BIOCHEMISTRY

INTRODUCTION

Biochemistry, as the name implies, is the chemistry of living organisms. It has its origin in chemistry and biology. It tries to explain life processes at molecular level. There is a basic unity of biochemistry throughout nature. Although different organisms differ outwardly in their life processes, there are striking similarities in executing different tasks. Genetic code, metabolic pathways, enzymes, coenzymes and even regulatory mechanisms are similar to a large extent in all the living organisms. Living organisms have certain extraordinary properties. They can grow, respond to stimuli and replicate themselves with high fidelity. All these activities are ultimately interpretable in chemical terms. The lifeless organic molecules with appropriate complexity and properties make a living thing. The basic phenomena of biochemistry are to understand how the collections of inanimate molecules that constitute living organisms interact with each other to maintain life. The basic life processes or chemistry remains broadly the same whether it is an unicellular microorganism or the higher organisms such as human or plants. Life is nothing but thousands of ordered chemical reactions. In other words, chemistry is the logic of all biological phenomena.

Biochemistry is taught to the students of agriculture, because

1. Agriculture can be better managed with better varieties and practices.
2. Transgenic plants which are high yielding, nutritionally more desirable and self fertilizing can be synthesized ie:- designer plants and animals
3. Pesticides which are more specific, biodegradable and non toxic to animals can be formulated.

History of biochemistry

Only during 17^{th} and 18^{th} centuries, important foundations were laid in many fields of biology. The 19^{th} century observed the development of very crucial concepts. Studies in biochemistry are so rapid that it is now a fore runner and language of biology. Louis Pasteur, during 1857, did a great deal of work on fermentations and pointed out categorically the central importance of enzymes in this process. The breakthrough in enzyme research and hence, biochemistry was made in 1897 by Edward Buckner when he extracted enzyme from yeast cells in crude form which could ferment a sugar molecule into alcohol. This was a final blow to vitalism. Wohler thus initiated the synthesis of organic compound from inorganic compound. Neuberg introduced the term biochemistry in 1903.

In 1926, James Sumner established the protein nature of enzyme. He was responsible for the isolation and crystallization of urease, which provided a breakthrough in studies of the properties of specific enzymes.

In 20th century, the growth was very fast in the field of biochemistry. Full structures of many compounds were formulated for eg: ATP structure by Fiske and Subbarow. The first metabolic pathway elucidated was the glycolytic pathway during the first half of the 20th century by Embden and Meyerhof. Otto Warburg, Cori and Parnas also made very important contributions relating to glycolytic pathway. Krebs established the citric acid and urea cycles during 1930-40. In 1940, Lipmann described the central role of ATP in biological systems.

The biochemistry of nucleic acids entered into a phase of exponential growth after the establishment of the structure of DNA in 1953 by Watson and Crick followed by the discovery of DNA polymerase by Kornberg in 1956. From 1960 onwards, biochemistry plunged into an interdisciplinary phase sharing much in common with biology and molecular genetics.

Frederick Sanger's contributions in the sequencing of protein in 1953 and nucleic acid in 1977 were responsible for further developments in the field of protein and nucleic acid research.

Some important scientists and their contribution to biochemistry you study

1828	Wohler	Synthesized the first organic compound, urea from inorganic components
1854-1864	Louis Pasteur	Proved that fermentation is caused by microorganisms
1877	Kuhne	Proposed the term 'Enzyme'
1894	Emil Fischer	Demonstrated the specificity of enzymes and the lock and key relationship between enzyme and substrate
1897	Buckner	Discovered alcoholic fermentation in cell-free yeast extract
1902	Emil Fischer	Demonstrated that proteins are polypeptides
1903	Neuberg	First used the term 'biochemistry'
1913	Michaelis and Menten	Developed kinetic theory of enzyme action
1926	Sumner	First crystallized an enzyme, urease and proved it to be a protein
1933	Embden Meyerhof and Parnas	Demonstrated crucial intermediates in the chemical pathway of glycolysis and fermentation
1937	Krebs	Discovered citric acid cycle
1940	Lipmann	Role of ATP in biological systems
1950	Pauling and Corey	Proposed the α-helix structure for keratins

1950-1953	Chargaff	Discovered the base composition of DNA
1953	Sanger and Thompson	Determined the complete amino acid sequence of insulin
1953	Watson and Crick	Proposed the double-helical model for DNA structure
1958	Meselson and Stahl	Confirmed the Watson-Crick model of semi conservative replication of DNA
1961	Jacob & Monod	Proposed the operon hypothesis and postulated the function of messenger RNA
1999	Ingo potrykus	Golden rice- rich in β-carotene

PLANT CELL

The word cell was coined by Robert Hooke with the help of compound microscope. Cell is the basic unit of life.

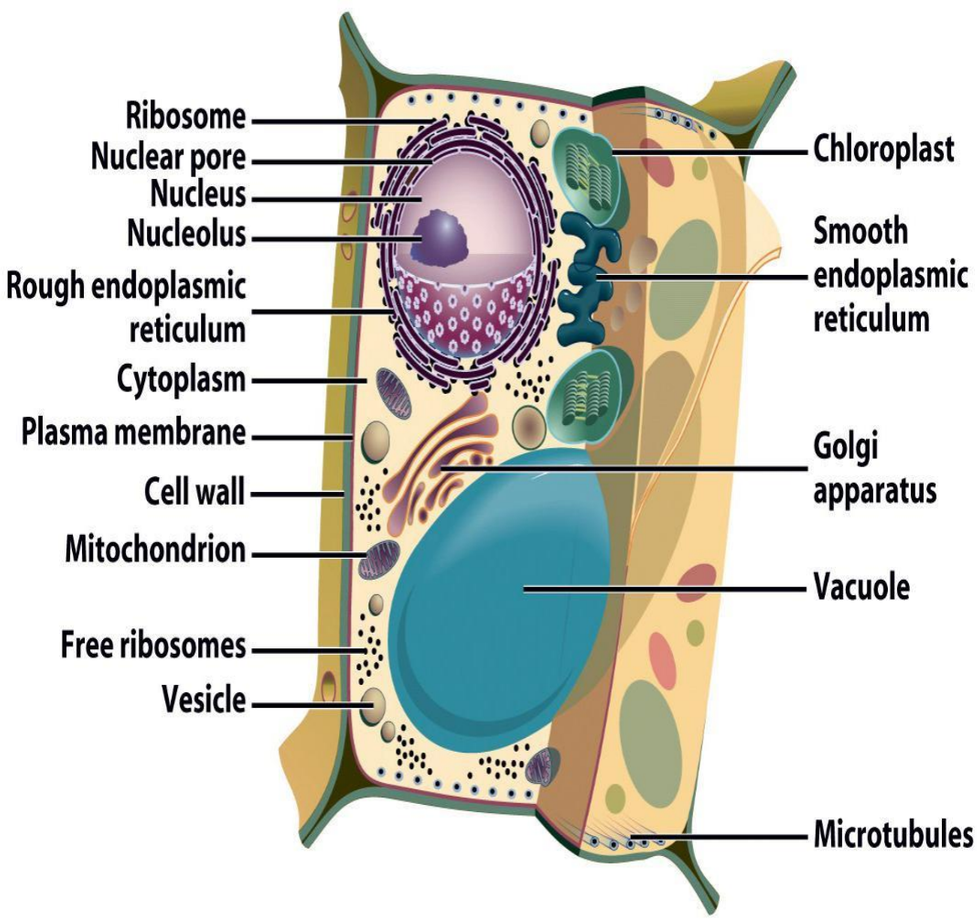
A plant cell has three distinct regions

a. Cell wall
b. Protoplasm
c. Vacuole

Cell wall and vacuole are considered as non-living substances. The protoplasm which is living has two components

1. Cytoplasm
2. Nucleus

The cytoplasm contains several organelles such as mitochondria, chloroplast, ribosomes, endoplasmic reticulum, golgi complex, lysosomes, plastids etc.



Plant Cell & organelles

The brief description of the plant cell and various organelles and their functions are as follows.

***Cell wall*:** Cell wall is a non-living component of the cell and is secreted and maintained by the living portion of the cell, called protoplasm. A typical cell wall is composed of three different regions

1. Middle Lamella
2. Primary cell wall (1-3 µm thick and elastic)
3. Secondary cell wall (5-10 µm thick and rigid)

Functions of cell wall

1. It protects the inner contents of the cell.
2. It gives definite shape to the cell.
3. It provides mechanical support to the tissues and act as a skeletal framework of plants.
4. It helps in transport of substances between two cells.
5. The cell wall is hydrophilic in nature and it imbibes water and helps in the movement of water and solutes towards protoplasm. It also acts as a permeable structure during absorption of minerals and solutes.

***Protoplasm*:** It is the living, colloidal and semi fluid substance. It is also called as cytoplasm. Cell devoid of cellwall is called protoplast. Protoplast is enclosed by a membrane called as cell membrane or plasma membrane

Cell membrane : All cells are enclosed by a thin, membrane called plasma membrane or plasmalemma. The plasma membrane and sub cellular membrane are collectively called biological membrane.

Cell membrane consists of proteins, lipids and other substances

1. ***Proteins***:- The proteins present in the membranes can be categorized into two types
   a. Intrinsic proteins or integral proteins: - Which are embedded or buried in the lipid layer. These proteins associate with hydrophobic interactions to the tails or fatty acid chains of the lipid layer. In addition to the hydrophobic associations, integral proteins also posses hydrophilic aminoacid residues which are exposed at the surface of the membrane. These proteins cannot be removed easily.
   b. Extrinsic proteins or peripheral proteins: - They are attached to the membrane surface by weak ionic interactions. These proteins are not much involved in the architecture of membrane. Peripheral proteins are bound to hydrophilic proteins of the integral proteins protruding from the lipid layer.
2. ***Lipids***: - The cell membrane consists of phospholipids and glycolipids. The fatty acid chains in phospholipids and glycolipids usually contain 16-20 even numbered carbon atoms. Fatty acids may be saturated or unsaturated.
3. Other substances like polysaccharide, salicylic acid etc. are found attached to the proteins or lipids on the membrane.

Functions of cell membrane:

1. The cell membrane surrounds the protoplasm of the cell, thus separating the intracellular components from the extracellular environment.
2. It anchors the cytoskeleton to provide shape to the cell, and in attachment to the extracellular matrix.
3. The plasma membrane is differentially permeable and able to regulate the transport across the membrane.
4. The cell membranes maintain the cell potential.

Cell nucleus:

It is oval or spherical in shape and is generally larger in active cells than in resting cells. A nucleus consists of three main parts viz. nuclear envelope, nucleolus and chromatin. The nucleus is separated from the cytoplasm by a double membrane called the nuclear envelope. The space between the outer and inner membrane is known as nuclear pores which provide direct connection between nucleus and cytoplasm.

Nucleolus is a spherical, colloidal body found in the nucleus and is the place where almost all DNA replication and RNA synthesis occur.

Chromatin is the basic unit of chromosome and contains genes which play important role in the inheritance of characters to offspring from parents.

Functions of cell nucleus :

1. It regulates growth and reproduction of cells.
2. The nuclear envelope allows the nucleus to control its contents, and separate them from the rest of the cytoplasm where necessary.
3. The DNA replication, transcription and post transcriptional modification occur in the nucleus.

Chloroplast : Chloroplasts are organelles found in plant cells and other eukaryotic organisms that perform photosynthesis because of the presence of green pigment, chlorphyll. They are flattened discs usually 2-10 micrometers in diameter and 1 micrometer thick. The chloroplast is surrounded by double layered membrane. The space between these two layers is called intermembrane space. Stroma is the aqueous fluid found inside the chloroplast. The stroma contains the machinery required for carbon fixation, circular DNA, ribosomes etc. within the stroma the stacks of thylakoids are arranged as stacks called grana. A thylakoid has a flattened disc shape and has a lumen or thylakoid space. The light reactions occur on the thylakoid membrane.

Functions of chloroplast :

1. The important processes of photosynthesis i.e, light and dark reactions occur within the chloroplast.
2. The granum is the site of NADP reduction forming $NADPH+H^+$ and photophosphorylation i.e., formation of ATP in presence of light. Thus, light reaction of photosynthesis takes place in the granum region.
3. The stroma is the main site for the dark reaction of photosynthesis.
4. The chloroplast has its own genetic system and is self replicating. Thus, associated with cytoplasmic inheritance.

Mitochondria : Mitochondria are rod shaped cytoplasmic organelles, which are main sites of cellular respiration. Hence, they are referred to as power house of the cell. Each mitochondrion is enclosed by two concentric unit membranes comprising of an outer membrane and an inner membrane. The space between the two membranes is called perimitochondrial space. The inner membrane has a series of infoldings known as cristae. The inner space enclosed by cristae is filled by a relatively dense material known as matrix. The matrix is generally homogeneous, but may rarely show finely filamentous or fibrous structures. The matrix contains several copies of round or circular DNA molecules.

Functions of mitochondria:

1. ATP, the readily available form of energy is produced in mitochondria.
2. Krebs cycle takes place in the matrix of mitochondria
3. The enzymes of electron transport chain are found in the inner membrane or cristae of mitochondria.
4. Heme synthesis occurs in mitochondria.
5. Controls the cytoplasmic Ca+2 concentration

Ribosomes : Chemically, ribosomes are ribonucloprotein complexes. Ribosomes are of two types. Ribosomes of prokaryotes have sedimentation coefficient of 70 S and consist of two sub units of unequal sizes 50S and 30 S subunits. Ribosomes of eukaryotes have 80 S sedimentation coefficient (40S & 60 S). The two or more ribosomes become connected by a single m RNA and then may be called polyribosome

The major function of the smaller subunit of ribosome is to provide proper site for binding of mRNA and its translation. The larger subunit of ribosome supports translation and translocation processes coupled with polypeptide synthesis.

Functions of Ribosomes :

1. They provide the platform for protein synthesis
2. They have the machinery for protein synthesis.

Golgi complex: Golgi bodies is an assemblage of flat lying cisternae one above the other in close parallel array. Each golgi complex has 3 to 12 interconnected cisternae which are composed of lipoproteins.

Functions of Golgi complex:

1. It helps in Packaging of proteins for exporting them.
2. It plays a role in sorting of proteins for incorporation into organelles.
3. It is involved in the formation of the cell wall of plant cells

Endoplasmic reticulum: Endoplasmic reticulum arises from the outer membrane of the nucleus forming an intermediate meshed network. It is of two types. The granular or rough endoplasmic reticulum in which the outer surface of endoplasmic reticulum is studded with ribosome and agranular or smooth endoplasmic reticulum in which the ribosomes are not attached.

Functions of Endoplasmic reticulum :

1. Rough endoplasmic reticulum is associated with the synthesis of proteins.
2. Smooth endoplasmic reticulum is associated with synthesis of lipids and glycogen.
3. It acts as an inter-cellular transport system for various substances.
4. It contains many enzymes which perform various synthetic and metabolic activities.

Vacuole: It is a membrane bound organelle found in plant cell and occupies most of the area in the plant cell. A vacuole is surrounded by a membrane called tonoplast. It is an enclosed compartment filled with water containing inorganic and organic molecules including enzymes in solution.

Functions of vacuole:

1. Isolating materials that might be harmful or a threat to the cell.
2. Stores waste products.
3. Maintains internal hydrostatic pressure or turgor within the cell
4. Maintains an acidic internal pH
5. Exports unwanted substances from the cell
6. Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole.
7. Most plants stores chemicals in the vacuole that react with chemicals in the cytosol.
8. In seeds, stored proteins needed for germination are kept in protein bodies which are modified vacuole.

Microbodies : Microbodies are ubiquitous organelles found in the majority of eukaryotic plant cells. They are mostly spherical and have a diameter ranging

from 0.2um to 1.5um.. Two types of microbodies, peroxisomes and glyoxysomes, have been characterized. These organelles differ in their distribution and enzyme composition, although both have the capacity to transform non-carbohydrate material into carbohydrate.

Peroxisomes : Peroxisomes are found in leaves of higher plants.
Functions of Peroxisomes: Peroxisomes act in parallel with chloroplast in higher plants and are believed to undertake photorespiration.

Glyoxysomes : Glyoxysomes are temporary in that they occur during transient periods in the life cycle of a plant such as in certain beans and nuts which store fats in their seeds as energy reserves. Glyoxysomes appear in the first few days after seed germination in endosperm cells and associate closely with lipid bodies. They disappear after the storage fats are broken down and converted into carbohydrate.

Functions of Glroxysomes: Glyoxysomes are involved in the formation of sugars by the breakdown of fatty acids in germinating seeds.

Cytoskeleton : The cytoskeleton is scaffolding contained within the cytoplasm and is made out of protein. The cytoskeleton is present in all cells. The cytoskeleton provides the cell with structure and shape

There are three main kinds of cytoskeleton filaments

1. Microfilament: - They are composed of actin subunits.
2. Intermediary filaments: - They function in the maintenance of cell shape by bearing tension. They also participate in the cell-cell and cell matrix junctions.
3. Microtubules: - They are like hollow cylinders mostly comprising of 13 protofilaments which in turn are alpha and beta tubulin. They are commonly organized by the centrosome.

Functions of cytoskeleton :

1. Provides mechanical support
2. Anchors organelles
3. Helps to move substances intra cellular

PLANT CELL WALL

Plant cells are surrounded by a rigid, semi-permeable cell wall. The cell wall is comprised of mainly polysaccharides with some proteins and lipids. The three main polysaccharide components of the cell wall are cellulose, hemicelluloses and pectin. Two types of proteins like expansin and extensin are present predominantly. Since cell wall also contains enzymes, it can be regarded as an organelle. Cell walls provide rigidity and mechanical strength, maintain cell shape and the direction of cell growth. The cell wall also prevents expansion when water enters the cell. Under the light microscope the walls separating the cells in a plant tissue are usually clearly visible. The walls of adjacent cells meet at a dividing line known as the middle lamella, which can be distinguished with the electron microscope.

Each cell wall performs certain specialized functions, such as

1. Structural function.
2. Affects developmental pattern

3. Defines cell's position within the plant
4. Cell- cell and cell- nucleus communication
5. Defense against pathogens
6. Recognises symbiotic nitrogen fixing bacteria
7. Recognises self

Cell wall is made up of three layers. They are

a) Middle lamella: This is the first layer formed during cell division. It makes up the outer wall of the cell. It is shared by adjacent cells. It is composed mainly of pectic compounds and proteins

b) Primary wall: Primary wall deposited by cells before and during active growth. Plant cells are surrounded by a polysaccharide rich primary wall. The primary walls of different plant cells differ greatly in appearance. Young cells have a very thin cell wall.

Composition of primary wall

Primary walls are composed predominantly of polysaccharides together with lesser amounts of structural glycoproteins, phenolic esters, enzymes, and calcium and boron minerals

Functions of primary wall:

- It gives structural and mechanical support
- It maintains and determines cell shape
- It resists internal turgor pressure of cell
- Controls rate and direction of growth
- Ultimately responsible for plant architecture and form
- Regulate diffusion of material through the apoplast
- Protects against pathogens
- Protects against dehydration and other environmental factors
- Source of biologically active signaling molecules
- Plays a major role in cell – cell interaction
- Participates in early recognition of symbiotic nitrogen fixing bacteria.

c) Secondary cell wall: Secondary cell wall is formed after cell enlargement is completed. Some cells deposit additional layers inside the primary wall. This occurs after growth stops or when the cells begin to differentiate (specializes). The secondary wall is mainly for support and is comprised primarily of cellulose, hemicellulose and lignin. It often can distinguish distinct layers, S1, S2 and S3 - which differ in the orientation, or direction, of the cellulose microfibrils. It is extremely rigid and provides compression strength.

Composition of secondary cell wall

The secondary cell walls are much thicker than the primary walls and consist of 40-45% cellulose, 15 – 35 % hemicellulose, 15 – 30 % lignin and negligible amounts of pectic polysaccharides.

Functions of secondary cell wall:

- It plays major role in providing mechanical support.
- It facilitates the transport of water and nutrients.
- It allows extensive upright growth

Formation of cell wall

A cell plate formed between the two daughter cells originates from microtubles, which act as the base for the construction of the new cell wall. The microtubules may direct the cell- wall forming materials to the cell plate which grows from the centre towards periphery of the cell and soon becomes the pectin rich middle

lamella. Above the middle lamella the primary wall formation takes place. The protoplasts of the cells of the primary wall secrete the secondary wall materials, when the cells have stopped enlarging. The protoplast then totally diminishes and only the wall remains. The rings, spirals or network in a mature stem cross section are due to secondary wall deposition.

Chemical changes in cell wall

Chemical changes take place in cell wall by accumulation of various depositions as the cell matures. These chemical changes bring corresponding change in the structure and function of the cell. The various depositions are

Lignification**:** The deposition of the lignin on cell walls is called lignification. As a result of lignification, the cell walls become hard, thick walled and dead. Usually after of the thickening of the cell wall, the protoplasm of the cell diminishes in size and the cell becomes dead and rigid. Cellulose microfibrils are impregnated in phenoloic polymer called lignin. Lignin displaces water in matrix and form hydrophobic meshwork that bonds tightly to cellulose and prevent wall enlargement. Lignins add up mechanical strength and reduce susceptibility of wall to attack by pathogens

Suberization**:** The cell wall of cork cells and casparian strips of endodermis get deposited with a layer of suberin by a process known as suberization.

Cutinization**:** In some cells, in the outer layers of the cell wall, the cellulose gets converted to cutin by a processs of cutinization. This forms a definite, impermeable membrane on the cell wall of cuticle. Cutinization helps in checking evaporation of water. It is found normally on the exposed parts of the plant.

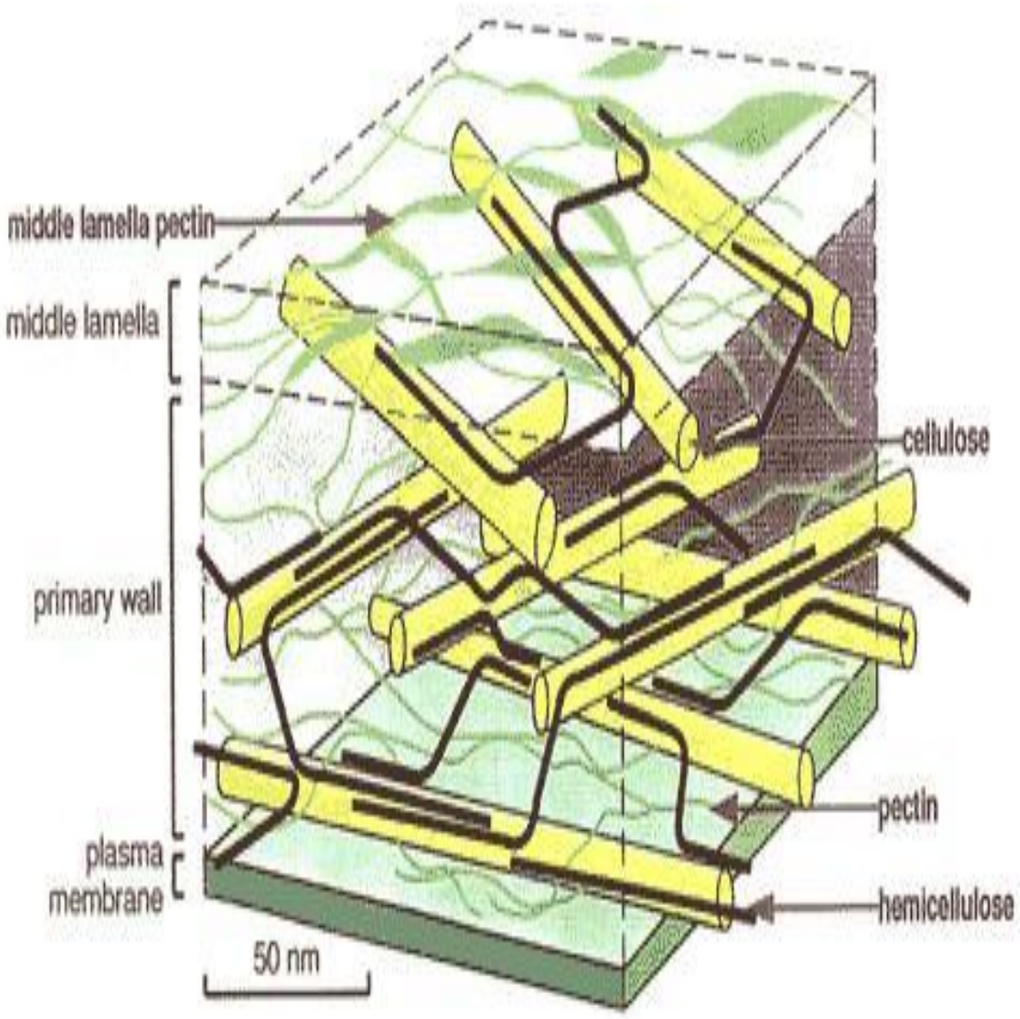
Mucilaginous changes: Sometimes the cellulose is changed to mucilage which has the property of absorbing and retaining water. This forms a viscous, mucilaginous coating on the cell walls and helps to tide ove dry conditions. Many sea weeds yield mucilaginous substances such as agar, alginic acid and carageenen of great commercial value.

Mineralization: The deposition of the minerals on the cell walls by the process of infilteration or by deposition of inorganic salts is known as mineralization. The minerals usually deposited are silica, carbonates and oxalates of calcium. The phenomenon of silica and calcium deposition are known as silicification and calcification respectively.

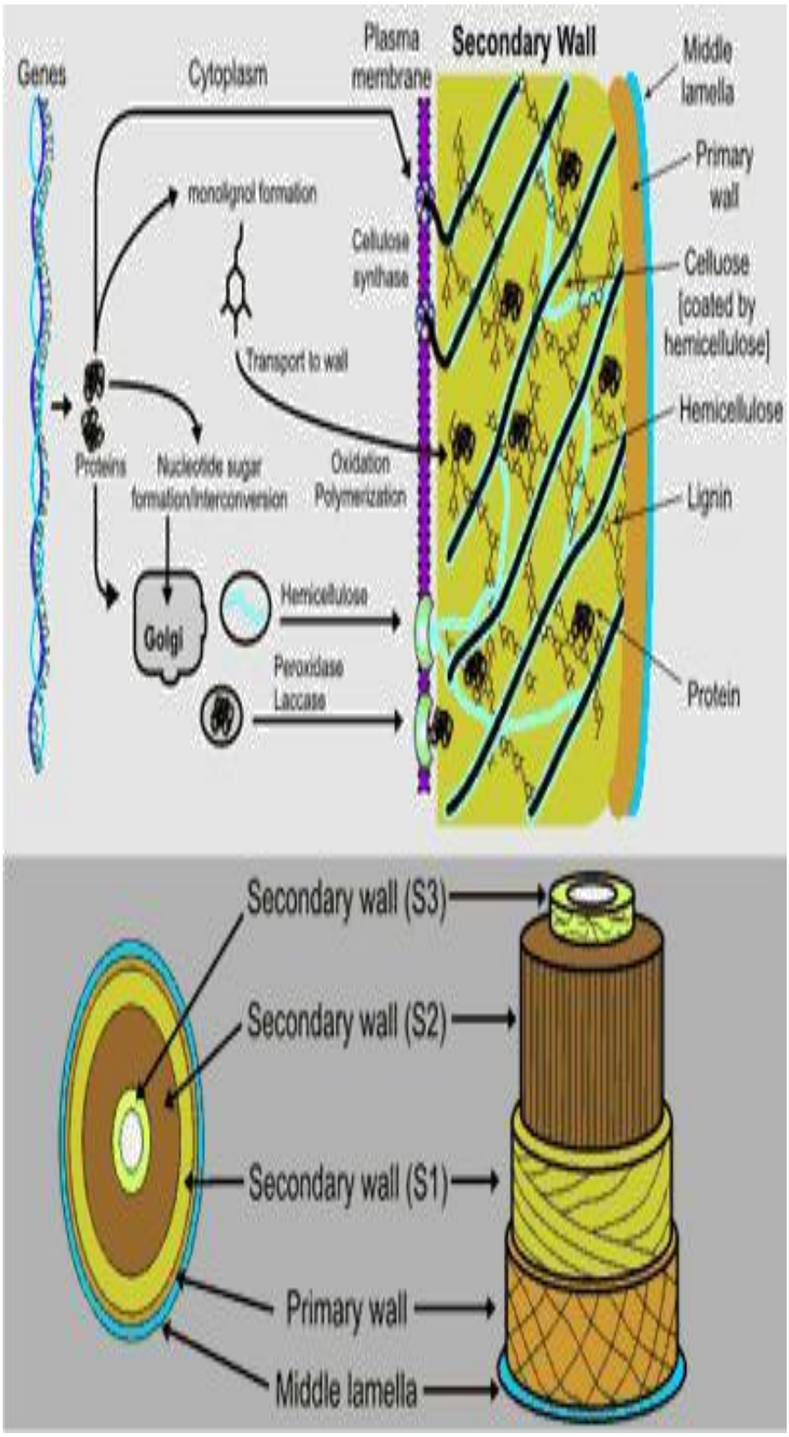
Role of plant cell wall in live stock, food and paper industry:

- Primary walls are the major textural component of plant-derived foods.
- Plant-derived beverages often contain significant amounts of wall polysaccharides. Some wall polysaccharides bind heavy metals, stimulate the immune system or regulate serum cholesterol.
- Wall polysaccharides are used commercially as gums, gels, and
- stabilizers.
- Cell wall structure and organization is of interest to the plant scientist, the food processing industry and the nutritionist.
- Cellulose plays a major role in paper industry.
- Secondary walls also have a major impact on human life, as they are a major component of wood
- It is a source of nutrition for livestock.
- The cell walls of fruits and vegetables are now recognized as important dietary components and may protect against cancer of colon, coronary heart disease, diabetes, and other ailments.

- Nevertheless, numerous technical challenges must be overcome to enable the efficient utilization of secondary walls for energy production and for agriculture.



Composition of cell wall



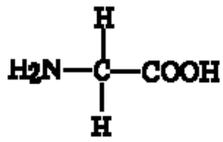
Layers of cell wall

PROTEINS

Proteins are made up of different amino acids.

Amino acids*:* In amino acids, there are two functional groups: an amino group and a carboxylic group. Both these groups are attached to the α carbon atom only. Amino acids are alpha (α) amino carboxylic acids. The carbon atom is tetrahedral in shape. The various groups attached to it are placed in different positions. Since the valence of the carbon atom is four, four groups can be attached to the carbon atom. Based on the groups attached to the carbon atom it may be of two types.

1. Symmetric carbon atom: When the valence of the carbon is satisfied by more than one similar atoms/ groups then the particular carbon atom is called as symmetric carbon atom. Eg : Glycine

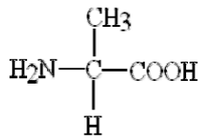


glycine

Compounds containing symmetric carbon atoms are optically inactive since they cannot rotate the plane of polarized light.

2. Asymmetric carbon atom: When the valence of the carbon is satisfied by four different groups, then that particular carbon atom is called as asymmetric carbon atom.

Eg: Alanine



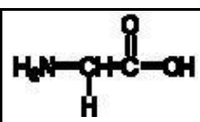
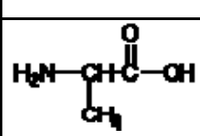
alanine

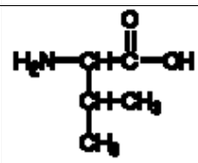
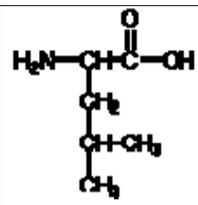
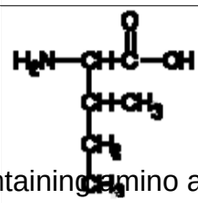
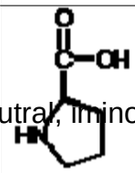
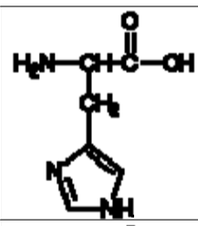
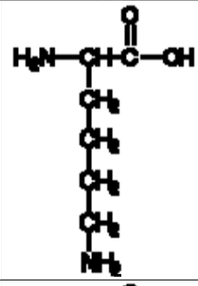
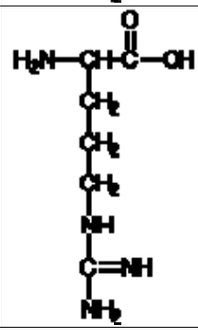
In amino acids, to α carbon atom, an amino group, a carboxylic group and a hydrogen atom are attached and the fourth group is the R group. This R group varies for each amino acid. All amino acids except glycine have at least one asymmetric carbon atom, hence they are optically active.

Classification of amino acids*:* Amino acids can be classified in various ways.

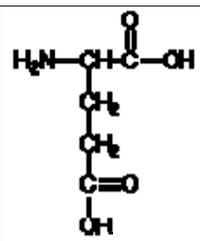
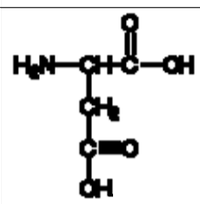
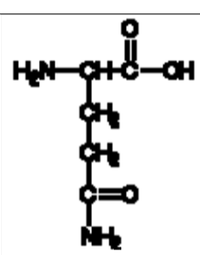
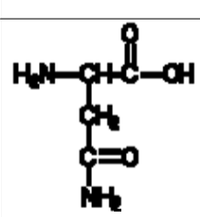
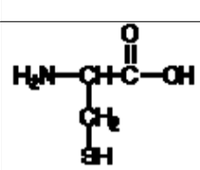
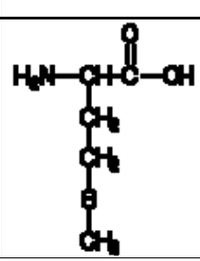
1. Based on side chains: Based on the structure of the R groups, all the amino acids are classified as aliphatic, aromatic and heterocyclic amino acids.

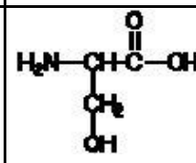
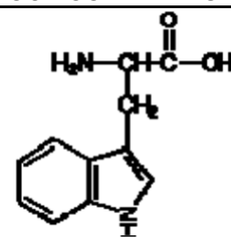
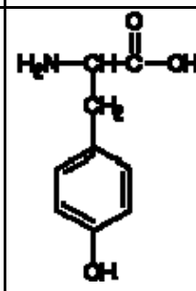
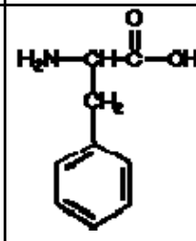
Structure of amino acids

Name of the amino acid	**3 letter code**	**Structure**	**Unique feature**
ALIPHATIC R GROUP CONTAINING HYDROPHOBIC AMINO ACIDS			
Glycine	Gly		symmetric amino acid, optically inactive amino acid
Alanine	Ala		Aliphatic hydrophobic neutral.

Valine	Val		Aliphatic, hydrophobic neutral, branched chain R group containing amino acid.
Leucine	Leu		Aliphatic, hydrophobic neutral, branched chain R group containing amino acid.
Isoleucine	Ile		Aliphatic, hydrophobic neutral, branched chain R group containing amino acid.
Proline	Pro		Hydrophobic, neutral, Imino amino acid.
ALIPHATIC R GROUP CONTAINING HYDROPHILIC, POSTIVELY CHARGED AMINO ACIDS			
Histidine	His		Aromatic, polar, hydrophilic, + ve charged, imidazole group containing amino acid
Lysine	Lys		Polar, hydrophilic, charged (+), ε amino group containing diamide amino acid
Arginine	Arg		Polar, hydrophilic charged (+), guanido group containing amino acid

ALIPHATIC R GROUP CONTAINING HYDROPHILIC,

NEGATIVELY CHARGED AMINO ACIDS			
Glutamatic acid	Glu		Polar, hydrophilic - ve charged R group containing amino acid α , γ dicarboxylic acid
Aspartatic acid	Asp		Polar, hydrophilic - ve charged R group containg amino acids . α ,β dicarboxylic acid
ALIPHATIC R GROUP CONTAINING HYDROPHILIC NEUTRAL AMINO ACIDS			
Glutamine	Gln		Polar hydrophilic neutral
Asparagine	Asn		Polar hydrophilic neutral It is a diamide
Cysteine	Cys		polar hydrophobic neutral
Methionine	Met		Hydrophobic neutral, sulphur containing amino acid.

Serine	Ser		Polar hydrophilic neutral
AROMATIC R GROUP CONTAINING AMINO ACIDS			
Tryptophan	Trp		Aromatic hydrophobic neutral, indole group containing amino acid
Tyrosine	Tyr		Aromatic polar hydrophobic phenol group containing amino acid.
Phenylalanine	Phe		Aromatic hydrophobic neutral amino acid

2.Based on their presence or absence in proteins: Amino acids are classified as protein amino acids and non protein amino acids.

a) *Protein amino acids:* - Amino acids that are used for synthesis of proteins are called protein amino acids. All the above mentioned 20 amino acids are present in proteins.

b) *Non protein amino acids:* Apart from the 20 amino acids that are present in proteins, several non protein amino acids are also present in nature. These are obtained by slight modification of 20 protein amino acids.
Eg:- beta alanine, hydroxy proline, N- acetyl glutamic acid etc

3. Based on requirement to the body as essential and non essential: Animals cannot synthesis all the 20 amino acids that are present in proteins. Some have to be provided to the body through external diet. The amino acids which cannot be synthesized by the body, which have to be supplied through diet are called essential amino acids. On the other hand, some amino acids can be synthesized by the body, and they are called as non essential amino acids.

