome C. Apart from that numbers of water molecules are found in the long inter-molecular span between cytochrome and Cytochrome c oxidase.

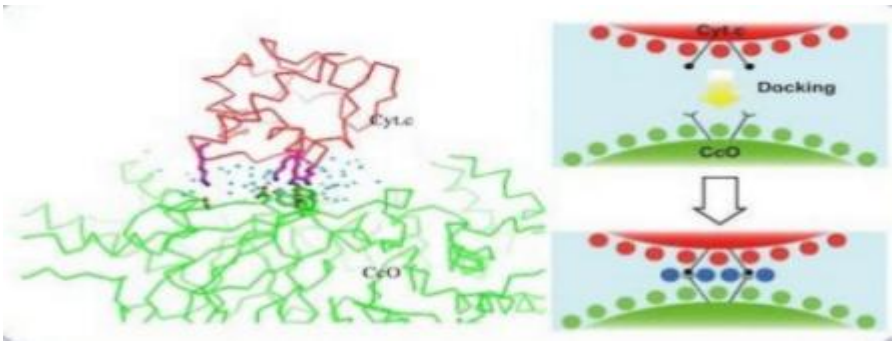


Fig.4.18: stable interactions in Cytochrome c oxidase

4.7.2. Methods of protein-protein interaction

The protein-protein interaction is carried out by several experimental *in vivo*, *in vitro* and computational (in silico) conditions such as:

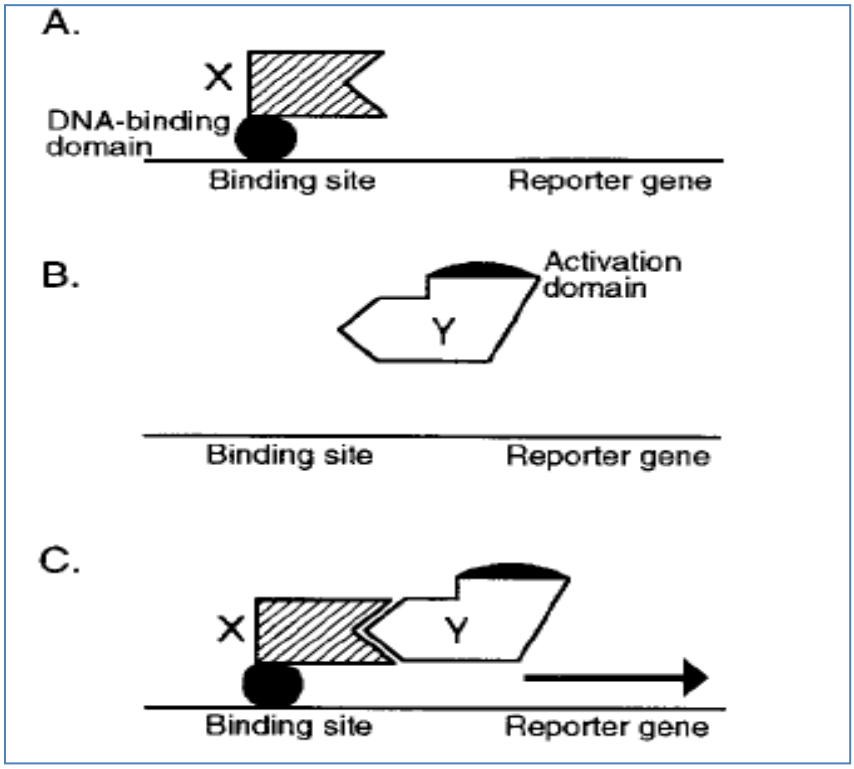
- Yeast 2 Hybrid Method
- Co-Immunoprecipitation (Co-IP)
- Protein affinity chromatography
- Protein microarray
- Pull down assay
- Fluorescence Spectroscopy

4.7.2.1. Yeast 2 Hybrid Method (Y2H)

Y2H is a molecular biology technique carried out to determine the protein–protein interactions and protein–DNA interactions by testing for physical interactions (such as non covalent binding) between two proteins or a single protein and a DNA molecule, respectively. For the discussion of two-hybrid screening method, the transcription factor can be split into two different fragments, called the binding domain (BD) and activating domain (AD). BD is the domain that is responsible for binding to the upstream activating domain (UAS) and AD is the domain which is responsible for the activation of transcription process. Hence Y2H is a protein-fragment complementation assay.

The key to the two-hybrid screen is that the activating and binding domains are indicated as the modular domains and can function in proximity to each other without direct binding. This is so called, as transcription factor is splitted into two fragments, it can still activate transcription when the two fragments are indirectly connected.

Y2H system is based on the observation where the two domains of the activator need not be covalently bonded and can be brought together by the interaction of any two proteins. The two-hybrid system has been applied successfully in the study of on cogenes and tumor suppressors and the related area of cell cycle control. The two-hybrid system has various useful features that make it easy for analysis of protein-protein interactions it is highly sensitive and detects interactions that are not detected by other methods.



Source: https://www.profacgen.com/Yeast-Two-hybrid-Screening-Technical.htm

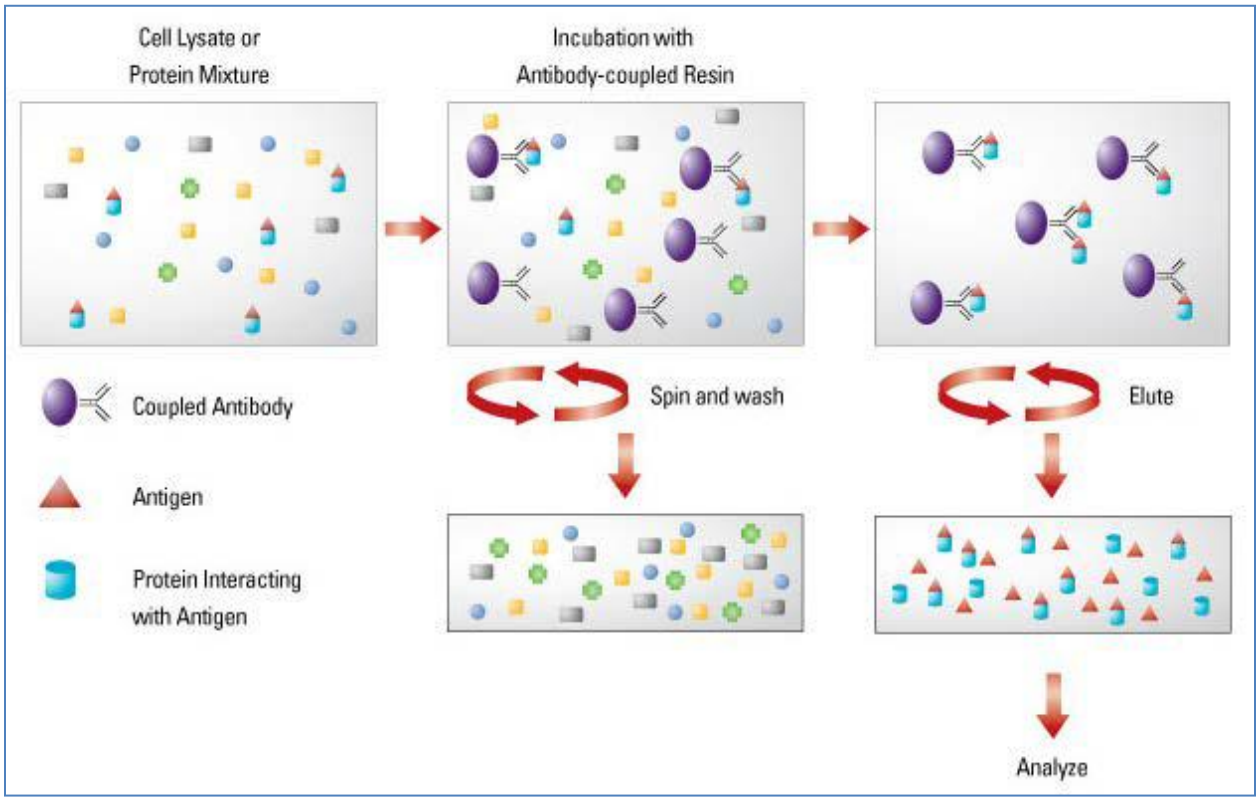
Fig. 4.19: Y2H: (A) The DNA-binding domain hybrid that does not activate transcription when protein X does not contain an activation domain. (B) The AD hybrid does not activate transcription as it lacks the efficiency to localize to the DNA-binding site. (C) Specific interaction between X and Y can bring the activation domain into close proximity to the DNA-binding site and results in transcription.

4.7.2.2. Co-Immunoprecipitation (Co-IP):

Co-immunoprecipitation (Co-IP) is a well-known methodology for protein interaction measurements. Co-IP is conducted in a similar fashion as an immunoprecipitation (IP) of a single protein done. Here the target protein is precipitated by an antibody which is known as '*bait*', is

used to co-precipitate a binding protein complex or '*prey*', from a lysate cell. The interacting protein is bound to the target antigen, which is bound by the antibody that is immobilized to the support surface. Immunoprecipitated proteins and their binding partners are normally detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses.

The assumption is generally made when the associated proteins are co-precipitated with these proteins that are related to the function of the target antigen at the cellular level. This is just an assumption; one can either model this for further verification.



Source: https://www.thermofisher.com/in/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/co-immunoprecipitation-co-ip.html

Fig. 4.20: Flow chart of Co-immunoprecipitation

4.7.2.3. Protein affinity chromatography:

Extract of different proteins are passed through a column that contained immobilized protein. Protein molecules that do not bind are easily eluted through the column and ligand proteins that

bind are attached in the column. Strongly bound proteins have more affinity with the immobilized protein than the weakly binding proteins.

Affinity purification generally involves the following steps:

A. Incubate crude sample with the affinity supported matrix to allow the target molecule present in the sample to bind with the immobilized ligand.

B. Then wash out the unbound sample components from the support.

C. Elute (dissociation and collection) the target molecule from the immobilized ligand by the buffer of interest so that the binding interaction can be overcome.

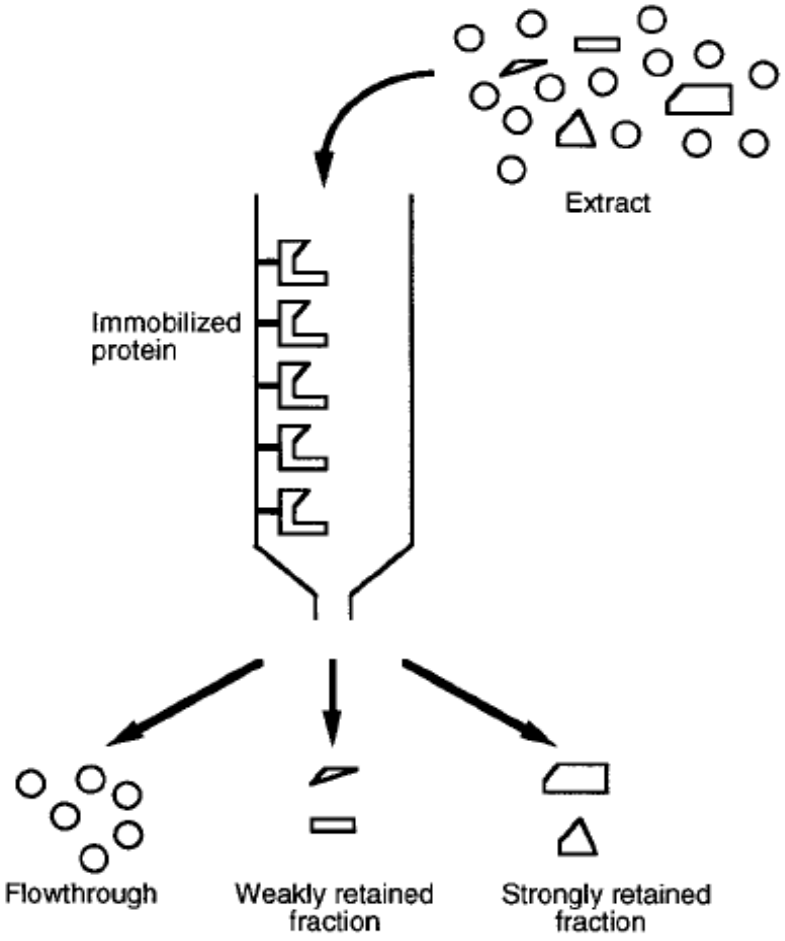


Fig.4.21: Protein affinity chromatography.

Applications:

a. Enzyme purifications

b. DNA-binding protein purifications

c. Purifications of glycoconjugates

d. Isolation of nucleotides

4.8. Protein–Nucleic Acid Interaction

The term protein nucleic acid interaction is defined when the proteins molecules is bind with nucleic acids to regulate the function of living cells. Late 19 century the proteins interaction with DNA is observed. After that, lots of *in vitro* and *in vivo* experiments were carried out to understand the interaction between protein and nucleic acids. The protein-nucleic acids interaction or complexes play in the regulation of transcription, translation, DNA replication, repair and recombination, RNA processing and translocation. It continues to revolutionize our understanding of cell biology, normal cell development and the mechanisms of disease. There are several physical forces such as electrostatic interaction, dipolar interactions and entropic effects are generally found to be responsible for protein- nucleic acids interaction. Proteins bind with DNA and RNA in discrete conserved domains along its tertiary structure. Proteins may also have multiple nucleic acid binding domains of the same kind or several different domains along their structure. The identity of the individual nucleic acid binding domains and their arrangement helps determine their functional importance within the protein.

4.8.1. Interaction types

The interaction occurs in a sequence-specific or non-sequence specific manner due to cumulative contribution of these physical forces. For example, specific protein–DNA interactions are commonly mediated by an α-helix motif in the protein that inserts itself into the major groove of the DNA, recognizing and interacting with a specific base sequence through Hbonds and salt bridges.

Specific types

The sequence of nucleotides directly affects the interaction outcome. It control transcription in prokaryotes and eukaryotes mediated by ionic interaction and van der Waals forces. In specific interaction the transcription factors influence the interaction by the Helix loop helix, Leucine zipper, Zinc figure motif and Lambda repressor. In specific interaction, the DNA binding protein includes these transcription factors. These binding seem to stabilize single standard by nucleases. The specific interaction process is use in DNA replication, transcription and repair. The level of specificity can be considered on the following basis.

- Site specification

- Recognition
- Affinity
- Equilibrium selection

Transcription factors:

Helix loop-A basic helix loop helix (bHLH) is protein structural motif that characterizes finally family of transcription factors. In general one helix is smaller and due to their flexibility of loop, allows dimerization by folding and packing against another helix. The large helix typically contains the DNA binding region. bHLH proteins typically bind to a consensus sequence called an E box (CACGTG). Example of transcription factor containing a bHLH, include BMAL-1 CLOCK, C-MyC, MyoD, Myf5, Pho4, HIF 1CE1 etc.

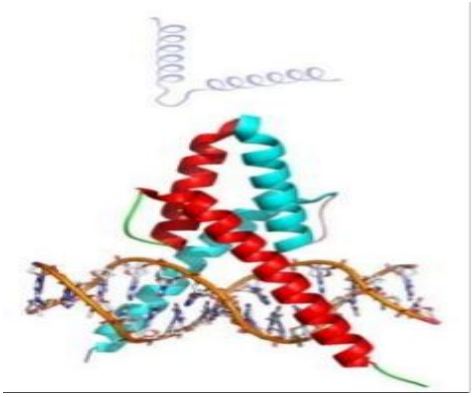


Fig. 4.22: Structure of Helix loop

- **Leucine Zipper (LZ)** – LZ formed by the dimerization of two specific alpha helix monomers bond to DNA, the bZIP interact with DNA via its N- terminal, where as lysine and arginines are located, these basic residue interaction in the major grove of the DNA forming sequence-specific interactions.

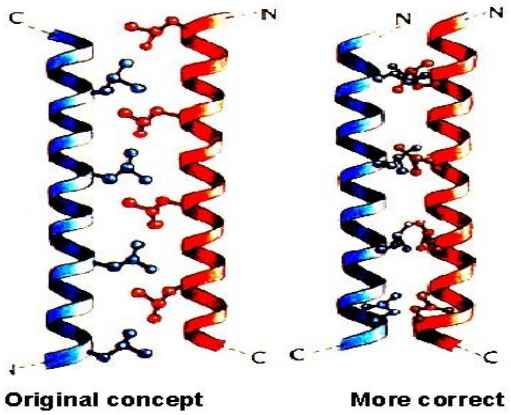


Fig.4.23: Structure of Leucine Zipper

Zinc finger- Conserved pair of Cys and His residue bind a Zn atom a stem loop structure bind to DNA in major grove. This finger mostly occur group of three structures the two His residue will be Cys (i.e. four Cys total) for example TFIIIA.Zn^{2+} ion is held between a pair of β strands and α helix.

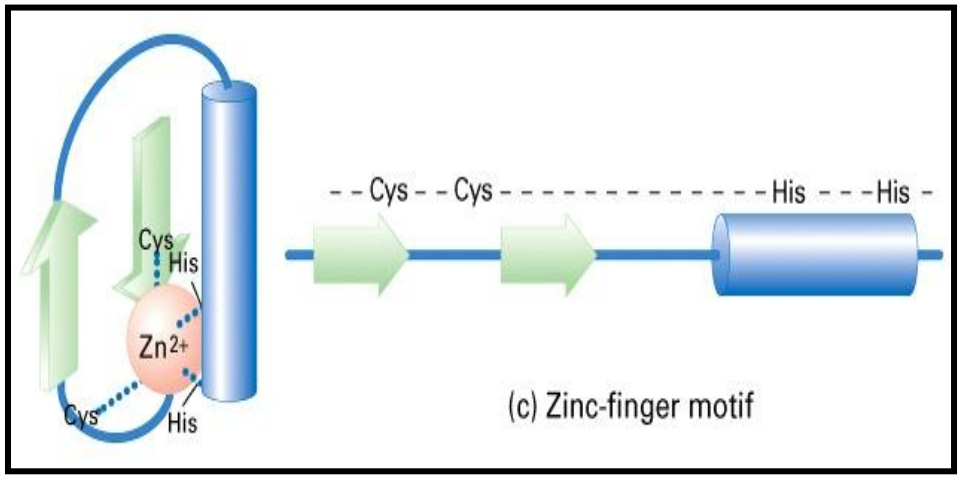


Fig.4.24: Structure of Zinc finger

- **Helix-turn helix-** It consist of two alpha helics connected a fixed angle by short stretch of polypeptiede chain. Recognition helix recognized and bind to specific DNA sequence by forming hydrogen with bases located in groove. Second helix stablize the overall configuration through hydrophobic interaction with the recognition helix.