Essential amino acids	Non essential amino acids
Methionine	Alanine
Arginine	Asparatic acid
Threonine	Glutamatic acid
Tryptophan	Cysteine
Valine	Glycine
Isoleucine	Proline
Leucine	Serine
Phenylalanine	Tyrosine
Lysine	
Histidine is essential for children only	

*4. Based on polarity of the side chains***:** This is the most accepted form of classification of amino acids which is based on polarity and hydrophobic nature of R groups.

a) Nonpolar or hydrophobic: The R groups of these amino acids are less soluble in water, or hydrophobic, than those of polar, because they contain bulky side chains. These amino acids play a major role in promoting hydrophobic interactions within protein structures. Eg: Glycine, Alanine, Valine, Leucine, Isoleucine, Proline. Phenylalanine, Tyrosine and Tryptophan.

b) Polar uncharged amino acids: The R groups of these amino acids are more soluble in water, or hydrophilic, than those of non polar, because they contain functional groups that form hydrogen bonds with water. These amino acids possess oxygen, sulfur and/or nitrogen in the side chain and are therefore polar. The R group of these amino acids cannot be ionised and thus do not carry an overall charge. These amino acids readily interact with water. Eg: Cysteine, Methionine, Serine, Threonine, Asparagine and Glutamine

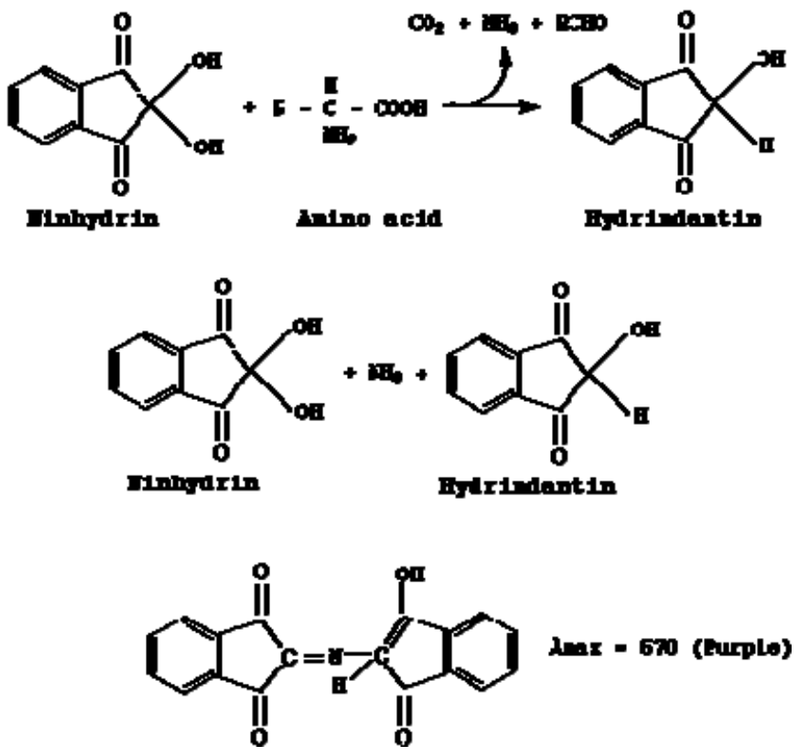
c) Polar amino acids with positively charged side chains: The R groups of these amino acids are not only polar but they also carry a positive charge and are therefore highly hydrophilic. Eg: Lysine, Histidine and Arginine. They are also called as basic amino acids as they can easily accept a proton.

d) Polar amino acids with negatively charged side groups: The R group of these amino acids is not only polar but they also carry a negative charge. Eg : Aspartic acid and Glutamic acid. They are also called as acidic amino acids as they can easily donate a proton.

Reactions of amino acids

1.Ninhydrin test: Ninhydrin is an oxidizing agent which oxidatively deaminates the alpha-amino groups of amino acids. It is very important for the detection and the quantitative analysis of amino acids. Ninhydrin also reacts with primary amines. However the formation of carbon dioxide is quite diagnostic for amino acids. Alpha amino acids yield a purple substance (Ruhemann's purple**)** that absorbs maximally at 570 nm. Imino acids (proline) yield a yellow product.

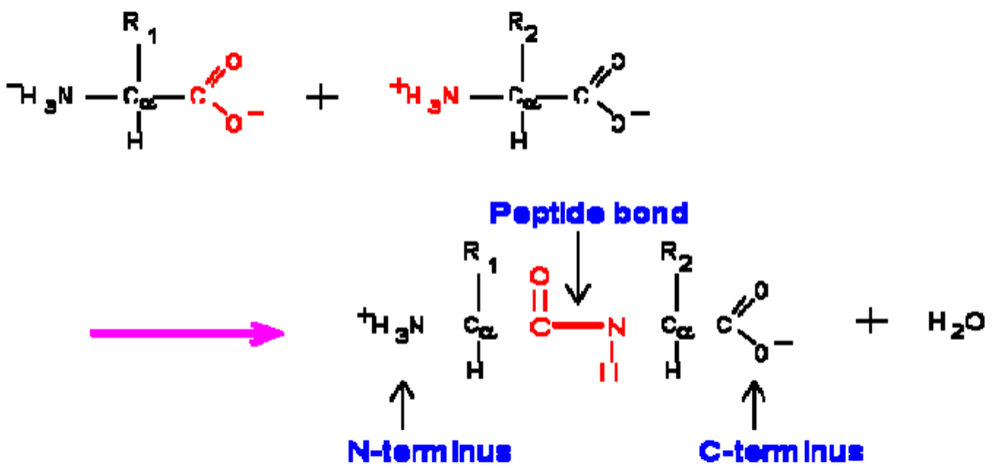
When a solution of amino acid is heated with ninhydrin, the amino acid is oxidatively deaminated to produce ammonia and a ketoacid. The keto acid is decarboxylated to produce an aldehyde with one carbon atom less than the parent amino acid. The net reaction is that, ninhydrin oxidatively deaminates and decarboxylates α-amino acids to CO_2, NH_3 and an aldehyde. The reduced ninhydrin then reacts with the liberated ammonia and another molecule of intact ninhydrin to produce a purple colored compound known as Ruhemann's purple.



RUHEMANN'S PURPLE

This ninhydrin reaction is employed in the quantitative determination of amino acids. Proteins and peptides that have free amino group(s) (in the side chain) will also react and give color with ninhydrin

*2. Peptide bond formation:-*Amino acids are linked together by formation of covalent bonds. The covalent bond is formed between the α-carboxyl group of one amino acid and the α-amino group of the next amino acid. The bond so formed between the carboxyl and the amino groups, after elimination of a water molecule is called as a peptide bond and the compound formed is a peptide.

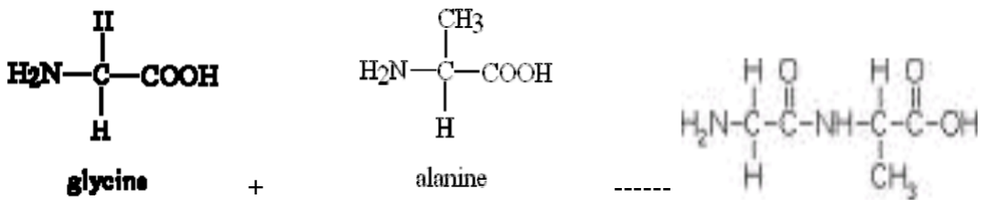


A peptide is a chain of amino acids linked together by peptide bonds. Proteins on partial hydrolysis yield the polypeptides and oligopeptides. Polypeptides are usually long peptides whereas oligopeptides are short (< 10 amino acids). Proteins are made up of one or more polypeptides with more than 50 amino acids.

Nomenclature of the peptides:-

In a peptide, always the first amino acid has its N terminal free. It will not be involved in the formation of a peptide bond, but the carboxyl group of first

amino acid and amino group of second amino acid are involved in the formation of the peptide bond. While naming a peptide, the first amino acid is usually named by adding yl and the second amino acid as it is.
Example:



Glycyl alanine

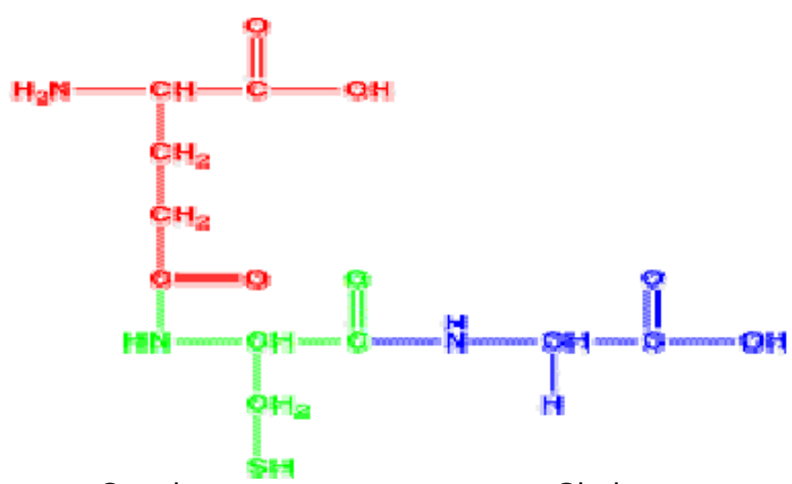
Peptides can be classified based on the structure as linear or cyclic peptides and based on number of amino acids involved in the formation of the peptide.

Based on structure:

1. Linear peptide: - When a peptide has its structure in the form of linear structure it is called as linear peptide. Eg: Glutathione and insulin. Number of peptide bonds present in a linear peptide = (n – 1) where n represents the number of amino acids.

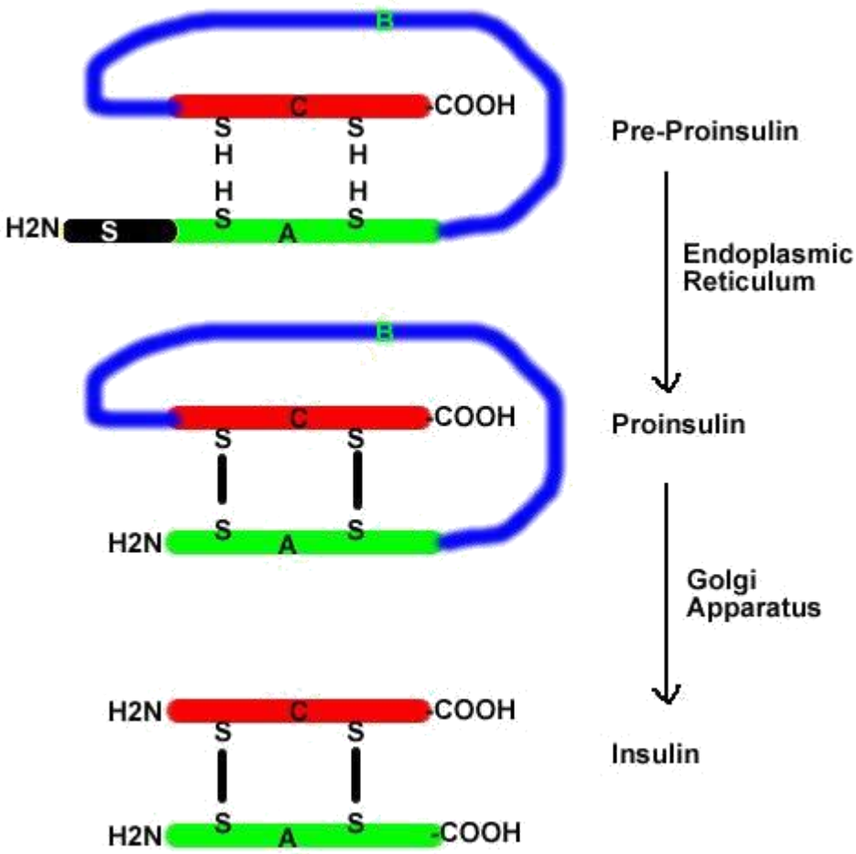
a) Glutathione: It is a small molecule made up of three amino acids, which exists in almost every cell of the body. This is called as natural redox tripeptide. There is an unusual peptide bond present between glutamic acid and cysteine and glycine. It is chemically called as gamma glutamyl cysteinyl glycine.

Glutamic acid



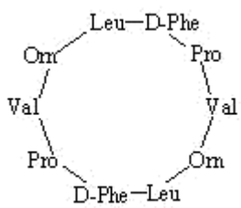
Cysteine Glycine

b) Insulin: The peptide hormone insulin is produced by clusters of specialized cells called the beta cells of islets of Langerhans of pancreas. It is synthesized as large precursor molecule pre-proinsulin. Pre-proinsulin undergoes partially hydrolysis and forms proinsulin with the removal signal peptide. Proinsulin is made up of three chains, chain A, B and C. In proinsulin, Chain A and C are connected by chain B which is made up of 30 amino acids. It undergoes a proteolytic cleavage and forms insulin with the removal of chain B. Chain A and chain C are connected by means of two disulphide bonds in insulin molecule.

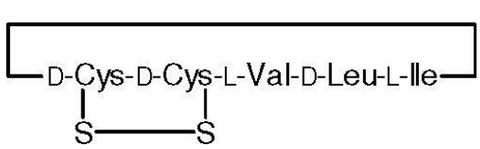


Cyclic peptides*:* If the carboxyl group at the C-terminus of a peptide forms a peptide bond with the N-terminal amino group, a cyclic peptide is formed. Cyclic peptide has its structure in the form of a ring. The number of peptide bonds in a cyclic peptide can be calculated by the formula
No of peptide bonds = n, where n= no of amino acids present in a peptide
Cyclic peptides are most commonly found in microorganisms, and often incorporate some D-amino acids as well as unusual amino acids such as ornithine (Orn).

a) Gramicidin-S: This antibiotic cyclic peptide is produced by a strain of *Bacillus brevis*. Gramicidine – S is a decapeptide made up of two pentapeptides. It has non protein amino acids such as D-Phenyl alanine, which is synthesized by microbes and ornithine. The structure of gramicidine –S is shown below



b) Malformin: - Malformin is another example of a cyclic peptide. Source is mycelium of fungus Aspergillus niger. Malformin promotes cell elongation, causes several malformations on the stem and petioles of several plant species and also causes root curvatures.
Chemically it is cyclo-D-cysteinyl-D-cysteinyl-L-valinyl-D-leucinyl-L-isoleucine



PROTEINS

The word protein was first coined in 1838 to emphasize the importance of this class of molecules. The word is derived from the Greek word proteios which means "of the first- rank. Proteins are polymers of several amino acids. They are folded into specific defined structures, which are maintained by large number of relatively weak bonds. Very small changes in the structure can modify the function. Hence, it is important to study the structure of protein in detail. The biological activity of proteins depends on maintenance of folded conformation. Proteins fold into well defined three dimensional shapes and they are able to recognize their corresponding substrates or antigen molecules and bind them tightly.

The protein structure has been classified into four different levels based on the folded confirmation of the protein.

Primary structure
Secondary structure
Tertiary structure
Quaternary structure

Primary structure: Primary structure is the simplest level of structural organization. The sequence of the different amino acids in a protein is called the primary structure of the peptide or protein. Though it is the simplest level of structural organization, in some aspects it is very important. The conformation and function of a protein are determined by the primary structure. Even a change in one amino acid residue may adversly affect the biological activity of protein. Eg: Hemoglobin is made up of four polypeptide chains, two chains of α type and two chains of β type. When glutamic acid present at the 6th position from N terminal end in the β chain is replaced by valine, Hemoglobin-S is formed and causes sickle cell anemia by which there is reduction in capacity to carry oxygen by hemoglobin. In a polypeptide, numbering of residues always starts at the N-terminal end (NH_2-group), where the amino group is not involved in a peptide bond formation.

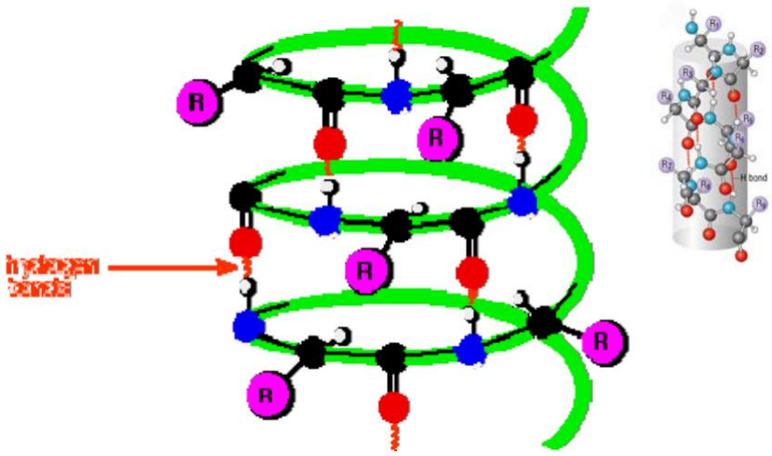
Secondary structure: The secondary structure of protein refers to the conformational patterns of the polypeptide chain. Different types of secondary structures occur widely in proteins. The most prominent are the alpha helix and beta conformations. Linus Pauling and Robert Corey predicted the existence of these secondary structures in 1951. In general, proteins fold into two broad classes of structures, namely globular proteins and fibrous proteins. Globular proteins are compactly folded and coiled, whereas, fibrous proteins are more filamentous or elongated.

The α-Helix: The α-helix is a common secondary structure encountered in proteins of the globular class. The formation of the α-helix is spontaneous and is stabilized by H-bonding between amide nitrogen and carbonyl carbons of peptide bonds spaced four residues apart.

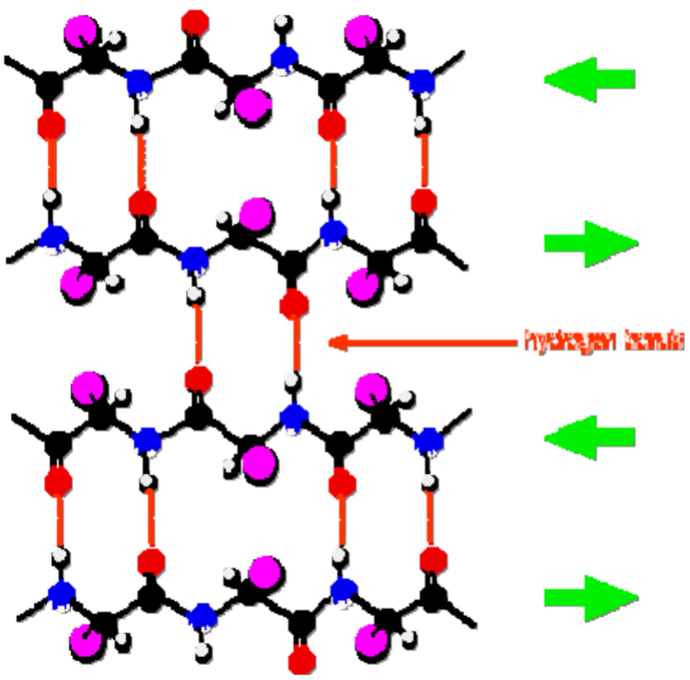
Features of α-Helix:

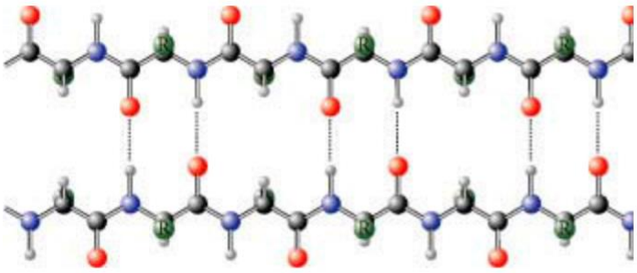
a) In this, the polypeptide backbone is tightly wound around an imaginary axis drawn longitudinally through the middle of the helix and R groups protrude outward from the helical back bone.
b) Single turn of the helix has 5.4 Amino acids and it is called as the pitch of the helix.
c) There are 3.6 amino acids residues per turn of the helix.
d) Distance between the peptide bonds is 1.5 A
e) The helix structure is maintained by the hydrogen bond formed between every first and the fourth amino acids of the helix
f) The hydrogen bonds are intra molecular and are parallel to the central axis

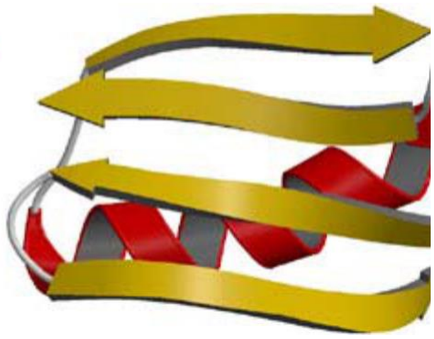
There are three types of α- helices based on the direction and the nature i.e.: left handed α- helix, right handed α- helix and triple helix Eg: collagen



β- Pleated Sheets: A α-helix is composed of a single linear array of helically disposed amino acids and β-sheets are composed of 2 or more different regions of stretches of at least 5-10 amino acids. The folding and alignment of stretches of the polypeptide backbone aside one another to form β-sheets is stabilized by hydrogen-bonding between amide nitrogen and carbonyl carbons. However, the hydrogen-bonding residues are present in adjacently opposed stretches of the polypetide backbone as opposed to a linearly continuous region of the backbone in the α-helix. B-sheets are said to be pleated. This is due to positioning of the α-carbons of the peptide bond which alternates above and below the plane of the sheet. B-sheets are either parallel or antiparallel. In parallel sheets, adjacent peptide chains proceed in the same direction (i.e. the direction of N-terminal to C-terminal ends is the same), whereas, in antiparallel sheets adjacent chains are aligned in opposite directions. B-sheets can be depicted in ball and stick format or as ribbons in certain protein formats.







Representation of a β pleated-Sheet Ribbon Depiction of β pleated -sheet

Tertiary structure : Tertiary structure refers to the complete three-dimensional structure of the polypeptide units of a given protein. It is the spatial relationship of different secondary structures to one another within a polypeptide chain and how these secondary structures themselves fold into the three-dimensional form of the protein. The tertiary structure is maintained by different forces. These include hydrogen bonding, hydrophobic interactions, electrostatic interactions and Van der Waals forces.

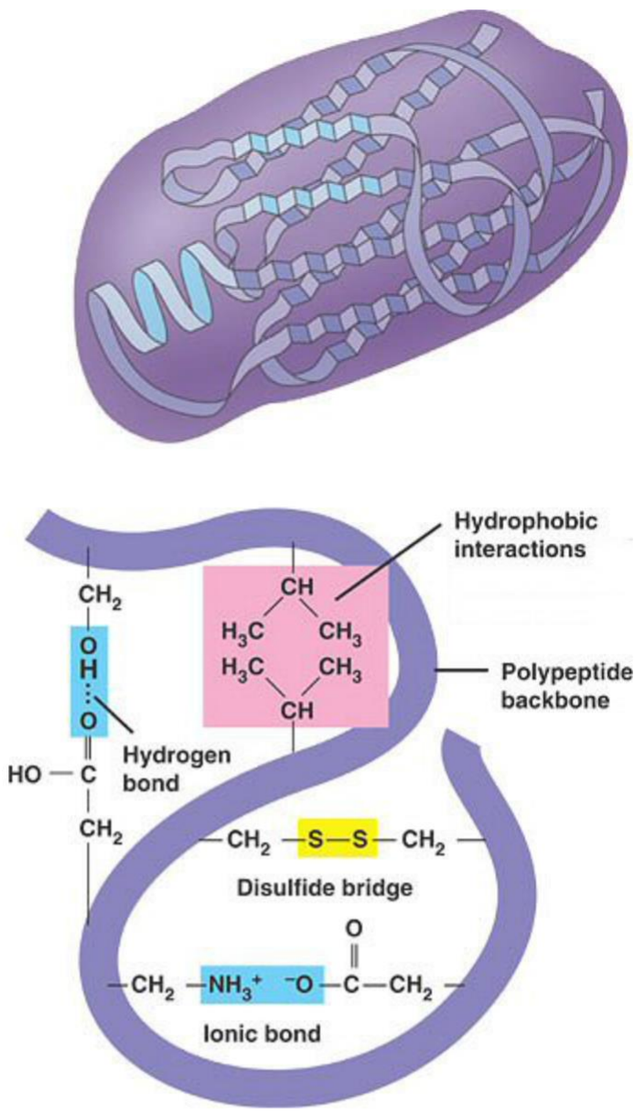
Hydrogen Bonding: Polypeptides contain numerous proton donors and acceptors both in their backbone and in the R-groups of the amino acids. The environment in which proteins are found also contains the ample H-bond donors and acceptors of the water molecule. H-bonding, therefore, occurs not only within and between polypeptide chains but with the surrounding aqueous medium.

Hydrophobic forces: Proteins are composed of amino acids that contain either hydrophilic or hydrophobic R-groups. It is the nature of the interaction of the different R-groups with the aqueous environment that plays the major role in shaping protein structure. The hydrophobicity of certain amino acid R-groups tends to drive them away from the exterior of proteins into the interior. This driving force restricts the available conformations into which a protein may fold.

Electrostatic forces**:** Electrostatic forces refer to the interaction of ionized R-groups of amino acids with the dipole of the water molecule. The slight dipole moment that exists in the polar R-groups of amino acid also influences their interaction with water. It is, therefore, understandable that the majority of the amino acids found on the exterior surfaces of globular proteins contain charged or polar R-groups.

Van der Waals forces: There are both attractive and repulsive Van der Waals forces that control protein folding. Attractive Van der Waals forces involve the interactions among induced dipoles that arise from fluctuations in the charge densities that occur between adjacent uncharged non-bonded atoms. Repulsive van der Waals forces involve the interactions that occur when uncharged non-bonded atoms come very close together but do not induce dipoles. The repulsion is the result of the electron-electron repulsion that occurs as two clouds of electrons begin to overlap.

Although Van der Waals forces are extremely weak, relative to other forces governing conformation, it is the huge number of such interactions that occur in large protein molecules that make them significant to the folding of proteins.

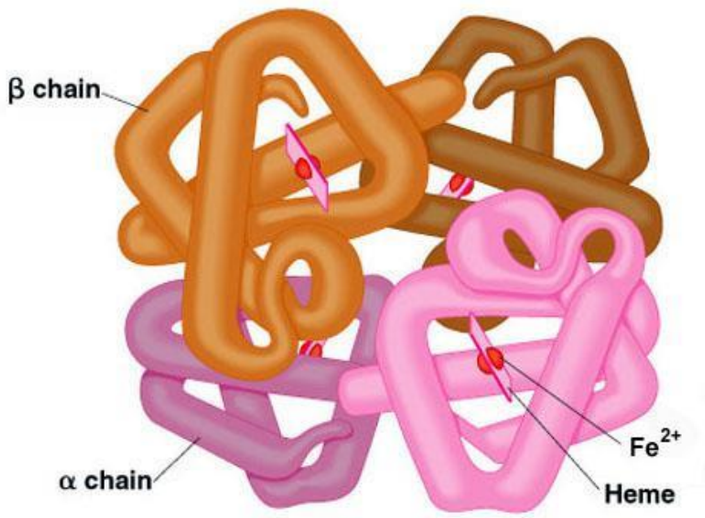


The tertiary structure of a protein

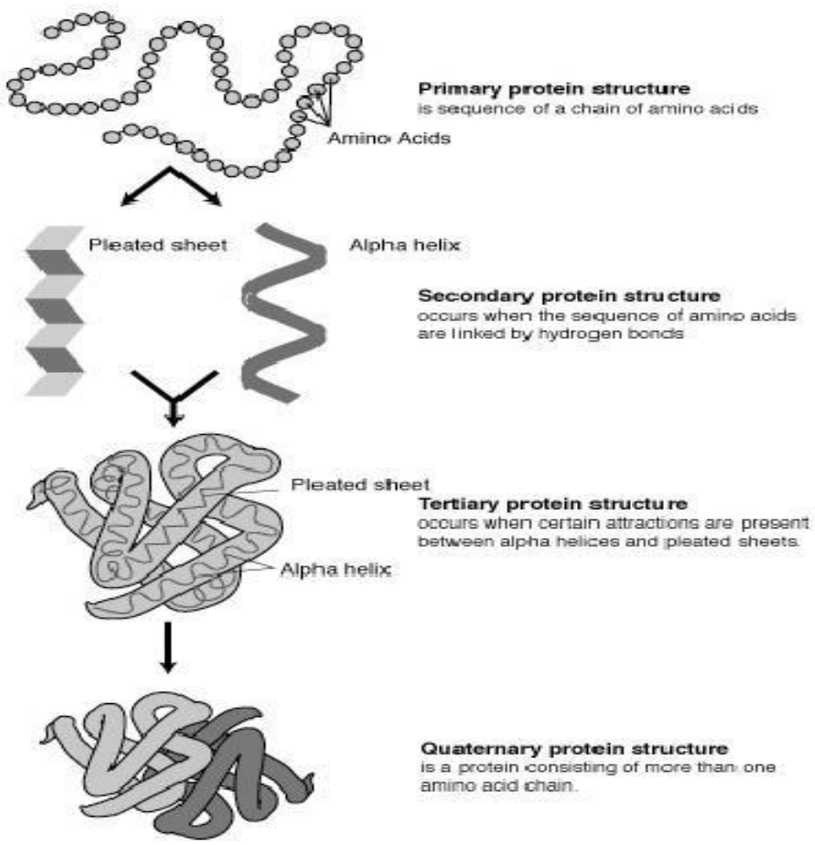
Quaternary Structure: Many proteins contain 2 or more different polypeptide chains that are held in association by the same non-covalent forces that stabilize the tertiary structures of proteins. Proteins with multiple polypetide chains are

oligomeric proteins. The structure formed by monomer-monomer interaction in an oligomeric protein is known as quaternary structure.

Oligomeric proteins can be composed of multiple identical polypeptide chains or multiple distinct polypeptide chains. Proteins with identical subunits are termed homo-oligomers. Eg: Acetylcholine receptor. Proteins containing several distinct polypeptide chains are termed hetero-oligomers. Hemoglobin, the oxygen carrying protein of the blood, contains two α and two β subunits arranged with a quaternary structure in the form, $\alpha_2\beta_2$. Hemoglobin is, therefore, a hetero-oligomeric protein.



Structure of Hemoglobin molecule



Levels of organization of protein structure

Properties of proteins:

1. U.V absorption: Proteins absorb U.V radiation at 280 nm because of the presence of aromatic amino acids like tryptophan and tyrosine. This property is used in estimation of proteins.
2. Isoelectric point: Isoelectric point is also called as isoelectric pH. This is the pH at which the number of positive and negative charges is equal in the protein and they are electrically neutral. Solubility of proteins is least at isoelectric pH.
3. Zwitterions: Proteins contain both positive and negative charges and hence they are called as zwitterions. Amino acids will act as zwitterions as they can donate a proton and forms cation. They can as well accept a proton and forms an anion. Each amino acid can act as an anion, cation, neutral species and as zwitterion.
4. Immunological properties: Proteins exhibit a special property called immunological property, which is useful in defense mechanism. When ever any antigen enters into the body, immediately body releases a special class of proteins called as defense antibodies. The interaction of antigen and antibody to form the antigen-anti body complex is called immune reaction. Antigen may be a protein (protein coat of virus), or a carbohydrate (sugars on the bacterial outer coat) or nucleic acid. Antibodies are special glycoproteins which will recognize and bind antigens.
5. Denaturation: It is a physical change in which there is a collapse of protein structure. Due to denaturation, there is a decrease in solubility and loss of biological activity of proteins. Denaturation occurs at extreme temperatures and pH and also by many chemicals like organic solvents, urea, ionic detergents etc. On denaturation, non covalent bonds in the protein are broken and its primary structure remains intact. When the favorable conditions are provided, some peoteins ie: a reversibly denatured protein,will spontaneously return to its native biologically active form and this process is called renaturation.
6. Protein folding: Many proteins fold to their native conformation on their own by self assembly. However several other accessory proteins help in this process. They include
    a) Enzymes: Eg: Peptidyl prolyl cis-trans isomerase introduces reverse bends.
    b) Molecular chaperons: They are a special class of proteins which will help in the folding of other proteins. Molecular chaperons will identify the improperly folded proteins and provide a microenvironment in which a polypeptide can progressively fold itself. Molecular chaperons will not impose a structure to proteins but only provide the required environment to the protein. They belong to heat shock protein family which protects polypeptide from denaturation and aggregation at high temperature.

7. Solubility: Protein solubility is influenced by pH, heavy metals, salts and organic solvents.

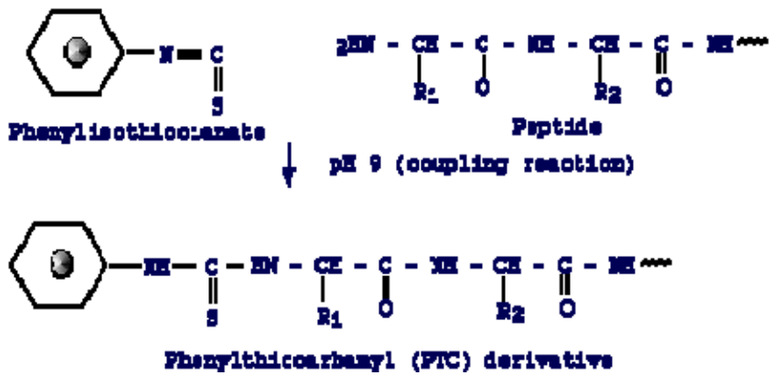
    a) pH: Solubility of proteins is minimum at isoelectric point (PI). At acidic PH, proteins behave like cations. Anion forms of some compounds like Trichloroacetic acid are effective in precipitating the proteins. Solubility is influenced by the presence of polar hydrophilic groups on the surface.
    b) Heavy metals: At alkaline pH, proteins behave like anions. Cationic forms of some heavy metals such as Mercury and Lead are attracted by the negative charges present on the free side chains of proteins and precipitate them.

c) Salts: By the addition of small quantity of salt like ammonium sulphate or sodium chloride, solubility of protein is usually increased due to increased ionic strength of the solution. On further increase of salt, the solubility decreases.

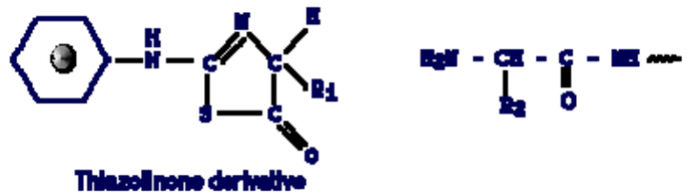
d) Organic solvents: Organic solvents like ethanol, acetone and butanol lower the dielectric constant of the medium and decreases the solubility.

Sequencing of amino acids*:* There are several effective methods by which a polypeptide end groups may be identified. The most effective method in identification of N- terminal residue is Edman degradation method named after its inventor Pehr Edman.

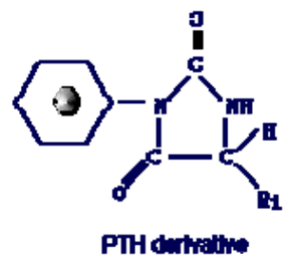
Phenylisothiocynate reacts with the N- terminal amino groups of proteins under mildly alkaline conditions to form their phenylthiocarbamyl derivative.



This product on reaction with an anhydrous strong acid such as trifluoroacetic acid (TFA, F_3CCOOH), forms thiazolinone derivative with the cleavage of a peptide bond involving the carboxyl group of the N-terminal residue. The treatment with TFA does not affect the other peptide bonds leaving a peptide chain with n-1 amino acid residues. The Edman degradation there fore releases the N- terminal amino acid residue but leaves intact the rest of the polypeptide chain.



The treatment of the thiazolinone derivative with aqueous acid leads to the formation of Phenylthiohydantoin (PTH) derivative of N-terminal amino acid which can be identified by chromatography. The residual peptide chain can be now submitted to a new coupling reaction and the next amino acid can be identified.

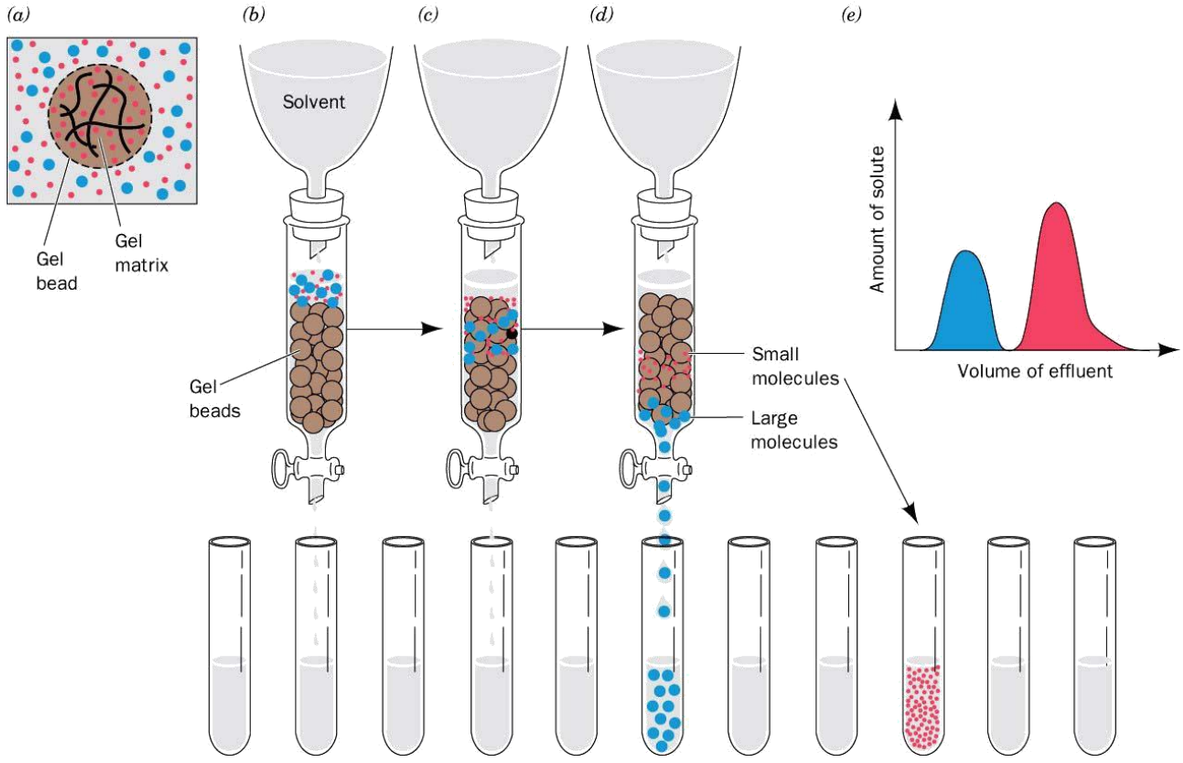


Purification techniques: The following techniques are used for purification of proteins.