Non specific integration

In non specific interaction, the sequence of nucleotides does not matter, non specific interaction effected chromatin, nucleosome and chemically modify by methylation, demethylation, acetlation and phosphorylation etc. within chromosomes, DNA is held in complex with structural proteins. These proteins organize into chromatin. In eukaryotes this structure involves DNA binding to complex of small basic protein. While in prokaryotes multiple types of proteins make ionic bond to the nucleic acidic sugar phosphate of the DNA and independent of the base sequence.

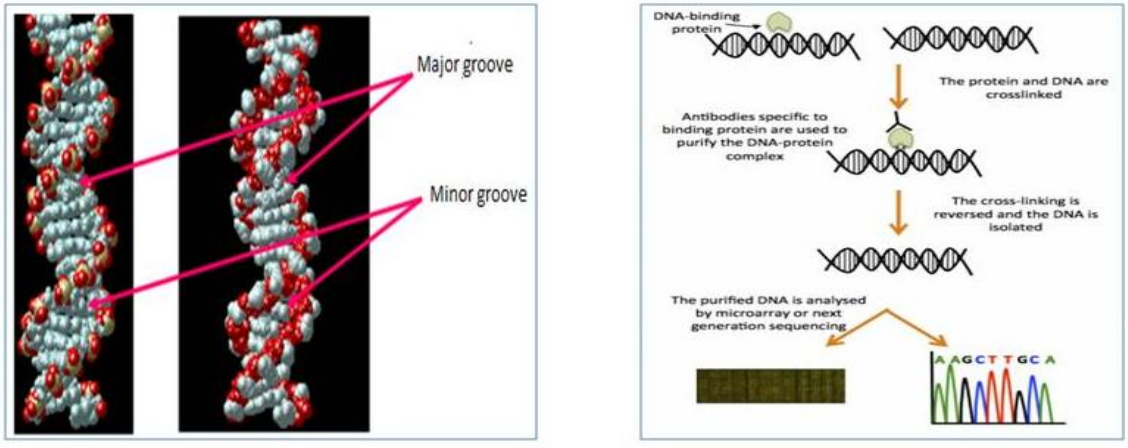
4.9. DNA - Protein Interactions

As discussed above, the interaction between nucleic acid (DNA, RNA) and protein is either specific or non specific. When the interaction occurs in non-specific manner, the sequence of nucleotides does not matter, as far the binding interactions are concerned (Fig.4.25). In specific protein-DNA interaction, the sequence of nucleotides is considered. In addition, the functional groups present on both entities are responsible for association i.e. functional groups on the protein and the sugar-phosphate backbone of DNA is actively participates in interaction. These DNA - protein interactions are strong, and are mediated by:

Hydrogen bonding: Can be direct H-bonds, or indirect, mediated by water molecules

Ionic interactions: Salt bridges; protein side chains - DNA backbone interaction

Other forces: van der Waals, hydrophobic



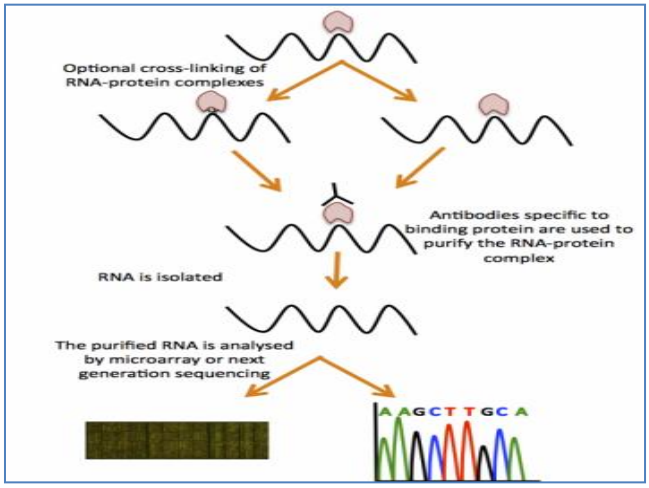
Source: ebi.ac.uk/training/online/course/functional-genomics-ii-common-technologies-and-data-analysis-methods/dnarna-protein

Fig.4.25: Protein DNA interaction

Protein–DNA interactions occur when a protein binds to a molecule of DNA, often to regulate the biological function of DNA, usually the expression of a gene. Among the proteins that bind to DNA are transcription factors that activate or repress gene expression by binding to DNA motifs and histone that form part of the structure of DNA and bind to it less specifically. DNA binding proteins 'see' the edges of the base pairs in the major or minor groove. DNA–protein analyses are referred to as ChIP-chip or ChIP-seq, and require the crosslinking of DNA-protein with formaldehyde before the immunoprecipitation.

4.10. Protein–RNA interactions

Proteins interact with RNA in order to splice, protect, translate or degrade the message. The first interaction occurs just after transcriptional initiation, when the complement to the promoter sequence is cleaved out of the mRNA and the capping machinery incorporates a "GpppN" cap at the 5' end of the mRNA. This results in recruitment of elongation factors that regulate the reset of mRNA transcription. Elongation is followed by 3'-end processing and splicing, resulting in a mature RNA transcript that is exported to the cytoplasm for translation. All of these processes require significant protein–RNA interactions and are highly regulated and complex(Fig.4.26).



Source: ebi.ac.uk/training/online/course/functional-genomics-ii-common-technologies-and-data-analysis-methods/dnarna-protein

Fig. 4.26: Protein RNA interaction

For RNA-protein analyses the cross-linking step is optional. RNA-immunoprecipitation (RIP)-chip/seq analyses can be performed with or without formaldehyde-based cross-linking

before immunoprecipitation. Without cross-linking, this method is not well suited for detecting transient RNA-protein interactions and is prone to high background.

4.11. Function of protein

Proteins are large, complex molecules that play many critical roles in structure, function, and regulation of the body's tissues and organs. Proteins are made up of hundreds or thousands of smaller units called amino acids, which are attached to one another in long chains. There are 20 different types of amino acids that can be combined to make a protein. Protein has several functions in which some are summarized:

- **Growth and Maintenance:** Some amount of protein molecules are break down under normal condition and that is useful to build and repair tissues. Other times, it breaks down more protein than it can create, thus increasing your body's needs.
- **Causes Biochemical Reactions**: We know the enzymes are proteins which take part of lots of biochemical reactions that take place within and outside of your cells. Thus several functions such as Digestion, Energy production, Blood clotting, Muscle contraction etc. depend on enzymes.
- **Acts as a Messenger:**We also know that some proteins are considered as hormones, the hormones are chemical messengers that aid communication between your cells, tissues and organs.
- **Provides Structure:**Protein play important role in regulation and structure maintenance of cells and tissues with stiffness and rigidity. These proteins include keratin, collagen and elastin, which help form the connective framework of certain structures in your body.
- **Maintains Proper pH:** Protein plays a vital role in regulating the concentrations of acids and bases in your blood and other bodily fluids such as-
  - **pH 2:** Stomach acid
  - **pH 4:** Tomato juice
  - **pH 5:** Black coffee
  - **pH 7.4:** Human blood
  - **pH 10:** Milk of magnesia

- **pH 12:** Soapy water

- **Balances Fluids:** Proteins regulate body processes to maintain fluid balance.Albumin and globulin are proteins in your blood that help maintain your body's fluid balance by attracting and retaining water
- **Bolsters Immune Health:** Proteins help form immunoglobulins, or antibodies, to fight infection
- **Transports and Stores Nutrients:** Transport proteins carry substances throughout your bloodstream into cells, out of cells or within cells.
- **Provides Energy:** Protein contains four calories per gram, the same amount of energy that carbs provide. Fats supply the most energy, at nine calories per gram.

Protein main function

We should learn that the protein's function depends on its shape, and size and their distributions. Unfortunately when protein is disformed, the resulting proteins causes anumber of problems called malfunction. Protein formation is an error-prone process, and mistakes along the way have been linked to a number of human diseases. There are several malfunction that occurs due to protein misfolding is believed to be the primary cause of Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt-Jakob disease, cystic fibrosis, Gaucher's disease and many other degenerative and neurodegenerative disorders.By changing a gene's instructions for making a protein, a mutation

4.12.Summary

In this unit you have learn that-

Proteins are building block of organism formed by combination of amino acids through peptide. In the formation of proteins there are 20 amino acids used that is called essential amino acids. Some are essential amino acids for examples Theonine, Valine, Leucine, Lysine, Isoleucine, Methionine, Phenylalanine, Tryptophan, Arginine, and Histidine. Whereas Arginine and Histidine are semi essential amino acids. The amino acids are the monomeric unit of proteins. The amino acid is composed of two groups i.e. NH_2 and COOH is responsible for linking of two amino acids. The amino acids come together and form peptide bonds and release water molecules in this process. Thus protein is also called polypeptide,protein is abundant

naturally occurring organic molecules found in every part of every cell. It exists as fundaments in aspects of cell structure and functions. Different kind of protein is specialized for various biological functions. In this unit you have studied various types of proteins structure such as primary, secondary, tertiary and quaternary. Hemoglobin is also example of oligomeric protein which consists protein goblin and pigments heme. Hemoglobin is red in color and an oxygen carrying pigment in red blood cells of vertebrates. Transport of oxygen in RBCs is the main function of hemoglobin and it also play role as acid base buffer.

4.13. Terminal questions

Q.1: What are amino acids? Discuss essential and non essential amino acids.

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

Q.2: How acid acts as zwitter ions and how acts as a buffer?

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

Q.3: Write the classification of amino acids and their functions.

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

Q.4: Write in brief on acid base reaction on amino acids.

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

Q.5: Discuss the types of proteins and peptide bond.

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

Q. 6: Write notes on hemoglobin and their functions.

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

Q.7: What is protein-protein interaction? Write the types and methods of protein-protein interaction.

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

4.14. Further readings:

6. Principles of Biochemistry: Lehninger, Nelson and Cox. Student edition, CBS 1439 Publishers and Distributors, Delhi.
7. General biochemistry: J.H. Weil, New Age International (P) Limited, 5th edition, 2013,
8. Biochemistry: T.A. Brown, Viva book publication. First edition, 2018.
9. Elements of biochemistry: J.L. Jain, S. Chand publication, Seventh edition.
10. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

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PGBCH-101

Cell Biology and Bio-molecules

Block- III

Biomolecules part -II

UNIT -5

Lipids

UNIT-6

Nucleic acid

Introduction

This is the third block of Cell Biology and Bio-molecules. It consists of following two units:

Unit-5: In this unit the lipids biomolecules is discussed. It deals with the basic definition, structure, types and function of lipids. The essential, saturated and unsaturated fatty acids structure and their physical properties are also discussed. Nomenclature of fatty acids is also discussed briefly. Different types of complex lipids for example wax, phospholipids, glycerophospholipids, sphingophospholipids, glycolipids, glyceroglycolipids, sphingo-glycolipids and simple lipids such as terpenes, steroids, cholesterol, steroid hormones and bile salts are also briefly discussed. Lipids bilayer as a, component and structure of biological membrane is discussed.

Unit-6: This unit provides an insight into the structure and components of nucleotides and nucleosides. Nucleic acid definition and double helical structure of DNA, Different forms of DNA, RNA and its forms are discussed briefly in this chapter. The basic introduction of central dogma of life, denauration kinetics of DNA, nucleic acid sequencing along with biological function of nucleic acid is discussed.

Unit-5: Lipids

Structure

5.1. Introduction

Objectives

5.2. About lipids

5.2.1. Biological functions of lipids

5.2.2. Physical properties of fatty acids

5.3. Fatty acids

5.3.1. Saturated fatty acids

5.3.2. Unsaturated fatty acids

5.3.3. Nomenclature of fatty

5.3.4. Essential fatty acids

5.4. Waxes

5.5. Phospholipids

5.6. Glycerolphospholipids

5.7. Sphingophospholipids

5.8. Glycolipids

5.9. Glyceroglycolipids

5.10. Sphingo-glycolipids

5.10.1. Function of sphingo-glycolipids

5.11. Simple lipids

5.11.1. *Terpenes*

5.11.2. *Steroids*

5.11.3. Cholesterol

5.11.4. Steroid hormones

5.11.5. Bile salts

5.12. Lipid bilayer structure

5.13. Biological membrane: components and structure

5.13.1. Functions of membrane

5.14. Summary

5.15. Terminal questions

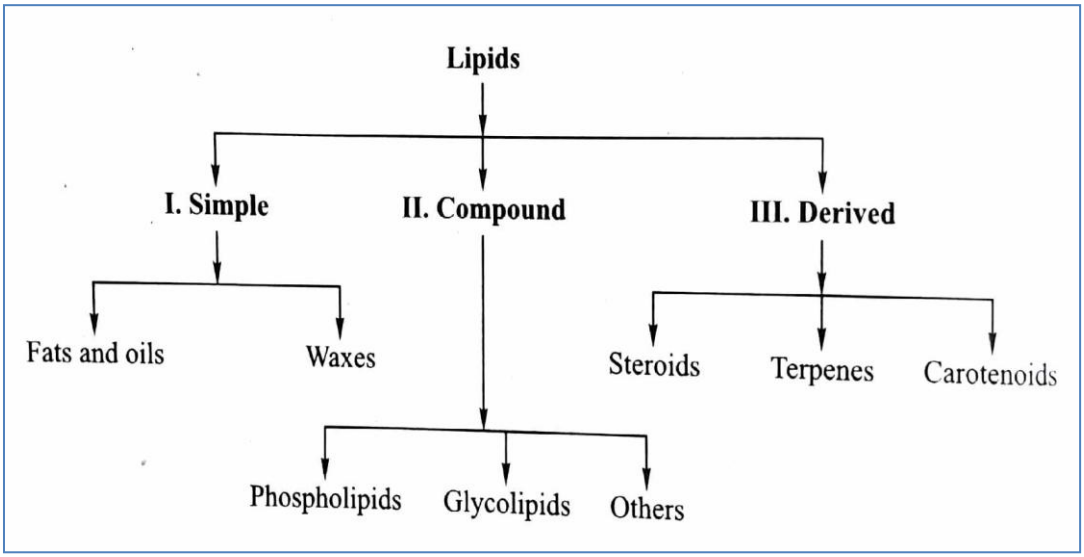
5.16. Further readings

5.1. Introductions:

This unit covers types, structure and function of lipids. It also deals in brief about fatty acids, structure and sources. Fatty acids are unbranched chain of carboxylic acids and key components of lipids. Fatty acids have long chain of hydrocarbon these. Fatty acids are classified as saturated and unsaturated fatty acids on the basis of type of bond. Lipids are water insoluble organic molecules. The lipids are extracted from cells and tissues by non polar solvent. Lipids are present as important constituents in cell membrane, cell surface components and are also useful in storage and transport of metabolic fuels. Lipids are classified as special class of biomolecules that is linked with member of other class of molecules either covalently or through the weak bond. The glycolipids and lipoproteins are considered as hybrid molecules which contain both proteins and lipids and are important components of cell membrane. Different kinds of lipids and their structure such as glycerol lipids, phospholipids, glycerolphospholipids, sphingophospholipids and glycolysis etc are discussed. The lipid which is not having fatty acid in their structure is called simple lipids. They occur in smaller amount in cells and tissue as compared to other complex lipids that contains fatty acids as building block. The lipids are universal component of all cell membranes. The cell membrane not only consists of lipids but also contains proteins and carbohydrate in variable amount. The biological membrane which is present in eukaryotic and prokaryotic cells is also known as plasma membrane is also the subject matter of this chapter.

Objectives:

- To describe types of fatty acids
- To understand Nomenclature of fatty acids
- To study the function of lipids in body
- To study simple and complex lipids
- To study the lipid bilayer structure
- To study the components and structure of biological membranes



5.2. About lipids

Lipids are essential components of all living organisms. It is present naturally in plants and animals. Lipids are chemically diverse group of compound which is insoluble in water and soluble in organic solvents. Lipids consist of large hydrocarbon (non polar group) which defines solubility behavior of lipids. Lipids are hydrophobic (nonpolar) or amphipathic (contacting both nonpolar and polar regions) in nature. Due to the presence of major portion of hydrocarbon in lipids it is classified in several forms and each shows distinct properties. Fig. 1 illustrates some examples of compounds that contain lipids. The chemical structure of lipids drives several biological functions. Lipids are important

structural component of membrane, helps in storage and transport of fuels, present as protective coating on the surface of many organisms. Lipids are also found in cells that provide cell recognition, species specificity and tissue immunity. The lipids are combined either covalently or through weak bonds with member of other class of biomolecules and produces hybrid molecules. Although, lipids are present in relatively small quantities they play important roles as enzyme cofactors, electron carriers, light absorbing pigments and hydrophobic anchors for proteins. The main function of lipid is energy storage, structural components of cell membrane, and as important signaling molecules.

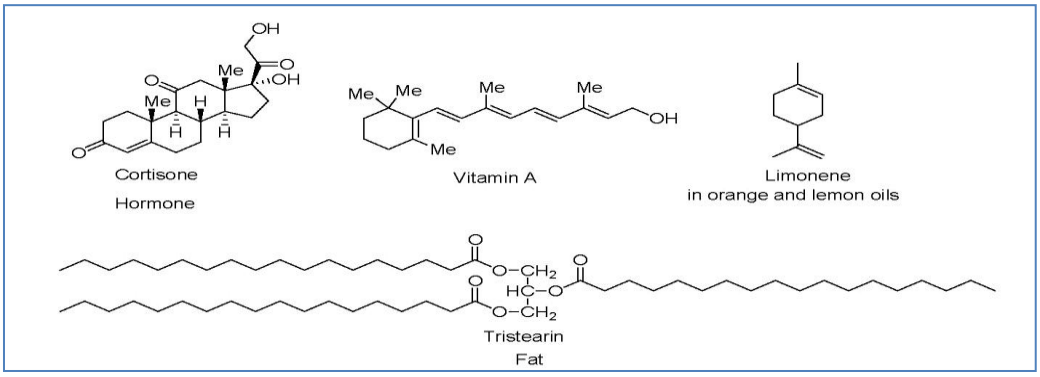


Fig.5.1: Examples of compound that have lipids

On the basis of backbone structure, the lipids are classified as complex lipids and simple lipids. The complex lipids are those lipids which contains fatty acids as components. The lipids which have fatty acid components differ in their backbone structure and are covalently joined together such as acylglycerole, phosphoglycerides, sphingolipids and waxes etc.