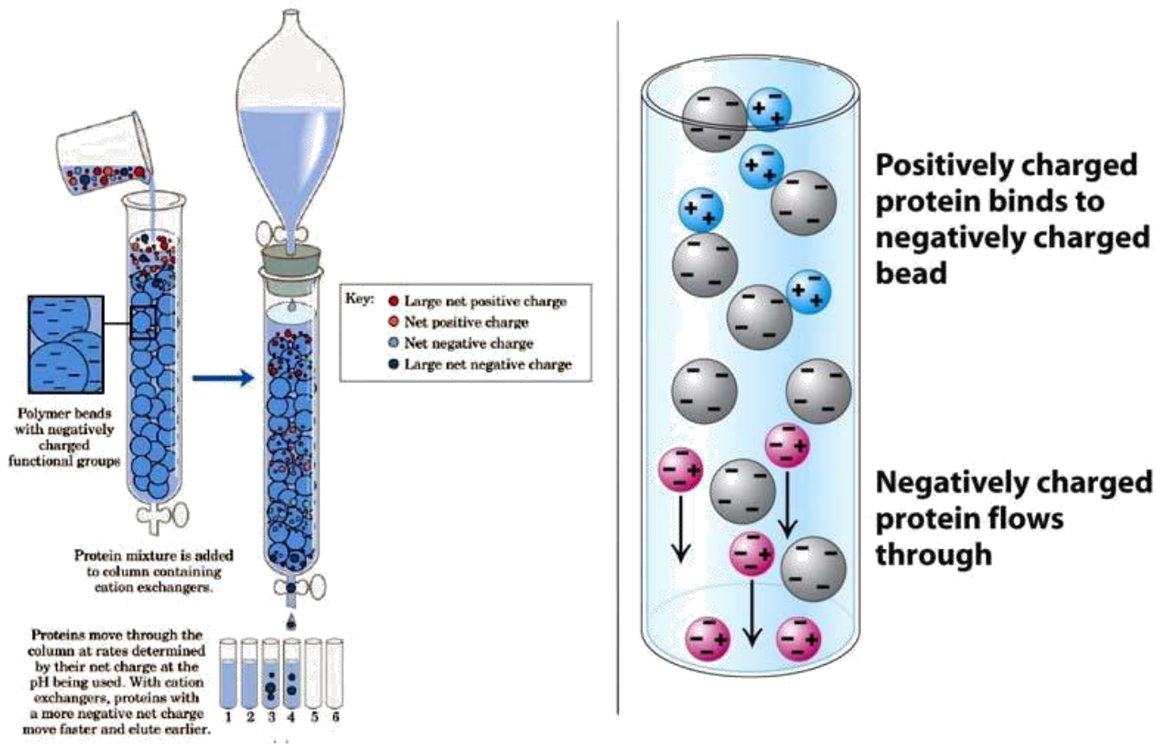
a) Salting in and salting out: Increase in the solubility of a protein by addition of small quantities of sodium chloride is called as salting in and this is due to increasing the ionic strength of the solution. On the other hand, when excess of salt is added to the solution, there is decrease in the solubility and this is called as salting out. Salting out occurs due to hydrophobic effect. There are hydrophobic amino acids and hydrophilic amino acids in protein molecules. The hydrophobic amino acids generally are present in the interior of the protein but some are present on the surface also in small patches. Water molecules become ordered, when they are forced to interact with these patches. When the salt concentration is increased, a competition develops for the water between the protein and the salt. Some of the water molecules are attracted by the salt ions, which decreases the number of water molecules available to interact with the charged part of the protein. As the salt concentration increases, the water on protein is removed thus exposing the hydrophobic area of protein molecule. These hydrophobic areas on the protein molecule get attracted to each other by hydrophobic effect. This results in increase in weight of the molecule and its aggregation. Larger the surface hydrophobic area on a protein molecule, quicker will be the precipitation of the protein at a lower concentration of the salt.

b) Dialysis: Proteins can be separated by dialysis through a semi permeable membrane such as cellulose membrane which has pores in it. Bigger molecules are retained in the bag and smaller molecules pass through the membranes.

c) Gel filtration or Size Exclusion Chromatography: This chromatographic technique is based upon the use of a porous gel in the form of insoluble beads placed into a column. When a solution of proteins is passed through the column, a protein of small size is likely to enter the pores. Small proteins can penetrate into the pores of the beads and, therefore, are retarded in their rate of travel through the column. The larger proteins will move through the gaps present in the column and are likely to move fast and are collected first when compared to the smaller ones. Different beads with different pore sizes can be used depending upon the desired protein size separation profile. Polymer beads made up of dextrose, agarose or polyacrylamide are generally used and the commercial names for few polymer beads are Sephadex, Sepharose and Biogel respectively.



d) Ion Exchange Chromatography: Each individual protein exhibits a distinct overall net charge at a given pH. Some proteins will be negatively charged and some will be positively charged at the same pH. This property of proteins is the basis for ion exchange chromatography. The basic principle is, like charges repel and unlike charges attract each other. Fine cellulose resins are used that are either negatively (cation exchanger) or positively (anion exchanger) charged. Proteins of opposite charge to the resin are retained as a solution of proteins is passed through the column. The bound proteins are then eluted by passing a solution of ions bearing a charge opposite to that of the column. By utilizing a gradient of increasing ionic strength, proteins with increasing affinity for the resin are progressively eluted. CM-Cellulose and DEAE-Cellulose are examples of negatively charged and positively charged resins.



Classification of proteins: Proteins are conveniently classified on the basis of their functions

Classification of proteins based on function:

a) Catalytic proteins: These are enzyme proteins that catalyze chemical and biochemical reactions within living cell and outside. This group of proteins probably is the biggest and most important group of the proteins. Enzymes are responsible for all metabolic reactions in the living cells. Eg: DNA and RNA polymerases, dehydrogenases etc.

b) Regulatory proteins: Few hormones are examples of this class of proteins that are responsible for the regulation of many processes in organisms. Eg: Insulin

c) Transport proteins: These proteins are involved in transporting some chemical compounds and ions. Eg: Haemoglobin, myoglobin

d) Defense proteins: These proteins are involved in the defense mechanism of the cell. Eg: Gamma globulins.

*e) Structural proteins**:*** These proteins are involved in maintaining the structure of other biological components like cells and tissues. Eg: Collagen, elastin.

*f) Contractile proteins**:*** These proteins are involved in contraction of the tissues. Ex: Actin and myosin are responsible for muscular motion.

*g) Storage proteins**:*** These proteins contain energy, which can be released during various metabolic processes in the organism. Ex: Egg ovalbumin, milk casein

h) Receptor proteins: These proteins act as receptor molecules. They are responsible for signal detection and translation into other type of signal.
Ex: GTPases.

Proteins are also divided into two classes based on the solubility in water.

a) Globular proteins which are soluble proteins are made up both hydrophobic and hydrophilic amino acids. In these proteins, hydrophobic amino acids are present internally.

b) Fibrous proteins which are insoluble proteins are made up of mostly hydrophobic amino acids.

Protein quality evaluation methods*:* Proteins are vital to the living processes and carry out a wide range of functions essential for the sustenance of life. In judging the adequacy of dietary proteins to meet the human needs, not only the quantity, but also the nutritional quality of the dietary protein is important. Proteins present in different foods vary in their nutritional quality because of differences in their amino acid composition. The human body derives the essential amino acids from the dietary protein. The quality of the dietary protein depends on its essential amino acid composition and its digestibility. The best quality protein is the one which provides essential amino acid pattern very close to the pattern of reference proteins such as egg protein or milk protein. The quality of a protein is evaluated by the following methods.

Biological methods: Proteins of raw grains (particularly legumes) are less digestible than that of animal foods. Generally, the low digestibility of plant proteins is due to the presence of trypsin inhibitors which are destroyed on cooking. The overall quality of a protein can be determined by biological methods with laboratory animals like rats as follows;

a) *Protein efficiency ratio (PER):* The gain in weight of young animals per unit weight of protein consumed is measured and the value obtained is designated as PER. Protein efficiency ratio is defined as gain in body weight by protein in take.

$$PER = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

b) *Digestibility coefficient (DC):* Dietary proteins are hydrolyzed to amino acids on digestion by various proteolytic enzymes. Proteins differ in the digestibility depending on their source. Since only amino acids are absorbed into the blood stream, undigested portion of the protein is excreted in the fecal matter. The term digestibility coefficient of protein refers to the percentage of the ingested protein absorbed into the blood stream after the process of digestion is complete. When an animal is fed on nitrogen free diet, certain amount of nitrogen is excreted in the fecal matter. This is derived mainly from the digestive juices. This is called endogenous faecal nitrogen. When a protein food is given, the nitrogen found in faeces consists of both endogenous nitrogen and food nitrogen lost in digestion. To find out nitrogen lost in digestion, endogenous faecal nitrogen should also be determined. For the determination of the digestibility coefficient, the data required are

1. Food nitrogen intake - In
2. Total fecal nitrogen excreted - Fn
3. Endogenous fecal nitrogen - Fe

$$DC = \frac{In - (Fn - Fe)}{In} \times 100$$

Where Fn – Fe is the food nitrogen lost in digestion.

The digestibility coefficient of proteins is influenced by several factors, such as indigestible carbohydrates and proteolytic enzyme inhibitors etc.

c) *Biological value (BV):* The amount of nitrogen (N) in the diet and in excreta of adult animals is measured and the percentage of N retained by animals from out of N absorbed from the diet is calculated. The value thus obtained is the biological value of the protein. It measures the quantity of dietary protein utilized by the animal for meeting its protein needs for sustenance and growth.

1. Food nitrogen intake - In
2. Total fecal nitrogen excreted - Fn
3. Endogenous fecal nitrogen - Fe
4. Total urinary nitrogen excreted - Un
5. Endogenous urinary nitrogen - Ue

$$BV = \frac{In-(Fn-Fe) - (Un-Ue)}{In - (Fn-Fe)} \times 100$$

ENZYMES

Enzymes are organic catalysts produced by living organisms. Hence they are called biocatalysts. Previously it was thought that all the enzymes are protein in nature but later it was proved that there are some non protein enzymes like ribozymes, which are RNAses. An enzyme may be a simple protein or a complex protein.

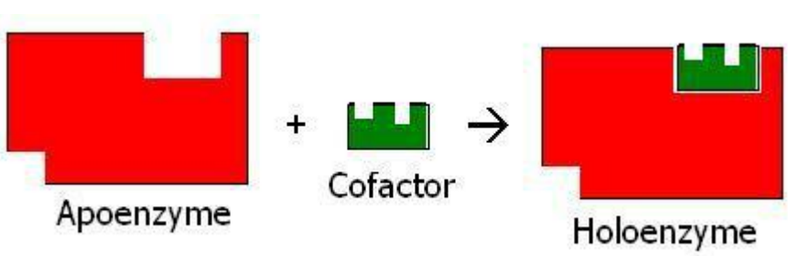
Characteristics of enzymes

1. Enzymes are biocatalysts. Hence they are not destroyed during an enzyme action. In other words, they are regenerated at the end of the reaction.
2. They can only speed up the reaction but can't initiate the reaction. They speed up the reaction by 10^{7} to 10^{14} times. These reactions occur even without enzymes but at a very slow pace. Time factor is very critical for biological reactions. Hence enzymes play a vital role.
3. Enzymes do not alter the equilibrium constant of a reaction but alters the rate at which equilibrium is reached. A non enzymatic reaction may take several years to reach equilibrium, while an enzymatic reaction takes a fraction of a second.
4. Enzymes are very specific. Their specificity is with regard to substrate and the reaction they catalyse.

Chemical nature of enzyme

J.B Sumner explained that enzymes are protein in nature. Many enzymes require the presence of other compounds such as cofactors, coenzymes and metal ions for their catalytic activity. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal ion or activator).

Apoenzyme + Cofactor = Holoenzyme



Apoenzyme is a protein substance which is thermo labile

According to Holum, the cofactor may be:

1. A coenzyme - a substance which is thermo stable and loosely attached to the protein part.
2. A prosthetic group - an organic substance which is thermo stable and is firmly attached to the protein
3. A metal ion or activator – such as K^{+}, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++}, and Mo^{+++}.

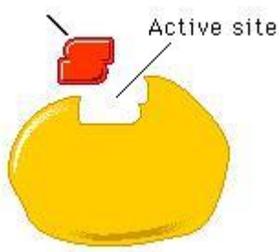
Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

1. *Absolute specificity* - The enzyme will catalyze only one reaction by acting on a single type of substrate . Eg: Glucokinase which converts only glucose to glucose 6- phosphate.
2. *Group specificity* - The enzyme will act on a group of related molecules. Eg : Hexokinase which acts on all hexoses and converts to their hexose phosphates.
3. *Linkage specificity* - The enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure. Eg: Proteases acting on various proteins.
4. *Stereochemical specificity* - The enzyme will act on a particular steric or optical isomer. Eg: L-Amino acid oxidases acting only on L- Amino acids.

Active site

It is part of an enzyme where substrates bind and undergo a chemical reaction. The active site of an enzyme is usually found in a cleft or pocket that is lined by amino acid residues (or nucleotides in ribozymes) that participates in recognition of the substrate. Residues that directly participate in the catalytic reaction mechanism are called active site residues.

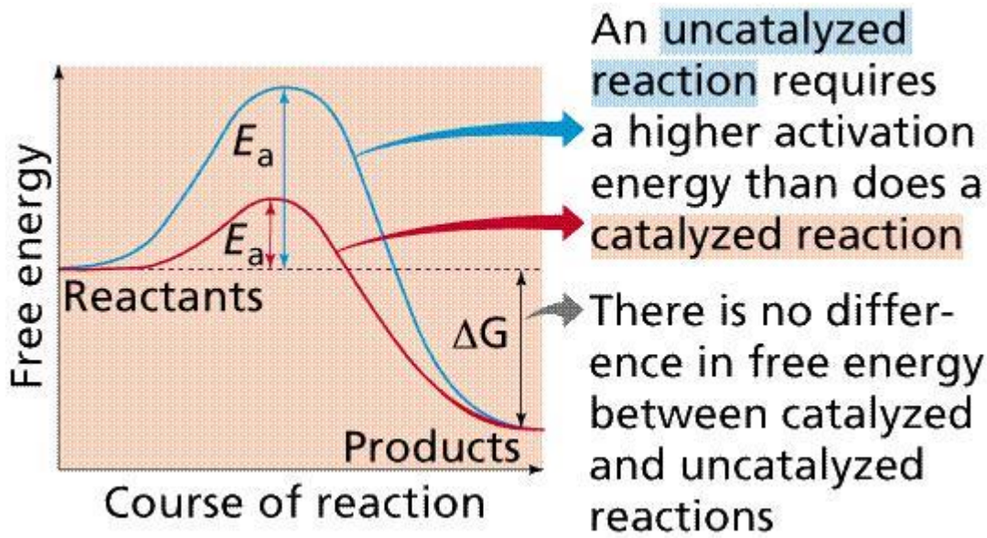


Mode of enzyme action

A chemical reaction such as A → P takes place because a certain fraction of the substrate possesses enough energy to attain an activated condition called the transition state. The minimum energy required by the substrate to cross the energy barrier and there by form products is called as activation energy. Every reactant has the required amount of energy but during the course of time some energy is lost when the reactants undergo collisions in the form of heat. Hence enzymes decrease the activation energy so that more number of reactants cross the energy barrier and products are formed.

The transition state of substrate is at the top of the energy barrier separating the reactants and products. The rate of a given chemical reaction is

proportional to the concentration of this transition state species. The energy of activation is the amount of energy required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state. Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the uncatalysed reaction. Thus they accelerate chemical reactions by lowering the energy of activation



Example:

H_2O_2 ----------→ H_2O + (O)
Catalase

Reaction condition	**Activation energy (KCal mol^{-1})**
Uncatalysed reaction	18
Catalysed by catalase	7

It is generally believed that the catalytic reactions occur in at least two steps.

Step 1: A molecule of enzyme (E) and a molecule of substrate (S) collide and react to form an intermediate called the enzyme-substrate complex (ES).

Step 2: The decomposition of ES complex to give product(s) and the active enzyme

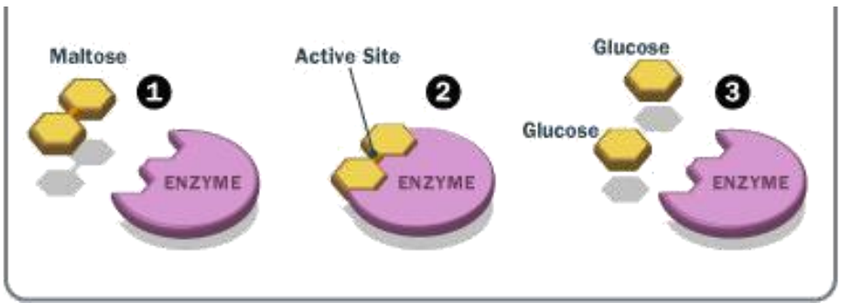
[S] + [E] ----------→ [ES] ----------→ P+ [E]

The formation of an ES complex favors lower activation energy.

Two theories were proposed to explain the mechanism of enzyme action

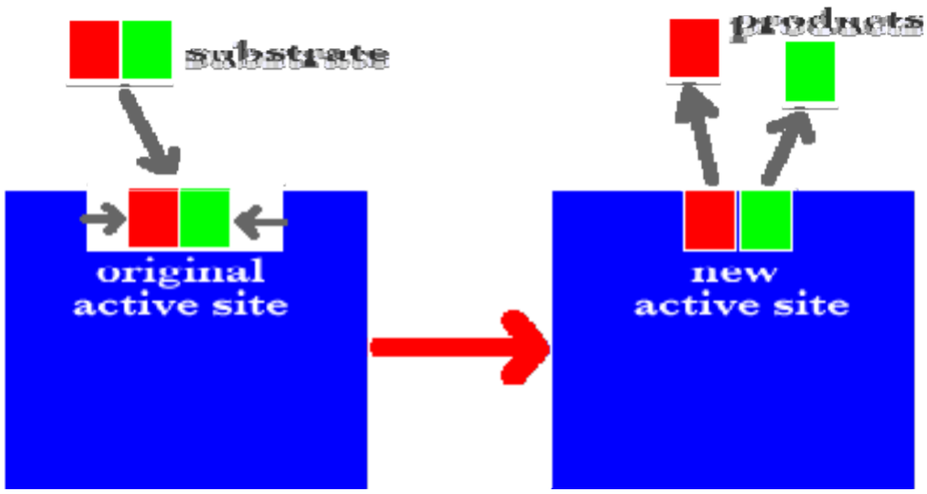
1. *Fischer's lock and key theory (Rigid template model)*

According to this theory proposed by Emil Fischer during 1890s, the active site possesses a unique conformation which is complementary to the structure of the substrate thus enabling the two molecules to fit together in much the same way as a key fits into a lock. An unfortunate feature of this model is the implied rigidity of the catalytic site.



2. *Koshland's induced-fit theory*

Koshland had advocated a theory to account for the specificity of enzymes. He postulated that the essential functional groups on the active site of the free enzyme are not in their optimal positions for promoting catalysis. When the molecule is bound by the enzyme the catalytic groups assume of favorable geometrical position to form the transition state. The enzyme molecule is unstable in this active conformation and tends to revert to its free form in the absence of substrate. In the induced fit model, the substrate induces a conformational change in the enzyme which aligns the amino acid residues or other groups for substrate binding, catalysis or both



Measurement of enzyme activity

The presence or absence of an enzyme is typically determined by observing the rate of
the reaction(s) it catalyzes. The goal of most enzyme assays is to quantitatively measure the amount of enzyme activity present in a sample. Thus, assay results are typically reported in "activity" units. A unit of activity may be defined in various ways, but all such units are ultimately based on rates of substrate consumption and/or product formation. The most common units for expressing catalytic activity are the International Unit (U) and the Katal (Kat). The International Union of Biochemistry (IUB) originally defined a standard unit of enzyme activity (1 U) as that amount of enzyme that catalyzes the formation of 1 μmol product (or the conversion of 1 μmol substrate) per minute under standard conditions. In 1979, the Nomenclature Committee of the IUB recommended the use of the Katal as the fundamental unit of enzyme activity. The Katal is defined as that amount of enzyme that catalyzes the formation of 1 mol product (or the conversion of 1 mol substrate) per second under defined conditions. 1 U = 1/60 micro katal = 16.67 nano katal.

Enzyme kinetics

Factors affecting enzyme activity:

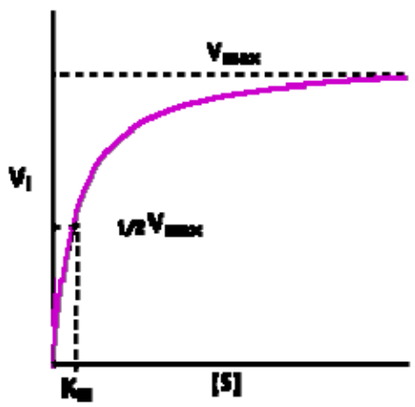
The factors that mainly influence any enzyme-catalyzed reaction are

1. Substrate concentration
2. Enzyme concentration
3. Temperature
4. pH
5. Inhibitors

Other factors such as state of enzyme (oxidation), time and activators also affect enzyme-catalyzed reaction to certain extent.

1. *Substrate Concentration:* It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity. Keeping the factors such as PH, temperature and enzymes concentration at optimum levels, if the substrate concentration is increased, the velocity of the reaction also increases to a certain extent. At very low substrate concentration, the initial reaction velocity (v) is nearly proportional to the substrate concentration (first order kinetics). However, if the substrate concentration is increased, the rate of increase slows down (mixed order kinetics). With a further increase in the substrate concentration the reaction rate approaches a constant (zero order-reaction where velocity is independent of substrate concentration). At initial point, even though the substrate molecules are present in excess than enzyme on molar basis, not all the enzyme molecules present combine with the substrate. Hence, increasing the substrate concentration will increase the amount of enzyme associated with substrate as ES and thus v will depend on [S]. At Vmax, all the enzyme molecules are saturated with substrate molecules so that further increase in [S] cannot result in increased reaction rate. Michaelis-Menten derived an equation to explain this type of behavior.

$$v = \frac{V_{max}[S]}{K_m + [S]}$$



V_i = initial velocity (moles/time)
[S] = substrate concentration (molar)
V_{max} = maximum velocity
K_m = substrate concentration when V_i is one-half V_{max} (Michaelis-Menton constant)

At half maximal velocity [S] = Km

i.e $\frac{Vmax}{2} = \frac{Vmax\,[S]}{Km+[S]}$

$\frac{Km + (S)}{2} = \frac{Vmax\,(S)}{Vmax}$

Km + (S) = 2 (S)

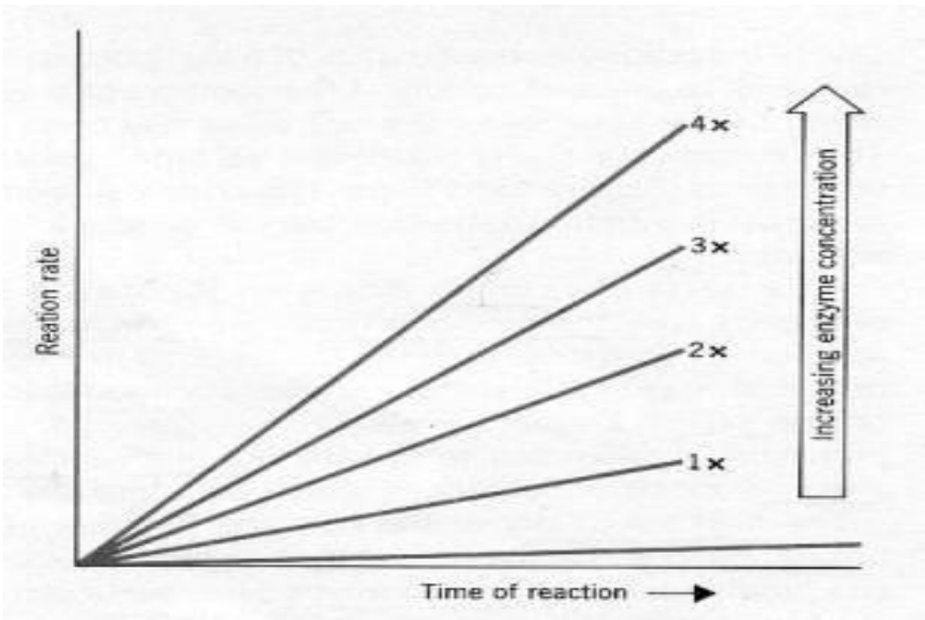
Km = 2 (S) - (S) = (S)

Km = (S)

Hence, Michaelis - Menten constant, Km, is defined as the substrate concentration at half maximal velocity and is expressed as mole per litre.

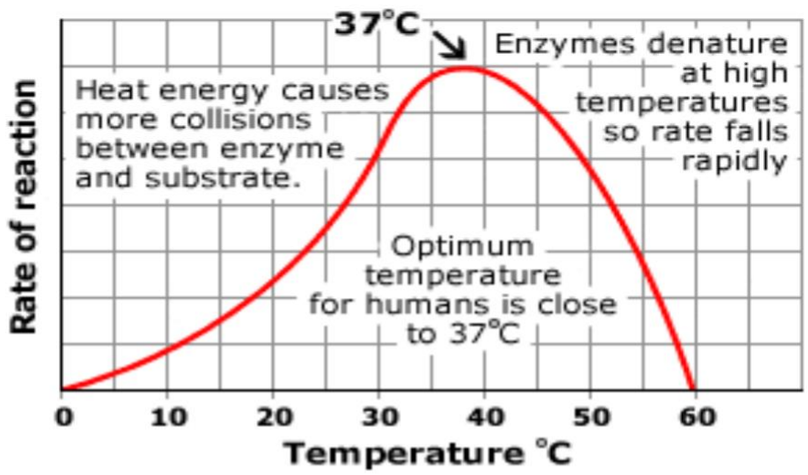
Significance of Km: A small Km indicates that the enzyme requires only a small amount of substrate to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations. A large Km indicates the need for high substrate concentrations to achieve maximum reaction velocity. The substrate with the lowest Km upon which the enzyme acts as a catalyst is frequently assumed to be enzyme's natural substrate, though this is not true for all enzymes.

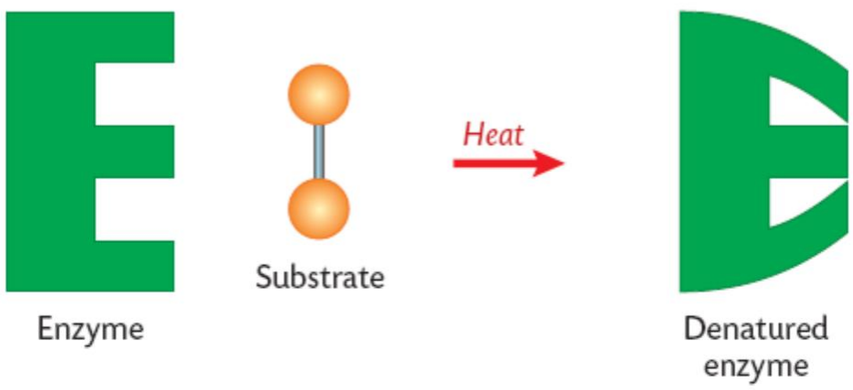
2. Enzyme Concentration: When compared to substrate concentration, the concentration of enzyme is always very low on molar basis. Hence, increasing the enzyme concentration will always increase the reaction rate unless until that the substrate becomes a limiting factor. In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present. Graphically this can be represented as: if substrate concentration is kept constant then the reaction may end as all the substrate is converted to product and no reaction takes place.



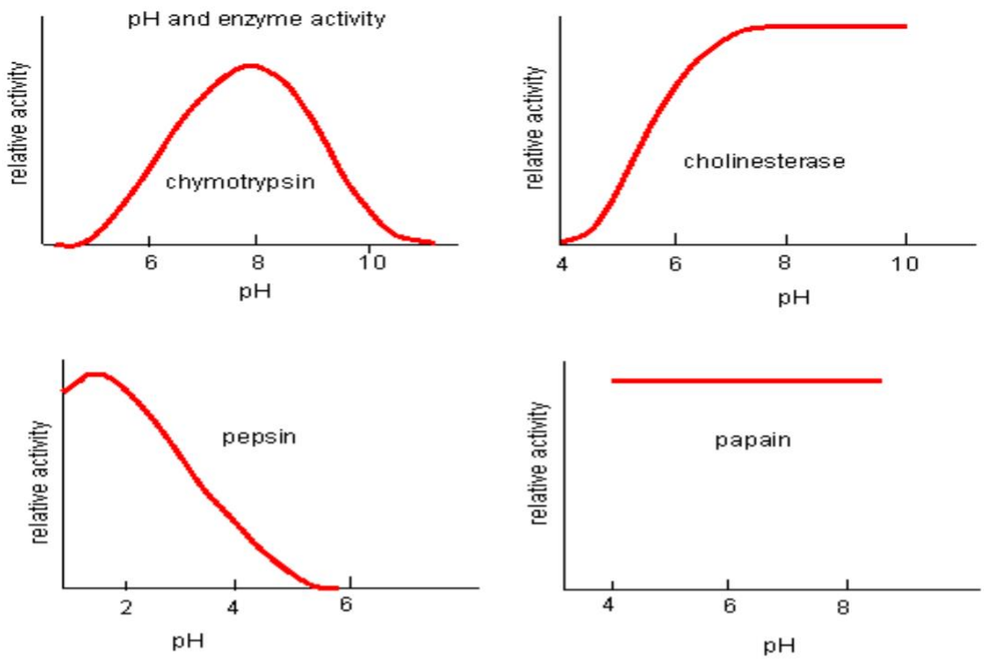
3. Temperature Effect: Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised, because enzymes are inactive below their optimum temperature. The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C.

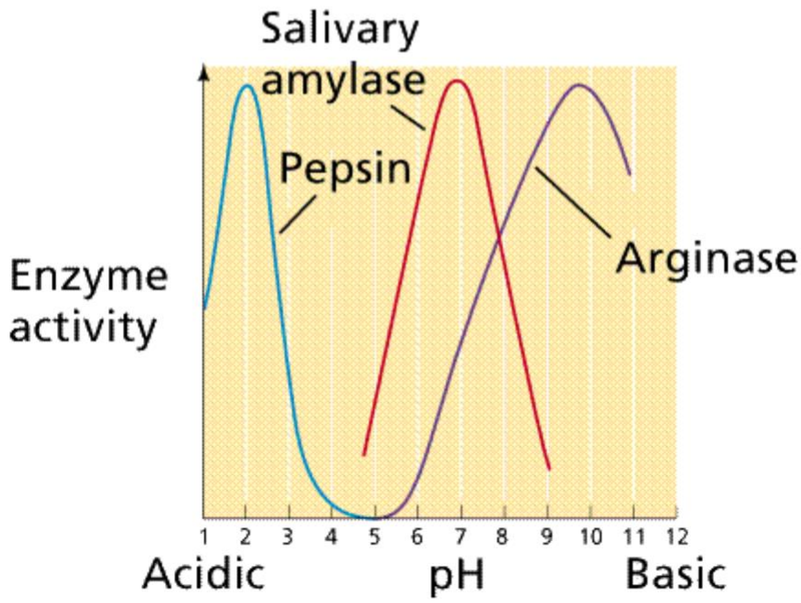
Over a limited range of temperature, the velocity of enzyme-catalyzed reactions roughly doubles with a 10^{o} C rise in temperature.





4. *Effect of pH*: Enzymes are affected by changes in pH. The most favorable pH value, the point where the enzyme is most active is known as the optimum pH. Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. For each enzyme, there is also a region of pH optimal stability. The optimum pH value will vary greatly from one enzyme to another.





In addition to temperature and pH there are other factors, such as ionic strength, which can affect the enzymatic reaction. Each of these physical and chemical parameters must be considered and optimized in order for an enzymatic reaction to be accurate and reproducible.

Enzymes undergo physical changes during the reaction but revert to their original form at the end of the reaction. Enzymes exhibit enormous catalytic power. The rates of enzymatically catalyzed reactions are 10^{7} to 10^{14} times greater than those of the corresponding uncatalyzed reactions and several times greater than those of the corresponding chemically catalyzed reactions. Eg: Carbonic anhydrase enzyme catalyses the conversion of carbon dioxide to carbonic acid.

$$CO_2 + H_2O \rightarrow H_2CO_3$$

In this reaction, each enzyme molecule can hydrate 10^{5} molecules of carbon dioxide per second.

Enzyme activity is regulated in a variety of ways, ranging from control over the amount of enzyme protein synthesized by the cell or modulation of

activity through reversible interaction with metabolic inhibitors and activators or through isoenzymes.

Cofactors

A large number of enzymes require an additional non-protein component to carry out their catalytic functions. Generally these non-protein components are called as cofactors. The cofactors may be either one or more inorganic ions such as Fe^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} or a complex organic molecules called coenzymes. Some enzymes require both coenzyme and one or more metal ions for their activity. Metals are required as cofactors in approximately two thirds of all enzymes. Metalloenzymes contain a definite quantity of functional metal ion that is retained throughout. Metal-activated enzymes bind metals less tightly but require added metals. The distinction between metalloenzymes and metal activated enzymes thus rests on the affinity of a particular enzyme for its metal ion.

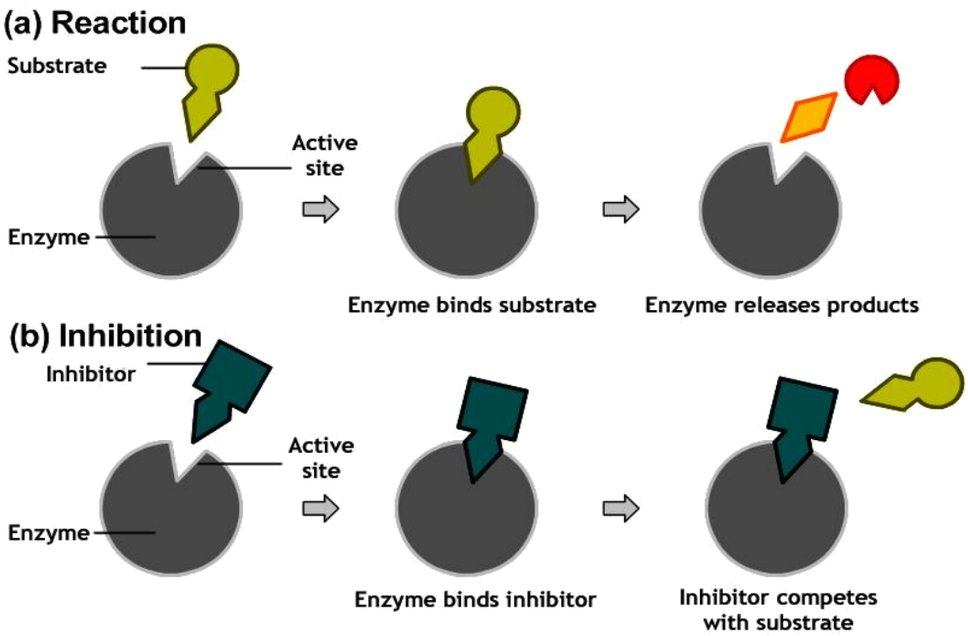
The iron-sulfur enzymes are unique class of metalloenzymes in which the active centre consists of one or more clusters of sulfur-bridged iron chelates. These are of greater importance in plant systems.

Inhibitors

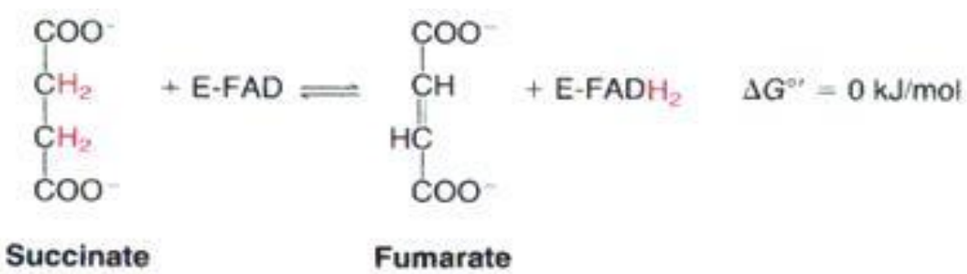
An enzyme inhibitor is a molecule that binds to enzymes and decreases their activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. They are also used as herbicides and pesticides. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically. These inhibitors modify key amino acid residues needed for enzymatic activity. In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind the enzyme, the enzyme-substrate complex, or both.

Enzyme inhibitors also occur naturally and are involved in the regulation of metabolism. For example, enzymes in a metabolic pathway can be inhibited by downstream products. This type of negative feedback slows flux through a pathway when the products begin to build up and is an important way to maintain homeostasis in a cell. Other cellular enzyme inhibitors are proteins that specifically bind to and inhibit an enzyme target. This can help control enzymes that may be damaging to a cell, such as proteases or nucleases. A well-characterized example is the ribonuclease inhibitor, which binds to ribonucleases in one of the tightest known protein–protein interactions. Natural enzyme inhibitors can also be poisons and are used as defenses against predators or as ways of killing prey.

a) *Competitive inhibitor:* Any compound which possesses a close structural resemblance to a particular substrate and which competes with that substrate for the same active site on the enzyme is called as competitive inhibitor. The inhibitor is not acted upon by the enzyme and so remains bound to the enzyme preventing the substrate to bind. This is a reversible process. It depends upon the relative concentration of substrate and inhibitor. Competitive inhibition can be completely reversed by addition of large excess of substrate.



The enzyme, succinate dehydrogenase converts succinic acid to fumaric acid. For this reaction, malonic acid is a competitive inhibitor as it structurally resembles that of succinate.



Glyphosate is a herbicide which resembles phospho enol pyruvate. Glyphosate inhibits EPSP (enoyl pyruvyl shikimate 3 phosphate) synthase involved in shikimate pathway. If shikimate production is affected, lignin synthesis is affected and hence weed is killed.

Kinetics: In competitive inhibiton Vmax is not altered but Km is increased

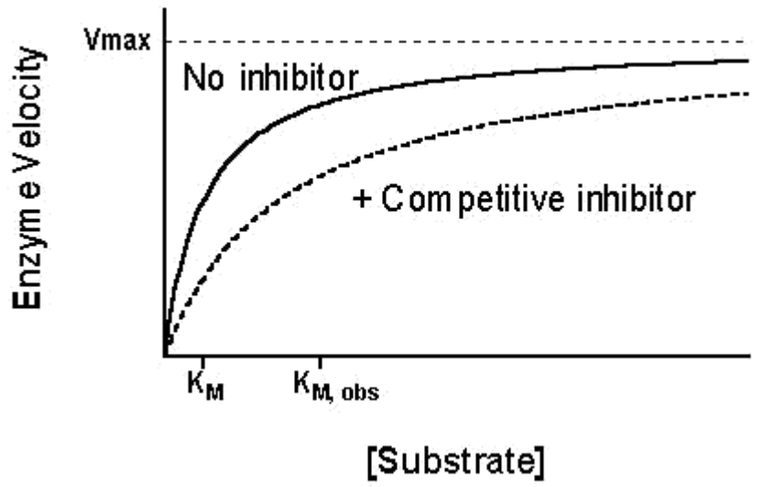
$$E + S \xrightarrow{\quad\quad} [E S] \xrightarrow{\quad\quad} E + P$$

high inhibitor concn.

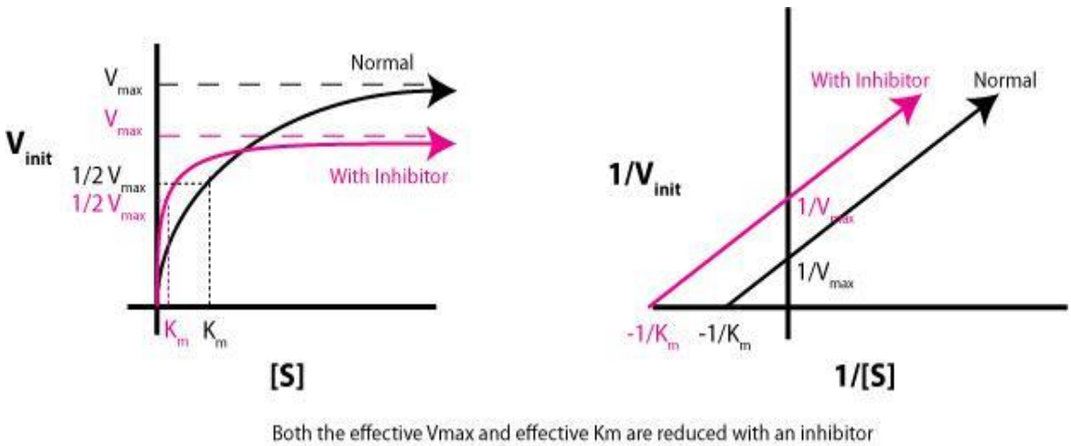
$$E + I \xrightarrow{\quad\quad} E I$$

$$\xleftarrow{\quad\quad}$$

high substrate concn.



b) Non-competitive inhibitor: Non-competitive inhibitors bind to a site other than the active site on the enzyme often to deform the enzyme, so that it does not form the ES complex at its normal rate. Once formed, the ES complex does not decompose at the normal rate to yield products. These effects are not reversed by increasing the substrate concentration.