The complex lipids are also called saponifiable lipids and they are obtained from soaps on alkaline hydrolysis while simple lipids are that lipids which do not contain fatty acids and are also called non saponifiable lipids. Lipids are generally classified into following groups:

Acyl gycerols

Phospholipids

Sphingolipids

Glycolipids

Alkyl glyceryl ethers

Terpenoids

Wax

5.2.1. Biological functions of lipids

- Lipids are main source of storage energy in living cells.
- Due to hydrophobic nature of lipids, it serve as a good packing material.
- Lipids has low thermal conductivity and hence work as an insulator form environment.
- Lipids have good mechanical protection (can absorb shocks).
- Due to hydrophobic nature, the lipids are good water repellant and hence provides dry surface to organisms.
- The hydrophobic nature of lipids prevents excessive wetting and also loss of water via evaporation.
- Lipids are also associated with buoyancy control and acoustics in marine mammals.
- Lipids are main components of cell membrane structure.
- Lipids are important integral part of many cofactors of several enzymes, such as ATP synthesis in mitochondria, vitamin K for blood clotting.
- Lipids also used in signaling molecules (hormones) and growth factor, such as paracrine hormones acts locally whereas the steroids hormones acts on whole body.
- Lipids are also components of pigments giving specific colors. Color of tomatoes, pumpkins, carrots and some birds are mainly due to different pigments which may include lipids.
- Some lipids are also used as antioxidants.

5.3. Fatty acids

Fatty acids are unbranched chain of carboxylic acids and are key components of lipids. Fatty acids are aliphatic monocarboxylic acids with mostly unbranched carbon chains as summarized in Table 5.2. The term "fatty acids" is based on the recognition that natural fats and oils consist of the esters of long-chain carboxylic acids with glycerin. There are more than 100 different types of fatty acids associated with various lipids of plants and animals. Fatty acids exists either saturated or unsaturated form.

5.3.1. Saturated fatty acids

Saturated fatty acids have general formula $C_nH_{2n+1}COOH$. The fatty acids consist of even numbers of carbon present in straight chain. The saturated fatty acids are found both in plants and animals. In animal fats, palmitic and stearic acids (C16 to C18) are the most abundantly found saturated fatty acids. Apart from straight chain, some branched chain fatty acid having even or odd number of carbon is considered as natural fats and oils. For example isopalmic acid [$C_{16}H_{32}O_2$], Anteisopalmitic acid [$C_{16}H_{32}O_2$].

5.3.2. Unsaturated Fatty acids

The unsaturated fatty acids have less intermolecular interactions compared to saturated fatty acids and hence have lower melting point. In other hand saturated fatty acids have no double bonds between carbon molecules as they are saturated with hydrogen molecules. The saturated fatty acids exits as solid at room temperature. On the basis of saturated or unsaturated hydrocarbon fatty acids lipids are recognized as oils and fats. A mixture of triglycerols containing high proportion of unsaturated fatty acids are referred as oils while mixture of triglycerols with relatively high proportion of saturated fatty acids are known as fats and are solid in nature. In unsaturated fatty acids, the double bonds between carbon molecules have cis-configuration and are separated by CH_2 group. Examples of unsaturated fatty acids are as follows

Monoethenoid acids:

It contains general formula $C_nH_{2n-1}COOH$ for example Oleic acid.

Diethenoid acids:

It has two double bond and also contains general formula $C_nH_{2n-3}COOH$ for example Linoleic acid.

Triethenoid acids:

It has triple double bond and also contains general formula $C_nH_{2n-1}COOH$, for example, Linolenic acid.

Tetraethenoid acids:

It has four bond and also contains general formula, $C_nH_{2n-7}COOH$ for example Arachidonic acid

Table 5.1: Different kinds of naturally occurring fatty acids.

Symbol	Common name	Structure	Melting point
Saturated fatty acids			
12:0	Luaric acid	$CH_3(CH_2)_{10}$ COOH	44.2
14:0	Myristic	$CH_3(CH_2)_{12}$ COOH	53.9
16:0	Palmitic	$CH_3(CH_2)_{14}$ COOH	63.1
18:0	Stearic	$CH_3(CH_2)_{16}$ COOH	69.6
20:0	Arachidic	$CH_3(CH_2)_{18}$ COOH	76.5
24:0	Lingoceric	$CH_3(CH_2)_{22}$ COOH	86.0
12:0	Dodecanoic acid	$CH_3(CH_2)_{10}$ COOH	43.2
Unsaturated fatty acids			
16: $1^{\Delta 09}$	Palmitoeic	$CH_3(CH_2)_5CH= CH(CH_2)_7COOH$	-0.5
18: $1^{\Delta 09}$	Oleic	$CH_3(CH_2)_7CH= CH(CH_2)_7COOH$	13.4
18: $2^{\Delta 09, 12}$	Linoleic	$CH_3(CH_2)_4CH= CHCH_2CH= CH(CH_2)_7COOH$	-5
18: $3^{\Delta 09, 12,15}$	Linolenic	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	-11
Some unusual fatty acids			
18: $1^{\Delta 09,trans}$	Elaidic	$CH_3(CH_2)_7CH= CH(CH_2)_7COOH$ (trans)	
16: $1^{\Delta 09,trans}$	trans-Hexadecenoic	$CH_3(CH_2)_5CH= CH(CH_2)_7COOH$ (trans)	

Source: Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439

5.3.3. Nomenclature of fatty

Fatty acids differ from each other by the primary chain length, by the number and position of their unsaturated carbon bonds. The fatty contains long hydrocarbon chain

ranging from 4 to 36 carbons (C4 to C36). On the basis of number of chain length and number of double bond the nomenclature of fatty acids has been carried out. In this nomenclature the carbon chain length and number of double bond is separated by colon; for example the 12-carbon, with single bond (saturated) of Lauric acid is abbreviated as 12:0 and the 18- carbon oleic acid, with double bond, is abbreviated as 18:1 (where 12 and 18 represents number of carbon, and 0 and 1 represents number of carbon double bonds in respective compounds). In case of long chain fatty acids having double bonds at different positions of carbon chain, the position of carbon double bonds are specified by superscripted number following Δ (delta); for example a 20- carbon fatty acid with double bond between C-9 and C-10 and another between C-12 and C-13 is designated as 20:2(Δ9'12). In most of the monounsaturated fatty acids double bonds exist between C-9 and C-10, whereas in polyunsaturated fatty acids the double bonds exits between C-12 and C-15 ($\Delta^{12'15}$). The double bonds of polyunsaturated fatty acids are almost never conjugated but are separated by a ethylene group for example, -CH=CH-CH_2-CH=CH-; only few plants have conjugated double bonded fatty acids. Fatty acids with odd number of carbon atoms occur in trace amount in terrestrial animals but bacteria contains simple types of saturated fatty acids having carbon number C_{12} to $C_{18.}$ The physical properties of fatty acids are determined by length and degree of unsaturation of the hydrocarbon chains. Lower solubility of fatty acids in water occurs due to the presence longer chain and fewer numbers of double bonds. The carboxylic group being polar in nature accounts for the slight solubility of short-chain fatty acids in water.

5.3.4. Essential fatty acids:

Fatty acids required in the diet of mammals are called essential fatty acids. The essential fatty acid starts with the short chain polyunsaturated fatty acids (SC-PUFA). The most essential fatty acids in mammals is linoleic acid, which makes about 10 to 20 present of total fatty acids of diacylglycerols and phosphoglycerides. Some of the fatty acids are not synthesized in mammals and hence must be obtained from plants such as Linoleic and ϒ-Linoleic acid. The Linoleic acid is essential for biosynthesis of chidonic acid, which is not found in plants. Only two fatty acids α-linolenic acid (omega-3 fatty acid) and linoleic acid (omega-6 fatty acid) are known to be essential for human (Fig.5.2). Both essential fatty

acids cannot be synthesized by humans because human lack the desaturase enzymes required for their production. The α-linolenic acid is used as a precursor for the biosynthesis of long chain omega fatty acids eicosapentaenoic acid and docosahexaenoic acid. Linoleic acid, arachidonic acid, and DHA are the most common polyunsaturated fatty acids (PUFA) present in tissues. Linoleic acid and α-linolenic acid are important structural component of membrane and serve as precursors to bioactive lipid mediators, and also provide a source of energy. Omega-6 and omega-3 fatty acids are polyunsaturated fatty acids (PUFA). The parent fatty acid of the omega-6 series is linoleic acid, and the parent fatty acid of the omega-3 series is α-linolenic acid and give rise to longer chain derivatives inside the body as shown in Table 1. The first double bond exist between C-7 to C-7 form the methyl end of the fatty acids(n-6) for omega-6; and between C-3 to C-4 for the methyl end of the fatty acid(n-3). Double bonds introduce kinks in the hydrocarbon chains that influence the structure and physical properties of the fatty acid molecule. Some fatty acids are classified as conditionally essential fatty acids under some developmental or disease conditions such as docosahexaenoi acid and ϒ- linoleic acid. However, the specific functions of essential fatty acids are not clear but it is observed that it plays important role as a precursor in biosynthesis of group of fatty acid derivatives called prostaglandins.

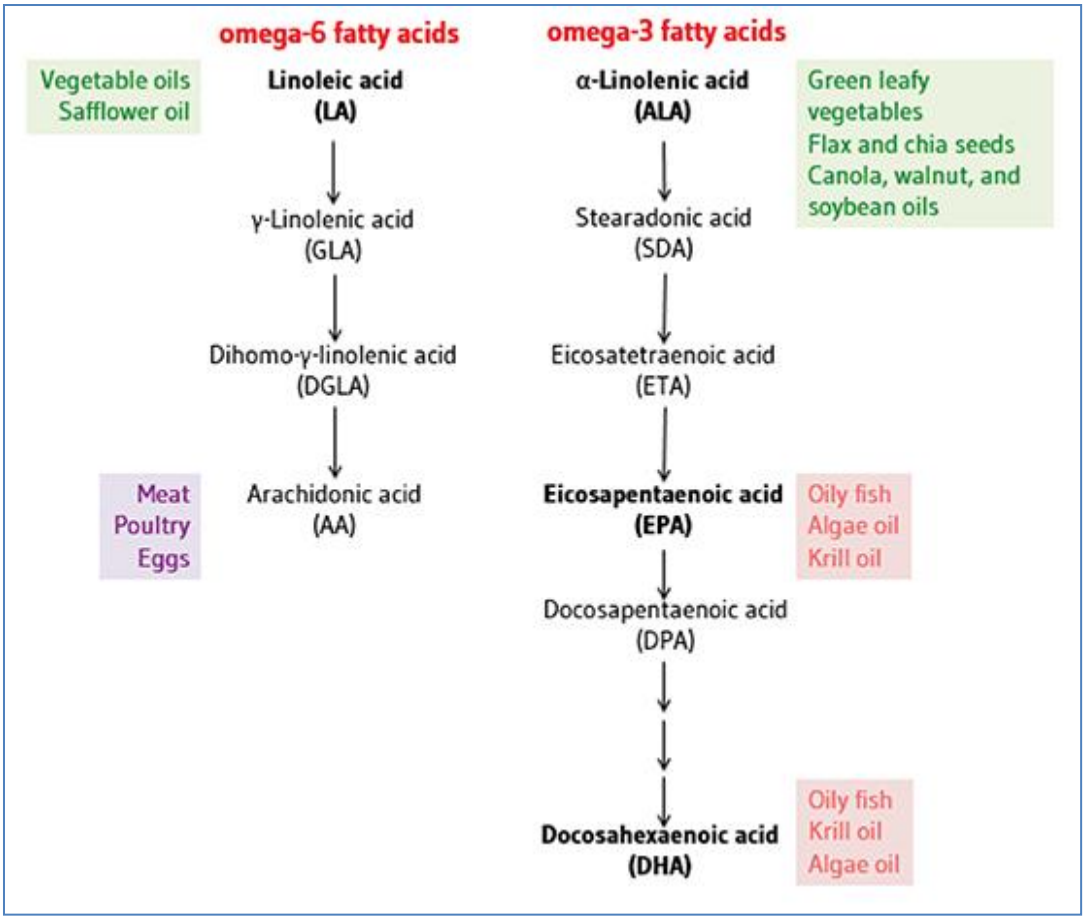


Fig.5.2: Classes of essential fatty acids

Table 5.2: Names and Abbreviations of the Omega-6 and Omega-3 Fatty Acids

Omega-6 Fatty Acids			Omega-3 Fatty Acids		
Linoleic acid	LA	18:2n-6	α-Linolenic acid	ALA	18:3n-3
γ-Linolenic acid	GLA	18:3n-6	Stearadonic acid	SDA	18:4n-3
Dihomo-γ-linolenic acid	DGLA	20:3n-6	Eicosatetraienoic acid	ETA	20:4n-3
Arachidonic acid	AA	20:4n-6	Eicosapentaenoic acid	EPA	20:5n-3
Adrenic acid		22:4n-6	Docosapentaenoic acid	DPA (n-3)	22:5n-3
Tetracosatetraenoic acid		24:4n-6	Tetracosapentaenoic acid		24:5n-3
Tetracosapentaienoic acid		24:5n-6	Tetracosahexaenoic acid		24:6n-3
Docosapentaenoic acid	DPA (n - 6)	22:5n-6	Docosahexaenoic acid	DHA	22:6n-3

Source: Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439

A fatty acid is one of the major components of a triglyceride, which is a form of lipid that is used in the body to store energy. The simplest lipids constructed from fatty acids are the triacylglycerols. Sometime triacylglycerols are also referred as triglycerides, fat or neutral fats, which consists of a glycerol esterified with three fatty acids. Triacylglycerol are a major form of energy storage in animals and plants. Animals have specialized fat storage cells called adipocytes, which are present in white and brown fat tissue. The energy is produced from triacylglycerols through its complete oxidation. About 9 Kcal (38 KJ) energy is produced by complete oxidation of 1 g of triacylglycerols compared to about 4 k cal g^{-1} of sugar, glycogen and amino acids. Triacylglycerols are oxidized when the energy demand increases or when other energy, such as sugar and fat are unavailable. In some triacylglycerols the three fatty acids are identical for example tripalmitin, which has three 16: 0 chains and triolein in which three 18: 1 (delta 9) chains are observed as shown in Table 2 are called as simple triacylglycerols. The triacylglycerols which have chains of different fatty acids are called complex triacylglycerols.

Table.5.3: Composition of some common fatty acids

Common name	Fatty acid composition
Simple triacylglycerols	
trilaurin	12:0, 12:0, 12:0
Tripalmitin	16:0, 16:0, 16:0
Tristearin	18:0, 18:0, 18:0
Triolein	18:1 (Δ9), 18:1(Δ9), 18:1(Δ9)
Complex triacylglycerols	
Component of olive oil	18:1 (Δ9), 18:1(Δ9), 16:0

5.3.5. Physical properties of fatty acids

- Fatty acids are weak acids (pka = 4.5-5) ionized at physiochemical pH.
- Saturated fatty acids are solid at room temperature and its melting point depends on the chain length and degree of unsaturation.

- Cis-double bonds disrupt intermolecular packing and therefore have lower melting point(M.P).
- Polyunsaturated fatty acids are readily oxidized by exposure to air.
- Fatty acids form micelles, which are nanosized colloidal dispersions prepared from amphiphilic molecules (having both hydrophobic tail and hudrophilic head).

5.3. Waxes

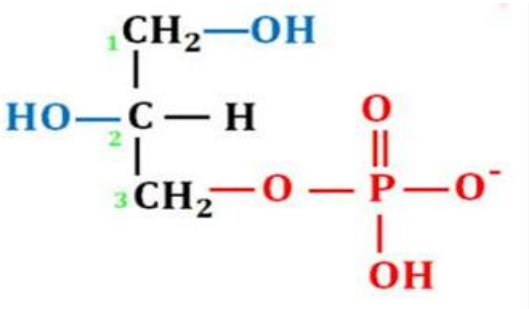
Biological waxes consist of a long chain (C_{14} to C_{36}) saturated and unsaturated fatty acid linked through ester oxygen to long chain alcohol (C_{16} to C_{30}). It is water insoluble and has high melting points ranging from 60 to 100 ˚C which is generally higher than those of triacylglycerols. Waxes are long chain nonpolar lipids and exist in solid state at room temperature. Waxes are soft and pliable when hot but becomes hard when cold. Waxes are generally synthesized by animals and plants. The composition of waxes depend on the presence of varieties of carboxylic acids and alcohol fatty acids like animal waxes. The composition of wax components also depends on the species and geographical location of the organism. The best known animal wax is bees wax. The bees wax is naturally produced by honeybees of the genus *Apis*. Chemically, bees wax consists mainly of esters of fatty acids and various long-chain alcohols but major component of bees wax is the ester myricyl palmitate, which is being used by bees for constructing honeycombs. Waxes forms protective coating on skin, fur and feathers, leaves and fruits of higher plants and on the exoskeleton of many insects. Waxes are also the chief storage form of metabolic fuel. Several animals like vertebrates secrete waxes to protect hair and skin and keep it pliable, lubricated and waterproof. The Birds, particularly waterfowl, secrete waxes from their preen glands to keep their feathers water-repellent. Plants waxes along with cutin forms cuticle which control evaporation, wetting and hydration from their surface. The epicuticular waxes of plants are mixtures of substituted long-chain aliphatic hydrocarbons, containing alkanes, alkyl, esters, fatty acids, primary and secondary alcohols, diols, ketones, aldehydes. Biological waxes have different functions like

- Storage of metabolic fuel in planktons

- Protection and pliability for hair and skin in vertebrates
- Waterproofing of feathers in birds
- Protection from evaporation in tropical plants and ivy
- Used by people in lotions, ointments, and polishes

5.4. Phospholipids

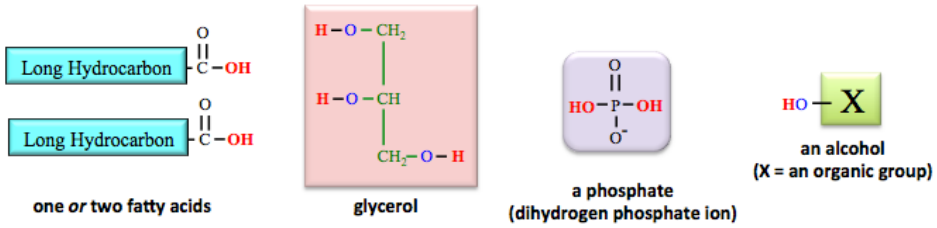
Phospholipids are types of lipid molecule and constitute major components of cell membranes. Each phospholipid is made up of two fatty acids in which one is phosphate group and another is glycerol molecules. In otherwords, glycerol-3 phosphate is a back bone of phospholipids. In phospholipids, the phosphate group is the negatively charged polar head, which is hydrophilic in nature and is located at the end of the molecule where two fatty acid chains are attached. Another end of phospholipids is hydrophobic or "water fearing", which consist of uncharged fatty acid. A phospholipid molecule is repelled by water. Phospholipids resemble a triacylglycerol but one of the fatty acid is replaced by hydrophilic group attached to the glycerol component by phosphodiester bond. When many phospholipids line up they form double layer which is characteristic of all cell membranes. Since phospholipids tails are hydrophilic the water molecules will be attached to inner membrane. While the heads are hydrophobic in nature they are faced outward and are attracted to the intracellular and extracellular fluid. If phospholipids are placed in water, they form into micelles, which are lipid molecules that arrange themselves in a spherical form in aqueous solutions. The phospholipids are classified into two types such as gylcerophospholipids and sphingophospholipids.