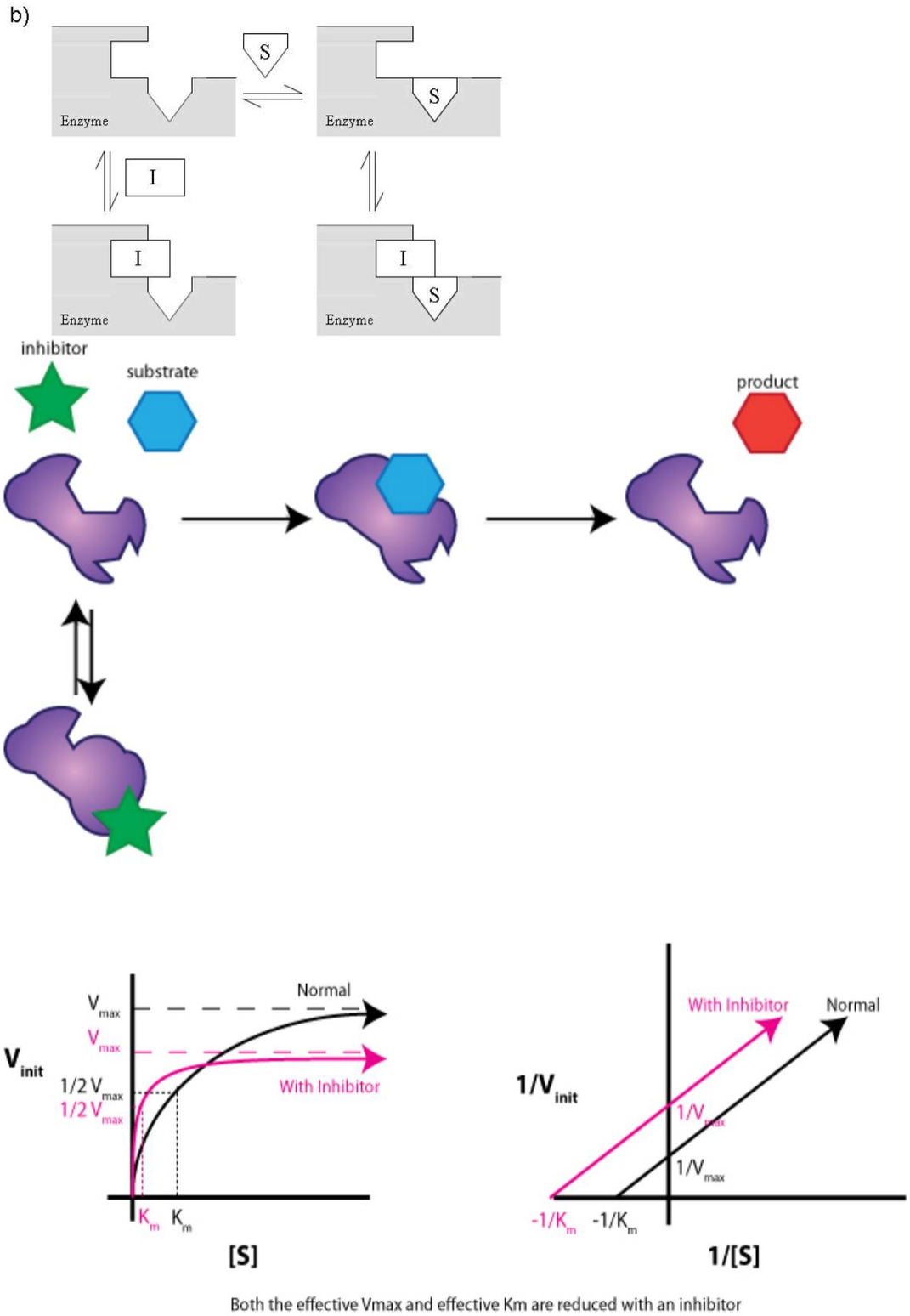


Both the effective Vmax and effective Km are reduced with an inhibitor

E + I -------- EI
ES+I ------- ESI

Some enzymes possessing an essential -SH group are non-competitively inhibited by heavy metal ions (Hg^{2+}, Pb^{2+}). Some metalloenzymes are inhibited non competitively by metal chelating agents like ethylene diamine tetraacetic acid (EDTA).

c) Uncompetitive inhibitors : In this type of inhibition the inhibitor binds to enzyme substrate complex only and not to the free enzyme. It distorts the active site. The dissociation of ES to form product is affected. H_3P0_4 is uncompetitive inhibitor to the enzyme succinyl CoA synthetase which acts on the substrate succinyl CoA.
Kinetics: Both Vmax and Km are decreased.
Inhibitors are used as tools to probe the mechanism of enzyme - catalysed reactions and as therapeutic agents.



Both the effective Vmax and effective Km are reduced with an inhibitor

Isoenzymes

Enzymes which exist in multiple forms within a single species of organism or even in a single cell are called isoenzymes or isozymes. Such multiple forms can be detected and separated by gel electrophoresis of cell extracts. Since they are coded by genes, they differ in amino acid composition and thus in their isoelectric pH values. Lactate dehydrogenase is an example for the isoenzymes which occur as five different forms in the tissues of the human and other vertebrates. All the five isozymes catalyze the same reaction.

$$\text{Lactate} + \text{NAD}^{+} \text{----------} \rightarrow \text{Pyruvate} + \text{NADH} + \text{H}^{+}$$

They have the molecular weight of about 134,000 and contain four polypeptides. The five isozymes consist of five different combinations of

two different kinds of polypeptides M and H. Kinetic study of lactate dehydrogenase isozymes has revealed that although they catalyze the same reaction, they differ significantly in their Km values for their substrates as well as Vmax values. The two polypeptide chains in LDH are coded by two different genes. Skeletal muscle contains four identical M chains and designated as M4; whereas heart muscle contains four identical H chains and designated as H4. LDH of other tissues are a mixture of the five possible forms H4, H3M, H2M2, HM3 and M4. Isoenzymes can be separated by electrophoresis.

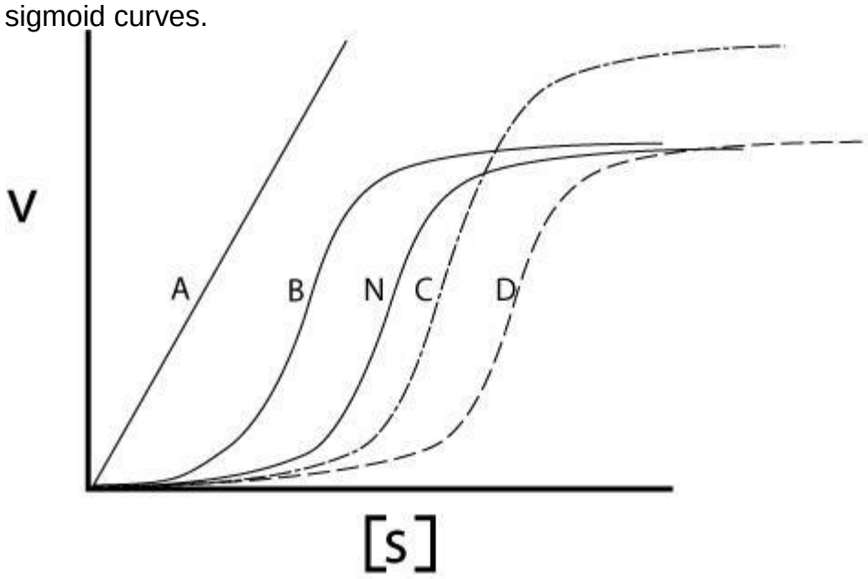
Multienzyme complexes:

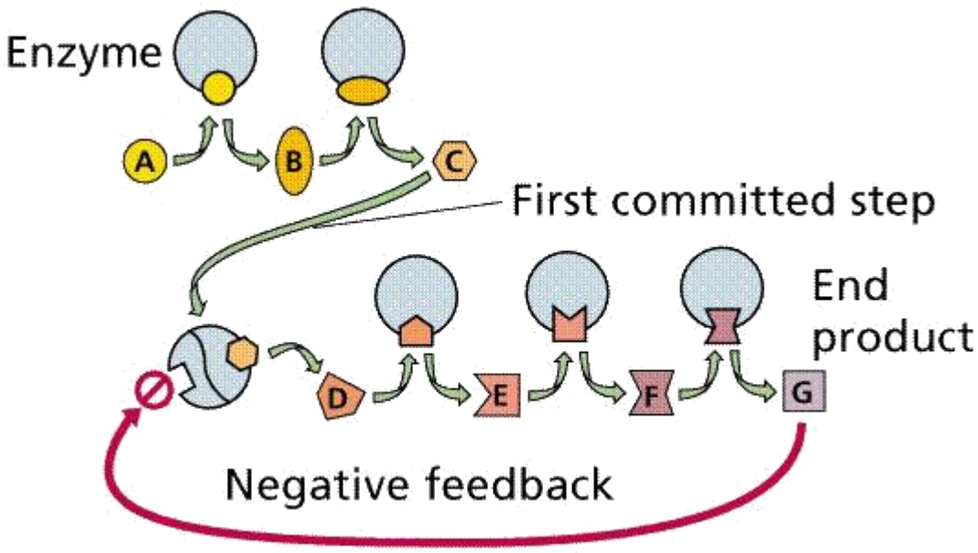
A group of related enzymes participating in a given metabolic pathway is called as multienzyme complex. This system catalyses sequentially consecutive reactions. The reactions are linked by common intermediates so that the product of first enzyme is substrate for the second enzyme. Ex: All the enzymes catalyzing glycolysis will form into a complex called metabol. The enzymes catalyzing synthesis of fatty acid will form a complex of seven proteins ie: Fatty acid synthetase complex

Allosteric enzymes

These enzymes are specialized to serve a regulatory function in addition to their catalytic activity. They have distinct regulatory and catalytic sites. In many multienzyme systems, the end product of the reaction sequence may act as a specific inhibitor of an enzyme at or near the beginning of the sequence. This type of inhibition is called end product inhibition or feed back inhibition or retroinhibition. The first enzyme in this sequence that is inhibited by the end product is called an allosteric enzyme. The term allosteric denotes another space or another structure. Ex: L-Threonine dehydratase, the first enzyme in the synthesis of L-isoleucine is inhibited by the L-isoleucine. This inhibition can adjust continually the rate of synthesis of metabolic intermediates.

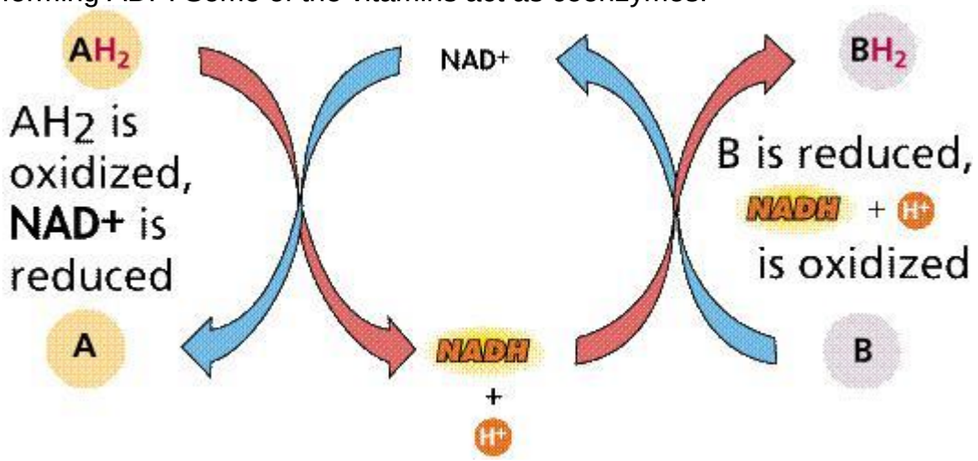
Another interesting feature of allosteric enzymes is that they usually do not show classical Michaelis – Menten kinetic relationships between substrate concentration, Vmax and Km because their kinetic behaviors is greatly altered by variations in the concentration of the allosteric modulator. On the other hand some allosteric enzymes show sigmoid curves.





Coenzymes

The non protein component of the enzyme is called as coenzyme. They are loosely bound to the enzyme or non covalently bound. What ever changes occur on the substrate the opposite changes occur on the co-enzyme. Hence co enzyme are also called as co- substrates. Eg: Glucose is converted to glucose 6 phosphate which is catalysed by hexokinase in presence of co enzyme ATP. Here glucose is gaining a phosphate molecule and ATP is loosing a phosphate and there by forming ADP. Some of the vitamins act as coenzymes.



Vitamins and their coenzyme with their metabolic roles

Vitamin	Coenzyme	Metabolic role
Thiamin B_1	TPP (Thiamin pyro phosphate	Aldehyde group transfer, oxidative decarboxylation
Riboflavin B_2	FMN, FAD flavin mononucleotide, flavin adenine dinucleotide	Hydrogen atoms or electron transfer
Nicotinic acid (niacin)	NAD,NADP nicotinamide adenine dinucleotide/phosphate	Hydrogen atoms or electron transfer
Pantotheni c acid	Coenzyme A	Acyl group transfer
Pyridoxine B_6	PLP	Amino group transfer decarboxylation racemization etc
Biotin	Biocytin	Carboxylation

Folic acid	THFA, FH_4	One carbon transfer
Cobalamin B_{12}	Coenzyme B_{12}	1,2 shift of hydrogen atoms intramolecular migration transmethylation
Ascorbic acid	Ascorbic acid	Cofactor in hydroxylation
Lipoic acid	Lipoic acid	Hydrogen atom and acyl group transfer

Classification of enzymes

The International Union of Biochemistry (IUB) established a commission on enzyme nomenclature to adopt a systematic classification and nomenclature of all the existing and yet to be discovered enzymes. This system is based on the substrate and reaction specificity. Although, this International Union of Biochemistry system is complex, it is precise, descriptive and informative.

IUB system classifies enzymes into six major classes (should be written in specific order only)

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

Again each class is divided into subclasses according to the type of reaction catalysed. Each enzyme is assigned an enzyme commission number (E.C), a recommended name usually a short for everyday use, a systematic name which identify the reaction it catalyses and a classification number which is used where accurate and unambiguous identification of an enzyme is required.

1.Oxidoreductases: These are the enzymes which catalyse oxidation-reduction reactions between two substrates.

Example: Alcohol dehydrogenase enzyme catalyses the conversion of ethyl alcohol to acetaldehyde

$$CH_3\text{-}CH_2\text{-}OH + NAD^+ \dashrightarrow CH3\text{-}CHO + NADH + H^+$$

(reduced) (oxidised) (oxidised) (reduced)

Enzyme: Recommended name Alcohol dehydrogenase

Systematic name Alcohol:NAD^+ oxido-reductase

Enzyme Commission number E.C.1.1.1.1

First digit 1 indicates oxido-reductase (Major class)

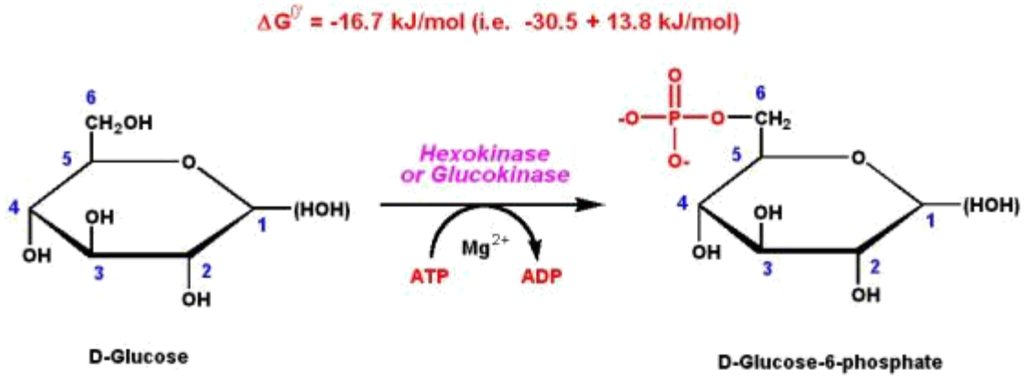
Second digit 1 indicates enzymes acting on CH-OH group of donors (Sub-class)

Third digit 1 indicates NAD^+ as the electron acceptor (Sub-sub class)

Fourth digit 1 indicates the specific enzyme

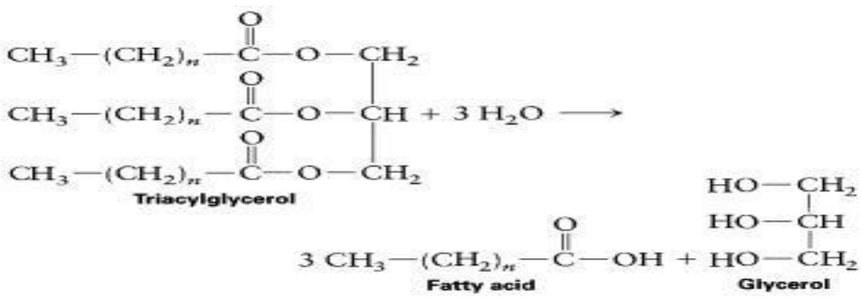
2. *Transferases:* These are the enzymes which catalyze the transfer of a functional group other than hydrogen between substrates.

Example: Phosphorylation of glucose by hexokinase



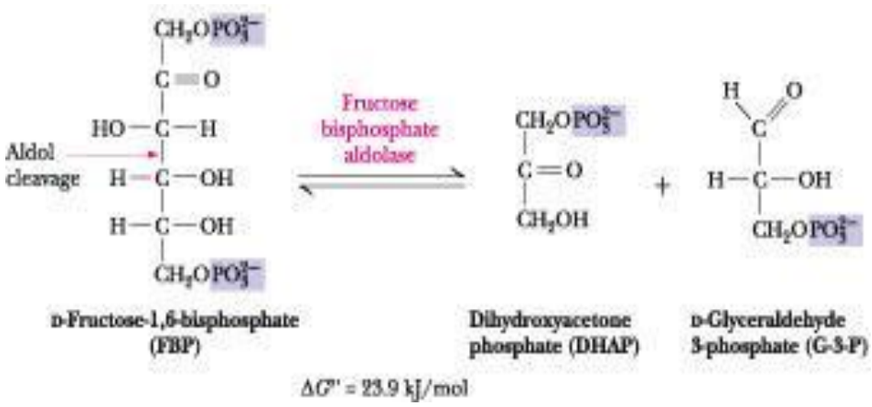
3. Hydrolases: These are the enzymes which catalyze the removal of groups in presence of water. Eg: Hydrolysis of ester, peptide or glycosidic bonds.

Eg : Hydrolysis of triacylglycerol by lipase



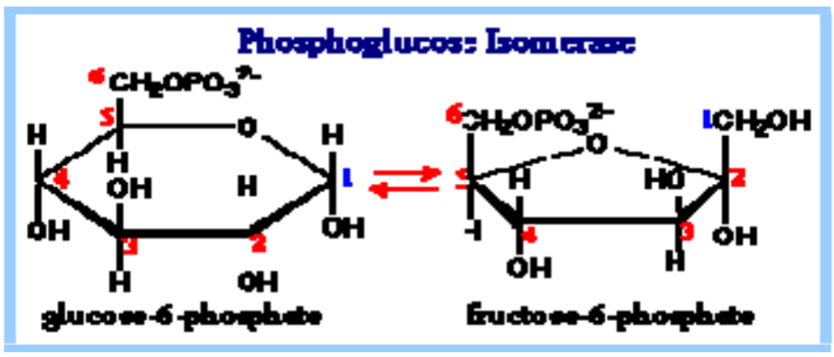
4.Lyases: These are the enzymes which catalyse the removal of groups from substrates by mechanism other than hydrolysis leaving a double bond in one of the products.

Example: Aldolases, decarboxylase, etc

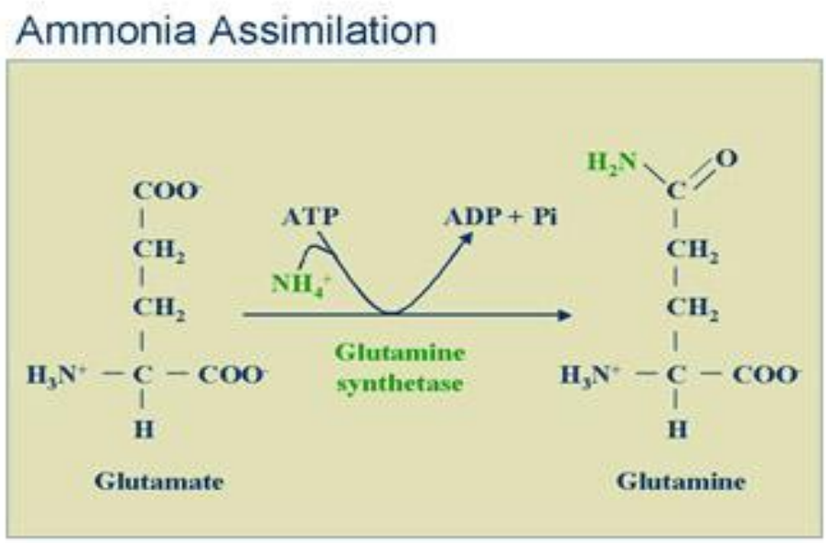


5. Isomerases : These are the enzymes which catalyze the inter conversion of optical, geometrical or positional isomers.

Example:



6. *Ligases:* These are the enzymes which catalyze the joining together of two compounds with the hydrolysis of a high energy compound.



Immobilization of enzymes: Immobilized enzymes are enzymes which may be attached to each other, or to insoluble materials, or enclosed in a membrane or gel. This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again.

Advantages of immobilisation:
1. Easier purification of the product as the separation of the enzymes from the products is easily accomplished.
2. It is easy to recover and recycle the enzymes. This leads to a more economical process.
3. The enzymes remain functional for much longer time as it is a gentler process.
4. Immobilised enzymes are used in bioreactors.

Methods of Immobilization: When immobilizing an enzyme to a surface, it is most important to choose a method of attachment that will prevent loss of enzyme activity. The surface on which the enzyme is immobilized is responsible for retaining the structure in the enzyme through hydrogen bonding or the formation of electron transition complexes. The following methods of immobilization of enzymes are generally used.

a) Carrier-Binding
b) Cross-Linking
c) Entrapping
d) Adsorption

a) *Carrier-Binding:* In this method, the amount of enzyme bound to the carrier and the activity after immobilization depend on the nature of the carrier. The selection of the carrier depends on the nature of the enzyme itself, as well as the particle size, surface area, molar ratio of hydrophilic to hydrophobic groups and chemical composition. Some of the most commonly used carriers for enzyme immobilization are polysaccharide derivatives such as cellulose, dextran, agarose, and polyacrylamide gel.

b) *Cross-Linking:* Immobilization of enzymes has been achieved by intermolecular cross-linking of the protein, either to other protein molecules or to functional groups on an insoluble support matrix. The most common reagent used for cross-linking is glutaraldehyde.

c) *Entrapping:* The entrapment method of immobilization is based on the localization of an enzyme within the lattice of a polymer matrix or membrane. It is done in such a way as to retain protein while allowing penetration of substrate**.**

d) *Adsorption :*In this method the enzyme is attached to a support. Supports can be ceramics, glass, or plastics.

Industrial application of enzymes

1. *Starch hydrolysis*: The traditional acid hydrolysis of starch was completely replaced by α -amylases and glucoamylases, which could convert starch with over 95% yield to glucose. α-amylase cuts rapidly the large alpha- 1, 4-linked glucose polymers into shorter oligomers in high temperature. Glucoamylase hydrolyses the oligomers into glucose. Sometimes additional debranching enzymes like pullulanase are added to improve the glucose yield. β-amylase is commercially produced from barley grains and used for the production of the disaccharide maltose.

2. *Baking:* α -amylases have been most widely studied in connection with improved bread quality and increased shelf life. In addition to starch, flour typically contains minor amounts of cellulose, glucans and hemicelluloses like arabinoxylan and arabinogalactan. The use of xylanases decreases the water absorption and thus reduces the amount of added water needed in baking. This leads to more stable dough. Proteinases can be added to improve dough-handling properties. Glucose oxidase has been used to replace chemical oxidants and lipases to strengthen gluten, which leads to more stable dough and better bread quality.

3. *Detergents:* Detergents were the first large scale application for microbial enzymes. Bacterial proteinases are still the most important detergent enzymes. Lipid degrading enzymes were introduced in powder and liquid detergents. Lipases decompose fats into more water soluble compounds by hydrolyzing the ester bonds between the glycerol backbone and fatty acid. Amylases are used in detergents to remove starch based stains. In textile washing, cellulases remove cellulose microfibrils.

4*. Pulp and Paper:* The major application is the use of xylanases in pulp bleaching. Xylanases liberate lignin fragments by hydrolyzing residual xylan. This reduces considerably the need for chlorine based bleaching chemicals. Paper making enzymes are used especially in modification of starch, which is used as an important additive. Starch improves the strength, stiffness and erasability of paper. The starch suspension must have a certain viscosity, which is achieved by adding amylase enzymes in a controlled process.

5. *Leathe:* Leather industry uses proteolytic and lipolytic enzymes in leather processing. The use of these enzymes is associated with the structure of animal skin as a raw material. Enzymes are used to remove unwanted parts. Alkaline proteases are added in the soaking phase. This improves water uptake by the dry skins, removal and degradation of protein, dirt and fats and reduces the processing time. In dehairing and dewooling phases enzymes are used to assist the alkaline chemical process. This results in a more environmentally friendly

process and improves the quality of the leather (cleaner and stronger surface, softer leather, less spots). The used enzymes are typically alkaline bacterial proteases.

6*. Animal feed industry:* Enzymes are used in the preparation of poultry feed, pig feed and turkey feed.

LIPIDS

Lipids are non polar substances of biological origin that are sparingly soluble in water, but soluble in organic solvents like chloroform, hexane, benzene etc

Classiciation of lipids:

Lipids can be classified based on various criteria. A widely accepted criterion for lipid classification is their constituent units. Accordingly, the lipids are classed into two major groups.

I. Simple or non saponifiable lipids: Lipids belonging to this class can not be hydrolyzed (saponified) to form simpler units. They do not have any backbone too.

S. No.	Sub-Class
1	Terpenes
2	Steroids
3	Prostaglandins

Terpenes

Terpenes are made up of isoprene units. Terpenes are the most widespread class of biochemical substances.

Steroids

Steroids are predominant mostly in eukaryotes. They are derivatives of a ring referred to as cyclopentano perhydro phenanthrene made up of three six and one five membered fused rings. Substitution and opening up of the five membered ring forms different steroids.

The most talked about steroid is cholesterol because of the direct relationship between the incidence of cardiovascular problems and the level of serum cholesterol in human beings. Plants however, contain very little cholesterol. Cholesterol is the metabolic precursor of many sex hormones

Prostaglandins

Prostaglandins are synthesized from the USFA arachidonic acid (20:4, $\Delta^{5,8,11,14}$ all *cis*). Prostaglandins are of significance in mammals in lowering blood pressure, controlling inflammatory response in skin and joints, and induction of labor etc.

II. Complex or saponifiable lipids - Lipids belonging to this class can be hydrolyzed (saponified) into two or more constituent units. They are further sub-classified depending upon their backbone constituent.

S. No.	Sub-Class	Backbone
1	Acyl glycerols	Glycerol

2	Phosphoglycerides	Glycerol-3-phosphate
3	Sphingolipids	Sphingosine
4	Waxes	Long chain monohydroxy alcohols

Acyl glycerols:

Acyl glycerols are made up of the alcohol glycerol and fatty acids. Glycerol is a trihydroxy alcohol with the following structure.

Fatty acids

Fatty acids are the carboxylic acids with a hydrocarbon chain. They form the most important constituent of lipids. They contain normally 12 to 26 C atoms; however, 16 and 18 C fatty acids are the most common. Most fatty acids contain an even number of carbon atoms. They may be linear or branched or may have cyclic groups. They may have additional functional groups like OH, C=O (keto) etc. They may be saturated (SFAs) with no double bond or unsaturated (USFAs) containing up to six double bonds. SFAs up to 8 C atoms are liquids at room temperature, while those with more than 8 C atoms are solids. The melting point of fatty acids therefore increases with increase in chain length.

In USFAs, the double bonds are of *cis* type and separated from each other by one or more CH_2 groups. Those USFAs with more than one double bond are referred to as polyunsaturated fatty acids (PUFA). The position of the double bond is indicated by a superscript Δ followed by the number of C atom counting from the carboxyl end. Among the USFAs, oleic and linoleic acids are the most abundant.

The USFAs are more condensed in length than the SFAs of equal chain length. The double bonds give the fatty acid molecules a sharp bend. The double bonds thus prevent tight packing within the membranes. This behavior of USFAs does have profound biological significance. Further, the introduction of double bonds in fatty acids decreases the melting point(M.P) of the fatty acids. Thus,

Fatty acid	**M.P**
18 C saturated fatty acid – Stearic acid	$69^{o}C$
18 C unsaturated fatty acid – Oleic acid (one double bond)	$13^{o}C$
18C unsaturated fatty acid – Linoleic acid (two double bonds)	$-17^{o}C$

Chemical structure & Names of Some common fatty acids

Fatty acid and chemical Structure	**Abbreviation**	**Systematic name**
Myristic acid $CH_3(CH_2)_{12}COOH$	14:0	Tetradecanoic acid
Palmitic acid $CH_3(CH_2)_{14}COOH$	16:0	Hexadecanoic acid
Stearic acid $CH_3(CH_2)_{16}COOH$	18:0	Octadecanoic acid
Arachidic acid $CH_3(CH_2)_{18}COOH$	20:0	Eicosanoic acid

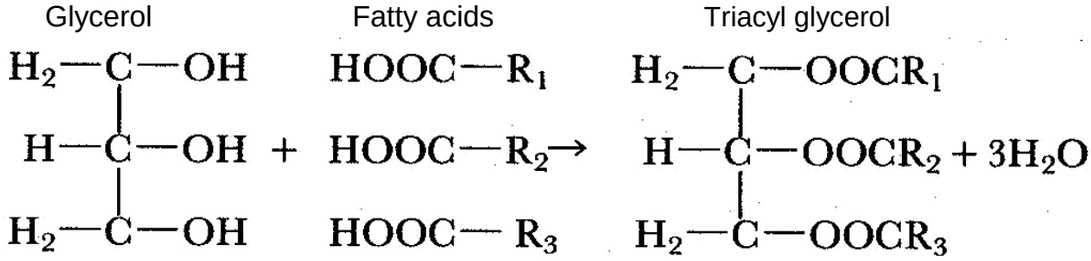
Palmitoleic acid $CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	16:1, $\Delta^{9\ cis}$	9 – Hexadecenoic acid
Oleic acid $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	18:1, $\Delta^{9\ cis}$	9 – Octadecenoic acid
Linoleic acid $CH_3(CH_2)_4CH=CH\ CH_2CH=CH\ (CH_2)_7COOH$	18:2, $\Delta^{9,12}$ both *cis*	Octadecadienoic acid
α- Linolenic acid $CH_3\ CH_2\ CH=CH\ CH_2CH=CH\ CH_2CH=CH\ (CH_2)_7COOH$	18:3, $\Delta^{9,12,15}$ all *cis*	Octadecatrienoic acid
Ricinoleic acid $CH_3(CH_2)_5CHOHCH_2CH=CH(CH_2)_7COOH$	18:1 $\Delta^{9\ cis}$ 12-OH	12- Hydroxyoctadecenoic acid

Two USFAs viz. linoleic and linolenic acid are not synthesized by mammals and are therefore important dietary requirements for good health and growth. They are called Essential Fatty Acids. They have to be derived from plants sources.

Acyl glycerols:

In acyl glycerols, one or two or three OH groups of glycerol remain esterified by fatty acids (RCOOH) to form mono -, di -, and triacyl glycerols (MAG, DAG and TAG), respectively.

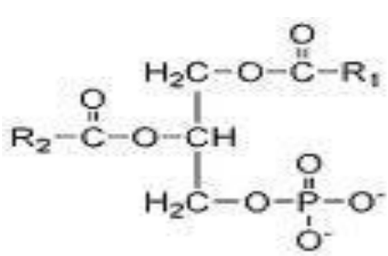
Glycerol Fatty acids Triacyl glycerol



Mono-, di- and triacyl glycerols are commonly referred to as mono-, di-and triglycerides, respectively. They occur as liquids or solids depending upon their constituent fatty acids. Triacyl glycerols include fats (solids at room temperature) and oils (liqiuds at room temperature). MAG and DAG are components of many biological membranes.

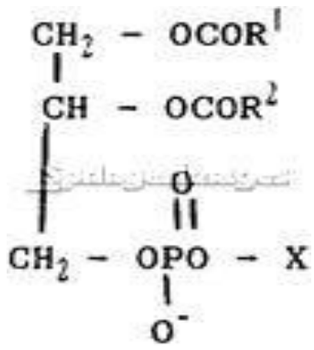
Phosphoglycerides

They are widespread in nature. The parent compound is phosphatidic acid – glycerol 3- phosphate in which esterification of C1 and C2 OH are by fatty acids and that of C3 OH by phosphoric acid.



Phosphatidic acid (R^1 and R^2 are fatty acid derivatives)

The phosphate in turn remains joined (esterified) to a range of substances containing OH groups forming various phosphoglycerides. Thus,



if X is	the phophoglyceride is
H	phosphatidic acid
choline $HOCH_2CH_2N^+(CH_3)_3$	phohatidyl cholines (lecithins)
ethanolamine $HOCH_2CH_2NH_2$	phohatidyl ethanolamines (cephalines)
serine $HOCH_2CHNH_2COOH$	phohatidyl serines
Glycerol $CH_2OHCHOHCH_2OH$	Phohatidyl glycerols

Phosphoglycerides are amphipathic molecules with a non polar aliphatic tail and a polar phosphoryated head. In a bilayer arrangement these form the major components of biological membranes within which proteins remain embedded.

Sphingolipids

Sphingolipids are also important components of membranes. They however, do not contain glycerol. Instead they contain an 18 C amino alcohol called sphingosine or its few derivatives. Sphingolipids are important components in nerve cell membranes where they protect and insulate the nerve fibres.

Waxes

Waxes are water insoluble substances present on the surface of plant and animal bodies. They include a variety of compounds such as hydrocarbons, alcohols, ketones, and carboxylic acids and most importantly the esters of long the chain monohydroxy alcohols (24 to 28 C) with fatty acids (20 to 24 C). Waxes are used as carriers in cosmetics and a variety of coloring materials. Waxes contain many unusual fatty acids.

Waxes are water repelling and impart a protective function against degradation by water. Shining appearance of fruits, leaves, and flower petals is often due to the presence of waxes. Lotus leaves contain high amounts of waxes. Examples of natural waxes are bees wax, jojoba wax, ear wax etc.

Functions of lipids:

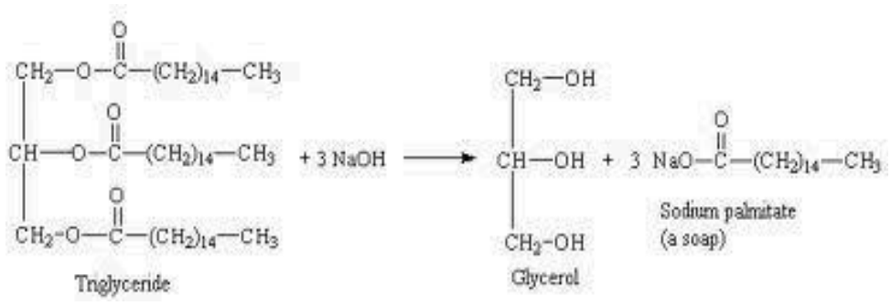
Lipids encompass a very large and diverse group of biological substances and so they perform a wide ranging functions too. Some major functions of lipids are as follows.

1. Lipids act as reservoir of energy in biological systems. Being more reduced than carbohydrates, lipids can store more energy. The most important storage form of lipids is the triacyl glycerols stored in the oil bodies in plant seeds and adipose tissues in animals.
2. Lipids act as the major components of biological membranes. The most Important class of lipids in this regard is the amphipathic phospholipids with a small hydrophilic head and a long hydrophobic tail arranged in a bilayer form.
3. Some lipids act as members of electron transport system in inner mitochondrial membrane viz. ubiquinone and also phosphorylation systems in thylakoid membrane.
4. Lipids act as carriers of sugars viz. dolichol in the biosynthesis of glycoproteins.
5. Lipids materials are used for the biosynthesis of certain hormones in animals & plants.
6. Lipids in the form of bile acids (e.g. cholic acid) help in the digestion and absorption of other lipids.
7. Triacyl glycerols act as heat insulating materials.

Analytical properties of lipids:

Saponification

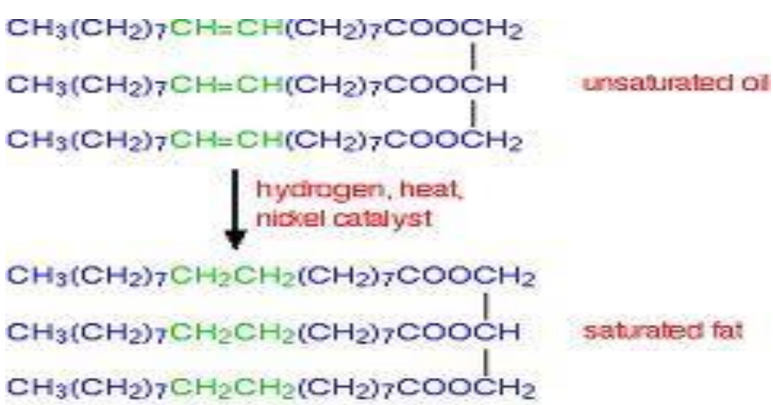
The term 'Saponification' refers to the alkaline hydrolysis of saponifiable lipids - most importantly the fats and oils. In the case of fats and oils, the products are glycerol and the salts of the respective fatty acids. Thus,



R^1 COONa, R^2 COONa, and R^3 COONa, are the sodium salts of the fatty acids. The reaction is utilized in the making of soaps.

Hydrogenation

Hydrogenation refers to the addition of hydrogen to the double bonds of the fatty acids in acyl lipids like triacyl glycerols. The double bonds thus get saturated and the constituent fatty acids are converted to the appropriate saturated fatty acids. Thus,



The addition of hydrogen makes the unsaturated fatty acids saturated and the liquid oil gets converted to a solid fat. The reaction process is used industrially in the making of Vanaspathi from edible or even unconventional oils.

Saponification number

Saponification Number is defined as the number of milligrams of KOH required to saponify 1 g fat or oil. Saponification Number indicates the molecular weight or chain length of the fatty acids present. If the number is high, the molecular weight or chain length of the fatty acids is low and *vice versa*.

Iodine value / number

Fats and oils do have double bonds in their fatty acids and thus respond to the addition of iodine. The latter adds to the double bonds as follows.

Iodine Number is defined as the number of grams of iodine absorbed by 100 g fat or oil. Iodine Number of fats / oils / acyl lipids indicates their degree of unsaturation. If the number is high, the degree of unsaturation is also high i.e. more USFAs are present.

Acid value / number

Acid Number/Value is defined as the number of milligrams of KOH required to neutralize the free fatty acids present in 1 g fat or oil. The free fatty acids may arise due to chemical or microbiological decomposition of the oils or fat during storage i.e. rancidity. Acid Number / Value is used to assess the degree of spoilage (rancidity) of a fat or oil.

Industrial applications of acyl lipids:

1. Soaps and detergents: Soaps and detergents are the most important cleansing agents in personal hygiene, hair care, dish washing and laundry. . Soaps are the alkali metal salts of fatty acids. Since all of them contain an acyl group, acyl lipids mostly in the form of unconventional vegetable oils are used in the manufacture of soaps and detergents.

2. Paints: The paint industry uses acyl lipids in the form of vegetable oils as carriers of colorizing substances. Such oils are drying oils and the special feature is that coloured organic substances are readily dispersed / dissolved in such oils. After application to the surface, the oil gets oxidized by oxygen to form a thin film of solid layer keeping the colorizing matter also in the form of a thin film along with. Among such oils, the linseed oil is the most important. Such oils used in making paints must be highly unsaturated.

3. Rubber

Natural rubber is obtained from the latex (milky secretion) of a tree *Hevea brasiliensis*. There are few other species also. Rubber is highly elastic, water repellent, resistant to weak acids and alkalies, tough, impermeable, adhesive and insulator. These characteristics make rubber a very useful in industries.

Natural rubber in its latex form however, as such can not be used. To make it usable, it has to be vulcanized. In other words, the naturally occurring polyterpene rubber forms the basic material for the manufacture of better usable synthetic rubber.

Biodiesel

Biodiesel is a partial substitute for petroleum diesel and can be blended with the latter. Chemically, biodiesel is monoalkyl esters of fatty acids

produced from vegetable oils by transesterification. In India, biodiesel is produced from the oils of Jatropha (wild castor, *Jatropha* curcus), Karanj (*Pongamia pinnata*) and few other species. Soybean oil, rape seed oil etc are also used.

Major advantages of biodiesel

(1) Easily biodegradable

(2) Environmentally safe

(3) Low fume emissions

(4) Nontoxic and does not contain lead

(5) Low Green House Gas emissions

(6) The automobile engines need not be modified

(7) The byproduct is glycerol, an important industrial chemical

(8) Calorific value is comparable to petroleum diesel

Major constraints in the use of biodiesel

1. Availability of water for growing the crop, though water requirement is relatively less 2. Availability of land.

CARBOHYDRATES

Carbohydrates are the most abundant biomolecules on earth. Each year, photosynthesis converts more than 100 billion metric tones of carbon dioxide and water into cellulose and other plant products. Carbohydrates are polyhydroxy aldehydes or ketones or substances that yield such compounds on hydrolysis. Many but not all carbohydrates have the empirical formula $(CH_2O)n$. Some carbohydrates also contain nitrogen, phosphorous or sulfur. So, the carbohydrates are defined as polyhydroxy aldehydes or ketones or their condensation products or derived products. The basic condensing bond is glycosidic bond.

Functions of carbohydrates:

1. Carbohydrates such as sugar and starch are dietary staple in most parts of the world and the oxidation of glucose is the central energy yielding pathway in most cells.
2. They are the reserve or storage forms of energy in plants (starch, inulin) and in animals (Glycogen)
3. The insoluble carbohydrate polymers serve as structural and protective elements in the cell walls of bacteria and plants and in connective tissue of the animals. i.e., they give structural rigidity. Eg: cellulose in cell wall, chitin in insects and mucopolysaccharides in bacteria.
4. They are important components of nucleidc acids, coenzymes and flavoproteins (ribose)

Classification

Carbohydrates vary greatly in size ranging from smaller glyceraldehyde molecule to larger starch molecule. Large carbohydrate molecules are polymers of small molecules. The carbohydrates have been classified based on the degree of polymerization into

Monosaccharides: These are the simple sugars which cannot be further broken down.

Oligosaccharides: They are composed of short chains of monosaccharides.

Polysaccharides: They are composed of several monosaccharide units.
Conjugated polysaccharides: In addition to carbohydrates, these polysaccharides contain proteins and lipids also.

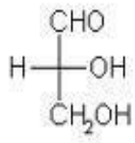
Monosaccharides: They cannot be further hydrolysed under normal conditions of hydrolysis. They are further classified based on the number of carbon atoms present in the monosaccharide into trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) and heptoses (7C).

Based on the functional group, they are classified as aldoses and ketoses depending on whether they have aldehyde or ketone as functional group.

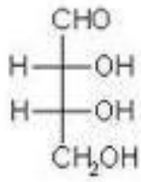
	Aldoses	**Ketoses**
Triose	Glyceraldehyde	Dihydroxy acetone
Tetrose	Erythrose	Erythrulose
Pentose	Ribose, Xylose, Arabinose	Ribulose, Xylulose
Hexose	Glucose, Galactose, Mannose	Fructose
Heptose		Heptulose

Pentoses and hexoses are the predominant type of monosaccharides found in nature.
Some of the biologically important aldoses and ketoses are shown in the figure.

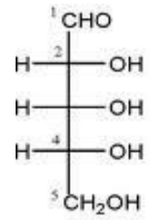
Aldoses



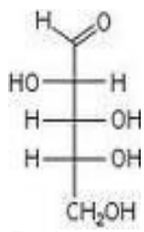
D-Glyceraldehyde



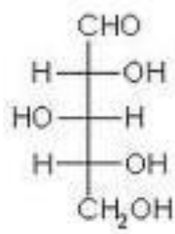
D- Erythrose



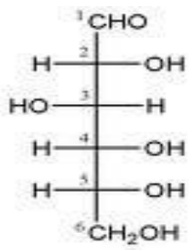
D-Ribose



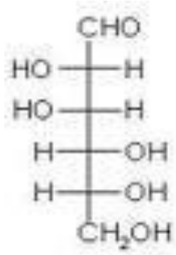
D-Xylose



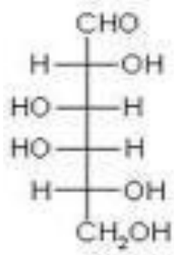
D-Arabinose



D-Glucose

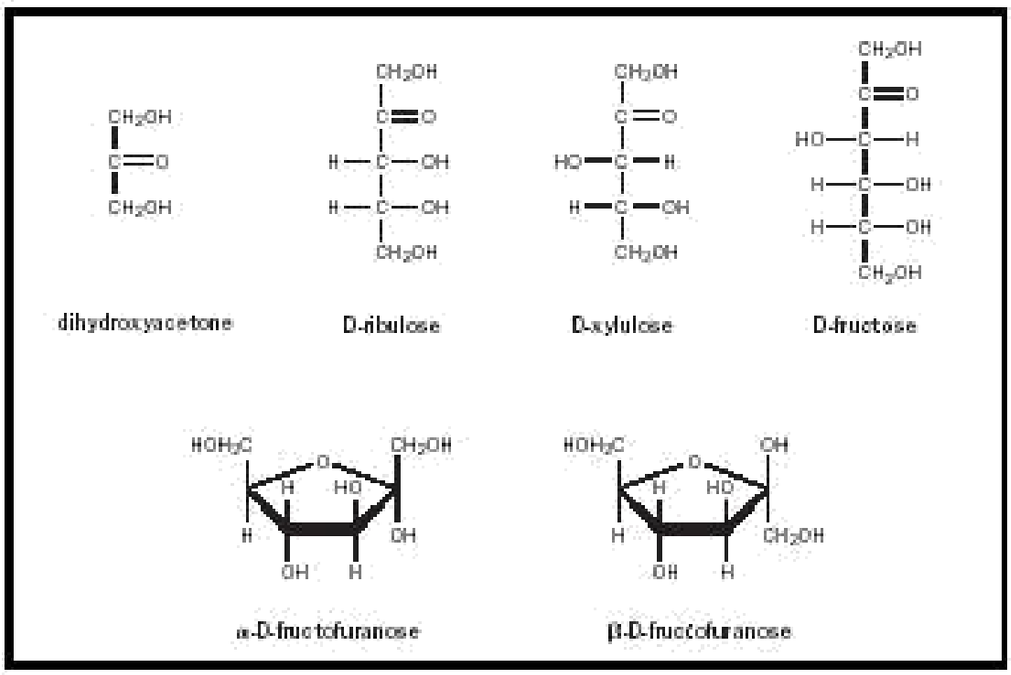


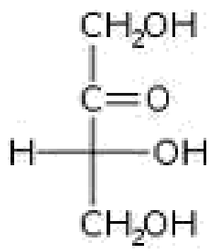
D-Mannose



D-Galactose

Ketoses



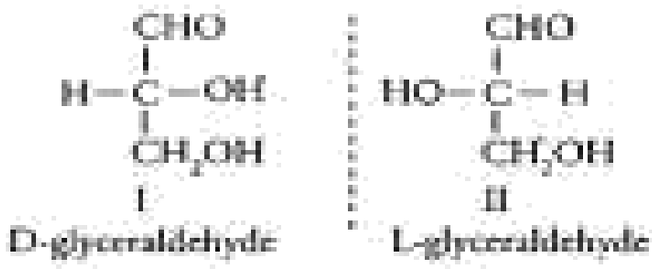


D- Erythrulose

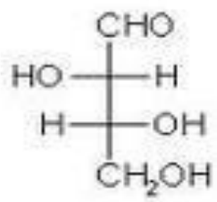
Isomerism

a) *Stereoisomerism:* Most of the monosaccharides contain the same number of atoms and the same kinds of groups, yet they are definitely distinct substance. For example, the formula $C_6H_{12}O_6$ represents 16 different simple sugars, all possessing the structure CH $_2$OH. CHOH CHOH CHOH CHOH CHO. This is due to different arrangement of the constituent groups of the molecule in space. This phenomenon is called as stereoisomerism and these sugars are called as stereoisomers. For example; Glucose, mannose & galactose are stereoisomers. When there are several asymmetric carbon atoms in a chain molecule and the end groups are not identical, the number of stereoisomers possible is equal to 2^n where n is the number of asymmetric carbon atoms. Thus there are 16 stereoisomers possible corresponding to the formula CH_2OH. CHOH CHOH CHOH CHOH CHO, which contains four asymmetric carbon atoms(2^n).

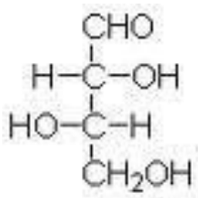
Monosaccharides belong to D or L series depending on the position of OH group on the penultimate carbon atom. If OH is towards right side of the penultimate carbon atom it is called as D sugar and if OH is towards left side of the penultimate carbon atom it is called as L sugar. Glyceraldehyde, the simplest sugar is used as a reference compound for representing D & L forms of sugars. The structures of D and L glyceraldehydes are shown in the figure.



D & L forms of sugars which are non super imposable mirror images of each other are called *enantiomers.* Eg: D& L Threose molecules as shown in figure



D-Threose

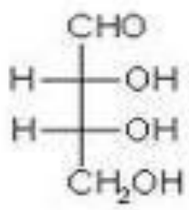


L-Threose

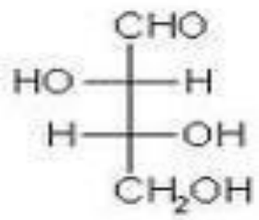
In nature, D-sugars are more widely distributed than L-sugars.

The stereoisomers which are not mirror images of each other are called *diastereomers*

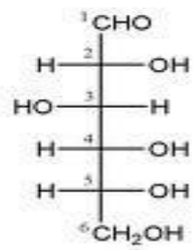
Eg: 1) D-Erythrose & D- Threose 2) D- Glucose, D- Mannose & D- Galactose as shown in figure



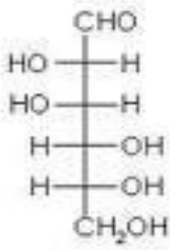
D-Erythrose



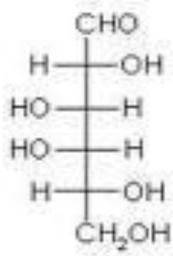
D-Threose



D-Glucose



D-Mannose



D-Galactose

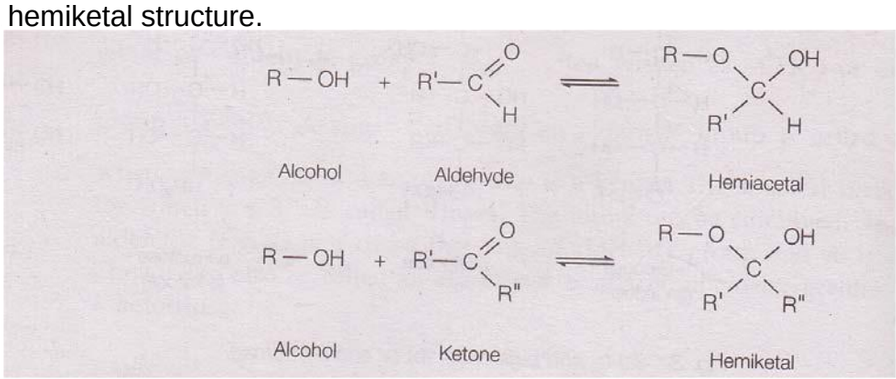
Among the diastereoisomers, those which differ in configuration at a single carbon atom are called *epimers*. Mannose is an epimer of glucose at 2 nd carbon atom whereas galactose is an epimer of glucose at 4th carbon atom whereas galactose & glucose bear no epimeric relationship. The structural formulae of these sugars are shown above.

a) *Structural isomerism:* Some compounds have same molecular formula but different structural formulae. For example Glucose, galactose & mannose have same molecular formula but different structures and hence they are called structural isomers.

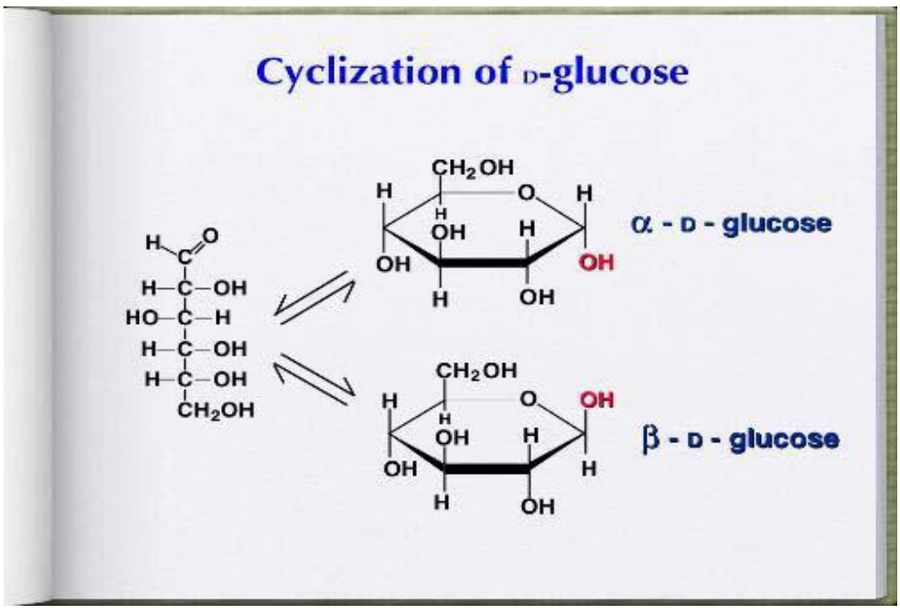
C)*Functional isomerism:* Glucose and fructose have same molecular formula but glucose is an aldose while fructose is a ketose. This kind of isomerism is called Functional isomerism.

d) *Optical isomerism:* Carbohydrates exhibit another kind of isomerism called optical isomerism. It is shown by the compounds having an asymmetric carbon. They have same molecular and structural formulae but differ in their behaviour towards plane polarized light. An optical isomer rotating the plane of polarized light toward right is called dextrorotatory 'd' (+) while one rotating the plane of polarized light toward left is called levorotatory 'l' (-).

Ring Structures: The aldehyde or ketone group of a monosaccharide can react with a hydroxyl group to form a covalent bond. Formally, the reaction between an aldehyde and the hydroxyl group of a sugar (an alcohol) creates a hemiacetal structure whereas a ketone reacts with hydroxyl group of a sugar (alcohol) to form a hemiketal structure.



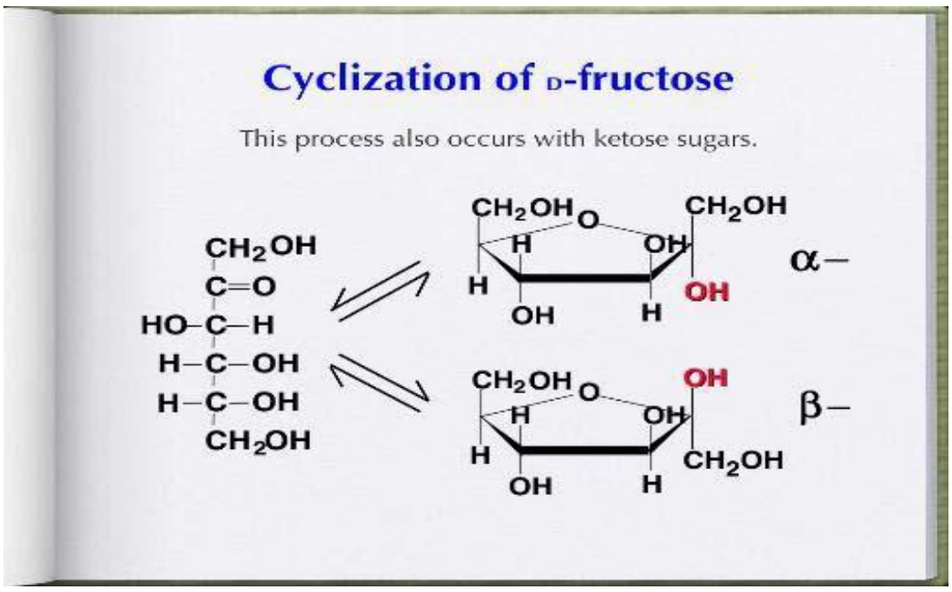
For tetroses and larger sugars, the reaction can take place within the same molecule so that the straight chain form of the sugar cyclizes. The following figure shows the cyclization of D-glucose to form a six-carbon ring.



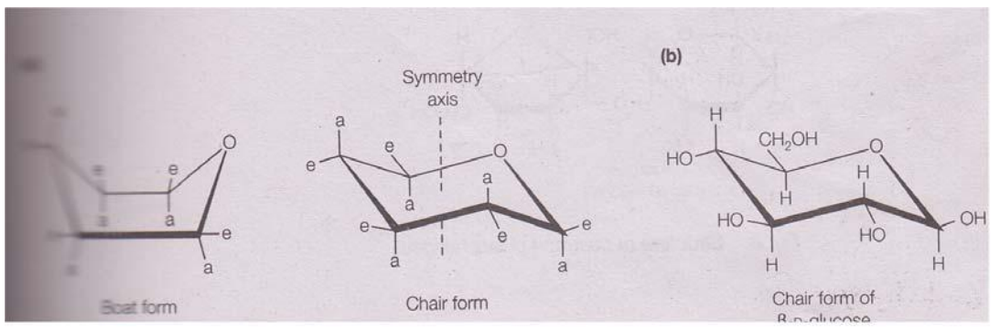
A new asymmetric center is formed during cyclization of an aldehyde at C-1. Thus two isomers of glucose exists as α – D-Glucose in which OH group at C-1 lies below the plane of the ring and β-D-Glucose in which the OH group at C-1 lies above the plane of ring. The C-1 carbon is called *anomeric* carbon atom and the alpha and beta forms are called anomers. In aqueous solution the alpha and beta forms are rapidly inter convertible via the open chain structure to give an equilibrium mixture and this is termed as *mutarotation*. Because of the structural similarity to the ring compound called pyran, the six membered ring structures of hexoses are called pyranoses.

Five membered sugars such as D-ribose and D-deoxyribose and six carbon ketose sugars such as D-fructose, form rings called furanoses as their structures are similar to the furan ring. Again the furanoses can exists both in

alpha and beta forms except here the nomeneclature refers to the hydroxyl group attached to C-2 which is the anomeric carbon atom.



The pyranose ring of a six-carbon aldose sugar can exist in either a **boat or a chair** configuration. The substituents attached to the ring carbons that extend parallel to the symmetry axis are said to be axial (a) whilst those that extend outward from this axis are said to be equatorial (b) . In the boat form, there is considerable steric hindrance between the various groups attached to the carbon atoms of the ring and therefore this form is less favorable energetically. Hence the chair form predominates, as shown for β-D-glucose where all the axial positions are occupied by hydrogen atoms.



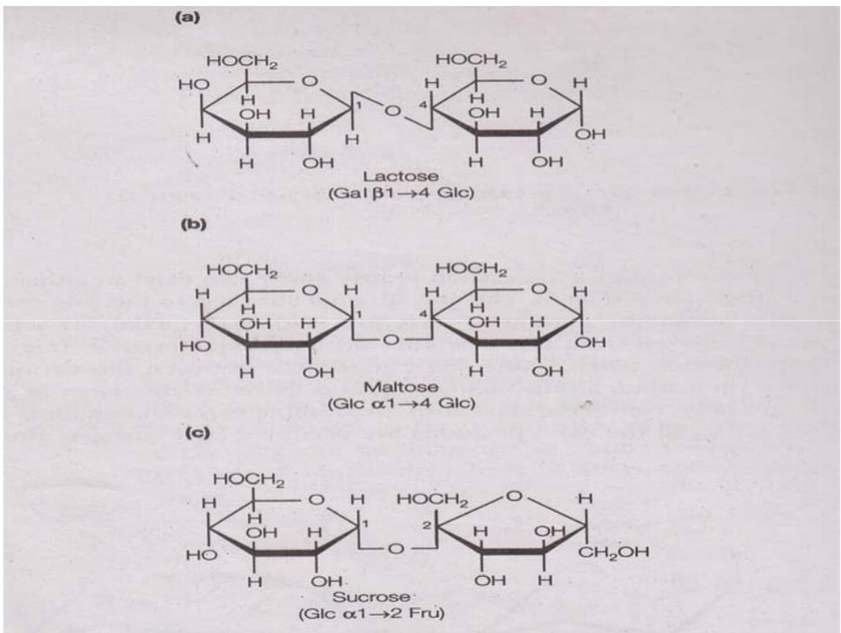
Oligosaccharides: The monosaccharides condense with each other through glycosidic linkage to form oligosaccharides. The oligosaccharides are further classified depending upon the number of monosaccharide units present.

Disaccharides: The hydroxyl group on the anomeric carbon atom of one monosaccharide can react with the hydroxyl group of a second monosaccharide to form a disaccharide. The covalent bond formed is called a glycosidic bond.

Eg: ***a)*** *Lactose*: It is a disaccharide formed between the anorexic carbon C-1 of β-D-galactose and C-4 of α- D-glucose. Since the anomeric carbon of galactose molecule is involved in the bond and is in the beta-configuration, this is called β (1↱4) bond which can be abbreviated as β1↱4.

b) *Maltose*: It is a disaccharide formed between the C-1 and C-4 positions of two α- D glucose units. However, here the configuration of the anomeric carbon atom involved is the alpha form and hence the bond is called an α (1↱4) bond or abbreviated as α1↱4. For lactose and maltose, one of the anomeric carbons has been used to form the bond, leaving the second anomeric carbon free. Thus both lactose and maltose have a reducing end. Hence they are called as reducing disaccharides.

c) Sucrose: It is a disaccharide formed by glycosidic bond formation between the anomeric C-1 of α- D - glucose and the anomeric C-2 of β-D fructose so that sucrose lacks a free reducing group. Thus sucrose is a non reducing disaccharide. It is formed by condensation of Glucose & Fructose



Trisaccharides: Three monosaccharide units condense with each other to form trisaccharides.
Eg: Raffinose is formed by condensation of Galactose , Glucose & Fructose

Polysaccharides: Many monosaccharide units condense to form polysaccharides through glysosidic linkage.

Polysaccharide classification:
They are classified depending on the function, nature of branching and repeating unit.

1. Functional classification:
a. Structural polysaccharide: Polysaccharides belonging to this class help in maintaining the cell structure.
Eg: Cellulose, chitin, Hemicellulose, pectin.
b. Storage polysaccharide: Polysaccharides belonging to this class help in storing carbohydrate material in the cell.
Eg: Starch, glycogen, inulin.

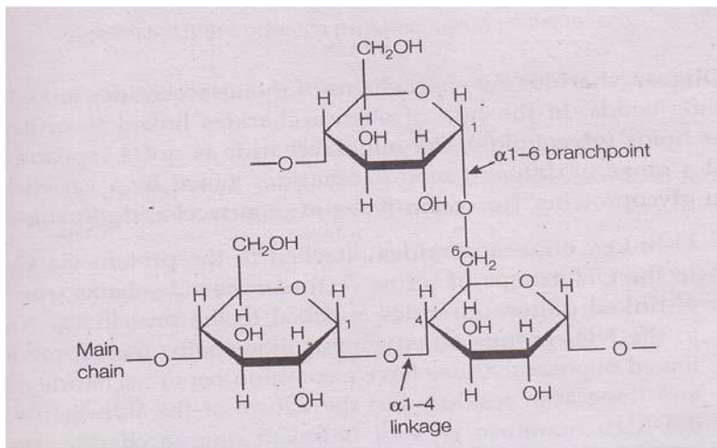
2. Nature of branching:
a. Linear: Polysaccharides belonging to this class have a linear glycosidic bonding only. Eg: Cellulose, chitin, amylose
b. Branched: Polysaccharides belonging to this class have a branched glycosidic bonding. Eg: Starch, amylopectin, glycogen.

3. Repeating Unit:
a. Homopolysaccharide: Polysaccharides belonging to this class contain the same basic repeating monosaccharide unit. Eg:Starch, glycogen, chitin, inulin
b. Heteropolysaccharides: Polysaccharides belonging to this class contain more than one basic repeating unit. Eg: Hemicellulose, pectin.

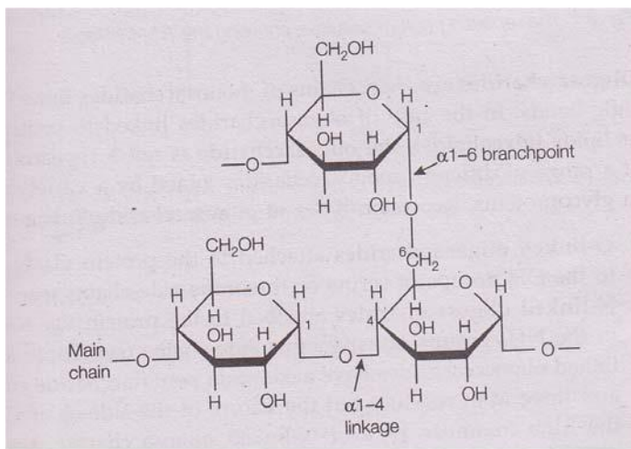
Polysaccharides are long chains of sugar units joined together. Depending on the polysaccharide, the chains may be linear or branched. In plants, the storage form of glucose is the polysaccharide called as starch where as in animals excess glucose is stored as a large branched polysaccharide called glycogen. These polysaccharides serve as nutritional reserves and when

required they are broken down and the monosaccharide products are metabolized to yield energy. In contrast, cellulose is present in cell walls and behaves as a structural polysaccharide.

***Starch*:** Starch exists in plants as insoluble starch granules in the cytoplasm. Each starch molecule contains a mixture of two polysaccharide forms, amylose and amylopectin. Amylose is unbranched polymer of glucose residues joined in α 1⇗4 linkages. Amylopectin is the branched form in which most of the glucose residues are joined in α 1⇗4 linkages but additional α 1⇗6 bonds occur at every 25-30 residues creating the branch points.

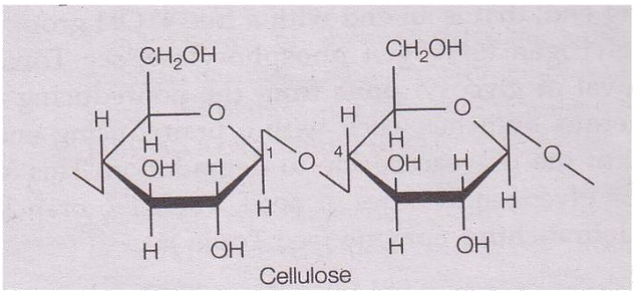


Glycogen: Glycogen molecule consists of glucose units which are linked in long chains by α1⇗4 bonds. For every 10 units or so, the chain is branched by the formation of α 1⇗6 glycosidic bond. The glycogen chain terminates in a non reducing end with a free 4'-OH group. Since the enzyme that degrades glycogen catalyzes the removal of glycosyl units from non reducing end of glycogen chain, the numerous branches, each with a non reducing end, greatly increase the accessibility of the polysachharide to degradation. The α1⇗6 branches are removed by debranching enzyme.



Dextran is a glucose polymer where the glucose residues are mainly linked by the α 1⇗ 6 bonds. A few branches also occur which is formed by α1⇗2, α1⇗3 or α 1⇗4 bonds depending on the bacterial or yeast species that is the source of dextran.

Cellulose: Cellulose is an unbranched polysaccharide of glucose units linked by β 1⇗4 bonds. The glucose residues in cellulose are arranged as straight fibrils. In plant cell walls, the cellulose fibrils are embedded in a matrix of other polysachharides. In wood, the matrix also contains lignin, a complex polymer of phenolic residues. Mammals including humans, lack enzymes capable of digesting the β1⇗4 linkages of cellulose and so cannot digest plant cell walls.



Conjugated polysaccharides: Besides occurring in free state, the carbohydrates occur in nature in conjugation with other biomolecules like lipids and proteins to form glycolipids and glycoproteins. Mucopolysaccharides are glycoproteins characterized by the presence of amino sugars like glucosamine, galactosamine.Eg: Hyaluronic acid & Heparin.

Industrial uses:

Monosaccharides, oligosaccharides and polysaccharides are used in number of industries as listed below:

Monosaccharides

1. Glucose and fructose are used as energy source
2. Liquid glucose is widely used in the confectionary, bakery, and jam preparation, canning and leather industries.
3. Glucose can be fermented to biofuel ethanol.
4. Liquid dextrose is used in fermentation industries, for the manufacture of dextrose monohydrates, fructose and sorbitol syrups.
5. Sorbitol syrup is widely used in tooth paste, pharmaceuticals, cosmetics and tobacco industries.
6. Fructose is used as sweetener in beverages, sport drinks and also used as a flavoring agent.
7. Fructose is used in cosmetic and pharmaceutical industry

Oligosaccharides

1. Sucrose is used in confectionery industry and in desserts.
2. Sucrose is used in preservation of foods.
3. Sucrose is used in cosmetic and pharmaceutical industry.
4. Maltose is used in baby food industry.

Polysaccharides

1. Food industry: Starch plays a leading role in determining the texture of many foods and texture is of vital concern to both the consumers and the manufacturers. Starch finds numerous uses in the baking industry for the production of cakes, cookies, in ice-cream preparations etc
2. Paper industry: In Paper industry, a large quantity of starch is consumed as a surface-sizing agent, as a binder, as a paper coating agent etc. Starch is used in the manufacture of various adhesives or glues for book-binding, wall paper adhesives, gummed paper, envelop adhesives, school glues and bottle labeling
3. Textile industry: In textile industry, starch is used in sizing to strengthen the warp yarn, in finishing and changing the appearance of fabric after it is bleached, dyed or printed. Starch is used as a component in finishing agent to glaze and polish sizing thread. Clothing starch or laundry starch is a liquid that is prepared by mixing a vegetable starch in water.
4. Pharmaceutical industry: Starch is used as an excipient, a binder in medications to aid the formation of tablets

5. Printing industry : In the printing industry, food grade starch is used in the manufacture of anti-set-off spray powder used to separate printed sheets of paper to avoid wet ink being set off.
6. Plastic industry: Starch is used to produce various bioplastics, synthetic polymers that are biodegradable.

NUCLEIC ACIDS

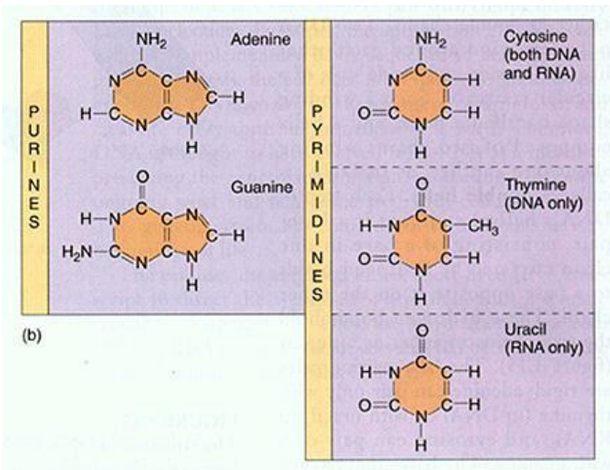
Nucleic acids were first discovered in 1868 by Friedrich Meischer. Nucleic acids are high molecular weight polymers which store and transfer genetic material from generation to generation. Knowledge of how genes are expressed and how they can be manipulated is becoming increasingly important for understanding nearly every aspect of biochemistry. These macromolecules are present in all living cells. Nucleic acids fall into two main classes according to the type of sugar they contain: the Deoxyribonucleic acids (DNA) & Ribonucleic acids (RNA).

Functions of nucleic acids:

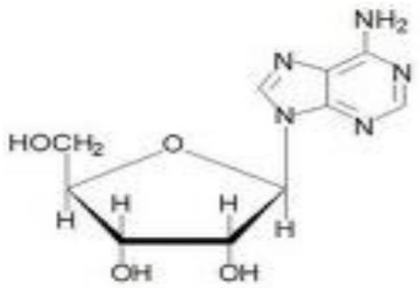
a) DNA stores and transmits genetic information
b) DNA expresses its encoded genetic information for the synthesis of RNA and protein for metabolic function.
c) DNA controls all cellular activities.
d) RNA is necessary for protein biosynthesis.

Nucleic acids are polymers of repeating units called nucleotides. Nucleotides are composed of Nitrogenous base, sugar and phosphoric acid. Nucleotides in nucleic acids are linked by 3'5'phospho diester linkages.

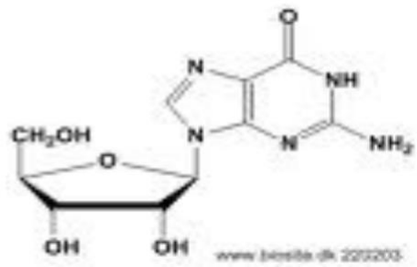
Nitrogen Bases: The five bases present in nucleic acids have carbon - nitrogen ring structures, hence they are called Nitrogen bases. There are two types of ring structures purines and pyrimidines. Adenine and Guanine are called purine nitrogen bases and Thymine, Cytosine and Uracil are called pyrimidine nitrogen bases. Pyrimidine nitrogen base present in both RNA & DNA is Cytosine, present only in RNA is Uracil and present only in DNA is Thymine.



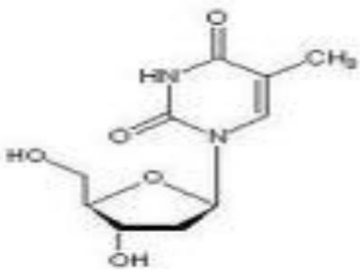
Nucleosides*:* The nitrogen bases are combined with ribose/deoxyribose to form nucleosides. In RNA, the nucleosides have ribose as sugar component and are called ribonucleosides, whereas they are called deoxyribonucleosides in DNA as the sugar in DNA is deoxy ribose. Nucleoside is formed by forming a bond between C_1 of β sugar and N_1 of the pyrimidine base or N_9 of the purine base. This linkage is called as β - N glycosidic linkage



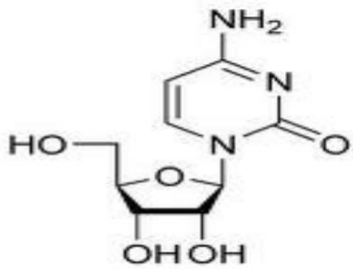
Adenosine



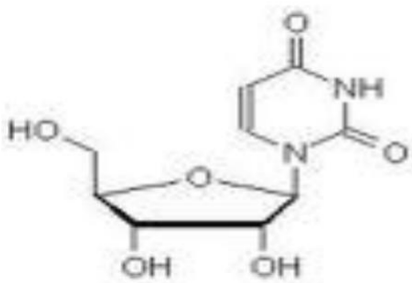
Guanosine



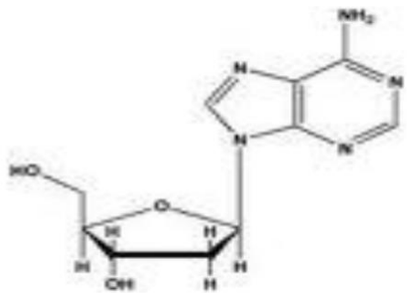
Thymidine



Cytidine

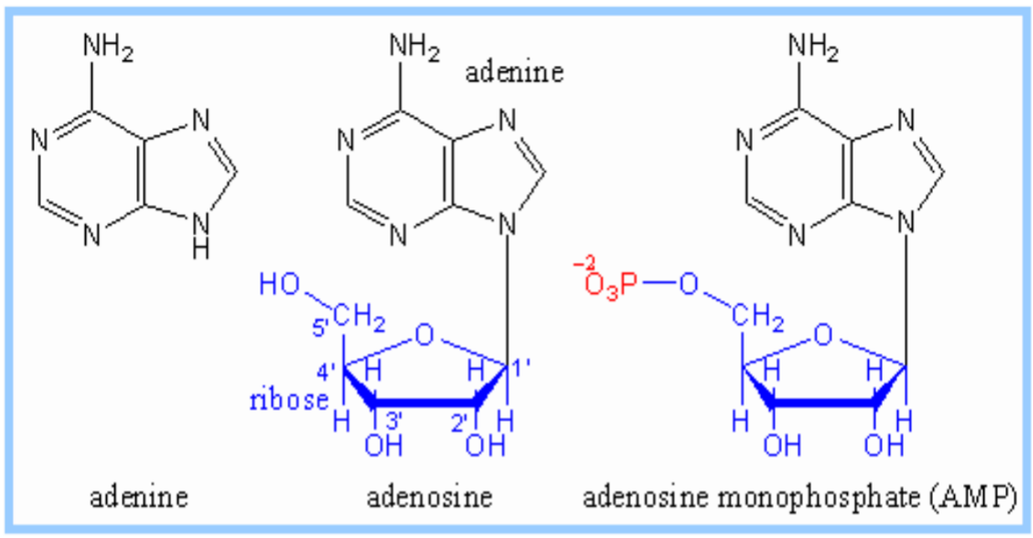


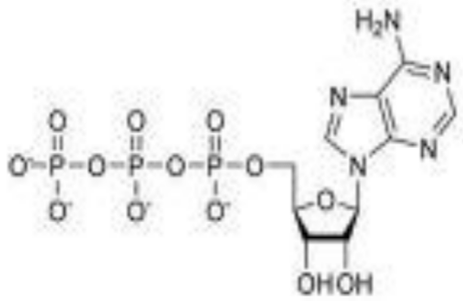
Uridine



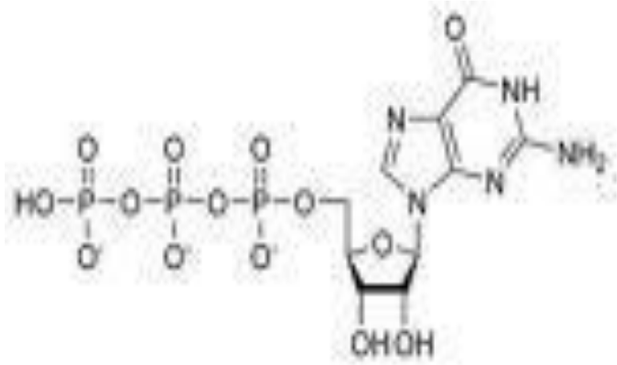
Deoxyadenosine

Nucleotide*:* A nucleotide is a phosphate ester of nucleoside. It consists of a phosphate group joined to a nucleoside at hydroxyl group attached to the C_5 group of the sugar that is 5'-nucleotide. In DNA, the nucleotides have deoxyribose as the sugar and hence are called deoxyribonucleotides. In RNA, the nucleotides have ribose as sugar moiety and hence are called ribonucleotides. Deoxyribonucleotides & ribonucleotides can have a single phosphate group, two phosphate groups or three phosphate groups.