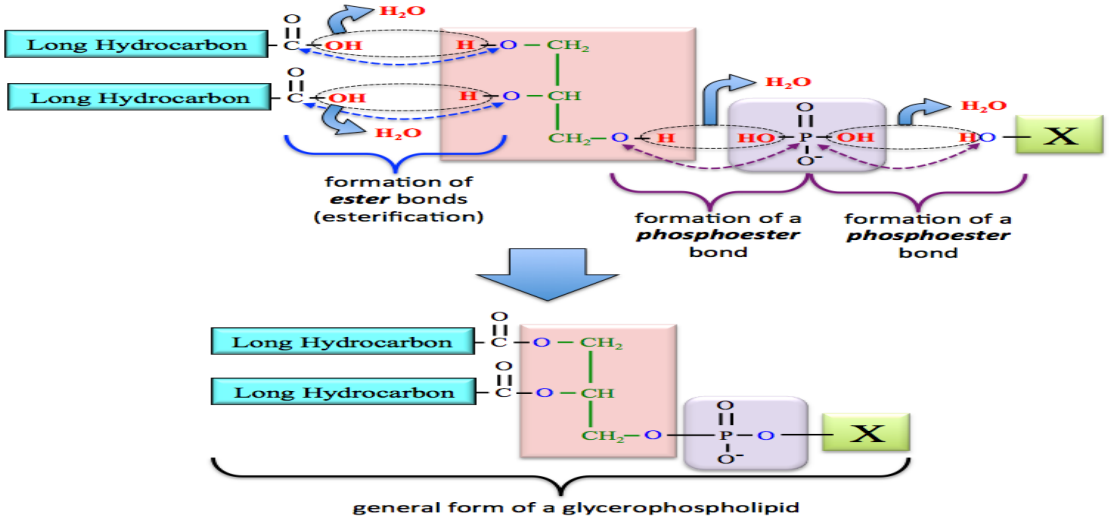
Glycerol-3-Phosphate

5.4.1. Glycerophospholipids

The glycerophospholipids are derivatives of glycerophosphoric acid that contains at least one O-acyl, or O-alkyl, or O-alk-1'-enyl residue attached to the glycerol moiety. In glycerophospholipids the glycerol is attached to two fatty acids and a phosphoric acid by the ester group. L-Glycerol 3-phosphate is the backbone of phospholipids. Here the glycerol is prochiral that can be converted to a chiral compound by adding a substituent such as phosphate to either of the CH_2OH groups. The polar tails i.e. hydrophobic exists due to the presence of two fatty acids in glycerol molecules, whereas the polar heads i.e. hydrophilic exists due to present of phosphate group attached to the carbon of glycerol molecules. Head group is charged at physiological pH. The glycerophospholipids are the primary constituents of the cell membranes and are asymmetrically distributed between the two bilayers membranes. The Glycerophospholipids provide the suitable environmental condition to membrane cells, fluidity and ion permeability. Besides this the glycerophospholipids are also involved in membrane fusion, apoptosis and regulation of the activities of membrane-bound enzymes and ion channels. The highly polar phosphate group may be further esterified by an alcohol; such substituent groups are called the head group.



Source:https://www.saddleback.edu/faculty/jzoval/mypptlectures/ch8_lipids_membranes/lecture_notes_ch8_lipids_current.pdf

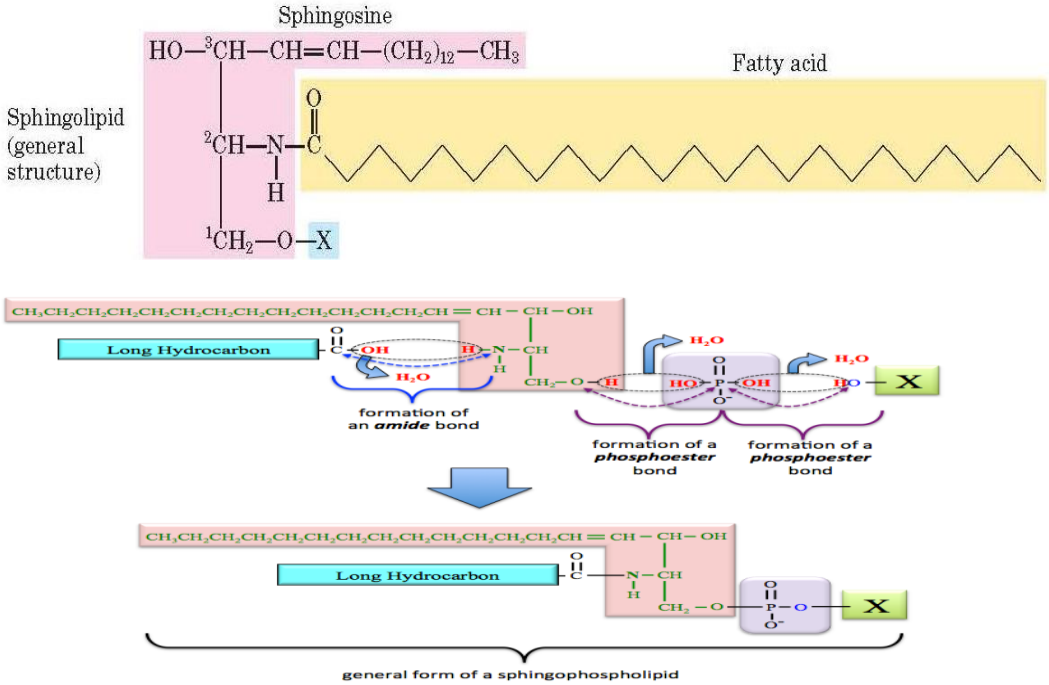


1.4.2. Sphingolipids

The sphingolipids are important lipids in cell membrane that contain sphingosine or related long chain, hydroxylated bases as back bone. Sphingolipids differs from glycerophospholipids on the basis of the backbone as sphingolipids comprises of hydroxylated base as backbone while in glycerophospholipids consist of glycerol. Sphingosine is 30 or more different long-chain amino alcohol found in sphingolipids. Sphingosine is an amino alcohol with a long unsaturated hydrocarbon chain. Sphingolipids are made by combining sphingosine, a fatty acid, a phosphate group, and an alcohol. In Sphingolipids the fatty acids are attached by sphingosine *via* an amide linkage. The Sphingolipids have long chain, nonpolar fatty acids tails and polar head groups. The polar head group is connected to sphingosine by a glycosidic or phosphodiester linkage. When the polar head is connected to sphingosine by phosphodiester linkage it is called Sphingophospholipids. However, other sphingolipids contain glycosidic linkages to sugars; any lipid linked to a sugar can be termed a *glycolipid*. Sphingolipids are divided into four major subclasses, differing by their head group.

. When the amino group of sphingosine is acylated with a fatty acid, the product is a ceramide. The primary hydroxyl group is substituted in one or two ways to give two classes of sphingolipids namely phosphosphingolipids and glycosphingolipids. In case of phosphosphingolipids, the primary hydroxyl group is esterified with choline phosphate and lipid is referred as sphingomyelin. Sphingomyelin head groups have no net charge. The

sphingomyelins is most abounded sphingolipids in tissue of higher animals. Sphingolipids with head groups composed of sugars bound by glycosidic linkages are considered *glycolipids*, as mentioned above, or, more specifically, glycosphingolipids. Cerebrosides have a single sugar, whereas globosides have two or more. These molecules are also referred to as neutral glycolipids because they have no net charge at physiological pH.



Source:https://www.saddleback.edu/faculty/jzoval/mypptlectures/ch8_lipids_membranes/lecture_notes_ch8_lipids_current.pdf

5.8. Glycolipid

Glycolipids are lipids that contain a sugar residue that means the carbohydrate attached by a glycosidic bond as shown in Fig.5.3. The sugar molecules that are attached to the sphingolipid or a glycerol group can be a monosaccharide, oligosaccharide, or polysaccharide. In the basic structure of glycolipids one or two molecule of fatty acids are attached with one sphingolipid or a glycerol and sugar molecule. Fatty acids are present in the back bone of glycolipids so that the lipids as whole have polar head and a non polar tail. Glycolipids are important component of cell membrane. The lipid bilayer of the cell membrane consists of two layers of lipids; the glycolipids interact and bind to the lipid-

bilayer through the hydrophobic nature of the lipid tail which anchors it to the surface of the plasma membrane.

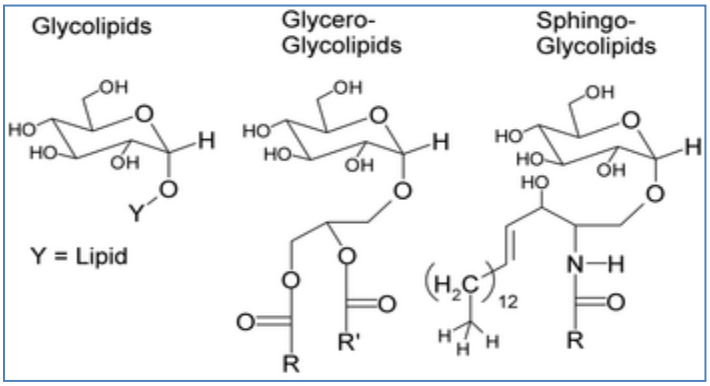


Fig.5.3: Structure of glycolipids

Glycolipids are generally classified into glyceroglycolipids and sphingo-glycolipids due to presence of glycerol and sphingosine back bone, respectively.

5.8.1.. Glyceroglycolipids

Gyceroglycolipid is sub class of glycolipids. It is made up of when the fatty acid and sugar residue are attached to glycerol. The acetylated or non-acetylated glycerol backbone is presented in glyceroglycolipid (Fig.5.4). Glyceroglycolipids is further classified into galactolipids and the sulfolipids on the basis of carbohydrate attached to the back bone. In the galactolipids, the one or two galactose residue is presented as sugar component which is attached to a C3 glycerol lipid by glycosidic linkage and the C1 and C2 of glycerol are esterified with fatty acid. The galactolipids naturally occurs in the chloroplast membranes of photosynthetic eukaryotes and photosynthetic bacteria.

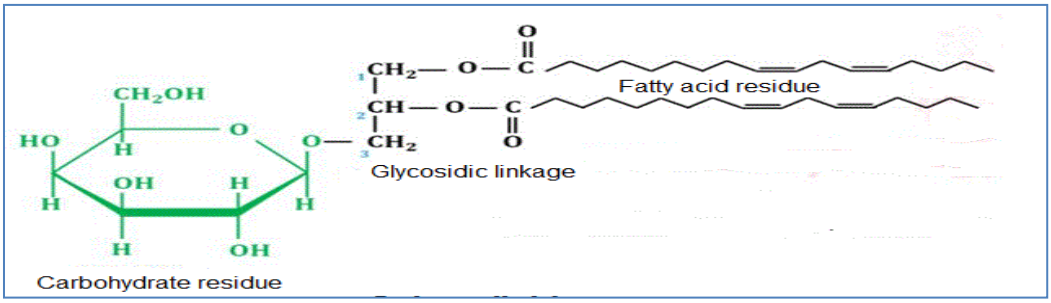
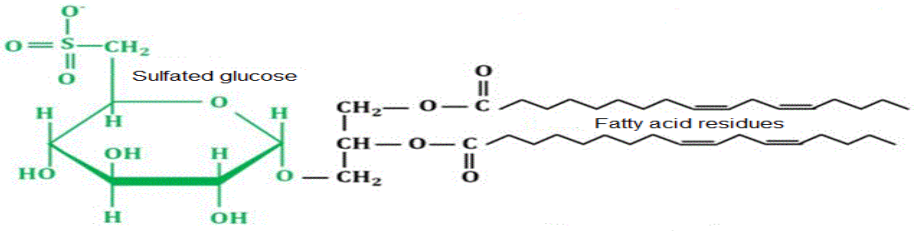


Fig.5.4: Structure of Gyceroglycolipid

Source: *www. esaybioligyclass.com*

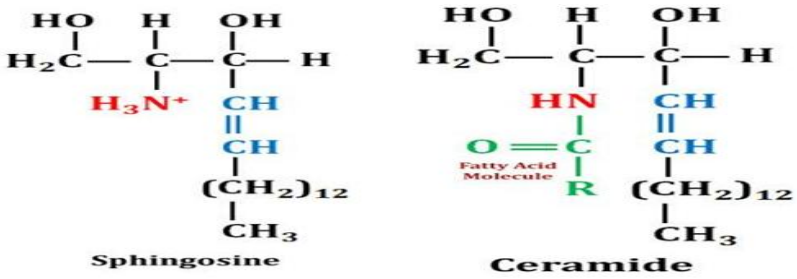
Sulfolipids is another class of glyceroglycolipids that has a carbohydrate moiety wherein the functional group contains sulfur in the sugar moiety attached to a lipid. An important group is the sulfoquinovosyl diacylglycerols which are associated with the sulfur cycle in plants.



Sulfolipid

5.8.2. Sphingo-glycolipids

Sphingo-glycolipids are another class of glycolipids it contains one or more neutral sugar residue as their polar head groups. Due to no electric charge in head of groups it is called neutral sphingo-glycolipids. We known when the sphingosine base connected to the N acyl fatty acid of 18 to 26 carbon atom it is known as ceramide. Sphingo-glycolipids are further classified in to Cerebrosides, gangliosides, globosides etc.

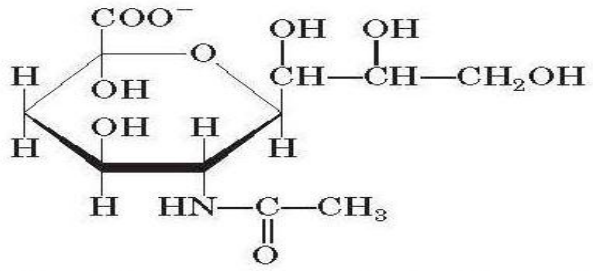


Sphingosine **Ceramide**

The ***cerebrosides*** are simple class of sphingo-glycolipids, in which the polar head group is a monosaccharide bound in β glycosidic linkage to the hydroxyl group of ceramide. A common fatty acid component of cerebrosides is cereboic acid. When the cerebrosides present in brain and nervous system contains D-galactose itis called as galactocerebrosides and when present in small amount in nonneural tissue of animal containing D glucose it is called as glucocerebrosides. The sphingo-glycolipids with disaccharides as their polar head groups are called dihexosides. Similarly trihexosides and tetrahexosides also exists. On the

basis of the identity of sugar attached to the ceramide unit, the sequence of sugar and the length of the oligosaccharide chains, the sphingo-glycolipids are of different types.

Gangliosides are most complex sphingolipids, in which the polar head group is a oligosaccharide bound in one or more residue of a sialic acid which give polar head group of the gangliosides a net negative charge at pH 7. It is most abundantly found in the grey matter of brain, where they constitute 6 percent of total lipids. Most known gangliosides have glucose residue in glycosidic linkage with ceramide, and the residue of D galactose are also present.



N-Acetylneuraminic acid (a sialic acid)
(Neu5Ac)

5.10.1. Function of sphingo-glycolipids:

- It plays important role in transmission of nerve impulses across synopses.
- These complex lipids are involved in tissue immunity and in cell recognition sites which is fundamental to the development and structure of tissue.
- Gangliosides Gm_{12} accumulate in brain in Tay-Sachs diseases, due to lack of the enzymes required for its degradation.

5.11. Simple lipids

The lipid which is not containing fatty acid in their structure is called simple lipids. They occur in smaller amount in cells and tissue as compared to other complex lipids that contains fatty acid as building block. The simple lipids are associated in different biological activities as vitamin, hormones, and other specialized fat soluble biomolecules. The terpenes and the steroids are considered to be the simple lipids; they have closely related structure and can be derived from five carbon building block. Simple lipids in large number are identified in plants and animals, in which many have characteristic order and flavor.