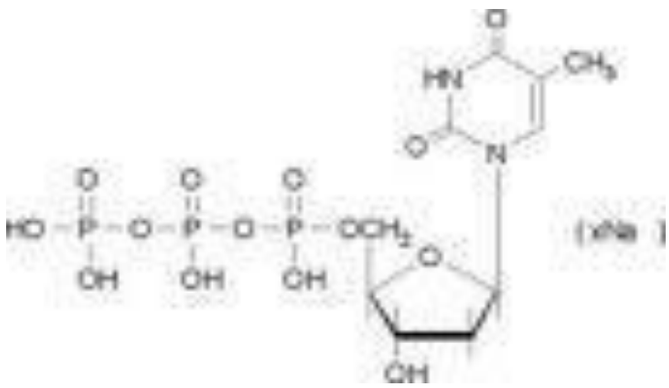




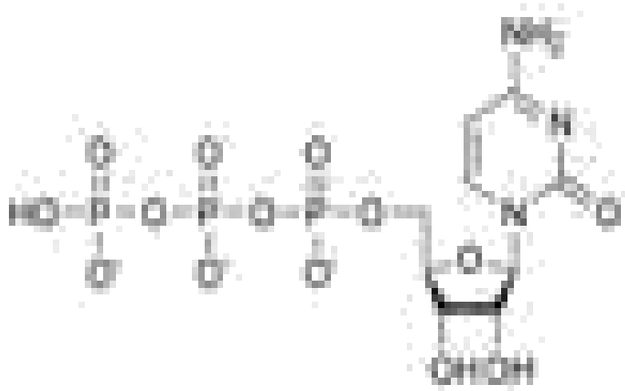
Adenosine triphosphate



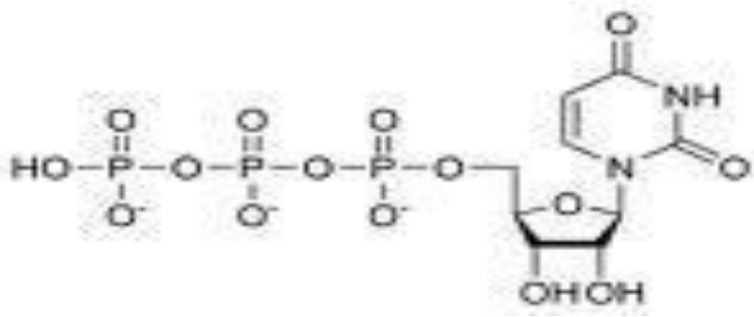
Guanosine triphosphate



Thymidine triphosphate



Cytidine triphosphate

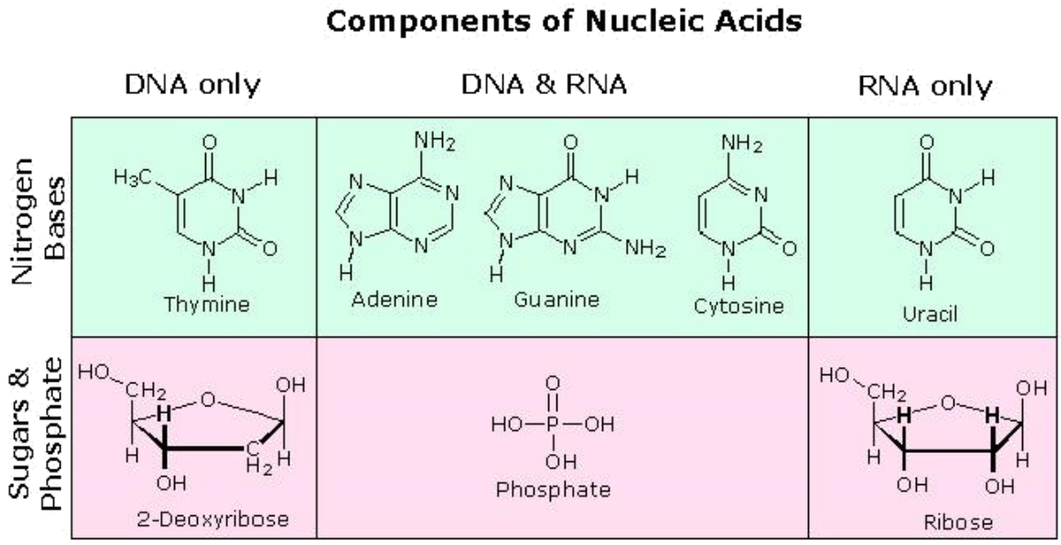


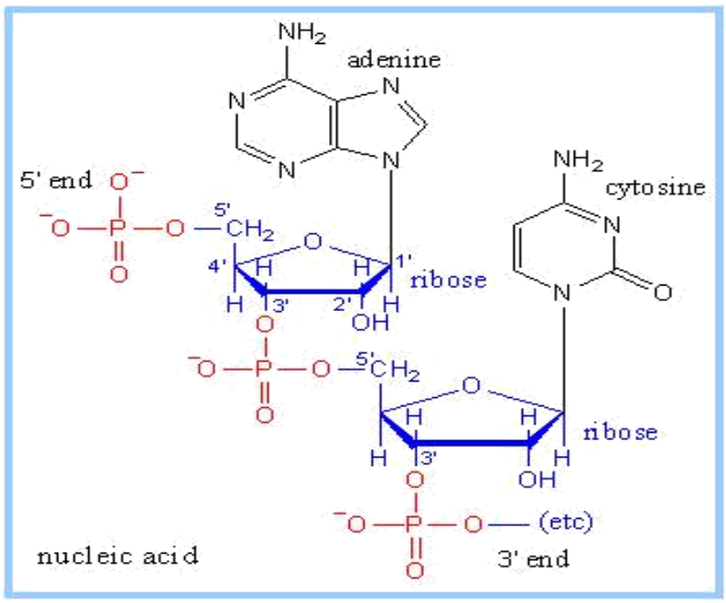
Uridine triphosphate

Functions of nucleotides

1. They play a major role in energy metabolism. Eg:ATP
2. They are the building blocks of nucleic acids.
3. They play a role as physiological mediators. AMP plays an important role as a secondary messenger
4. The nucleotides also serve as carrier of activated intermediates. Eg: UDP glucose, CDP choline
5. Many of the regulated steps of metabolic pathways are controlled by the intracellular concentration of nucleotides. Hence they behave as allosteric effectors.

Nucleic acids: In nucleic acids, the different nucleotides are joined to form a long polymer by 3'-5' Phosphodiester bond between phosphates and sugars. Each sugar has at least two hydroxyl groups, the 3' and 5' hydroxyl groups of ribonucleotides & deoxyribonucleotides. Each phosphoric acid has three esterifiable acidic groups. The 3'-5' Phosphodiester bond between nucleotides joins only the 3' hydroxyl of one nucleotide to 5' hydroxyl of another nucleotide. Hence each nucleotide in a polynucleotide chain has its 3' hydroxyl linked by a phosphate diester bond to the 5' hydroxyl of another nucleotide and the 3' hydroxyl of this nucleotide is linked to the 5' hydroxyl of still another nucleotide except at the end of the chain. The first nucleotide has a free 5' phosphate and the last nucleotide has a free 3' hydroxyl group

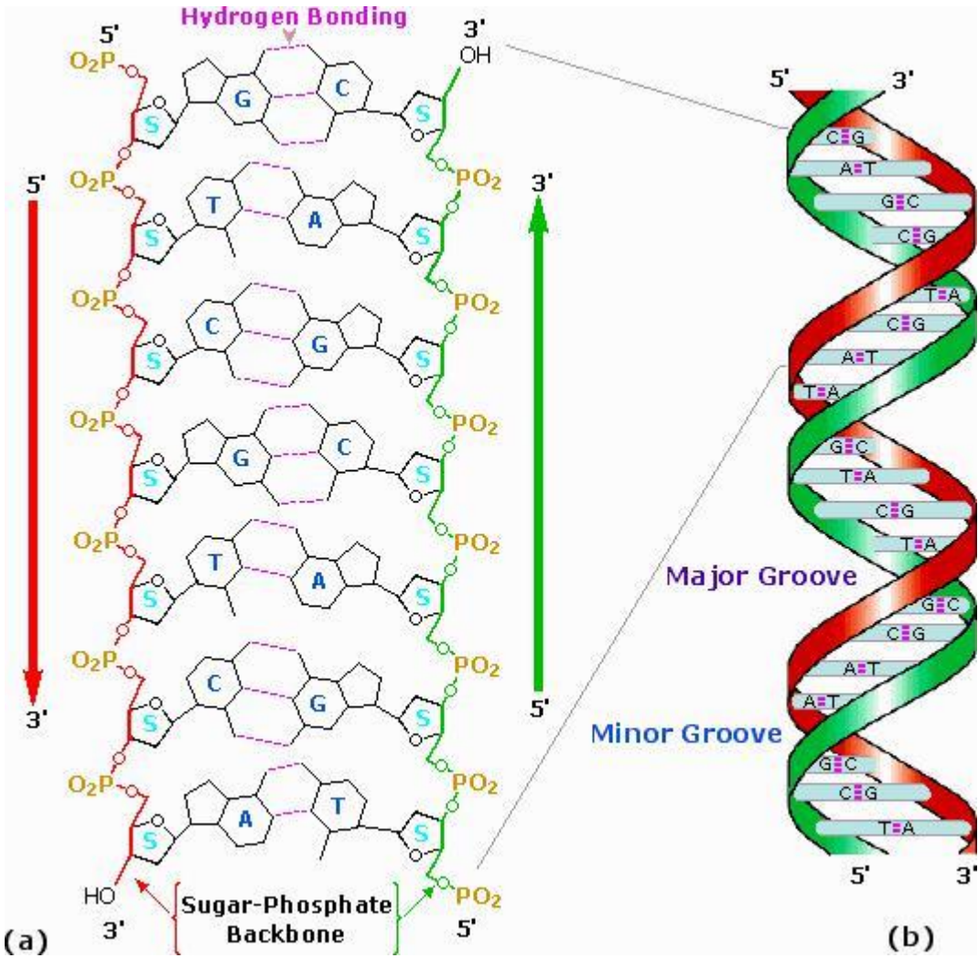




3'-5' Phospho diester bond formation

Structure of DNA: In 1953, Watson and Crick worked out the three-dimensional structure of DNA. DNA is composed of two strands of poly deoxyribonucleotide chains wound around each other to form a double helix, with the bases on the inside and the sugar-phosphate backbones on the outside. In the double helix, the two DNA strands are organized in an antiparallel arrangement (i.e. the two strands run in opposite directions, one strand is oriented 5' ⇗3' and the other is oriented 3'⇗5'). The nitrogen bases of the two strands form hydrogen bonds to each other; Adenine base pairs with Thymine and Guanine base pairs with Cytosine. This is called complementary base pairing. Thus a large two-ringed purine is paired with a smaller single-ringed pyrimidine and the two bases fit neatly in the gap between the sugar-phosphate strands and maintain the correct spacing. The G:C and A:T base pairing also maximizes the number of effective hydrogen bonds that can form between the bases. There are three hydrogen bonds between each G:C base pair and two hydrogen bonds between each A:T base pair. Thus A:T and G:C base pairs form the most stable conformation from

the point of view of maximizing hydrogen bond formation. The spatial relationship between the strands creates a major groove & a minor groove.



Forms of DNA:

Character	**A- DNA**	**B- DNA**	**Z- DNA**
Helical Shape	Right Handed	Right Handed	Left Handed
Number of base pairs	11	10	12
Helix pitch	2.8nm	3.4 nm	4.5 nm
Base tilted to normal axis	20 A^o	6 A^o	7A^o
Helix rise per base pair	2.4 A^o	3.4 A^o	3.7A^o
Occurance	Sprouting bacteria	Common	E. coli

Structure of RNA: Chemically, RNA is very similar to DNA but there are some important differences. RNA consists of the sugar ribose instead of deoxy ribose and nitrogen base uracil in place of thymine. Most RNA is single stranded.

There are three distinct types of RNAs: Messenger RNA, Transfer RNA and Ribosomal RNA. These differ from one another in size and function. The synthesis of these is catalysed by enzymes called RNA Polymerases.

Messenger RNA (mRNA): mRNA is the copy of the information carried by a gene on the DNA. The role of mRNA is to carry the information present in the DNA molecule to the translation machinery.This type of RNA makes up to 2% of the total RNA. It consists of the sequences of bases needed to determine the order in which the amino acids will be assembled in to each type of protein. There are several thousand types of mRNA in most cells. In eukaryotes, one type of mRNA corresponds to each type of protein but in prokaryotes, some mRNAs are polycistronic. In this case, an mRNA molecule may carry the code for several different proteins.

In prokaryotic cells, mRNA is made with in the nucleoid region of cytoplasm and is then used immediately to code for protein synthesis. In eukaryotes, most of the mRNA is made in the nucleus, from where it passes out through the nuclear envelope to the cytoplasm.

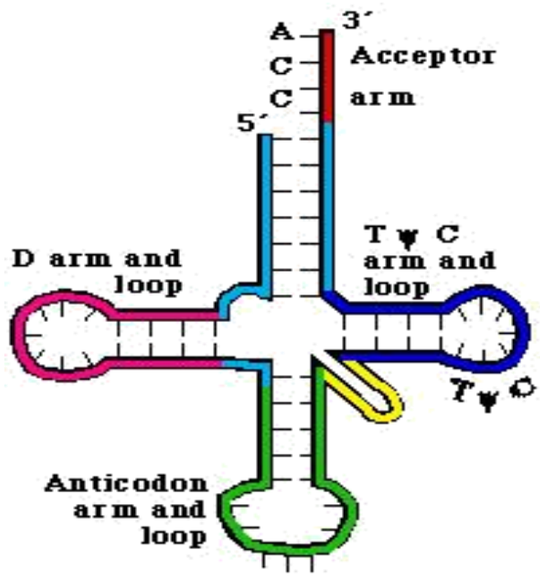
Ribosomal RNA (r RNA): Ribosomal RNA makes up to more than 80% of the total RNA of the cell. It is the major component of the ribosomes, the organelles on which protein synthesis takes place. Each ribosome consists of two unequal subunits, a small subunit and a large subunit each of which is a multi component complex of ribosomal RNAs and ribosomal proteins. A prokaryotic ribosome has a sedimentation coefficient of 70 S whereas the large and small subunits have sedimentation coefficients of 50 S and 30 S, respectively. In eukaryotes, the ribosomes are larger and more complex; the ribosome monomer is 80 S and consists of 60 S and 40 S subunits.

Transfer RNA (t RNA): tRNA is the information adaptor molecule. It is the direct interface between amino acid sequence of a protein and the information on DNA. There are more than 20 different t RNA molecules. Transfer RNA makes up about 16% of the total RNA. They are small, uniform in size having 75-90 nucleotides. These molecules play an important role in protein biosynthesis. Each tRNA becomes covalently bonded to a specific amino acid to form aminoacyl - tRNA which recognizes the corresponding codon in mRNA and ensures that the correct amino acid is added to the growing polypeptide chain. The tRNAs are small molecules, with a distinctive clover leaf secondary structures by internal pairing. The stem-loops of the cloverleaf are known as arms.

There are 4 arms and 3 loops in t RNA molecule.

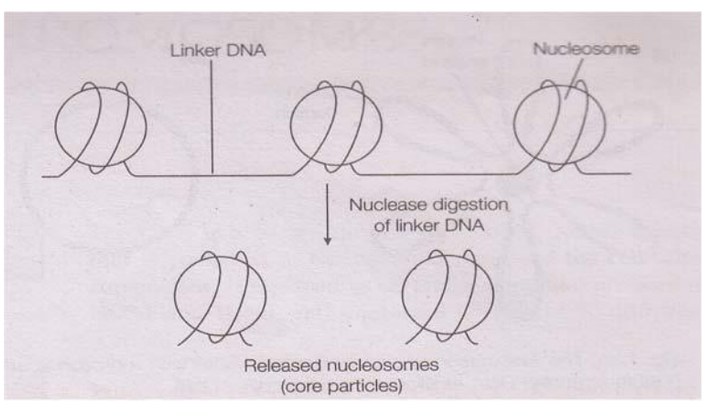
- The anticodon arm contains in its loop the three nucleotides of the anticocodon which will form base-pairs with the complementary codon in mRNA during translation;
- The D or DHU arm (with its D loop) contains dihydrouracil, an unusual pyrimidine;

- Some tRNAs also have a variable arm (optional arm) which is 3-21 nt size. The other notable feature is the amino acid acceptor stem. This is where the arnino acid becomes attached, at the 3' OH group of the 3'-CCA sequence. The three dimensional structure of tRNA*s is* even more complex as shown in figure because of additional interactions between the various units of secondary structure.
- The T or TψC arm (with its T loop) contains another unusual base pseudouracil (denoted ψ) in the sequence TψC



Packing of DNA into chromosomes:

DNA is a very long molecule and a cell cannot accommodate such long molecules. DNA does not normally exist as the simple double helix. Instead, eukaryotic DNA is packaged in a series of steps with protein, to form chromosomes. The amount of DNA in a single human cell, lined up end to end, would stretch nearly two meters. Hence DNA is compacted enough to fit into a single nucleus. The first level of packaging involves the binding of the chromosomal DNA to histones to from nucleosome. A nucleosome consists of a small amount of DNA wrapped up with protein. The proteins that interact with DNA to form chromatin comprise a family of basic (positively charged) proteins called histones. There are five different types of histone protein: H1, H2A, H2B, H3, and H4. Of these, two molecules each of H2A, H2B, H3, and H4 combine to form a histone octamer. DNA wraps around the octamer, making 1.8turns around the protein complex. The amount of DNA associated with the histone octamer is 146 base pairs (bp). The octamer plus the DNA comprise what is called the nucleosome core. A small stretch of DNA (60 bp) runs between adjacent nucleosome cores, and is known as the linker. A single nucleosome consists of one core plus a linker. The total amount of DNA involved in a single nucleosome is approximately 206 bp. Chromatin therefore consists of DNA wrapped around one histone octamer after another, like a long string of beads. This configuration is known as the **11 nm chromatin fiber**. Overall, the packing ratio is about 7 that is the DNA length is shortened about sevenfold by winding around the nucleosome. Complexing with histones protects DNA from the action of Deoxyribonuclease.



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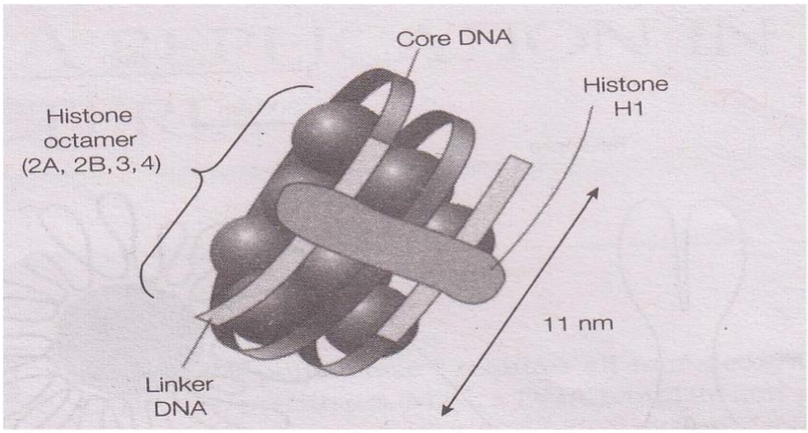
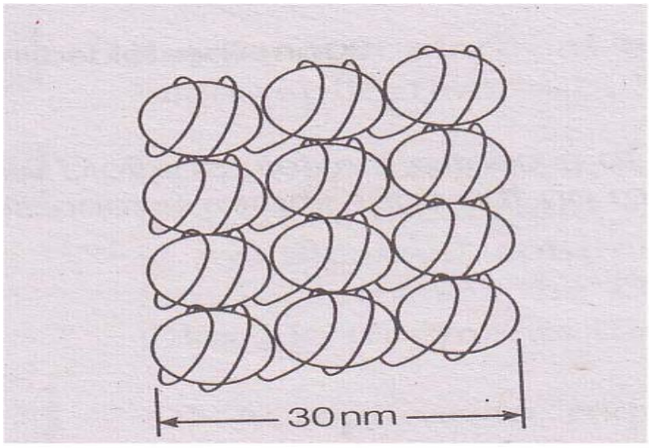
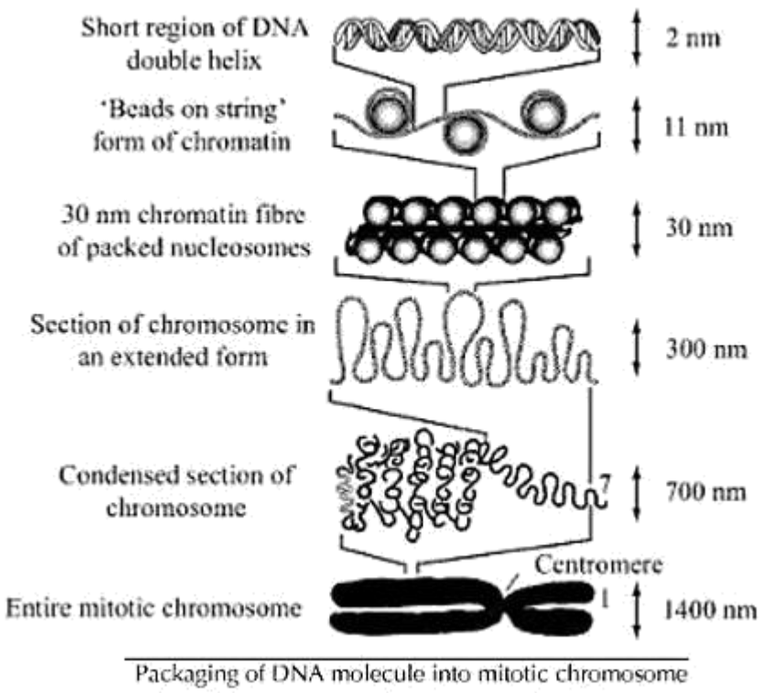
Chromatin can be packed further into higher-order structures. This involves the action of histone H1. H1 binds to DNA on the outside of nucleosomes (at a ratio of one H1 molecule per nucleosome), then H1 molecules interact with each other, causing the chromatin to form a spiral, with 6 to 8 nucleosomes per turn of the spiral. This structure is known as a **solenoid** or **30 nm chromatin fiber.**

Because the chromatin is so tightly packed, DNA in the 30 nm fiber is genetically inactive. The 30 nm fiber is folded up further to make metaphase chromosomes.

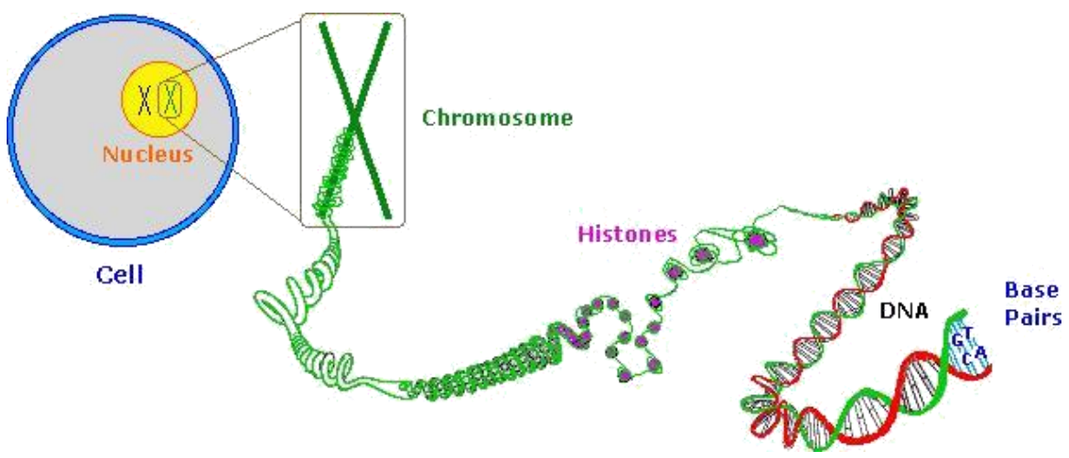




This 30 nm chromatin fibre is then folded into large looped domain with 300 nm diameter. In the next phase, this is further condensed to form 700 nm diameter looped domains. During mitiotic cycle, the looped domains are organized into distinct structures called the chromosomes with 1400 nm diameter.



Packaging of DNA molecule into mitotic chromosome



METABOLISM

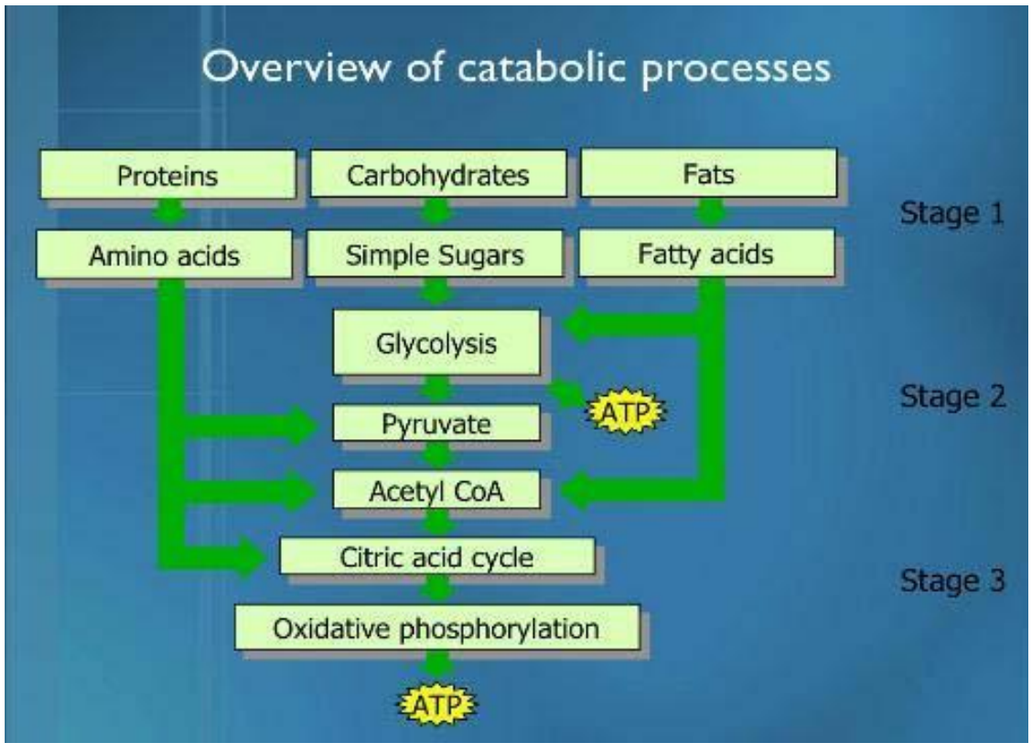
The sum total of all the enzymatic reactions occurring in the cell is collectively called as metabolism. The reaction sequence occurring with in organisms in an orderly and regulated way are known as metabolic pathways and the compounds formed as a result of metabolism are called metabolites. Reactions comprising the total metabolism of any cell can be broadly divided into two phases- catabolism and anabolism. Catabolism refers to the reactions involving the degradation or breakdown of various complex molecules to smaller and simpler molecules. The chemical energy of metabolites is conserved in the form of ATP. Anabolism refers to all the reactions involving synthesis of various molecules from smaller and simpler precursor molecules. This process requires the input of chemical energy in the form of ATP.

In living system, both catabolism and anabolism occur concurrently and simultaneously. The energy released during catabolism is required for

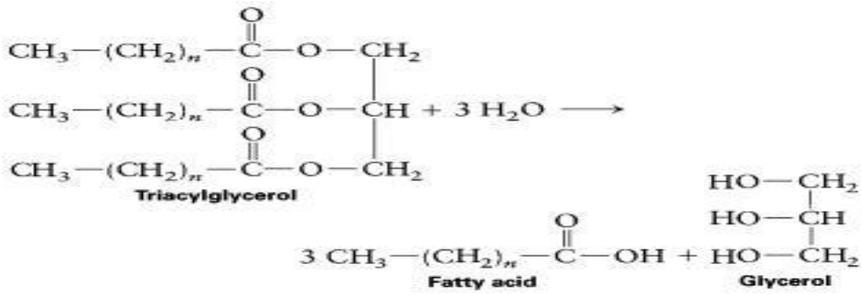
anabolic reactions and for many other cellular activities. Catabolic reactions are oxidative where as anabolic reactions are reductive in nature.

***Stages of metabolism*:**

Catabolic reactions take place in three major stages. Large molecules are broken down to their building blocks in the first stage of metabolism. Polysaccharides are hydrolyzed to yield monosaccharides, lipids are converted to glycerol and fatty acids, and proteins are broken down to amino acids. In stage II of catabolism, the various products of stage I are collected and converted to number of still simpler intermediates. Thus, the monosaccharides and glycerol are degraded via the three carbon intermediate pyruvic acid to yield a two carbon species, the acetyl CoA. Similarly, various fatty acids and amino acids are broken down to form acetyl Co A and few other products. Finally the acetyl Co A and other products of stage II are channelled in stage III in which they are oxidized into carbon dioxide and water followed by oxidative phosphorylation



Stage I : Eg: Hydrolysis of fat by lipase.



Stage II: Conversion of monomers into a form that can be completely oxidized.

a) Sugars are converted to puruvic acid and acetyl Co A.
b) Amino acids are deaminated and might enter at any stage in metabolic pathway.
c) Fatty acids are converted to acetyl Co A

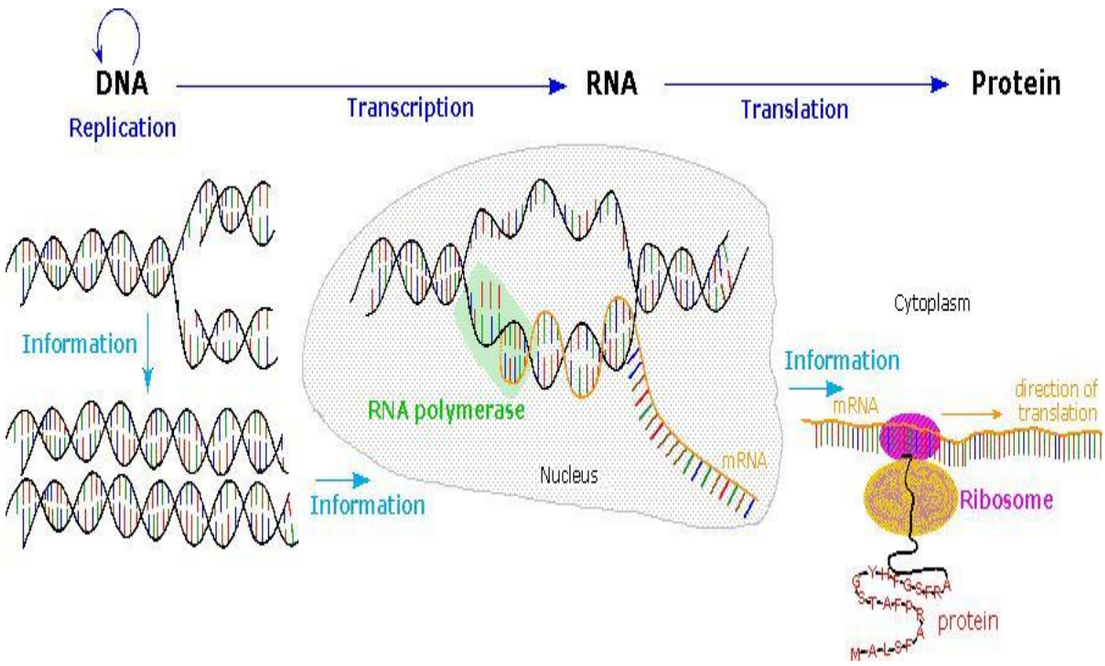
Stage III: Complete oxidation of carbohydrates, fats and proteins and the production of ATP

a) Carbohydrates, fats and proteins are converted to acetyl Co A.
b) The acetyl Co A units are processed in TCA cycle and converted to CO_2 and water with the liberation of ATP in oxidative phosphorylation

PROTEIN METABOLISM

Amino acids serve as the building blocks of proteins and as precursors of many other important biomolecules such as hormones, purines, pyrimidines, porphyrins and some vitamins. They also serve as source of energy when present in excess amounts and when they are used as fuel, amino acids undergo deamination reactions. The remaining carbon skeleton will either convert itself into glucose or undergoes oxidation to CO_2 via TCA cycle.

Central dogma: The Central dogma of molecular biology, which was formulated as a simple linear progression of information from DNA to RNA to Protein, is summarized in the following illustration. The replication process on the left consists of passing information from a parent DNA molecule to daughter molecules. The middle transcription process copies this information to a mRNA molecule. Finally, this information is used by the chemical machinery of the ribosome to make polypeptides.



Central dogma consists of 3 major processes in the preservation and transmission of genetic information.

a) Replication: It is the process by which each strand of parent DNA molecule is copied to form 2 identical daughter DNA molecules.
b) Transcription: It is the process by which genetic message in DNA is transcribed (copied) into the form of mRNA.
c) Translation: It is the process by which the information transcribed from DNA to mRNA directs the specific amino acid sequence and protein synthesis occurs.

The flow of genetic information as proposed by Crick is the central dogma of molecular biology.

The machinery of protein biosynthesis consists of DNA, mRNA, tRNA, ribosomes, and a large number of enzymes and cofactors. The whole machinery operates in cytoplasm and is closely connected to endoplasmic reticulum.

Ribosomes: They act as platform on which the protein synthetic machinery is assembled. They have two sites- amino acyl or A site and peptidyl or P site. The initiating codon AUG is positioned at P site. During elongation, all the incoming amino acyl t RNAs bind to A site. Ribosomes of prokaryotes have sedimentation coefficient of 70 S. During translation, there is continuous dissociation of 70S ribosome into 50S and 30 S subunits. mRNAs and tRNAs cannot bind directly to 70 S subunit but first bind to 30 S subunit which then combines with 50 S subnuits. Eukaryotes have 80 S sedimentation coefficient (40S & 60 S).

mRNA: The key component of translation is mRNA. It carries the genetic information from DNA to to the site of protein biosynthesis i.e ribosome. mRNA contains codons. Codon is the unit that codes for a given amino acid and consists of 3 nucleotides.

Genetic code: DNA replication is simply a complementary base pairing exercise, but the translation of the four letter (bases) alphabet code of RNA to the twenty letter (amino acids) alphabet of protein literature is far from trivial. Clearly, there could not be a direct one-to -one correlation of bases to amino acids, so the nucleotide letters must form short words or codons that define specific amino acids. The features of genetic code are

a) The code/codon is a triplet (as singles & doublets are insufficient to code 20 amino acids)
b) The genetic code is universal.The same code dictionary is used by all organisms (both prokaryotes & eukaryotes)
c) The genetic code is comma less. It is a series of codons without space or punctuations.
d) It is nonoverlapping. The nucleotide sequence of 2 consecutive amino acids donot overlap.
e) It is degenerate. With the exception of codes for Methionine & tryptophan, all the amino acids are encoded by more than one codon.

tRNA: tRNA has a clover leaf structure. The molecule is asymmetrically folded to yield a compact structure with anticodon arm at one end and amino acid arm at the other end. Anticodon is the nucleotide triplet in tRNA. It is present in the central petal of tRNA. Anticodon is complimentary to the corresponding codon in mRNA. tRNAs are small in size consisting of 70-90 nucleotides.

Translation (Protein biosynthesis):

Protein synthesis is the most complex biosynthetic mechanism. Despite the complexity, proteins are made at a high speed. A polypeptide chain of 100 amino acid residues is synthesized in few seconds. Protein synthesis is tightly regulated. Translation consists of 3 major steps. 1. Chain initiation 2 Chain elongation & 3. Chain termination. In addition to these, activation of amino acids prior to their incorporation into poly peptides and post translational processing of completed polypeptide constitute 2 additional stages.

Activation of amino acids: This process takes place in cytoplasm. Each amino acid is attached to a specific tRNA and the reaction is catalyzed by amino acyl tRNA synthetase.

Amino acid + tRNA + ATP _____ aminoacyl-tRNA + AMP + ppi

This reaction occurs in two steps.

ATP + amino acid ========= amino acyl adenylic acid + pyrophosphate.

Aminoacyl adenylic acid + t RNA ====== Amino acyl-t RNA + adenylic acid

Generally methionine is first attached to tRNA and then formyl group is transferred finally forming fMet tRNA.