5.11.1.Terpenes:

Terpenes and terpenoids are the most important constituents in essential oils. Terpenes molecules consist of isoprene which is five carbon hydrocarbons. Terpenes are repeating isoprene units classified according to the number of isoprene units they contain. When the two isoprene units are associated, it is called monoterpenes and those containing three isoprene unites are called sesquiterpenes. Similary those containing four, six and eight isoprence units are called as diterpenes, teraterpenes, and teraterpenes, respectively. The terpenes may exist either in linear or cyclic arrangement. In terpenes, the successive isoprene unites attached by head to tail arrangement, particularly in linear arrangement. But sometime, the isoprene unite are in tail to tail arrangement. The terpenes are found in many plants as an essential component of oil derived from such plants. The different types of monoterpenes such as menthol, pinene, camphor, a carvone are major component of oil of germanium. Farnesol and phytol oil are the example of sesquiterpenes and diterpenes, respectively. The triterpenes is also a good precursor for the biosynthesis of cholesterol. The natural rubber and gutta-percha are polyterpenes; they consist of long hydrocarbon chains which contains hundreds of isoprene unite in regular line order. The fat insoluble vitamin such as A, E and K are considered as simple class of terpenes, that is required in small amount in the diet of mammals. The polyprenols, another class of terpenes, is long chain linear polyisoprenoid compounds with a terminal primary alcohol. The most important is undecaprenyl alcohol also called bactoprenol, which contains 11 isomers uints. The polyprenols in the form of their phosphate esters, undecaprenyl phosphate and dolichyl phosphate, respectively have coenzymes like function in the reaction of enzymatic transfer of sugar groups form the cytoplasm.

Table5.4: Different types of terpenes and their number of isoprene unite.

Class	Isoprene units	C atoms
Monoterpene	2	10
Sesquiterpene	3	15
Diterpene	4	20
Sesterpene	5	25
Triterpene	6	30

Tetraterpene	8	40

5.11.2.Steroids:

Steroids are class of lipids molecules consisting of four fused rings sterol derivatives. It is important in biology, chemistry and medicine. The steroid group includes all the sex hormones, adrenal cortical hormones, bile acids, and sterols of vertebrates, as well as the molting hormones of insects and many other physiologically active substances of animals and plants. The steroids are structurally differ from other lipids but steroids are included in lipids categories because its molecules have hydrophobic and insoluble in water. In all steroids molecules the four carbon rings is arranged in a specific molecular configuration which gives several forms .

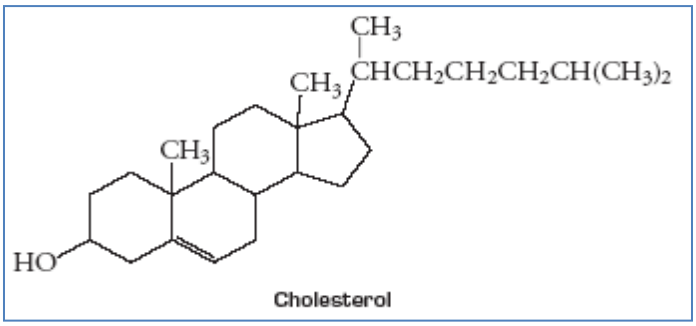
- **Ring structure of steroid**

The basic steroid unit is identical to sterols except that the hydroxyl group attached to the C3 carbon is replaced with a different chemical group because this group is variable in steroids. The different chemical group such as–OH functional group are attached at a particular site. The steroid core structure is composed of seventeen carbon atom, that atom is bonded with four fused three rings. In this three rings three six-member cyclohexane rings and one five-member cyclopentane ring are present. Steroids can be more radically modified, such as by changes to the ring structure, for example, cutting one of the rings. The steroids are classified into three types like cholesterol, sterols and bile salts based on the presence of different function group in their structure.

5.11.3Cholesterol:

Cholesterol is a type of steroid found mostly in humans and other animals. Cholesterol is synthesized in liver and it is the precursor of many steroids hormones. In the cholesterol, non polar rings and 8 member hydrocarbons exist as R group reveals its hydrophobic nature. Whereas the other short chain which consists of OH group show hydrophilic nature of molecules. Each hydrocarbon ring consists of six carbons in cholesterol molecules. Over all, the cholesterol is hydrophobic and insoluble in water. It is also the starting material for the synthesis of other compounds likes vitamin D, steroid

hormones (cortisol, cortisone, and aldosterone in the adrenal glands and of the sex hormones progesterone, estrogen, and testosterone) and bile acid. Cholesterol are also important component of cell membrane. Cholesterol travels in the blood stream in the form of lipoproteins.



Cholesterol

5.11.4. Steroid Hormones:

Steroid hormones are the steroids and acts as hormones. It regulates the wide range of physiological function of organs and tissues. Steroid hormones are secreted from three steroid glands and are transported through the blood stream to the cell of various targeted organs. All steroid hormones derived from cholesterol contains the characteristic five rings of carbon atoms of the parent molecule. They are transported through the bloodstream to the cells of various target organs where they carry out the regulation of a wide range of physiological functions. The steroids hormones can be grouped into two classes according to the organ that synthesize them namely adrenal steroids and sex steroids. Steroid hormones helps to control metabolism, inflammation, immune functions, salt and water balance, development of sexual characteristics, and the ability to withstand illness and injury. The adrenal cortex produces the adrenocortical hormones, which consists of the glucocorticoids and the mineral corticoids.

Table5.5: Different steroids hormones.

hormone class	target organs
Glucocorticoids	liver, retina, kidney, oviduct, pituitary
Estrogens	oviduct, liver
Progesterone	oviduct, uterus
Androgens	prostate, kidney, oviduct

5.11.5. Bile Salts:

Bile salts, produced from cholesterol, are glycocholate, taurocholate, and other bile salts are released from the gallbladder into the small intestine, where they aid indigestion by forming emulsions with dietary lipids (Fig.5.5).

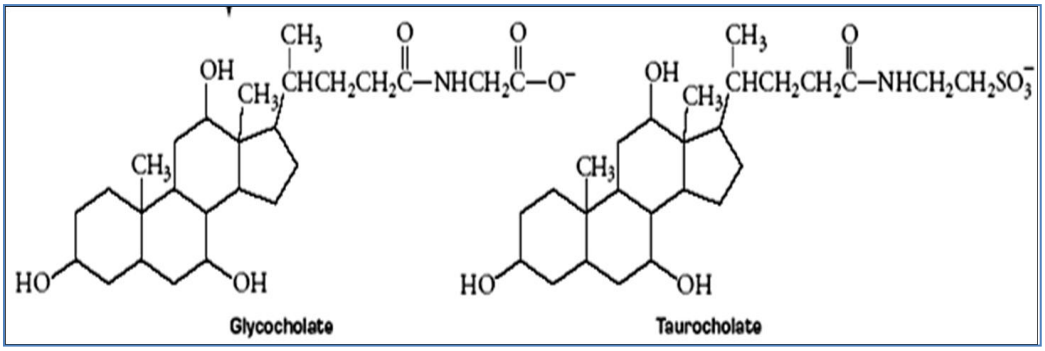


Fig.5.5: Structure of Bile salts

5.12. Lipid bilayer structure

The lipids are universal component of all cell membranes. The cell membranes not only consist of lipids but also proteins and carbohydrate in variable amount. The lipids structure in cell membrane provides the barrier that marks the boundaries of cell. The structure is called a lipid bilayer because it is composed of two layers of fat cells organized in to two sheets.

We knew that the lipids are water soluble because lipids have hydrophilic (polar head) and hydrophobic (polar tail) end in their structure. When the lipid contains both ends they are called amphipathic molecules. Glycerophospholipids, sphingolipids and sterol are having amphipathic nature. In the bilayer, the hydrophilic end or group is attached to aqueous water conditions while the hydrophobic region is repelled from such conditions. The hydrophobic tails of glycerophospholipids, sphingolipids, being water fearing prefer being embedded in lipid-rich environment away from water. The glycerophospholipids and sphingolipids could not form micelle just like soap molecules because the glycerophospholipids and sphingolipids has two tails. In these lipids, the two tails are existed at hydrophobic end of molecules that cannot form spherical micelles. These tails does not provide space to fit other molecules inside the micelles. Instead,

glycerophospholipids and sphingolipids protect their hydrophobic regions from water by aggregating into a bilayer (fig.5.6). In the bilayer, the hydrophobic tails get embedded inside the bilayer that is away from the surrounding water while the hydrophilic head groups are positioned on the upper and lower surface. Thus the biological membrane is made up of glycerolphospholipids, sphingolipids and other amphiphilic lipids such as sterols.

The lipid consists hydrophobic group keep away from the aqueous environment exists within the cell and between the cells. When the hydrophobic comes together, they have strong ability to hold two sheets of the bilayer close to one another, and thus they form stable, elastic and flexible structure of cell.

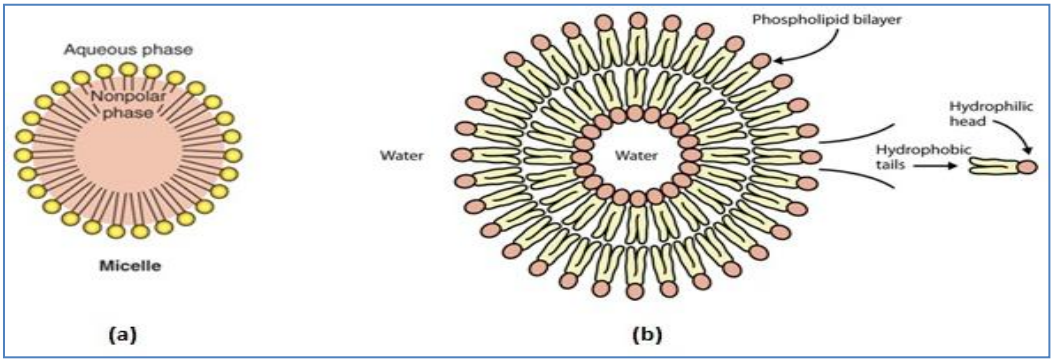


Fig.5.6: (a) micelle structure of lipid (b) aggregated structure of glycerophospholipids and sphingolipids

Source: V.W Radwell, D.A. Bender, K.M. Botham, P.J. Kenelly: Harpress illustrated biochemistry, 13th edition, www. accessmedicine.com.

The cell contain different class of lipids amongst which phospholipid is present in an abundant amount. In this phospholipids molecule the polar head group contains a phosphate group and polar tail contains two nonpolar fatty acid chain groups in their structure. The bilayer, that contain phospholipids organize them and form the layer that has inner and outer side of cell. In this layer, the phospholipids organize themselves in bilayer in such as way that the hydrophobic tails is hidden and hydrophilic groups is exposed to water. This organization is spontaneous, meaning it is a natural process and does not require energy. Thus, the lipids bilayer form highly impermeable structure that

create barrier for molecules to freely pass across it. While due to presence of hydrophilic group into outer side only water and gases can easily pass through the bilayer.

Table 5.6: Composition of lipids in bilayer.

Membrane	Lipids					protein	carbohydrate
	Total	GPP	Sphingolipids	Sterols	others		
Erythrocyte plasma membrane	43%	19%	8(%)	10(%)	6(%)	49(%)	8(%)
Liver cell plasma membrane	36%	23%	7(%)	6(%)	0(%)	54(%)	10(%)
Endoplasmic reticulum	28%	17(%)	1(%)	1(%)	9(%)	62(%)	10(%)
Outer mitochondria membrane	45%	41(%)	0(%)	0(%)	3(%)	55(%)	0(%)
Inner mitochondrial membrane	22%	20(%)	0(%)	0(%)	2(%)	78(%)	0(%)

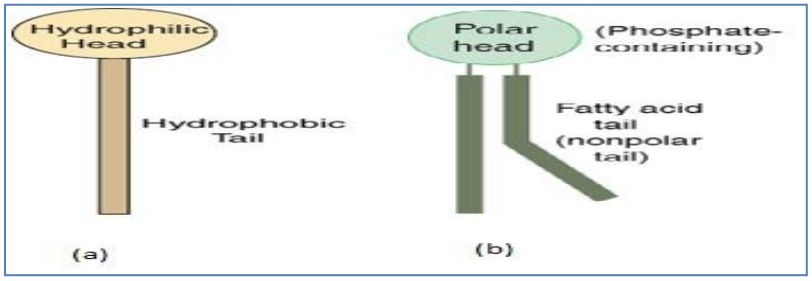


Fig.5.7 :(a) Basic Lipid and, (b) phospholipid Structure

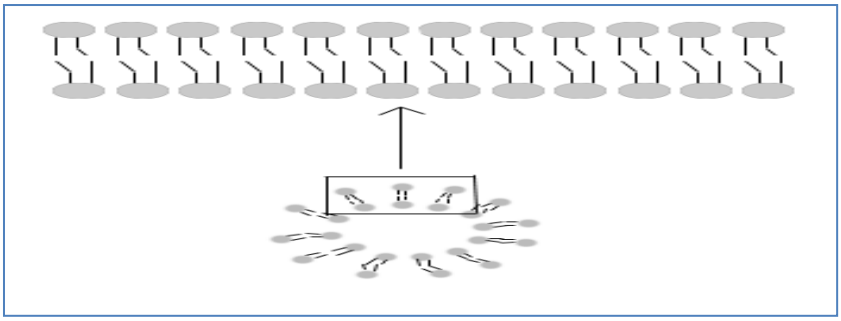


Fig. 5.8: Structure of Lipid Bilayer.

5.13. Biological membrane: components and structure

The biological membrane which is present in eukaryotic and prokaryotic is also known as plasma membrane. It works as a barrier between the inner and outer surface of a cell. In the case of animal cell, this membrane is present in the outer most layer of the cell and in the case of plant cells it is present just below the cell wall. As per earlier discussion we knew that membrane consists of various components in their structure that exists in various amounts.

Mostly membrane contains lipid, protein and rarely carbohydrate. The various lipids found in membranes exist in various amounts and have polar nature. The phosphoglycerides is predominantly present with much smaller amounts of sphingolipids. Most phospholipids contain two fatty acids, glycerol, a phosphate group, and a simple organic molecule such as choline. Apart from that nearly all the polar lipids of cells are localized in their membranes such as cholesterol or triglycerols relatively found in small amount in endoplasmic reticulum and in other cell organelle whereas, cholesterol is present in more amount in cells of higher animals. In the membrane system, the different kind of lipids determines the characteristic feature of membrane that has variability in different organ and the species.

The cell membrane contains more protein by mass, but the molar mass of a protein is about 100 times that of a lipid. Each types of membrane contain several proteins. Membrane proteins are either extrinsic or intrinsic in nature. Extrinsic membrane proteins are entirely outside of the membrane, but are bound by weak molecular attractions (ionic, hydrogen, and/or Van der Waals bonds). Intrinsic membrane proteinsare embedded in the membrane. Many of them extend from one side of the membrane to the other and are referred to as transmembrane proteins.

The extrinsic or peripheral, proteins are only loosely attached to the membrane surface and can be easily removed in soluble form by mild extraction procedure. The intrinsic or integral, proteins are tightly bound to the lipids portion and can be removed only by drastic treatment.

The unit-membrane hypothesis was given by J.D. Robertson, according to this hypothesis, the membrane consist of bilayer of mixed polar lipids, with their hydrocarbon chain. These hydrocarbon chains are oriented in inward and outward phase; the outward phase makes polar head due to presence of hydrophilic groups. The total thickness of unit membrane was suggested to be about 8.0 to 9.0 nm and the thickness of lipids bilayer is about 6.0 to 7.0 nm. However, this model could not explain many properties of membranes satisfactorily and was not accepted widely.

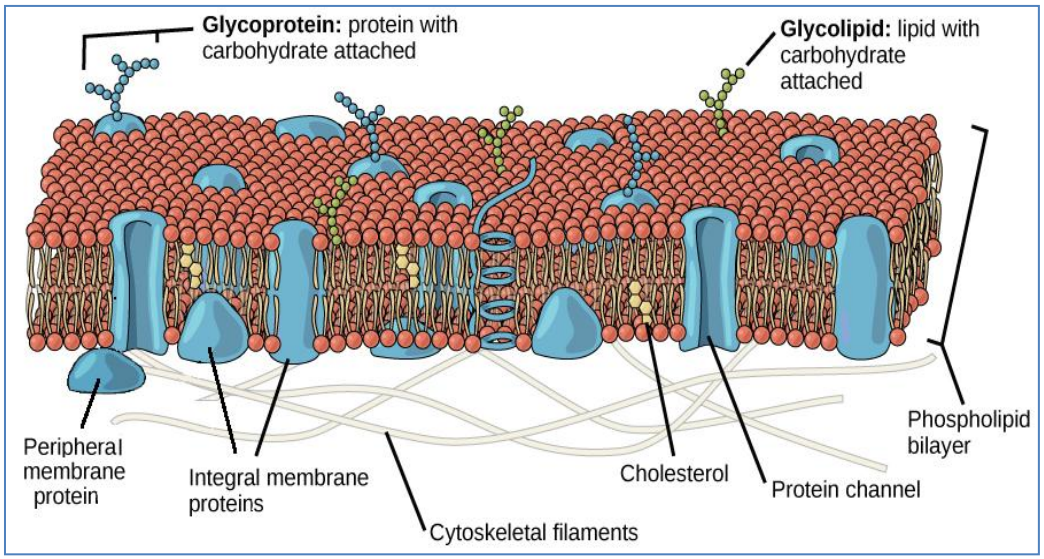


Fig.5. 9: Structure of plasma membrane.

Source:https://www.khanacademy.org/science/high-school-biology/hs-cells/hs-the-cell-membrane/a/structure-of-the-plasma-membrane

Another model for cell membrane commonly referred as "Fluid mosaic model" was proposed by S.J. Singer and G.L. Nicolson in 1972. According to this model, the phospholipid which is present in membrane are arranged in bilayer manner in which the nonpolar regions of lipid molecules in each layer face the core of the bilayer and their polar head groups face toward, interacting with the aqueous phase on either side. In this bilayer individual lipid molecules can move latterly, endowing the bilayer with fluidity, flexibility and high electrical resistance and relative impermeability to highly polar molecules. The fluid mosaic model appears with membrane that have mosaic components-primarily, phospholipids, cholesterol, and proteins-that move freely and fluidly in the plane of the membrane Fig.5.9. The membrane protein is generally globular protein molecule and is

embedded in the membrane. The mosaic model accounts satisfactory for many feature and property of biological membrane.

Table 5.7: The components of the plasma membrane.

Components	Locomotion
Phospholipids	Main fabric of the membrane
Cholesterol	Tucked between the hydrophobic tails of the membrane phospholipids
Integral proteins	Embedded in the phospholipid bilayer; may or may not extend through both layers
Peripheral proteins	On the inner or outer surface of the phospholipid bilayer, but not embedded in its hydrophobic core
Carbohydrates	Attached to proteins or lipids on the extracellular side of the membrane (forming glycoproteins and glycolipids)

Souces:https://www.khanacademy.org/science/high-school-biology/hs-cells/hs-the-cell-membrane/a/structure-of-the-plasma-membrane

5.13.1. Functions of membrane:

- It separates the contents of the cell from its outside environment and it regulates what enters and exits from the cell.
- Plasma membrane plays a vital role in protecting the integrity of the interior of the cell by allowing only selected substances into the cell and keeping other substances out.
- It also serves as a base of attachment for the cytoskeleton in some organisms and the cell wall in others. Thus the cell membrane supports the cell and helps in maintaining the shape of the cell.
- The cell membrane is primarily composed of proteins and lipids. While lipids help to give membranes their flexibility and proteins monitor and maintain the cell's chemical climate and assist in the transfer of molecules across the membrane.
- The lipid bilayer is semi-permeable, which allows only selected molecules to diffuse across the membrane.

5.14. Summary

In this unit you have learned that-

Lipids are essential components of all living organisms and it is present naturally in plants or animals. Lipids are chemically diverse group of compound which is insoluble in water and soluble in organic solvents. On the basis of backbone structure, the lipids are classified into complex lipids and simple lipids. The complex lipids are those lipids which contains fatty acids as components. A saponifiable lipid is types of complex lipids because it is obtained from soaps on alkaline hydrolysis. The simple or nonsaponifiable lipids include the terpenes and steroids. Terpenes are liner or cyclic compounds build of two or more isoprene. Steroids are other class of lipids molecules consisting of four fused rings and are sterol derivatives. The steroid group includes all the sex hormones, adrenal cortical hormones, bile acids, and sterols of vertebrates, as well as the molting hormones of insects and many other physiologically active substances of animals and plants. Most phospholipids contain two fatty acids, glycerol, a phosphate group, and a simple organic molecule such as choline. The biological membrane which is present in eukaryotic and prokaryotic is also known as plasma membrane. The fluid mosaic model accounts satisfactory for many feature and properties of biological membrane.

5.15. Terminal Questions

Q.1: What is the fatty acids, briefly describe the essential and non essential fatty acids?

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

Q.2: Write the biological function and properties of fatty acids?

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

Q.3: Write the characteristics of lipids and also discuss the classification of lipids.

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

Q.4: Describe the structure and function of Sphingo-glycolipids.

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

Q.5: What is the simple lipid? Give some examples.

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

Q.6: What do you understand about lipid bilayer structure?

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

5.16.Further readings:

1. Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Biochemistry: T.A. Brown, Viva book publication.
2. General biochemistry: J.H. Weil.
3. Wikipedia and internet sources.
4. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

Unit 7: Nucleic acids

Structure

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7.1. Introduction

This unit covers the structure and properties of nucleotides, nucleosides and nucleic acids. The nucleotides not only serve as building block of nucleic acid but also have

important function in intermediary metabolism. Nucleic acids are polymers consisting of nucleotides. The nucleic acids such as DNA and RNA are the major component of cell which together makes up to 5 to 15% of their dry weight. Nucleotides are the building block of nucleic acids which is formed by the association of three components namely nitrogenous base, pentose sugars and phosphate groups. The molecule without the phosphate group is called a nucleoside. Presence of sugar and phosphate in nucleotides makes the backbone of double helix DNA and nitrogenous bases are present in the centre. The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine. Purine may itself be regarded as derivatives of pyrimidine because it consists of pyrimidine ring and an imidazole ring fused together. The five nitrogenous bases present in nucleotides are adenine (A), uracil (U), guanine (G), thymine (T) and cytosine(C). DNA are present in chromosome which is considered to be carrier of biological information. Genes are defined as segment of DNA coding for biological informational molecules like RNA or proteins. Nucleic acids are means of storing and transferring genetic materials in living organisms.

Objectives:

- To understand the nucleotides and nucleosides
- To study of component of nucleotides
- To study of structure of DNA
- To study of structure and kinds of RNA
- To understand the denaturation of DNA

7.2. Overview on nucleic acids:

A substance isolated from nucleus of pus cells identified as an important characterisitic of the nucleus was called as nucleons by Fried rich Miescher in 1868. Later on it was observed that nuclein consists of a basic protein and a phosphorous-containing organic acid, hence it was referred as nucleic acid. The term 'nucleic acid' was coined by Richard Altmann in 1889. Nucleic acids are the main information carrying molecule of the cell and are composed of nucleotides, which are monomers made of three basic components: a 5-carbon sugar, a phosphate group and nitrogenous bases. Nucleic acids are

the large biopolymers or small bimolecules, essential to all known forms of life and exists either in free state or in combination with proteins to from nucleoproteins. Nucleic acids are polymers consisting of monomer unit called nucleotides and hence referred as polynucleotides. Nucleic acid has fundamental importance because they are either carrier of genetic information or serve as agents enabling the expression of information. A monomers unit of nucleotides consists of three basic subunits namely nitrogenous base, sugar and phosphate. The major nucleic acid in the nucleus of cells is deoxyribonucleic acid (DNA) having pentose sugar deoxyribose and is main genetic material while another type of nucleic acid, ribonucleic acid (RNA) contains ribose and is known to play a significant role in the transmission of the genetic information from DNA into protein.

A nitrogenous base is a nitrogen containing base having basic properties. The main biological function of nitrogenous base is to bond nucleic acids together. Generally the flat shape of nitrogenous base plays important role as the building blocks of DNA and RNA. The five nitrogenous bases of nucleotides are adenine (A), uracil (U), guanine (G), thymine (T) and cytosine(C). Thymine and uracil are distinguished by merely the presence or absence of a methyl group on the fifth carbon (C5) of these heterocyclic six-membered rings. Adenine is always paired with thymine, and guanine is always paired with cytosine. These are known as base pairs. Nitrogenous bases are of two types i.e. purine and pyramidine.

Purines consist of two ring i.e. one pyrimidine ring (2N+4C) and one imidazole ring (2N+3C). Adenine and Guanine are the example of purine base. Whereas the Pyrimidines consist of one pyrimidines ring skeleton composed of two nitrogen and four carbons. Cytosine, thymine and Uracil are the examples of pyramidine base. DNA and RNA are important component of nucleotide. DNA is the de-oxyribose nucleic acid and RNA is ribose nucleic acid.

Pentose sugar is the most important subunit of nucleic acid. The nitrogen base forms bond with first carbon of pentose sugar to form a nucleotide. Nitrogen of third place (N3) form bond with sugar in case of pyramidines while in purines nitrogen of ninth (N9) form bond with sugar. Phosphate forms ester bond (covalent bond) with fifth carbon of sugar to form a complete nucleotide, pentose, ribose, de-oxyribose and phosphate.

Nucleotides are organic molecules that act as the building blocks of the genetic materials. Nucleotides are the constituents of nucleic acids i.e. deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleotide play significant role in cellular metabolism such as energy currency in metabolic transactions, cellular stimuli, cell signaling, and also participate as structural components of an array of enzyme cofactors and metabolic intermediates.

Monomers units of nucleotides consist of three subunits namely nitrogenous base, sugar and phosphate.

7.3. Nucleotides

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

Nitrogenous base (Nitrogen containing base)

Pentose Sugars

phosphate group

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

7.3.1. Nitrogenous base

A nitrogenous base is a heterocyclic organic molecules in which the ring contain both nitrogen and carbon atoms. It has both acidic and basic properties due to presence of lone pair of electron on the nitrogen atom. The main biological function of nitrogenous base is to bond nucleic acids together. The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine.

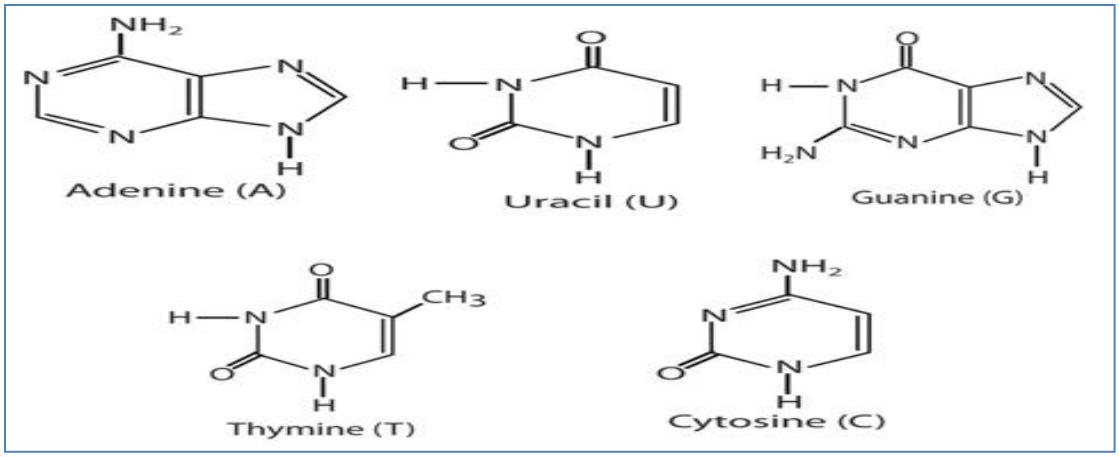


Fig.7.1: Structure of nitrogenous bases.

Both purine and pyramidine are non-polar. Purine may itself be regarded as derivatives of pyrimidine because it consists of pyrimidine ring and an imidazole ring fused together, purine comprises of adenine (A) and guanine (G) while pyrimidines includes . thymine (T), cytosine (C) and uracil (U). Thus these five nitrogenous bases are present in nucleotides.

Thymine and uracil are distinguished by merely the presence or absence of a methyl group on the fifth carbon (C5) of heterocyclic six-membered ring. Adenine is always paired with thymine, and guanine is always paired with cytosine.

The nitrogenous bases occur in cells in trace amount in free form as product of the enzymatic hydrolysis of nucleic acid and nucleotides. These free nitrogenous bases are relatively insoluble in water. The nitrogenous base that may exist in two or more tautomeric forms depends on the pH. The structure of five major bases is shown in Fig. 7.1 and types of nucleoside and nucleotide are representing in Table 7.1.

Table 7.1: Nucleosides, nucleotides and nucleic acids

Base	Nucleosides	Nucleotides	Nucleic acids
Adenine	Adenine + Ribose = Adenosine	Adenosine + Phosphate = Adenylic acid (AMP)	RNA
	Adenine + Deoxyribose = Deoxy adensine	Deoxy adensine + Phosphate = Deoxy adenylic acid (dAMP)	DNA
Guanine	Guanine + Ribose = Guanosine	Guanosine + Phosphate = Guanylic acid (GMP)	RNA
	Guanine + Deoxyribose	Guanosine + Phosphate	DNA

	= Guanosine	= Deoxy guanylic acid (GMP)	
Cytosine	Cytosine + Ribose = Cytidine	Cytidine + Phosphate = Cytidylic acid (CMP)	RNA
	Cytosine + Deoxyribose = Deoxycytidine	Deoxycytidine + Phosphate = Deoxycytidylic acid	DNA
Uracil	Uracil + Ribose = Uridine	Uridine + Phosphate = Uridylic acid (UMP)	RNA
Thymine	Thymine+ Deoxyribose = Deoxy thymidine	Deoxy thymidine + Phosphate = Deoxythymidylic acid (dTMP)	DNA

Apart from common nitrogenous bases (adenine, guanine, thymine, cytosine and uracil) large number of other purine and pyramindine derivatives are also observed These nitrogenous bases called as rare or minor bases occurs in small amounts in some nucleic acids. The minor bases are mostly methyl derivatives but contain acetyl, isopentenyl or hydroxymethyl group. Some of the minor bases are:5,6-Dihydrouracil

1-Methyluracil

3-Methyluracil

5-Hydroxymethyluracil

2-Thiouracil

N^4-Acetylcytosine

3- Methylcytosine

5- Methylcytosine

5- Hydroxymethylcytossine

1-Methyladenine

2-Methyladenine

7- Methyladenine

N^6-Methyladenine

N^6,N^6-Dimethyladenine

N^6-(Δ^2Isopentenyl) adenine

1-Methyguanine

N^2-Methyguanine

N^2, N^2-Dimethyguanine

7.3.2. Pentose Sugars

Pentose sugar is the most important component of nucleic acid. The nitrogen base makes bond with first carbon of pentose sugar to form a nucleotide. Nitrogen of third place (N3) makes bond with sugar in case of pyramidines. While in purines nitrogen of ninth (N9) makes bond with sugar. In nucleotides two types of pentose sugar such as ribose ($C_5H_{10}O_5$) and deoxyribose ($C_5H_{10}O_4$) occurs. The difference in RNA and DNA is due to presence of type of pentose sugar. RNA has ribose sugar while DNA has deoxyribose sugar. The ribose differs from deoxyribose in having a -OH group instead of -H at C-2 position. Ribose containing nucleotides are called ribonucleotides found only in RNA, while deoxyribose containing nucleotides are called deoxyribonucleotides .

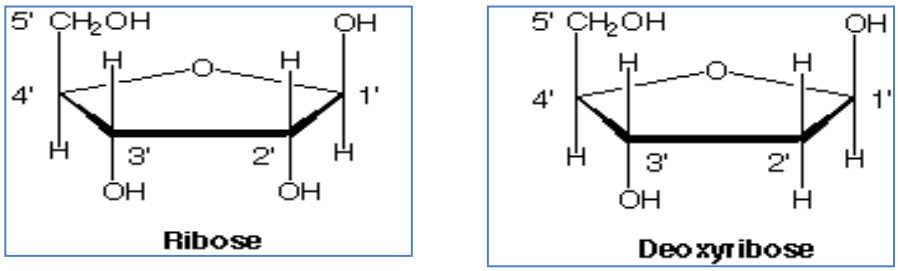


Fig.7.2: Pentose sugar: Ribose and deoxyribose

7.3.3. Phosphate group

A phosphate group is made by covalently attachment of one atom of phosphorous with four oxygen element. In phosphate group, two hydroxyl groups are present which is relatively reactive molecule that readily form phophoester bonds with other elements. Phosphate groups can be joined together to form phosphodiester bonds. Phosphate forms ester bond (covalent bond) with fifth carbon of sugar to form a complete nucleotide. Up to three individual phosphates can be attached in series. These phosphate groups are designated α, β and γ with the α-phosphate being the one attached directly to sugar. When

phosphate is added to a nucleoside, the molecule is called a nucleotide which is shown in Fig.7.3.

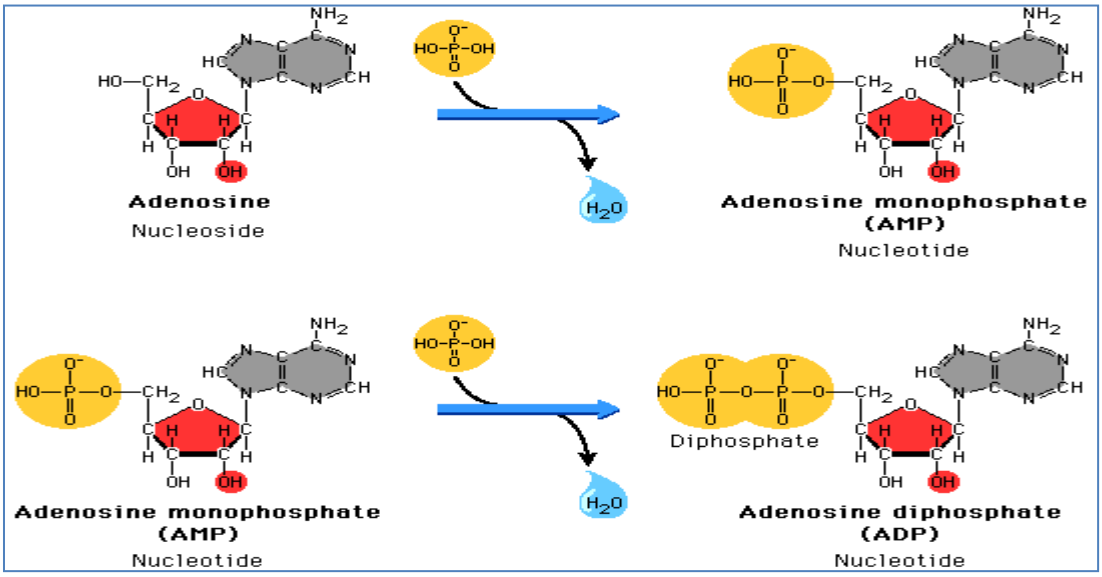


Fig.7.3 : Structure of phosphate group

Source:http://www.phschool.com/science/biology_place/biocoach/bioprop/ribose.html

The flow of genetic information from DNA-RNA-Protein is commonly referred as "Central Dogma of Life" comprising of transcription and translation process. Transcription is the first step in gene expression involving copying a gene's DNA sequence to make an information molecule i.e. RNA while translation is the process of converting the sequence of RNA (mRNA) molecule to a sequence of amino acids coding for a particular protein.

7.4. Nucleosides

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

The nucleosides are generally named for the particular purine or pyrimidine present. Nucleosides containing ribose are called Ribonucleoside while those having deoxyribose as Deoxyribonucleoside (Table 7.1)

7.5. Nucleic Acids

Nucleic acid is a key molecule for continuity of life. Nucleic acids are formed by association of nucleotides. Nucleic acids are the main information-carrying molecules in the cell and also the building blocks of living organisms. Deoxyribose nucleic acid (DNA) and Ribose nucleic acid (RNA) are two important nucleic acid present in the living organisms. RNA can be of different types namely *messenger* mRNA) and *transfer* (tRNA) and ribosomal (rRNA). DNA consists of covalently linked chains of deoxyribonucleotides while RNA consists of ribonucleotides. DNA is considered to be the master blue print of life and constitutes the main genetic material in all free living organisms and most viruses. RNA is also present in free living organism as genetic material but found in few viruses.

DNA and RNA share a number of physical and chemical properties because successive nucleotides of both DNA and RNA are covalently linked through phosphate-group "bridges," in which the 5-phosphate group of one nucleotide unit contains some minor bases. Alternating phosphate and pentose group form backbone of DNA and RNA, in which phosphodiester bridges provides the covalent continuity.