Initiation: Polypeptide synthesis begins from the amino terminal end. This stage requires 1) 30S & 50S sub units 2) mRNA 3) fMet tRNA 4) a set of initiation factors IF1, 2 & 3 and 5) GTP, Mg^{++}. The formation of initiation

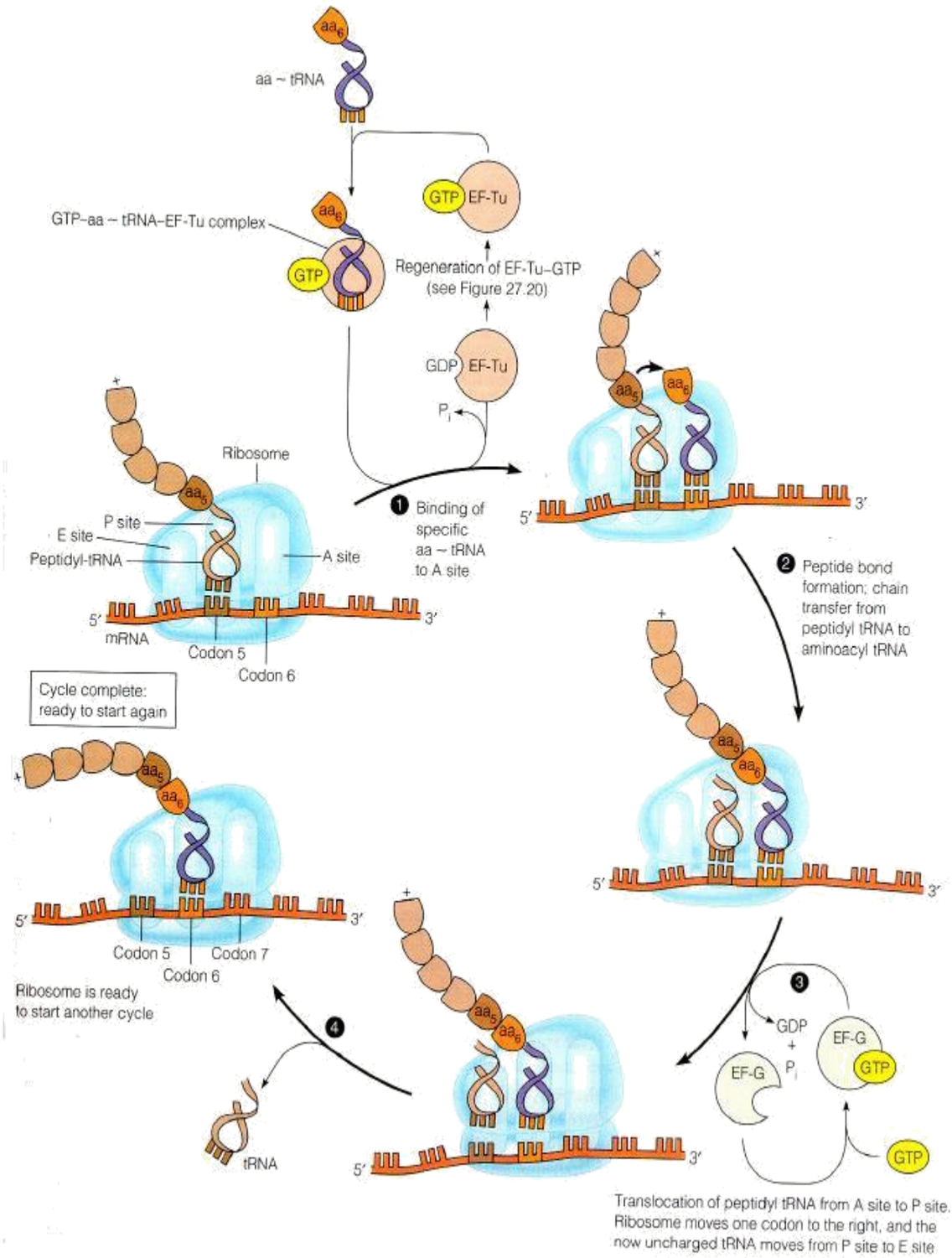
complex is divided into three steps. In the first step, 30S subunit binds to IF 3 which prevents the premature union of 30S & 50S. Then binding of mRNA to 30S sub unit takes place in such a way that the initiation codon AUG binds to a precise location on 30S. In the second step, complex of 30S, IF3 & mRNA forms a still larger complex by binding IF2 (which is already bound to GTP) & the fMet tRNA. The anticodon of this tRNA pairs correctly with the initiation codon. In the third step this large initiation complex of 30S subunit will combines with 50S subunit. Simultaneously GTP is hydrolyzed to GDP +Pi. IF2 & IF3 depart from ribosomes. This elaborate initiation process is required to ensure that the initiating aminoacyl- t-RNA is bound at the peptidyl site and positioned at the initiation codon AUG, so that the ribosomes start translation at the correct point on the mRNA. Translation of the codons of mRNA take place in the 5'___3' direction.

Elongation: This stage requires the complex formed above, the next amino acyl tRNA, a set of elongation factors EFTu, TS, G & GTP.

a) Binding of incoming amino acid: Elongation factor EF is made up of two subunits ie: Tu and Ts. As along as Tu and Ts are associated the next amino acid cannot bind to this complex. Once the two factors separate out then GTP will bind to Tu and forms a complex Tu-GTP and this complex will bind with the next amino acyl tRNA. This entire complex is bound to A site of ribosome and the energy released by GTP hydrolysis will facilitate the correct binding of the amino acid. GDP & EF are released.
b) Formation of the peptide bond: A new peptide bond is formed between the amino acids at A&P sites. This occurs by the transferring formylmethionyl group from its tRNA to the amino group of second amino acid, which is at A site. The A site alpha amnio acid acts as a nucleophile and formation of peptide bond takes place. The dipeptide thus formed is bound to A site leaving P site with uncharged tRNA at P site. Peptidyl transferase enzyme will participate in this reaction. For the formation of one peptide bond four ATP molecules are utilized.
c) Translocation: Finally the ribosome moves by a distance of one codon towards 3' end of mRNA, thus shifting dipeptidyl tRNA from A site to P site. Now A site is free for the incoming amino acyl tRNA. The uncharged tRNA is released from P site back into cytoplasm.

The ribosome moves from codon to codon along mRNA towards 3' end, adding one amino acid at a time to the growing chain.

Elongation continues until the ribosome adds the last amino acid, completing the polypeptide coded by the mRNA.



Termination: This stage is signaled by one of the 3 termination codons in mRNA – UAA, UAG, and UGA. The termination codon first occupies A site. Then the release factors 1, 2 & 3 contribute to the following:

a) Hydrolysis of terminal peptidyl tRNA bond
b) Release of free polypeptide and last tRNA from P site
c) Dissociation of 70S subunit into 30S & 50S which are ready to start a new cycle of protein biosynthesis.

In bacteria RF1recognizes the termination codons UAG and UAA, and RF2 reconizes UGA and UAA. RF1 or RF2 binds at a termination codon and induces peptidyl transferase to transfer the growing peptide chain. The specific function of RF3 has not been firmly established. In eukaryotes, a single release factor called eRF recognizes all three termination codons.

Inhibitors of protein biosynthesis: Protein synthesis being a long complex process is vulnerable to inhibition at many points. Some inhibitors of protein synthesis are

a) Streptomycin – inhibits initiation & causes misreading(binds to 30S subunit)
b) Tetracycline –in bacteria it inhibits by binding to amino acyl tRNA
c) Chloramphenicol – in bacteria it inhibits peptidyl transferase activity.
d) Puromycin – causes premature release of peptide, as it has a structure very similar to the 3' end of an aminoacyl-tRNA.
e) Cycloheximide-n blocks the peptidyl transferase of 80S eukaryotic ribosome
f) Ricin : inactivates 60S subunit of eukaryotic ribosome
g) Diphtheria toxin: it inactivates eukaryotic elongation factor eEF2

Post translational modifications: After synthesis, the nascent polypeptide chain is folded & processed into its biologically active form. It spontaneously assumes its native conformation i.e secondary, tertiary & quaternary structure. Some newly made proteins do not attain their final biologically active conformation until they have been altered by one or more processing reactions called posttranslational modifications. The post translational changes can be grouped under following sub headings

1. Amino terminal and Carboxyl terminal modification: Protein synthesis is initiated by formyl-methonine or methionine which has to be removed enzymatically, the N terminal amino acids is acetylated.
2. Loss of signal sequence: 15 to 30 residues at the amino-terminal end of some proteins play a role in directing the protein to its ultimate destination in the cell. Such signal sequences are ultimately removed by specific peptidases.
3. Modification of individual amino acids: The hydroxyl groups of certain amino acids like serine, threonine and tyrosine residues of some proteins are enzymatically phosphorylated by ATP. Carboxyl groups of glutamic acid are methylated, lysine is methylated Hydroxylation of Proline to hydroxy proline etc
4. Attachment of carbohydrate side chain: Carbohydrate side chain of glycoproteins is attached covalently during or after the synthesis of the polypeptide chain.
5. Addition of isoprenyl groups: A number of eukaryotic proteins are isoprenylated, a thioether bond is formed between the isoprenyl groups and a cysteine residue of the protein. In some cases, the isoprenyl group serves to help anchor the protein in a membrane.
6. Addition of prosthetic groups: Many prokaryotic and eukaryotic proteins require covalently bound prosthetic groups for their activity. These are attached to the polypeptide chain after it leaves the ribosome. Eg. The attachment of heme group to the cytochrome c
7. Proteolytic processing: Some viral proteins and proteases are initially synthesisied as larger inactive precursor proteins called as zymogens which have to be trimmed to ptoduce finally active forms Eg: trypsin is synthesized as trypsinogen
8. Formation of disulfide cross-links: Proteins to be exported from eukaryotic cells, after undergoing spontaneous folding into their native, conformations, are often covalently cross-linked by the formation of intrachain and interchain disulfide bridges between Cysteine residues. The cross links formed in this way help to protect the native conformation of the protein molecule from denaturation in an extracellular environment that can differ greatly from that inside the cell.

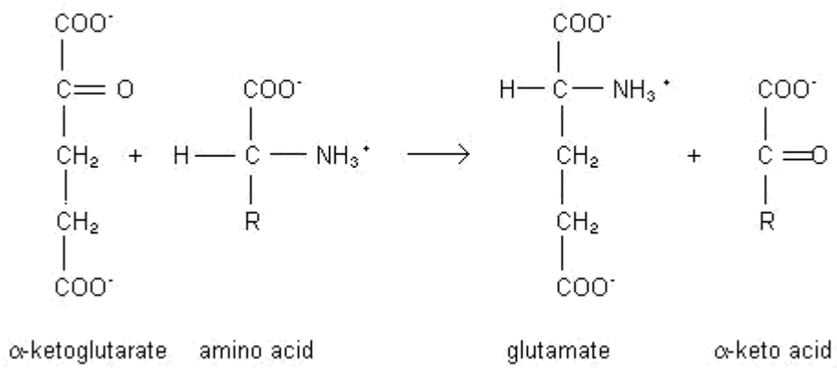
Degradation of proteins into amino acids: Proteins are hydrolyzed by proteases and the amino acids are released. These amino acids under go catabolism in the following ways.

a. Transamination

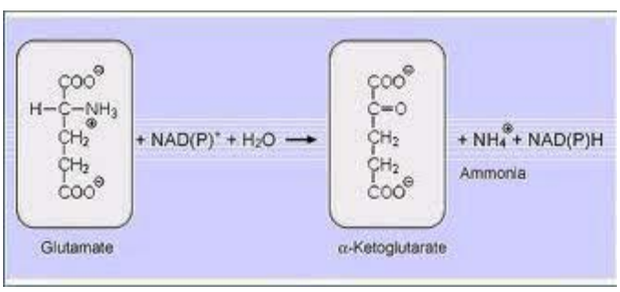
b. Oxidarive deamination.
c. Non oxidative deamination
d. Decarboxylation

Transamination : This reaction involves the transfer of an amino group from a donor amino acid to an acceptor α-keto acid to yield the α-ketoacid of the donor amino acid and the amino acid of the original α -keto acid. The reaction is catalyzed by an enzyme called aminotransferase or transaminase that requires PLP as co-enzyme for its activity.

TRANSAMINATION



Oxidative Deamination: Amino group from amino acid is oxidatively removed in this process. Glutamate dehrogenase enzyme catalyses the following reaction.

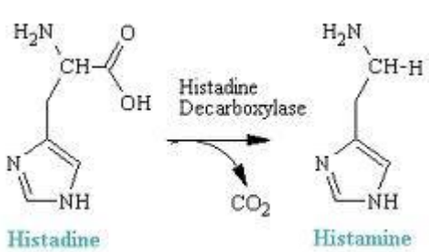


Non oxidative deamination:- Non oxidative deamination takes place in absence of water and the corresponding unsaturated dicarboxylic acid is formed

Aspartic acid + H_2 _____ Fumaric acid + NH_3

Structural formulae for the above reactions need to be written & explained in the class.

Decarboxylation:- In decarboxylation reactions, the CO_2 from the corresponding amino acids are removed and biologically active amines are formed.



Amino acid carbon skeletons:

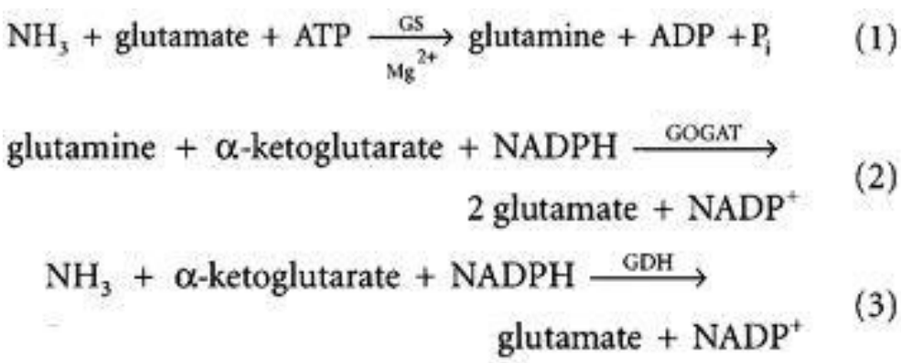
The remainder of the amino acid is referred to as the "carbon skeleton". Depending on the particular amino acid being catabolised, its carbon skeleton will be converted to acetyl coA, or pyruvate and or a citric acid cycle intermediate. Those carbon skeletons which end up as acetyl CoA are committed to energy production. They will be oxidised via the citric acid cycle.

Ammonia assimilation:

The ammonia released during metabolism of amino acids is toxic to the cells if it is not incorporated into synthetic processes. There are three reactions that can catalyze the incorporation of the nitrogen atom as NH3 into organic compounds. These reactions are those catalysed by glutamic dehydrogenase, glutamine synthetase and carbamyl phosphate synthetase.

Carbomyl phosphate Synthetase

CO_2 + 2ATP ---------- Carbomyl phosphate + 2ADP + H_3PO_4



Structural formulae for the above reactions need to be written & explained in the class.

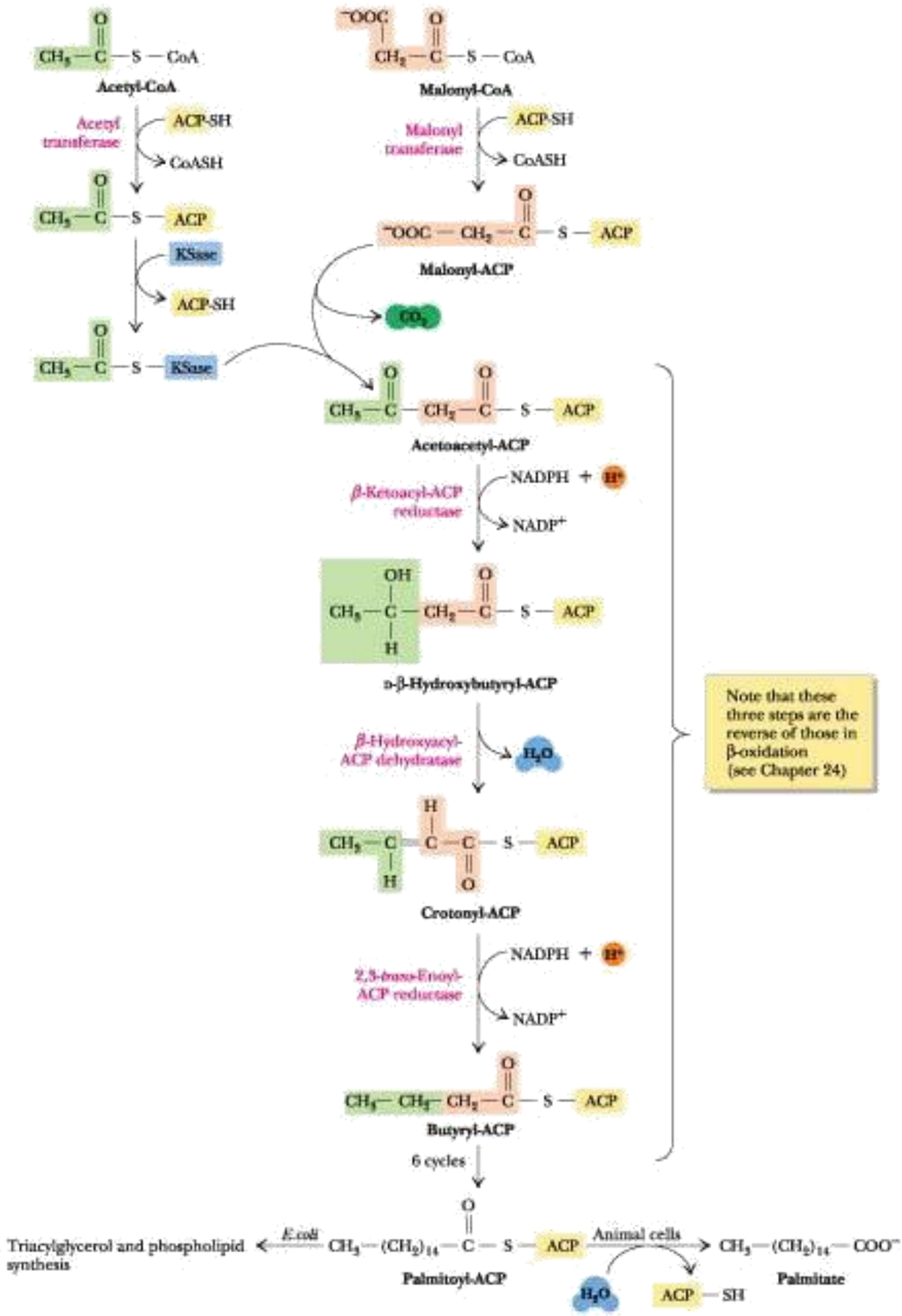
METABOLISM OF LIPIDS

The complete metabolism of fat leads to oxidation to C02 and water and the liberation of energy equivalent to 9 Calories /gram of fat.

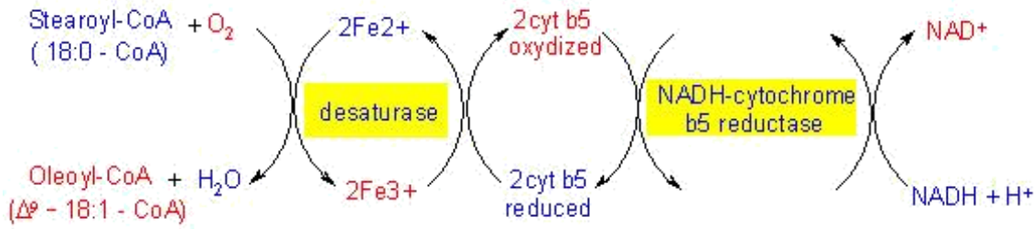
Anabolism of saturated fatty acids*:* Fatty acid synthesis is characteristic of all living organisms. Multienzyme complexes referred to as type I fatty acid synthases are essential for fatty acid synthesis. The first reaction of the sequence is carboxylation of acetyl coA to malonyl coenzyme A in the presence of the enzyme acetyl coA carboxylase.

Acetyl CoA +CO_2 + ATP ---> Malonyl CoA + ADP + Pi Acetyl CoA Carboxylase (Biotin)

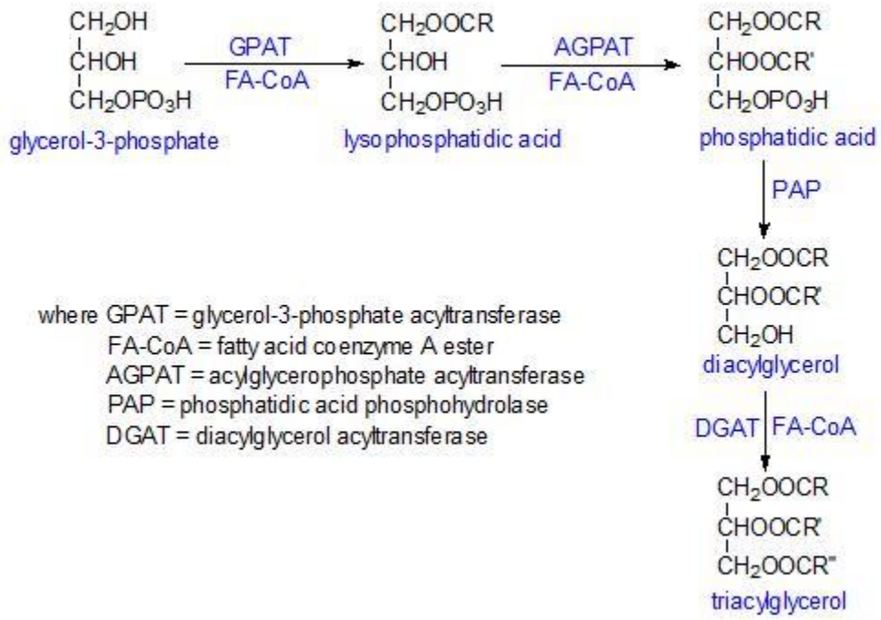
One molecule of acetyl CoA and one molecule of malonyl CoA are converted to their corresponding ACP derivatives in the presence of the enzymes transacylases.



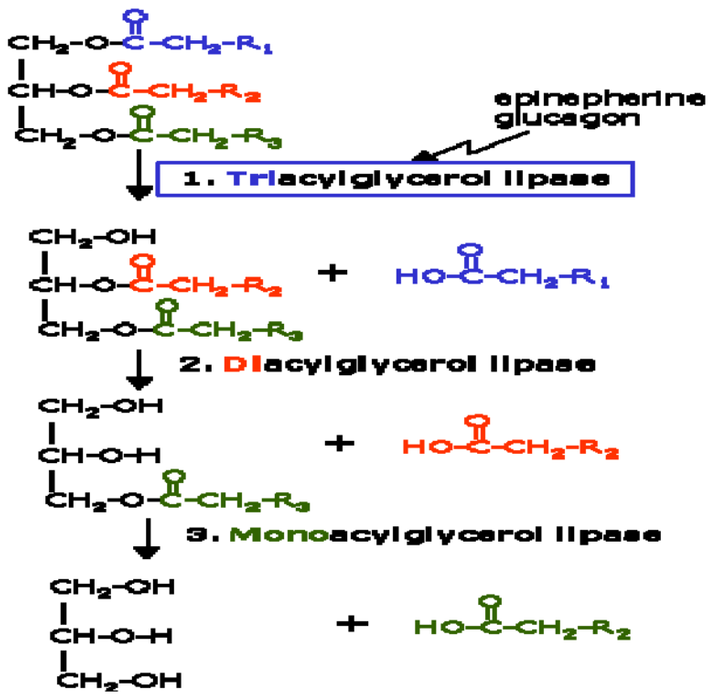
Anabolism of unsaturated fatty acids: The fatty acid synthesized generally is palmitate which is a 16:0 fatty acid. Unsaturation of fatty acids occurs in both the mitochondria and endoplasmic reticulum in presence of the enzyme f*atty acyl-CoA desaturases* .



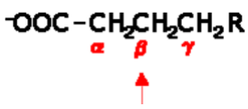
Anabolism of triacyl glycerol: The most important route to triacylglycerol biosynthesis is Kennedy pathway as illustrated below.



Catabolism of lipids: The first step in lipid catatabolism is the hydrolysis of the lipid in the cytoplasm to produce glycerol and fatty acids.



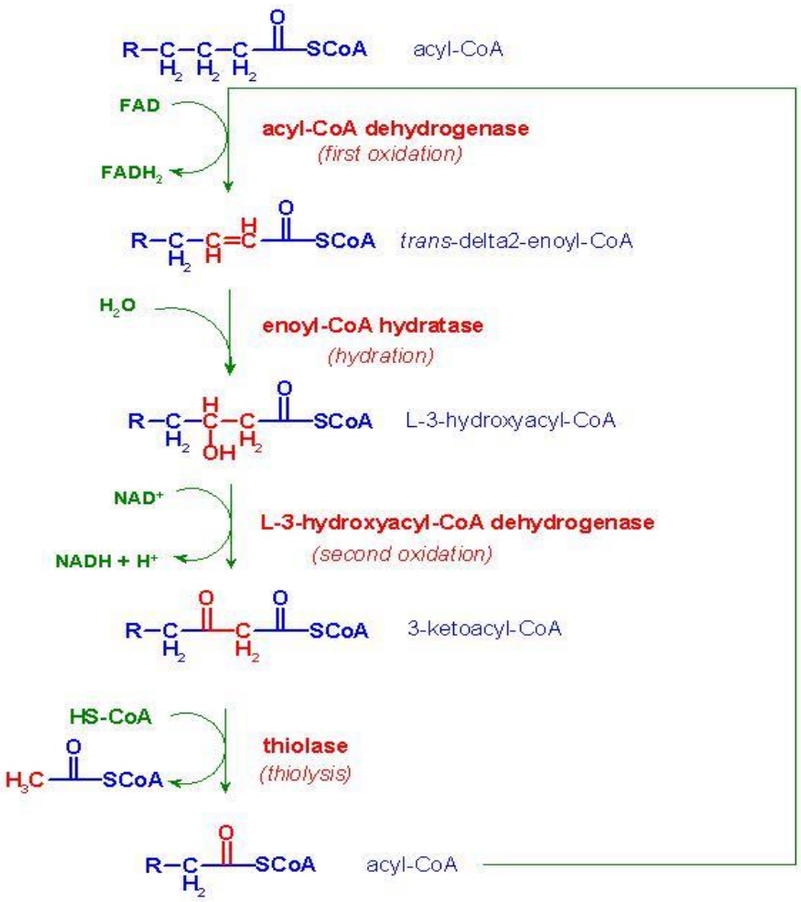
Catabolism of fatty acids: In the mitochondria, fatty acids are broken down by various types of oxidations such as α, β, and γ oxidation.



Alpha oxidation: alpha oxidation occurs in plants where odd number of carbon containing fatty acids are present. n carbon containing fatty acid gives rise to n-1 carbon containing fatty acid.

$R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$ ---------- $R\text{-}CH_2\text{-}CH_2\text{-}COOH$

β-oxidation: In β-oxidation, the fatty acid is broken down to release acetyl-CoA. The process involves 4 main steps: dehydrogenation, hydration ,oxidation ,thiolysis. The process repeats until the fatty acid has been completely degraded to acetyl-CoA. Each round of β -oxidation yields 1 molecule of acetyl CoA and requires 1 molecule of NAD^+ and 1 molecule of FAD^+ . Hence each round of β - oxidation releases 5 ATP molecules. For example, the β -oxidation of a C16 fatty acid will generate 8 molecules of acetyl CoA and 7 molecules of NAD^+ and FAD^+

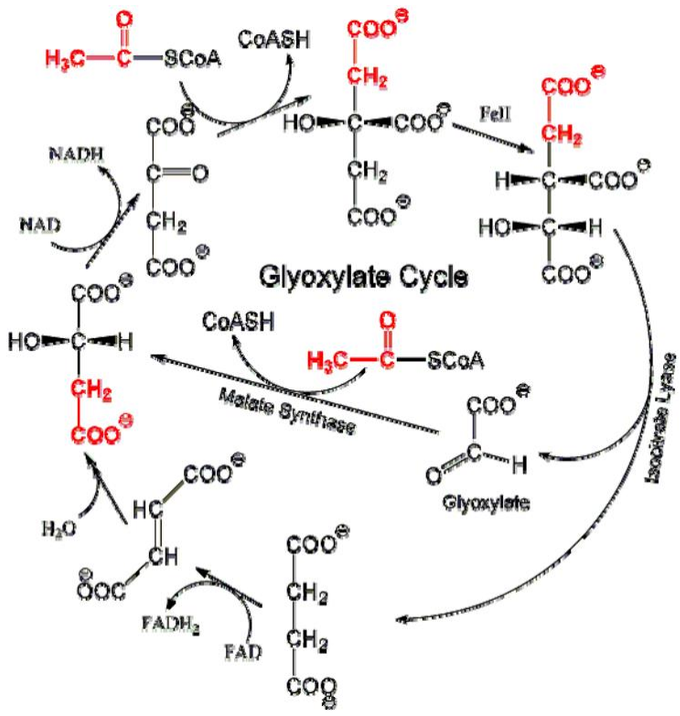


Omega oxidation: It occurs in the endoplasmic reticulum rather than the mitochondria, the site of beta-oxidation.The omega carbon in a fatty acid is the carbon farthest in the alkyl chain from the carboxylic acid. In the omega oxidation

pathway, this carbon is progressively oxidized first to an alcohol and then to a carboxylic acid, creating a molecule with a carboxylic acid on both ends.

$R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$ ---------- $COOH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$

Glyoxylate cycle: In plants, the glyoxylate cycle occurs in special **peroxisomes** which are called **glyoxysomes**. This cycle allows seeds to use lipids as a source of energy to form the shoot during **germination**. The lipid stores of germinating seeds are used for the formation of the carbohydrates that fuel the growth and development of the organism. The two enzymes which regulates this cycle are isocitrate lyases and malate synthase.



METABOLISM OF CARBOHYDRATES

Carbohydrate metabolism in the cell is essentially the metabolism of glucose and other substances related to glucose in their metabolic processes. The chemical reactions involved in carbohydrate metabolism in the body represent complex groups, sequences, and cycles of reactions which integrate at various points with the reactions concerned in the metabolism of lipids and proteins. The major types of chemical processes involved in carbohydrate metabolism may be grouped as follows:

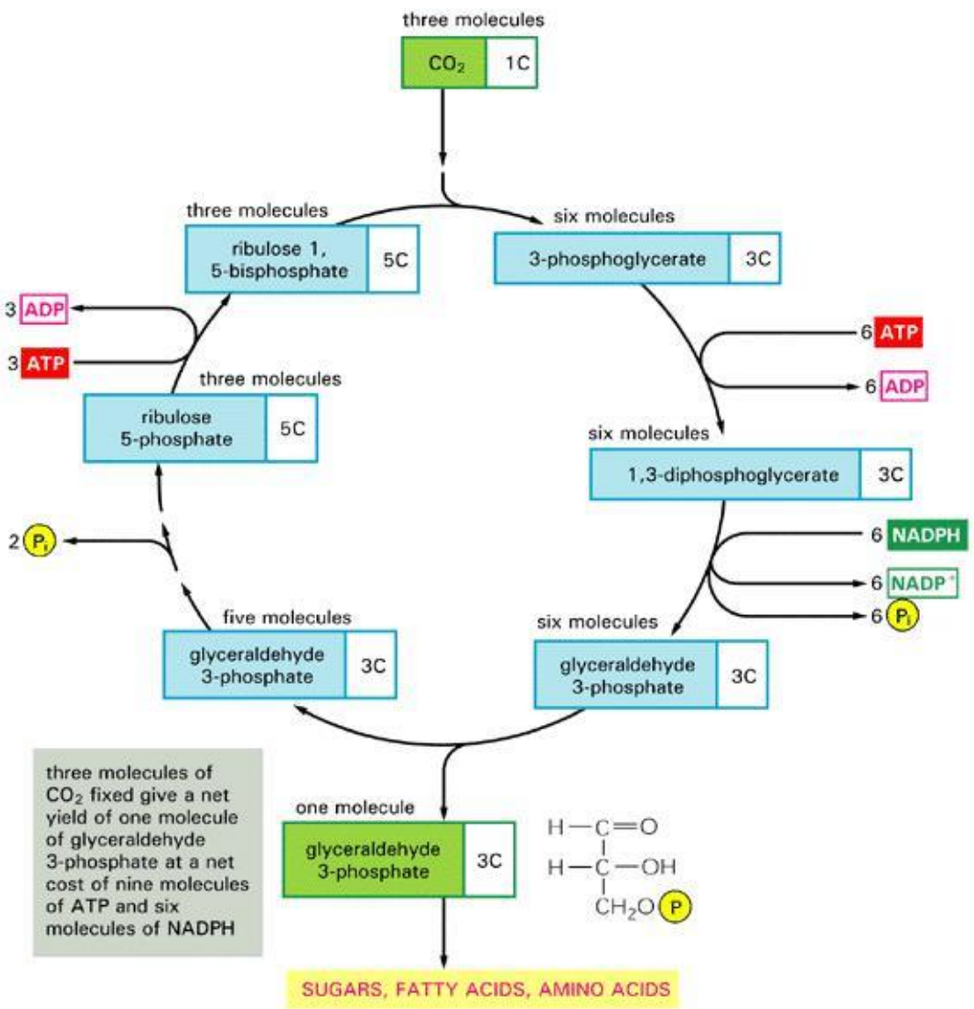
a) Anabolism of starch – Photosynthesis
b) Catabolism of starch
c) Catabolism of glucose – 1. Glycolysis & TCA cycle
   2. Oxidative pentose phosphate pathway

Photosynthesis: The mechanism by which green plants absorb light and convert carbon dioxide to carbohydrates is called as photosynthetic process. This process is composed essentially of two general processes. The first process consists of the absorption of light by the chlorophyll system, which provides energy in the form of activated chemical compounds. The reactions in this case are the so- called light reactions. In the second process carbon dioxide is reduced by the active molecules formed in the light reactions, and the production of carbohydrates takes place. Since these reactions do not require simultaneous illumination, the reactions involved are referred to as the dark reactions. Both the light and dark reactions represent complex interrelated processes under enzymatic control.

Light reaction (Hill reaction): Light reaction is the first stage of photosynthesis, the process by which plants capture and store energy from sunlight. In this process, light energy is converted into chemical energy, in the form of the energy-carrying molecules ATP and NADPH. The light reactions take place on the thylakoid membrane inside a chloroplast. The inside of the thylakoid membrane is called the lumen, and outside the thylakoid membrane is the stroma, where the light-independent reactions take place. The net-reaction of all light reactions in photosynthesis is:

$2H_2O + 2NADP^+ + 3ADP + 3P_i \rightarrow O_2 + 2NADPH + 3ATP$

Dark reaction(Calvin cycle): In this pathway, the free energy of cleavage of ~P bonds of ATP, and reducing power of NADPH, are used to fix and reduce CO_2 to form carbohydrate. Enzymes and intermediates of the Calvin Cycle are located in the chloroplast stroma**,** a compartment somewhat analogous to the mitochondrial matrix.

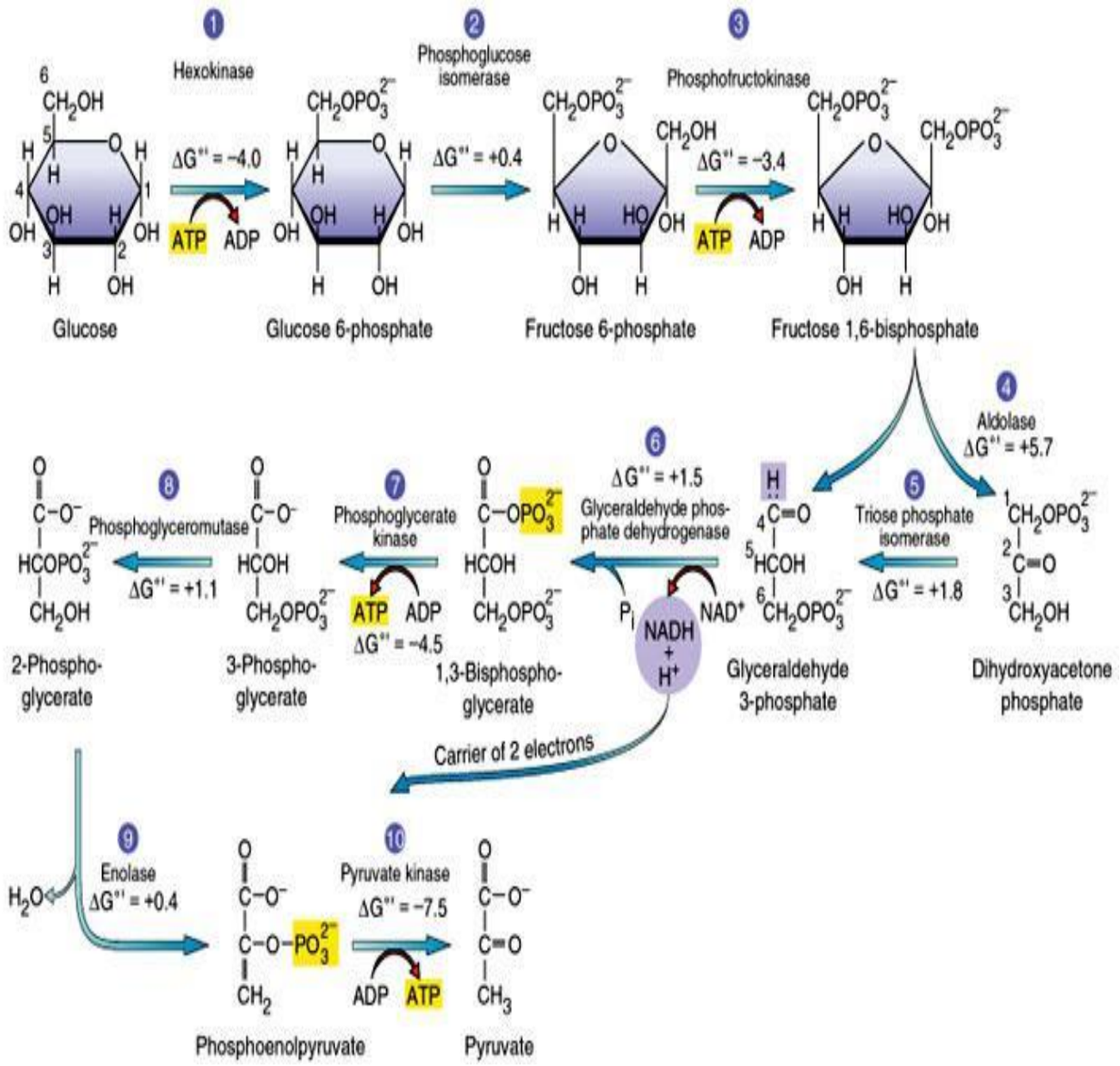


Catabolism of starch: Starch is hydrolyzed completely to glucose by different enzymes.