DNA and RNA are actually made up of chain of base pair of nucleic acids stretching from as few as three to millions. When these base pair combine in sugar chains, they make specific shape that looks like twisty ladder commonly referred as double helix.

7.5.1. Deoxyribonucleic acid (DNA):

DNA was fist isolated in 1869 form pus cells and salmon sperms by Friend Rich Miescher. Due to its occurrences in nucleus it is called *nuclein*. DNA is one type of nucleic acid which has deoxyribose sugar. DNA is a genetic material naturally found in human and almost all living organisms. Mostly DNA occurs in cell's nucleus and is called as nuclear DNA whereas few amount of DNA is also found in mitochondria and chloroplast. DNA present in chromosome are known to be associated with carrier of biological information.

Genes are defined as segment of DNA that codes for an informational molecules i.e. RNA or protein. The typical structure of DNA is shown in Fig. 7.4.

DNA is a type of nucleic acid comprising of nucleotides as monomeric units, which may vary from a minimum of three to thousands. A nucleotide is made up of three components; pentose sugar, nitrogenous base, and phosphate group. DNA molecule contains 2-Deoxyribose pentose sugar. Nitrogenous base, adenine and guanine which has double ring structure, and cytosine and thymine which has single ring structure are present in nucleotide of DNA. The base is joined to the sugar by β-n-glycoside bond attached to nitrogen number 1 of pyramidine or number 9 of purine.

The third part of nucleotide is phosphate group. The molecule comprising sugar and nitrogen base is called nucleoside. When nucleoside is attached to phosphate group it is called as nucleotide. Here phosphate group is attached to 5 carbon of the sugar, thus monomer unit of nucleotide is completed. Up to three individual phosphates group can be attached in series. Nucleotides are arranged in two long strands that form a spiral structure called as a double helix. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. The nucleotide of DNA can be represented with their abbreviated name dATP, dGTP, d CTP and dTTP.

The individual nucleotide attached together by phosphate groups form the polymer called as polynucleotide.During polymerization β and γ phosphate are cleaved off. The hydroxyl group attached to the 3’ carbon of the second nucleotide is also lost resulting a linkage called as phosphodiester bond. In the polynucleotide formation, the two same end of nucleotide are not combined together. In this combination, the nucleotide in which the triphosphate group attached to 5’ carbon has not participated in formation of phosphodiester bond and β and γ phosphate are still in place. This is called 5’ end or 5’ P end terminus. At the other end of the molecule, is the 3’ hydroxyl group called 3’ end or 3’-OH terminus. These two ends in polynucleotide define the direction, which can be marked as 5’ to 3’ or 3’to 5’.

In most of cells DNA is quite large and hence cannot be easily isolated. The single chromosome which exists in prokaryotic cells contains all DNA as single double helix. In

eukaryotic cells several chromosomes are present which carries the DNA molecules.. The extrachromosomal DNA in the form of plasmid DNA is often found in bacteria along with chromosomal DNA.

An important property of DNA is that it can replicate, or make copies of itself. During cell division each new cell obtains exact copy of the DNA present in the old cell. A DNA molecules in diploid eukaryotic cell nucleus is combined with basic protein called histones.

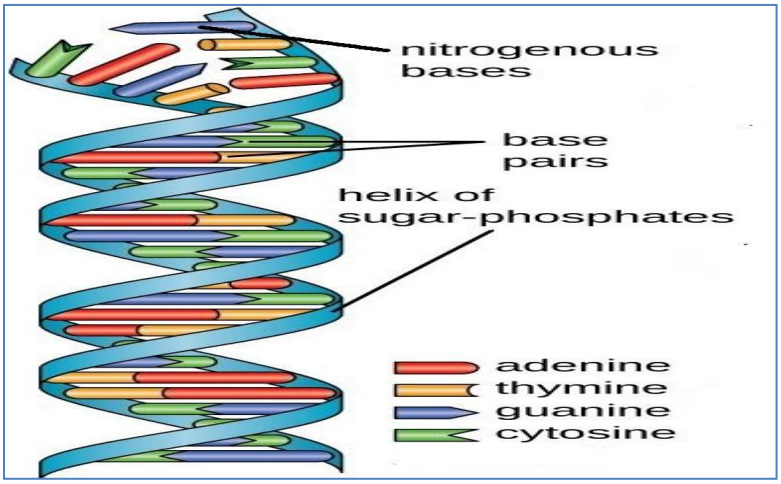


Fig.7.4: Structure of double stranded DNA

Source:*A. Rich. "The Era of RNA Awakening: Structural Biology of RNA in the Early Years." Quarterly Reviews of Biophysics 42 no. 2 (2009):117–137.*

Table 7.2: The nucleotide present in nucleic acid molecules.

Nucleotide	Base component	Abbreviations		Fund in
		3-letter	1-letter	
2'- deoxyadenosine 5'-triphasphate	Adenine	dATP	A	DNA
2'- deoxyguanosine 5'-triphasphate	Guanine	dGTP	G	DNA
2'- deoxycytidine 5'-triphasphate	Cytosine	dCTP	C	DNA
2'- deoxythymine 5'-triphasphate	Thymine	dTTP	T	DNA

Human DNA consists of about 3 billion bases, and more than 99 percent of these bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.

Table 7.3: The nucleotide present DNA and of various organism.

	Adenine	Guanine	Cytosine	Thymine	Uracil
DNA					
Human	30.9	19.9	19.8	29.4	
E.Coli	24.7	26.9	25.7	23.6	
Bacteriophase	21.3	28.6	27.2	22.9	
RNA					
Ox liver(total)$^{+}$	17.1	27.3	33.39		21.7
E Coli mRNA	25.1	27.1	24.1		23.7
Tobacco mosaic virus	29.8	25.4	18.5		26.7

7.5.2. Ribose nucleic acid (RNA)

RNA is high molecular weight single stranded nucleic acid made by nucleotides. Due to presence of ribose pentose sugar it is called as ribonucleic acid. Besides DNA and protein, RNA is one of major biological molecules that are essential for all kinds of life. RNA is present in all forms of organism and actively participates in cellular protein synthesis and also often replaces DNA as a carrier of genetic material in some viruses. RNA is an important component of central dogma of life and plays significant role in gene regulation by influencing transcription and translation process.

Like DNA, RNA also consists of nucleotides that are made up of four nitrogenous bases appended to a ribose sugar and phosphate group. The nitrogenous bases in RNA are adenine, guanine, cytosine and uracil. Each nucleotide in RNA contains a ribose sugar, with carbon number 1' and 5'. The nitrogenous bases purines and pyrimidines is attached to the 1' position, whereas the phosphate is attached at 3' position of one ribose and the 5' position of the next. The nitrogenous bases attached together with hydrogen bond. Second number of carbon of ribose sugar have hydroxyl group, this hydroxyl group causes the helix structure.

In RNA molecules, the nucleotides are covalently linked through phosphate-group and make bridge like structure in which the 5 -phosphate group of one nucleotide unit is joined to the 3-hydroxyl group of the next nucleotide resulting in aphosphodiester linkage (Fig. 7.5). Thus the covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to

the backbone at regular intervals. The back bones of RNA are hydroxyl groups of sugar residues that form hydrogen bonds with water. The phosphodiester linkage have same orientation along the chain that gives linear nucleic acid stands which have specific polarity and distinct 5' and 3' ends.

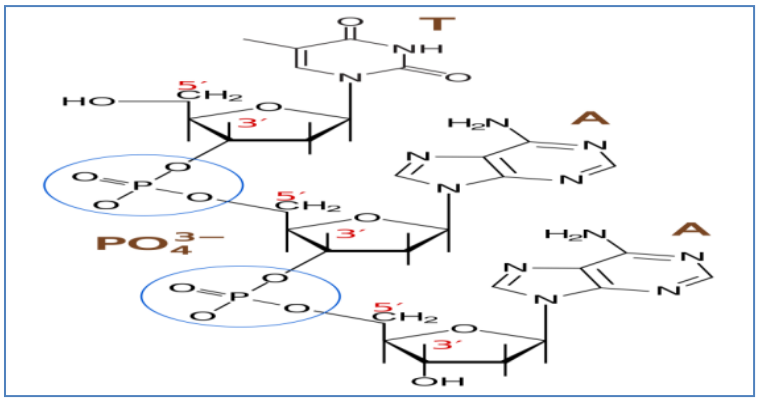


Fig.7.5: Phosphodiester linkages in the covalent backbone of RNA.

Source: From Wikipedia, the free encyclopedia

Although, single stranded RNA is not always linear. It has the ability to fold into complex three- dimensional shapes and from hairpin loops. In this three dimension shapes, adenine pairs with uracil (A-U) and guanine pairs with cytosine (G-C). Hairpin loops are commonly observed in RNA molecules such as messenger RNA (mRNA) and transfer RNA (tRNA).

In bacterial cells, most of RNA is found in the cytoplasm but during transcription process some of RNA is non-covalently attached to DNA. In eukaryotic cells, the various form of RNA has distinct intercellular distribution. In liver cell approximately 11 % of the total RNA is in nucleolus, about 15 percent in mitochondria, over 50 percent in the ribosomes, and about 25 percent in cytosol.

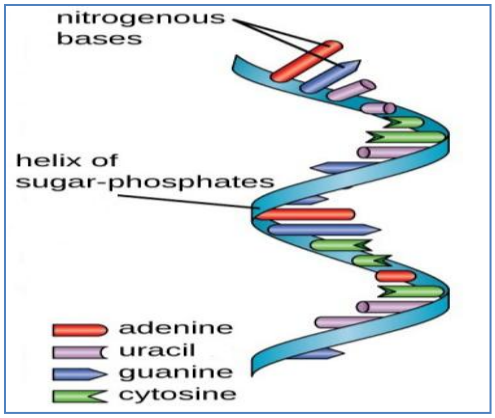


Fig. 7.6: Structure of single stranded RNA

Source: A. Rich. "The Era of RNA Awakening: Structural Biology of RNA in the Early Years." Quarterly Reviews of Biophysics 42 no. 2 (2009):117–137.

7.5.3. Types of RNA

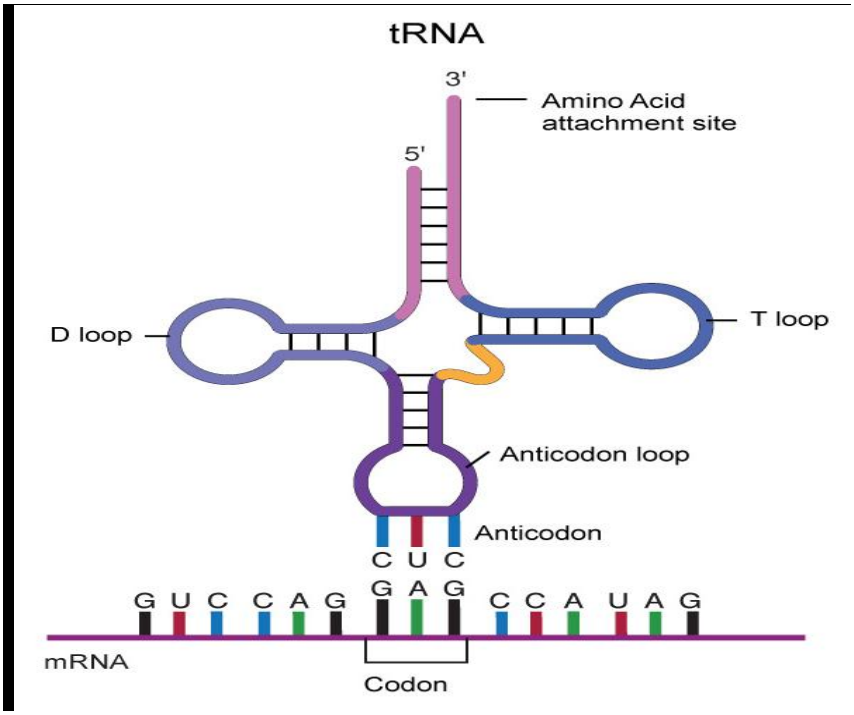
7.5.3.1. mRNA

The Messenger RNA (mRNA) is synthesized during the process of transcription. Here sequence of bases in one strand of chromosomal DNA is enzymatically transcribed into single strand of mRNA. In the process of transcription, DNA is transcribed into RNA which is further translated into protein by translation process resulting in copying the genetic information. After transcription, the mRNA passes into the cytoplasm and then onto ribosome where it serves as a template for the sequential ordering of amino acid during biosynthesis of protein. The bases pair in nucleotide (A-T and C-G) of DNA get new pair during transcription of DNA to RNA. It attends new base pair i.e. A- U and C-G means in mRNA adenine pair with uracil and cytosine pairs with guanine (A-U and C-G). At the end of transcription, mRNA is transported to the cytoplasm for the completion of protein synthesis. In eukaryotic cell, mRNAs contain long sequence of about 2000 successive adenylate residue at the 3 end which apparently plays an important roles in processing or transport of mRNA from nucleolus to ribosomes.

7.5.3.2. tRNA

Transfer RNA (tRNA) plays an important role in the translation portion 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. It contains an amino acid attachment site on one end and a special section in the middle loop called the anticodon site. The anticodon recognizes a specific area on mRNA called a codon. A codon consists of three continuous nucleotide bases that code for an amino acid or signal the end of translation. Transfer RNA along with ribosome read the mRNA codons and produces a polypeptide chain. The polypeptide chain undergoes several modifications before becoming a fully functioning protein.



7.5.3.3. *rRNA*

Ribosomal RNA (rRNA) is a component of cell organelle 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. During translation, a small ribosomal subunit attaches to a mRNA molecule. At the same time, an initiator tRNA molecule recognizes and binds to a specific codon sequence on the same mRNA molecule. A large ribosomal subunit then joins the newly formed complex. Both ribosomal subunits travel

along the mRNA molecule translating the codons on mRNA into a polypeptide chain as they go. Ribosomal RNA is responsible for creating the peptide bonds between the amino acids in the polypeptide chain. When the termination codon is reached on the mRNA molecule the translation process end. The polypeptide chain is released from the tRNA molecule and the ribosome splits back into large and small subunits.

7.6. Central Dogma:

The term Central Dogma was given by Crick. The formation of m-RNA form DNA and then synthesis of protein from mRNA is known as Central Dogma. In addition, the central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred back from protein to either protein or nucleic acid. It means it includes transcription and translation. The central dogma scheme of protein synthesis was presented by Jacob and Monad.

DNA $\xrightarrow{\text{Transcription}}$ **RNA** $\xrightarrow{\text{Translation}}$ **Protein**

We have studied that protein is made up of amino acids and every protein has unique amino acid arrangements in specific sequence. Information to synthesize proteins with unique amino acid sequence is provided by the nucleic acid present within the nucleus. In a preset sequence, DNA present in the nucleus give rise to the specific RNA sequence and that in turn guide the cellular machinery to synthesize protein Fig(7.7). Scientist considered this as the fundamental event to run the life and considered as “central dogma of life”. Thus it is considered that central dogma is the base of life where it regulate all biological process and makes the sequence of information in biological system.

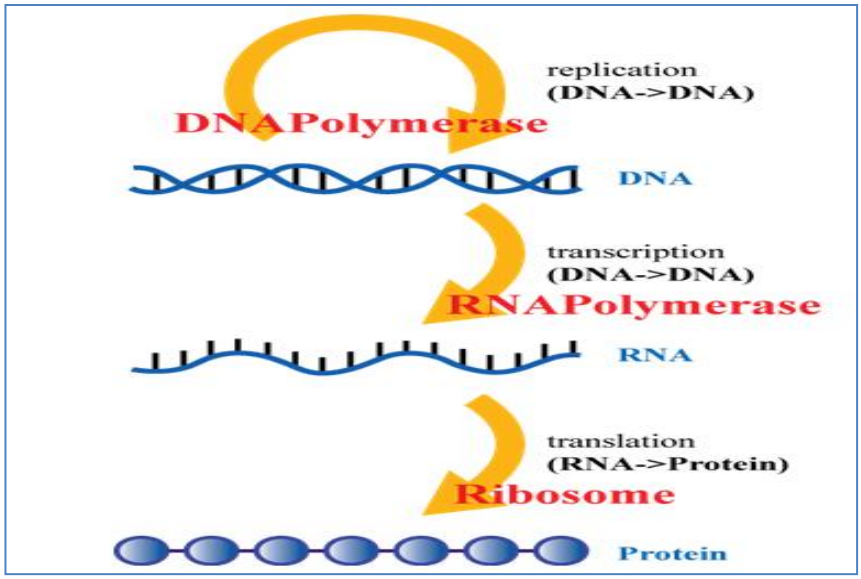


Fig. 7.7: Flow of information sequence-to sequence in biological system.

Souce:https://nptel.ac.in/courses/122103039/module4/lec25/2.html

Under normal conditions, the flow of information from sequence-to-sequence requires 4 processes. The four processes required for flow of information is as follows shown in Fig (7.8).

a) **Replication:** Sequence dependent synthesis of DNA occurs from pre-existing DNA. Here genomic content (DNA) in organism is duplicated by utilizing the sequence information of parent DNA. The enzyme used for this purpose is DNA dependent DNA polymerase.

b) **Transcription:** It is a sequence dependent synthesis of RNA from DNA. The DNA is present in nucleus whereas the protein synthesis machinery is present in cytosol. In transcription process the information present in DNA is used for synthesis of RNA which has the ability to transport outside the nucleus to participate into protein synthesis. The enzyme used for this purpose is DNA dependent RNA polymerases.

c) **Translation:** It is also sequence dependent synthesis of protein from mRNA The RNA present in cytosol is utilized by translation machinery to synthesize protein in a sequence dependent manner through a process known as translation.

d) **Reverse Transcription:** Reverse transcriptase, also called RNA-directed DNA polymerase, an enzyme encoded from the genetic material of retroviruses that catalyzes

the transcription of retrovirus RNA (ribonucleic acid) into DNA (deoxyribonucleic acid). It was discovered by Baltimore in Rous-sarcoma virus.

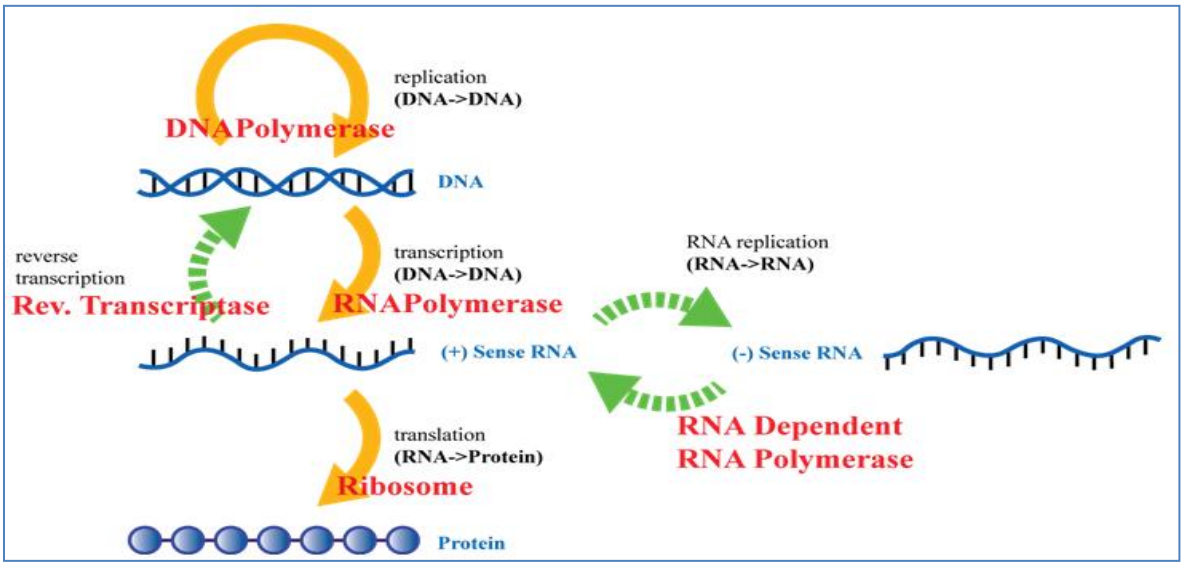


Fig.7.8 : The flow of information sequence-to-sequence for protein synthesis

Source: https://nptel.ac.in/courses/122103039/module4/lec25/2.html

7.7. Nucleic acid sequencing:

Nucleic acid sequencing is deciphering the arrangement of bases of DNA or RNA, which determines the uniqueness of an individual. A nucleic acid sequence is a succession of letters that indicate the order of nucleotides forming alleles within a DNA (using GACT) or RNA (GACU) molecule. By convention, sequences are usually presented from the 5' end to the 3' end. In addition, sequencing an entire genome (all of an organism's DNA) remains a complex task. It requires breaking the DNA of the genome into many smaller pieces, sequencing the pieces, and assembling the sequences into a single long .

DNA is a polymeric molecules made up of the monomeric constituent. The arrangement of four types of nitrogenous bases i.e. A, T, G and C present in DNA of an organism can be determined by different methods of DNA sequencing. In general, there are two common methods of DNA sequencing namely

7.7.1. Sanger's Methods:

Frederick Sanger in 1977 developed this method. It also called chain termination sequencing or enzymatic method of sequencing. According to this method a single stranded DNA is used as a template to synthesize the complementary strand with specifc arrangement of bases as shown in Fig. 7.9. The enzymatic polymerization reaction involves a primer, DNA polymerases, all four deoxyribonucleotides (dNTPs), and 2'3'-dideoxynucleotide triphosphate (ddNTPs). When DNA polymerase utilizes ddNTPs as nucleotide, it gets incorporated into the growing chain but instead of chain elongation, it stops the elongation due to absence of 3'-hydroxyl group. In a typical sequencing reactions, four separate tubes comprising of single stranded template DNA, all four dNTPs, one specific ddNTP (i.e. ddATP/ddGTP/ddTTP/ddCTP), primer and DNA polymerase is added and the reaction product is analyzed on high resolution polyacrylamide gel electrophoresis. The ratio of NTPs/ddNTPs is adjusted in such a way so that chain termination occurs at each position of the base in the template.

The DNA sequencing has following steps:

Step 1: A primer is added and annealed to the 3' of the DNA template.

Step 2: The radiolabeled ^{35}S ATP to label the primer.

Step 3: The polymerase reaction is divided into 4 reactions.

Step 4: DNA synthesis continues until terminated by the incorporation of the specific ddNTPs (either A, T, G or C).

Step 5: A change of polymerization reaction is performed in the presence of high concentration of NTPs to extend all non-terminated sequences into high molecular weight DNA. These sequences as fragments of DNA are separated by polyacrylamide sequencing gel.

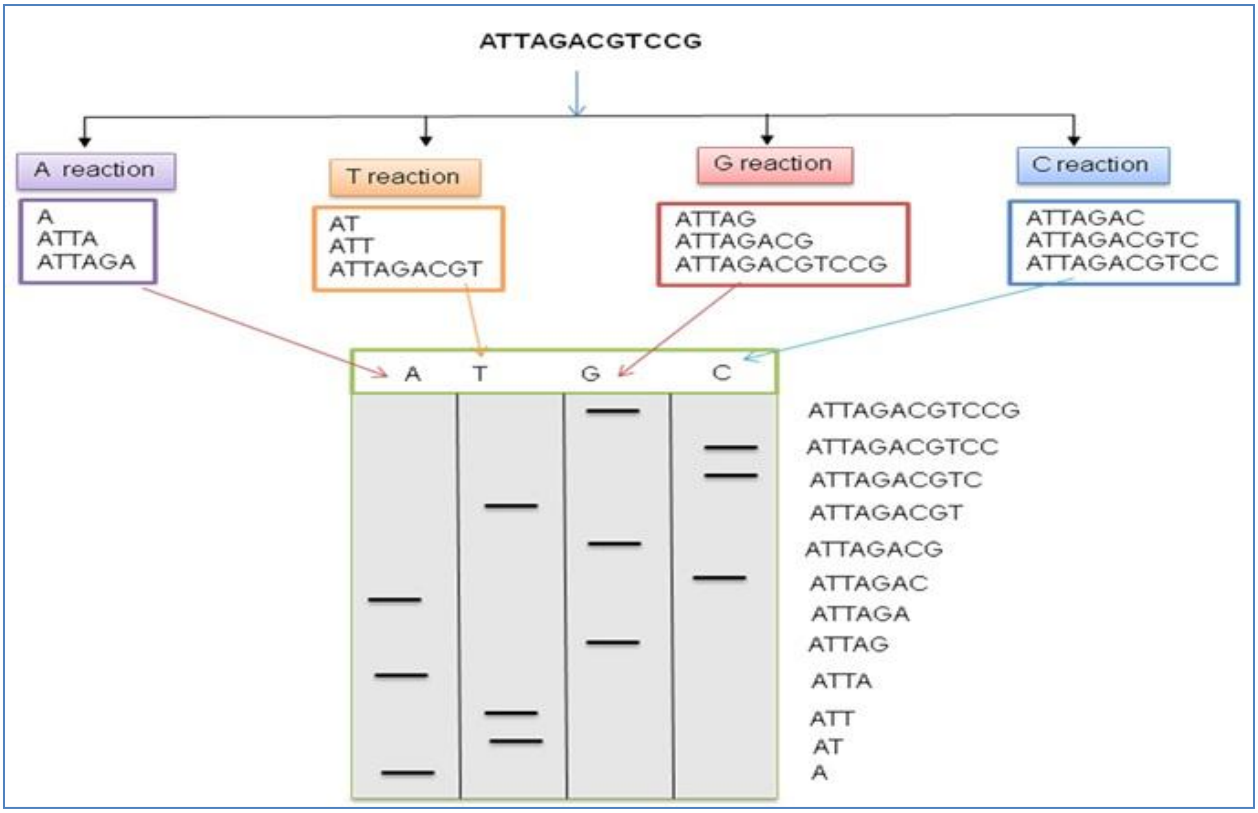


Fig.7.9: The Sanger DNA sequencing scheme.

7.7.2. Maxam-Gilbert method:

Allan Maxam and Walter Gilbert developed a different method for DNA sequencing in 1977 which is based on nucleobase-specific partial chemical modification of DNA followed by subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides. This results in generation of fragments which are subjected to its separation on polyacrylamide gel electrophoresis similar to Sanger's method. Here the template DNA is double stranded DNA and different chemicals are used for modification and cleavage. Specific chemicals that selectively attack purines and pyrimidines are used in this method. In this method, a 3' or 5' radiolabeled DNA is treated with a base specific chemicals which randomly cleaves the DNA at their specific target nucleotide. These fragments are analyzed on a high resolution polyacrylamide gel and an autoradiogram is developed (Figure 7.10). The fragment with terminal radiolabel appears as band in the gel. The chemical reactions are performed in two steps;

a. Base Specific Reaction: Different base specific reagents are used to modify the target nucleotide.

Reaction 1: Dimethylsulfate (DMS) modifies the **N7** of guanine and then opens the ring between **C8**and **N9**.

Reaction 2: Formic acid acts on purine nucleotide **(G+A)** by attacking on glycosidic bond.

Reaction 3: Hydrazine breaks the ring of pyridine.

Reaction 4: In the presence NaCl cytosine is break down.

b. Cleavage reaction: After the base specific reactions, piperidine is added which will replace the modified base and catalyzes the cleavage of phsophodiester bond next to the modified nucleotide.

Interpretation of the bands in autoradiogram: The fragment in G lane is read as "G" whereas fragment present in G+A but absent in G is read as "A". Similarly fragment in C is read as "C" whereas fragment present in T+C but absent in C is read as "T". To get the DNA sequence, the band with the lowest molecular weight is read followed by next band in the four lanes.

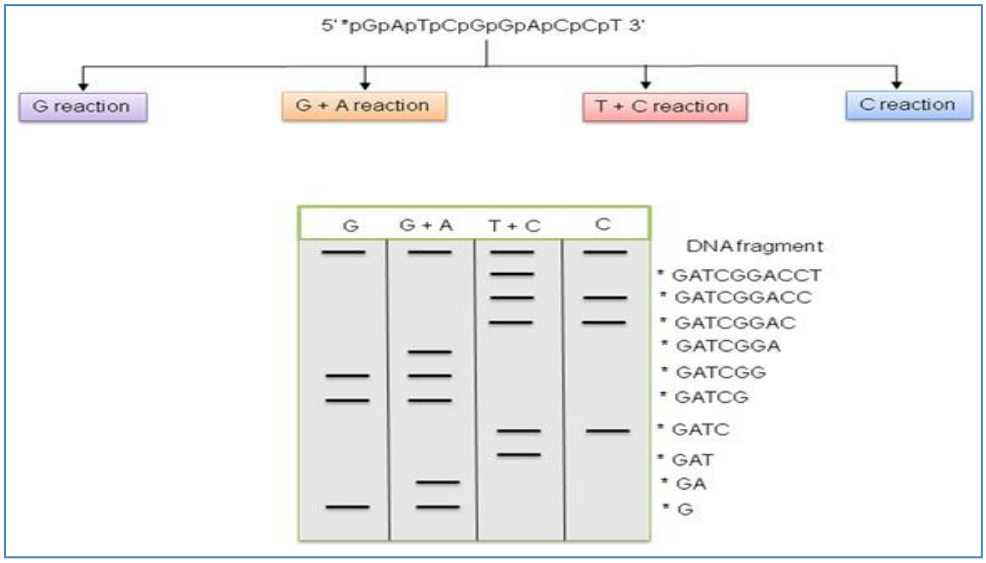


Fig. 7.10: The Maxam-Gilbert manual DNA sequencing scheme.

7.8. Denaturation of DNA

DNA denaturation refers to the melting of double-stranded DNA to generate two single strands. This involves the breaking of hydrogen bonds between the bases in the duplex. The denaturation occurs when the proteins and nucleic acid are subjected to either in elevated temperature or extreme of pH. In addition, non-physiological concentration of salt, organic solvents, urea or other agents subjected to DNA. The most common denaturation occurs due to thermal denaturation. The temperature at which DNA is half denatured is represent as Tm value, above the Tm the DNA is remained as single stand while below the Tm DNA is remained as double standed. At higher pH, the hydroxide ions (negative charge ions) can pull hydrogen ions form base pairs and forming hydrogen bonds between two stands causing them to separate. Low salt concentration could also denature DNA double stands by removing ions that stabilize the negative charge on the two stands from each other. From a thermodynamic point of view, the most important contribution to DNA helix stability is the stacking of the bases on top of one another. Thus, in order to denature DNA, the main obstacle to overcome is the stacking energies that provide cohesion between adjacent base pairs.

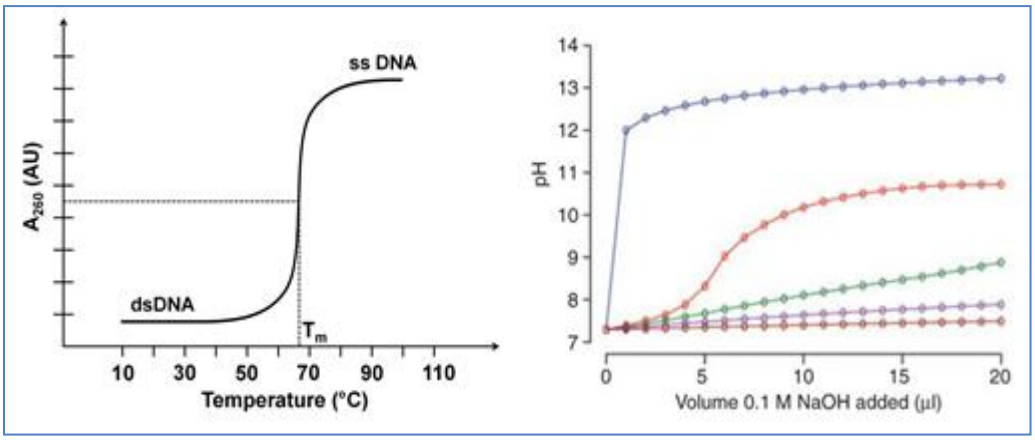


Fig.7.11: Effect of tem and pH on denaturation.

7.8.1. Characteristic of denatured DNA

- Hyper chronic- increase of absorbance (A260) upon denaturation
- The rate of increase in absorbance is directly proportional to the rate or denaturation
- Viscosity decrease upon denaturation

Effects of denaturation

Denaturation cause several diseases. DNA repair deficiency disorder is a medical condition due to reduce functionality of DNA repair mechanism. DNA repair defects can cause an accelerating aging diseases and an increased risk of cancer. Examples of DNA defects accelerated aging are bloom syndrome, Fanconi's anemia, Xeroderma pigmentosa etc.

7.9. Biological functions of nucleotides

We know the nucleotides are precursors of DNA and RNA molecules that are the fundament of central dogma. Apart from that the nucleotides has lots of biological functions. It is also useful in activation of intermediates in many biosynthesis for examples UDP-glucose, glycogen, CDP-diacylglycerol etc. It also participates in energy process of biological systems like ATP synthesis. Nucleotides may have one, two, or three phosphate groups covalently linked at the 5' hydroxyl of ribose. These are referred to as nucleoside mono-, di-, and triphosphates, respectively. Nucleoside triphosphates also serve as the activated precursors of DNA and RNA synthesis. Nucleotides are components of many enzyme cofactors. ATP is the central carrier of chemical energy in cells, probably reflecting the requirement for binding energy in catalysis. The presence of adenine in the structure of a variety of enzyme cofactors acts as coenzymes like NAD $(P)^+$, FAD, CoA and metabolic regulators. A variety of enzyme cofactors serving a wide range of chemical functions include adenosine as part of their structure. Some nucleotides are intermediates in cellular communication.

7.10. Summary

In this unit you have learned that,

Nucleic acids is formed by monomeric unit of nucleotides, the monomeric units of ribonucleic acid, and the ribonucleotides, are composed of one molecules of purine or pyrimidine base, one of D-ribose and one of phosphoric acids. The monomeric unit of Deoxyribonucleic acid contains the deoxyribonucleotides here purine or pyrimidine base are covalently lined in β-N Glycosyl linkage to carbon atom of D-ribose or 2-deoxy-D-ribose. Purines consist of two ring i.e. one pyramidine ring (2N+4C) and one imidazole ring

(2N+3C). Adenine and Guanine are the example of purine base. Whereas the Pyrimidines consist of one pyramidine ring skeleton of ring composed of two nitrogen and four carbons. Cytosine, thymine and Uracil are the example of pyramidine base. The nitrogen base forms bond with first carbon of pentose sugar to form a nucleotide. Nitrogen of third place (N3) form bond with sugar in case of pyramidines while in purines nitrogen of ninth (N9) form bond with sugar. Phosphate forms ester bond (covalent bond) with fifth carbon of sugar to form a complete nucleotide, pentose, ribose, de-oxyribose and phosphate. The denaturation of DNA occurs when nucleic acid are subjected to either in elevated temperature or extreme of pH. In this case, DNA is separated in to single strands due to breaking of hydrogen bonds.

7.11. Terminal questions

Q.1: What is nitrogenous base? How many types of nitrogenous bases are present in nucleic acids? Describe the structure of nitrogenous bases?

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

Q.2: How nitrogen base, pentose sugar and phosphate groups plays important role in the formation of nucleic acid?

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

Q.3: How do you differentiate nucleotides and nucleosides?

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

Q.4: What are nucleic acids? Write different types of RNA and their role in biological functions.

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

Q.5: What is DNA? Draw the typical structure of double strand DNA.

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

Q.6: What is the DNA denaturation? Discuss the factors that influence the denaturation of DNA.

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

Q. 7: What do you understand by central dogma of life? Discuss different methods of DNA sequencing.

7.12. Further readings

1. General biochemistry: J.H. Weil, New Age International (P) Limited, 5th edition, 2013,
2. Principles of Biochemistry: Lehninger, Nelson and Cox. Student edition, CBS 1439 Publishers and Distributors, Delhi.
3. Biochemistry: T.A. Brown, Viva book publication. First edition, 2018.
4. Elements of biochemistry: J.L. Jain, S. Chand publication, Seventh Edition**.**
5. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.