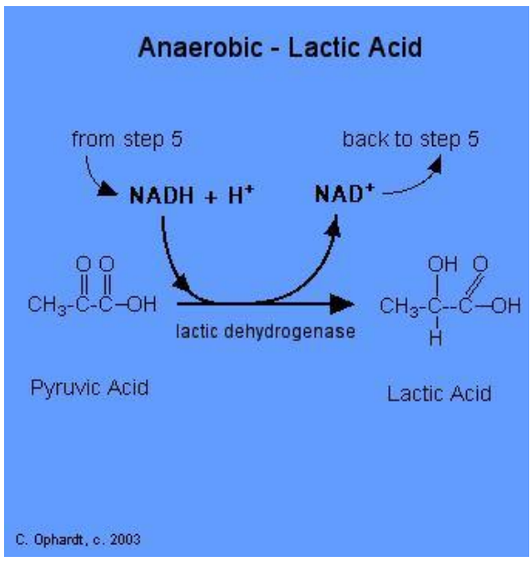
a) α-amylase hydrolyzes α 1-4 glycosidic bonds at random, internally and yields a mixture of dextrins.
b) β -amylase hydrolyzes maltosyl residues from non reducing ends and yield only maltose.
c) Debranching enzyme(α-1-6 glycosidase) acts at branch points of amylopectin
d) Maltase acts on maltose molecules and yields glucose.

Catabolism of glucose: The metabolism of sugars takes place through their phosphates to pyruvic acid (and lactic acid) in a process called Glycolysis. Pyruvate is the end product of aerobic glycolysis whereas lactate is the end product of anaerobic glycolysis. The enzymes of glycolysis are located in the cytoplasm of the cell and are associated into a unit called metabol for efficient functioning. Pyruvic acid is then converted to acetyl CoA, which is oxidized to $C0_2$ and H_20 in the citric acid cycle.

Glycolysis: The sequence of reactions from glucose to pyruvate is often called as Embden-Meyerhof-Parnas (EMP) pathway.



Under anaerobic glycolysis, pyruvic acid is converted to lactic acid as follows:



In a cell, the molecule which accepts energy from the breakdown of fuel molecules and donates this energy to cell functions is ATP. ATP is synthesized from ADP and inorganic phosphate and 7 kcal/mole must be available to achieve this reaction. This energy is obtained from the catabolism of carbohydrates, fats and proteins. There are two mechanisms by which energy is coupled to the formation of ATP. 1) Substrate level phosphorylation 2) Oxidative phosphorylation. Substrate level phosphorylation occurs when one of the phosphate groups in a metabolic intermediate is transferred to ADP to form ATP. In contrast oxidative phosphorylation couples inorganic phosphate to ADP directly. In the breakdown of glucose, many of the chemical reactions involve the removal of hydrogen atoms from various intermediates. These reactions require a coenzyme, to which the hydrogen atoms are transferred. As a result of such hydrogen transfers, some of the chemical energy in glucose molecule is transferred to the coenzyme molecule. The process of oxidative phosphorylation then uses this energy to synthesize ATP.

The energy release in glycolysis is as follows:

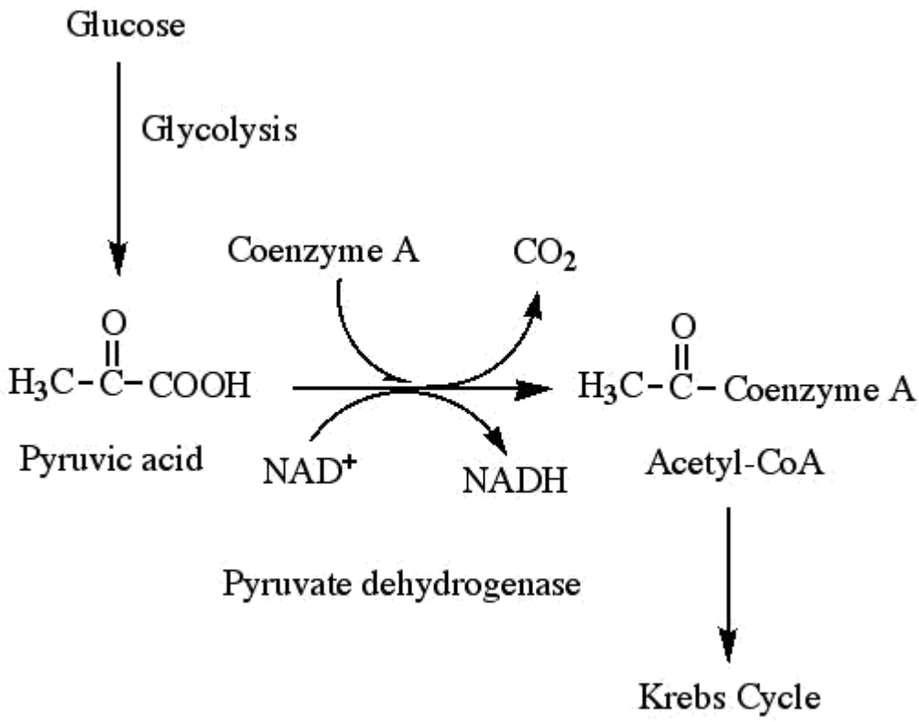
Aerobic glycolysis : Through substrate level phosphorylation =4ATP
Through oxidative phosphorylation =6ATP

Total	= 10 ATP
Input	= 2 ATP
Net energy yield	**= 8 ATP**

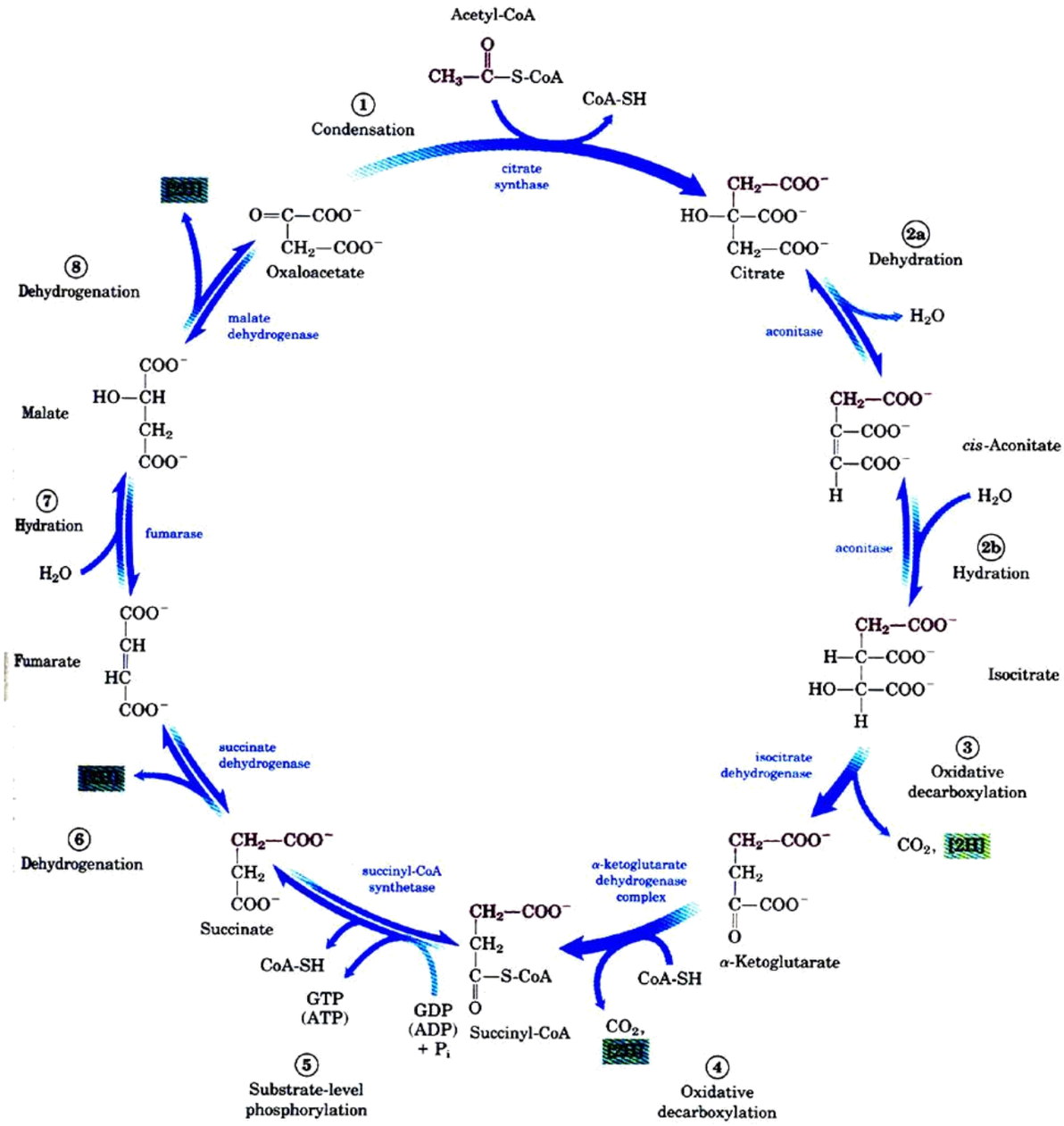
Anaerobic glycolysis : Through substrate level phosphorylation = 4 ATP

Input	= 2ATP
Net energy yield	**= 2 ATP**

The end product of glycolysis, pyruvic acid is converted to acetyl CoA.
The reaction is as follows:



TCA Cycle: The enzymes of TCA cycle are located in mitochondria.



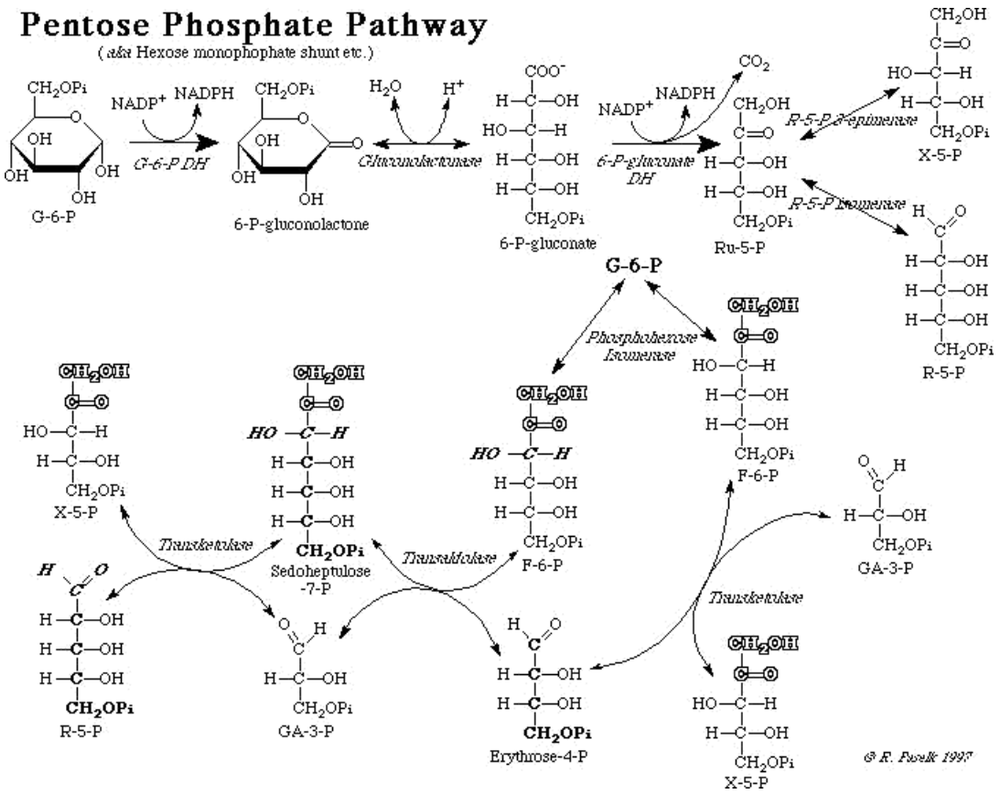
The energy release in TCA cycle is as follows:

a) Through substrate level phosphorylation = 1 ATP
b) Through oxidative phosphorylation = 11 ATP

Net energy yield = 12 ATP

Pentose phosphate pathway(PPP): This pathway, also known as the hexose monophosphate pathway produces reduced coenzyme in the form of NADPH, as well as ribose 5-phosphate for incorporation into nucleotides.
The net result of the PPP, if not used solely for ribose 5-phosphate production, is the oxidation of Glucose 6 phosphate, a 6 carbon sugar, into a 5 carbon sugar. In turn, 3 moles of 5 carbon sugars are converted, via the enzymes of the PPP, back into two moles of 6 carbon sugars and one mole of 3 carbon sugar. The 6 carbon sugars can be recycled into the pathway in the form of Glucose 6 phosphate, generating more NADPH. The 3 carbon sugar generated is glyceraldehyde-3-phsphate which can be shunted to glycolysis and oxidized to

pyruvate. This pathway has oxidative and non-oxidative reactions. The oxidative react ions are irreversible, and converts glucose 6-phosphate to five-carbon phospho sugars, with loss of CO_2 but concomitant production of NADPH for the reducing power needed for the biosynthesis of fatty acids.



Energetics of pentose phosphate pathway:

Glucose 6- phosphate + 12 $NADP^+$ + 7 H_2O _____ $6CO_2$ + 12 $NADPH+12H^+$ + Pi

The 12 molecules of NADPH on reoxidation to $NADP^+$ by NAD^+ give rise 12 units of NADH which through oxidative phosphorylation yields about the same number of ATP molecules (12 NADH = 36 ATP molecules).

Significance of pentose phosphate pathway: Apart from its energetics point of view, this pathway has other significant metabolic functions.

1. The NADPH which is generated through this pathway is required in reductive biosynthesis of fatty acids and steroids.
2. It supplies ribose needed for nucleic acid biosynthesis.
3. The ribose-5-phosphate which is produced as an intermediate is utilized for photosynthetic formation of glucose from CO_2 in some plants.
4. It also supplies erythrose-4-phosphate required for the biosynthesis of phenylalanine, tyrosine and tryptophan.
5. In humans, the shunt pathway is operative in liver where there is considerable biosynthetic activity and in red blood cells where NADPH is required to maintained high levels of reduced glutathione, a biological reducing agent
6. This pathway also makes possible for interconversion of various three, four, five, six and seven carbon sugars and connects all such sugars metabolically with the glycolytic sequence.

Electron transport chain in mitochondria: Reduced cofactors like NADH & FADH can be oxidized back to NAD & FAD and in the process energy liberated is used to form ATP molecules at electron transport chain. This chain is located in the mitochondrion on the inner membrane. All the enzymatic steps in the oxidative degradation of carbohydrates, fats and amino acids in aerobic cells converge at

the final stage of cellular respiration in which electrons flow from the catabolic intermediates to molecular oxygen. This yields energy for the generation of ATP from ADP + Pi.

ATP synthesis in mitochondrion and chloroplast is based on a hypothesis introduced by Peter Mitchell in 1961. It is now widely accepted and is called chemi-osmotic theory.
Mitochondrial electron flow: Here electrons pass from lower to higher reduction potential. Many of the proteins involved in electron flow are embedded in the inner mitochondrial membrane. They are organized into 4 respiratory complexes – I, II, III, & IV. Electrons are carried from I & II to III by Coenzyme Q (Ubiquinone) and from III to IV by Cytochrome c. Most of the electrons entering into mitochondrial respiratory chain arise from the action of dehydrogenases which collect electrons from oxidative reactions of Glycolysis,TCA cycle, β-oxidation and amino acid catabolism.

1. Complex I contains FMN & iron sulphur clusters also called NADH dehydrogenases. CompleX I catalyses the oxidation of NADH by Coenzyme Q (Co Q)
   NADH + Co Q (oxidized) _____ NAD^+ + CoQ (reduced)
2. Complex II contains Succinate dehydrogenase & 3 small hydrophobic units called SDH complex. Complex II catalyses the oxidation of $FADH^2$ by Coq. It passes the electrons from succinate to Co Q.
   $FADH^2$ + Co Q (oxidized) _____ FAD^+ + Co Q (reduced)
3. Complex III contains 2 cytochrome b molecules & 1 cytochrome c_1 called ubiquinone – cytochrome c oxidoreductase. Complex III catalyses the oxidation of CoQ by Cytochrome c
   Co Q (reduced) + Cyt c _____ Co Q (oxidized) + Cyt c (reduced)
4. Complex IV contains 2 cytochrome a molecules & 2 copper atoms called as cytochrome oxidase. Complex IV catalyses the oxidation of Cytochrome c by O_2 (the terminal electron acceptor of ETC)
   Cyt c (reduced) + ½ O_2 _____ Cyt c (oxidized) +H_2O

The proton consuming steps are located towards the matrix side and the proton releasing steps are located towards the inter membranes space. Protons accumulate in the inter membrane space whenever electrons flow through the chain. This proton gradient is dispersed by back flow of protons through ATP synthase. As a result, 3 ATP molecules are generated when NADH is oxidized and 2 ATP molecules are generated when FADH is oxidized. The terminal electron acceptor in ETC is O_2 or the common electron sink is O_2. Flow of electrons from cytochrome a_3 to O_2 is inhibited by cyanide. Cytochromes are iron containing electron transfer proteins of inner mitochondrial membrane.

Complex I

$NADH + H^+$	FMN	$Fe^{2+}S$	CoQ
NAD^+	$FMNH_2$	$Fe^{3+}S$	$CoQH_2$

Complex II

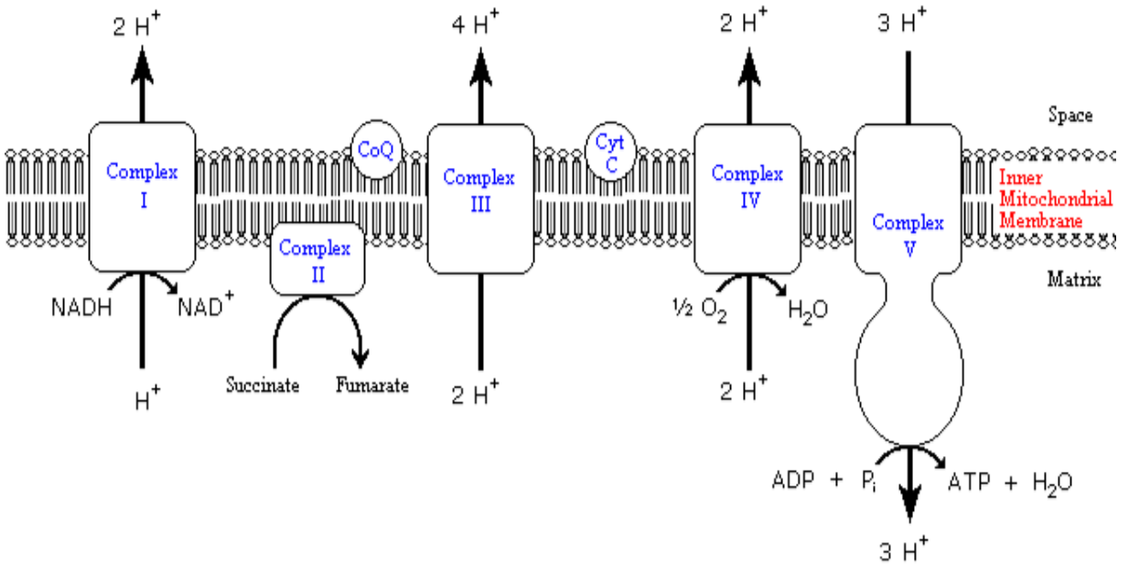
Succinate	FAD	$Fe^{2+}S$	CoQ
Fumarate	$FADH_2$	$Fe^{3+}S$	$CoQH_2$

Complex III

$CoQH_2$	cyt b ox	$Fe^{2+}S$	cyt c_1 ox	cyt c red
CoQ	cyt b red	$Fe^{3+}S$	cyt c_1 red	cyt c ox

Complex IV

cyt c red	cyt a ox	cyt a_3 red	O_2
cyt c ox	cyt a red	cyt a_3 ox	2 H_2O

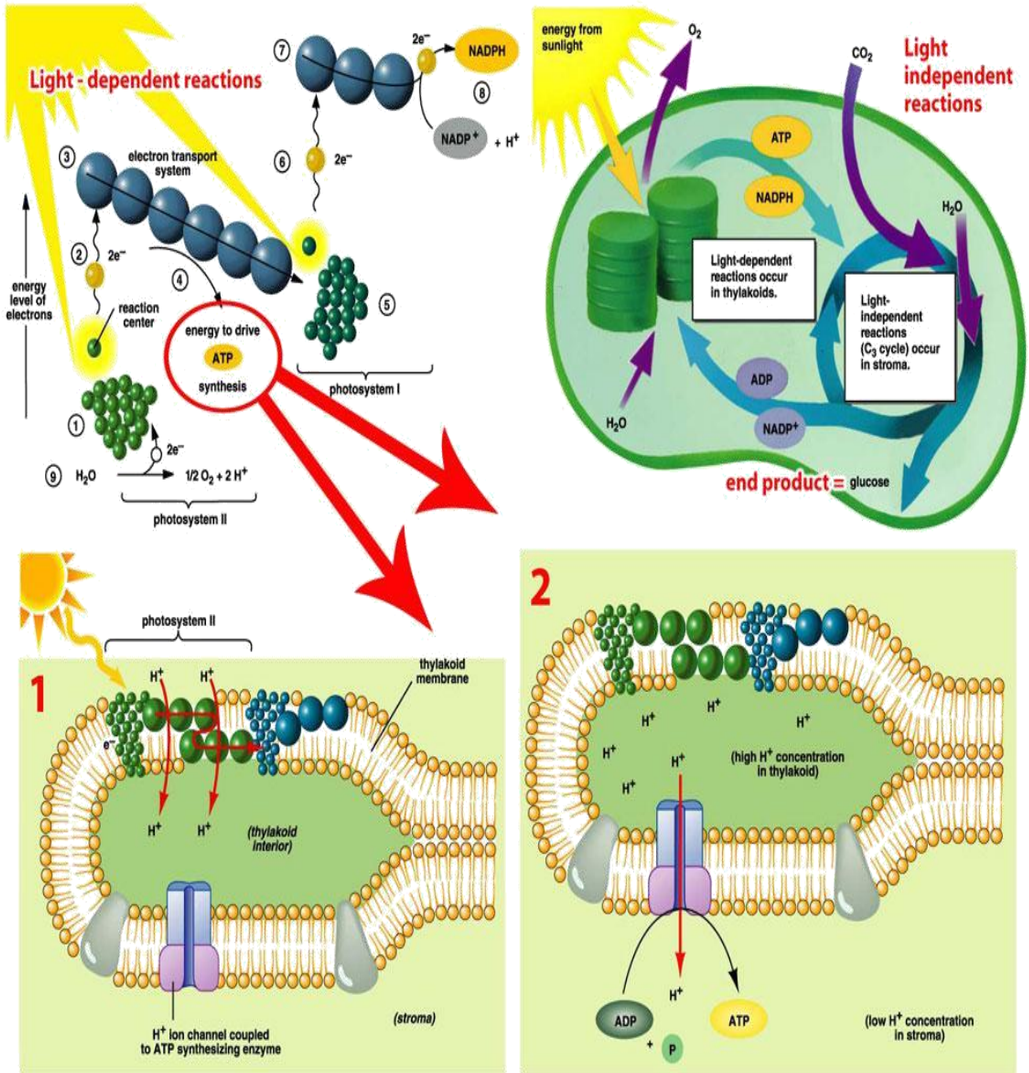


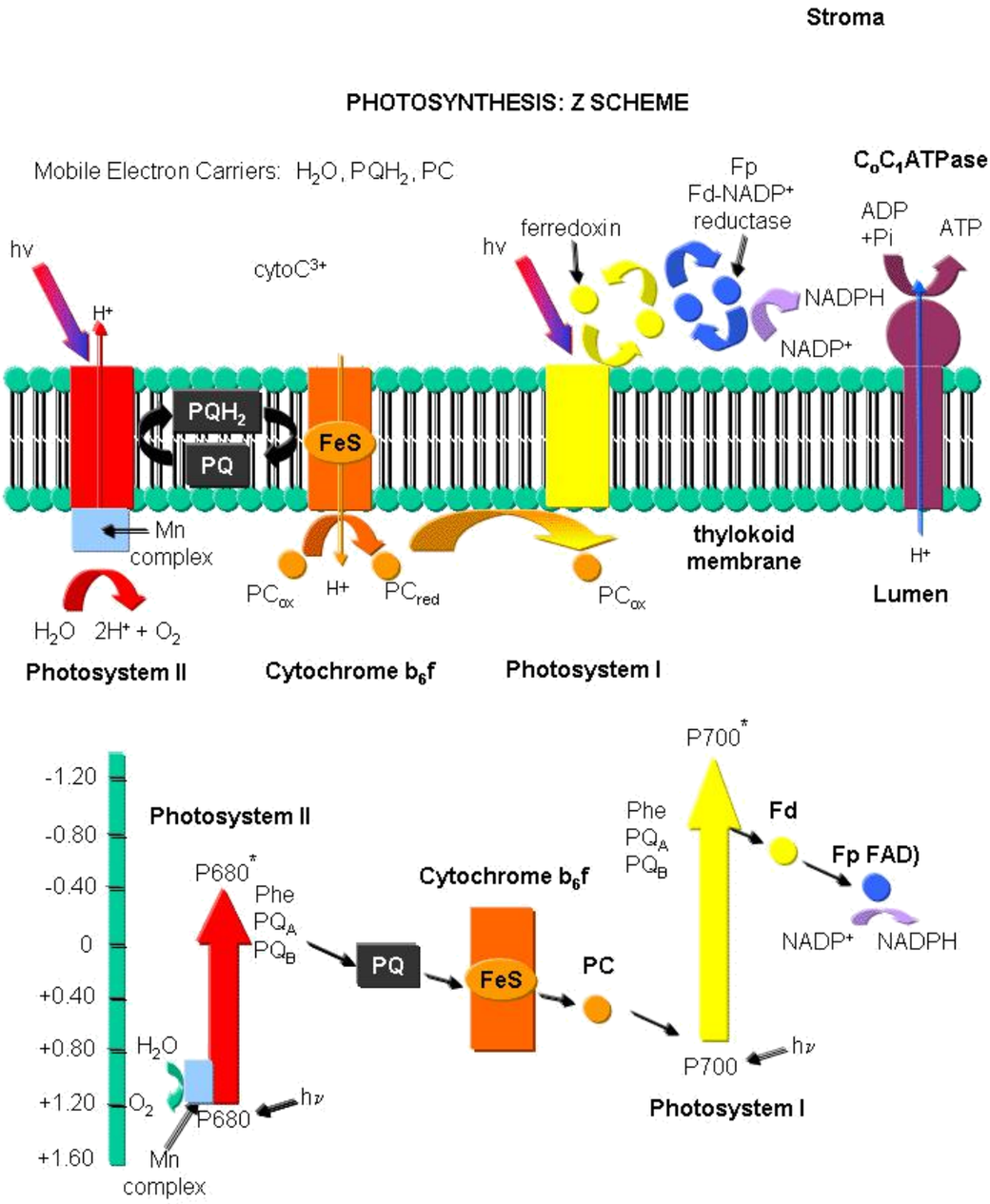
ETC in mitochondria

Electron transport chain in chloroplasts: The cells of all plants and photosynthetic algae contain chloroplasts, which produce ATP and NADPH using the energy of sunlight. The electron transport chain in chloroplasts is contained in two extremely complex transmembrane structures, *Photosystem II* (PS II) and *Photosystem I* (PS I). PS II and PS I are linked by a transmembrane proton pump, cytochrome b_6f, which is similar to mitochondrial *Complex III*. The overall process is the transfer of electrons from water to NADPH via a transmembrane proton pump:

H_2O → PS II → plastoquinone → *b_6f* → plastocyanin → PS I → NADPH

The resulting transmembrane proton gradient is used to make ATP via ATP synthase. NADPH is used by the Calvin cycle to make inorganic molecules from CO_2 and nitrates


Light - dependent reactions
electron transport system
energy level of electrons
reaction center
energy to drive
ATP
synthesis
photosystem I
photosystem II
NADPH
NADP+ + H+
2e−
H2O
1/2 O2 + 2 H+
energy from sunlight
O2
CO2
Light independent reactions
ATP
NADPH
H2O
Light-dependent reactions occur in thylakoids.
Light-independent reactions (C3 cycle) occur in stroma.
ADP
NADP+
end product = glucose
1
thylakoid membrane
(thylakoid interior)
(stroma)
H+ ion channel coupled to ATP synthesizing enzyme
2
(high H+ concentration in thylakoid)
ADP
+ P
ATP
(low H+ concentration in stroma)



Photosystem II

PS II is an extremely complex, highly organized transmembrane structure that contains a *water-splitting complex* , chlorophylls *a* and *b*, a *reaction center* (P680), pheophytin (a pigment similar to chlorophyll), and two quinones. It uses the energy of sunlight to transfer electrons from water to a mobile electron carrier in the membrane called *plastoquinone*:

$$H_2O \rightarrow P680 \rightarrow P680^* \rightarrow \text{plastoquinone}$$

Plastoquinone, in turn, transfers electrons to *b_6f*, which feeds them into PS I.

The water-splitting complex

It catalyzes a reaction that splits water into electrons, protons and oxygen:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

The electrons are transferred to special chlorophyll molecules (embedded in PS II) that are promoted to a higher-energy state by the energy of photons..

Link of water splitting complex and chlorophyll excitation

When the chlorophyll passes the electron to pheophytin, it obtains an electron from P_{680}^{*}. In turn, P_{680}^{*} can oxidize the Z (or Y_Z) molecule. Once oxidized, the Z molecule can derive electrons from the water splitting complex

PS II is a transmembrane structure found in all chloroplasts. It splits water into electrons, protons and molecular oxygen. The electrons are transferred to plastoquinone, which carries them to a proton pump. Molecular oxygen is released into the atmosphere.

Cytochrome *b_6f*

PS II and PS I are connected by a transmembrane proton pump, cytochrome *b_6f* complex (plastoquinol—plastocyanin reductase;. Electrons from PS II are carried by plastoquinone to *b_6f*, where they are removed in a stepwise fashion and transferred to a water-soluble electron carrier called *plastocyanin*. This redox process is coupled to the pumping of four protons across the membrane. The resulting proton gradient (together with the proton gradient produced by the water-splitting complex in PS II) is used to make ATP via ATP synthase.

Photosystem I

PS I accepts electrons from plastocyanin and transfers them either to NADPH (*noncyclic electron transport*) or back to cytochrome *b_6f* (*cyclic electron transport*):

plastocyanin → **P700** → **P700*** → **ferredoxin** → **NADPH**
↑ ↓
b_6f ← **plastoquinone** *b_6f*

There are two different pathways of electron transport in PS I. In *noncyclic electron transport*, ferredoxin carries the electron to the enzyme ferredoxin $NADP^+$ oxidoreductase that reduces $NADP^+$ to NADPH. Alternately, in *cyclic electron transport*, electrons from ferredoxin are transferred (via plastoquinone) to a proton pump, cytochrome *b_6f*. They are then returned (via plastocyanin) to P700.

NADPH and ATP are used to synthesize organic molecules from CO_2. The ratio of NADPH to ATP production can be adjusted by adjusting the balance between cyclic and noncyclic electron transport.

Metabolic regulation

Lac operon concept

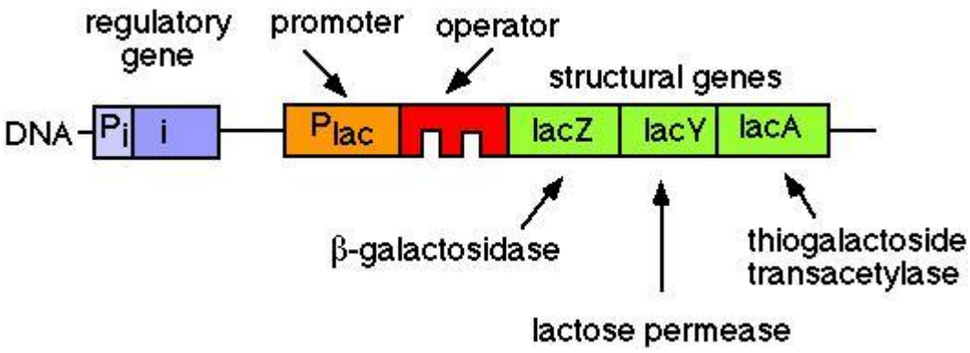
In its natural environment, *lac* operon is a complex mechanism to digest lactose efficiently. The cell can use lactose as an energy source only in the absence of glucose, but it must produce the enzyme β-galactosidase to digest it into glucose. It would be waste to produce enzymes when there is no lactose available, or if there is a more readily-available energy source such as glucose.

Structure of the operon

- The *lac* operon consists of three structural genes, a promoter, a terminator, regulator, and an operator genes. The three structural genes are: *lacZ*, *lacY*, and *lacA*.
    - *lacZ* encodes β-galactosidase (LacZ), an intracellular enzyme that cleaves the disaccharide lactose into glucose and galactose.

- *lacY* encodes β-galactoside permease (LacY), a membrane-bound transport protein that pumps lactose into the cell.
- *lacA* encodes β-galactoside transacetylase (LacA), an enzyme that transfers an acetyl group from acetyl-CoA to β-galactosides.

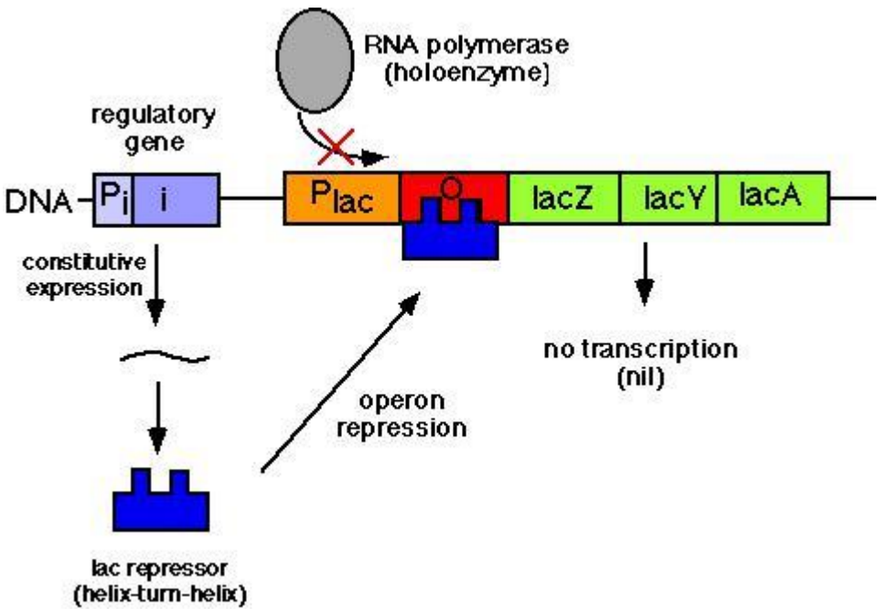
Only *lacZ* and *lacY* appear to be necessary for lactose catabolism.



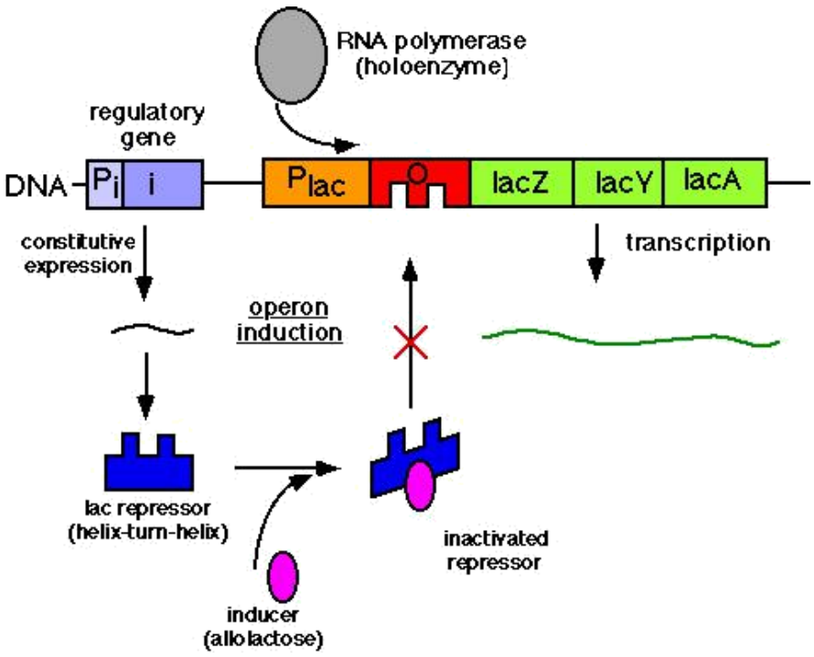
Mechanism of lac operon action:

This is divided into two phases ie:- Repression and Induction

Repression :- In absence of lactose, the repressor protein binds to DNA at the operator and blocks binding of RNA Polymerase enzyme at the promotor. Hence the corresponding RNA is not synthesised and the enzymes are not synthesised. So lactose is not utlisied.



Induction: - In presence of the lactose, lactose is converted to allolactose which acts as inducer and binds to the repressor protein and dissociates the repressor from the operator region. Now the RNA polymerase binds to the operator region and synthesizes m RNA. With the information present on the mRNA, the structural genes are synthesized and the three enzymes are released which act upon lactose and convert it into glucose and galactose.



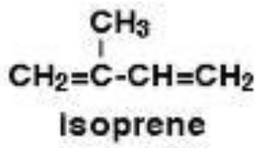
SECONDARY METABOLITES

Secondary metabolites unlike the primary metabolites are not essential for growth and development of living organisms. Many of them do not have any known function too. However, with the research results pouring in, more and more functions of secondary metabolites are being noticed and the differences between the primary and secondary metabolites are being narrowed down.

Among the different classes of secondary metabolites, Terpenes, Alkaloids,and Phenolics are the major ones.

TERPENES

Terpenes are hydrocarbon based natural products, the structures of which are derived from isoprene units. Isoprene is a 5 C unsaturated hydrocarbon i.e. 2 – Methyl 1,3 – butadiene.



Terpenes are thus referred to as Isoprenoids also. In simpler terms, terpenes are organic compounds containing an integral number of 5 C units.

The name ‘terpene’ has now almost been replaced by the more accepted term ‘terpenoids’, since a large number of their oxidized products like alcohols, aldehydes, ketones etc are derived from terpenes. Terpenes constitute one of the most diverse and numerous class of organic compounds. Also, they are very widespread and abundant too.

Classification

Terpenes are classified depending on the number of 5 C units they contain. Accordingly, they are classified as follows.

S. No.	No. of C atoms	No. of 5 C units	Class	Examples
1	5	1	Hemiterpene	isoamyl alcohol, isovaleric acid
2	10	2	Monoterpene	menthol (peppermint), geraniol (rose oil), limonene (orange lime), citronellal (oil of citronella)
3	15	3	Sesquiterpene	abscisic acid (plant growth regulator), zingiberene (ginger), farnesol (lily)
4	20	4	Diterpene	gibberellins (plant growth regulations, *Gibberella fujikuroi*), gossypol (cotton seed oil, toxic) phytol (component of chlorophyll), retinol (concerned with vision)
5	25	5	Sesterpene	very rare in nature
6	30	6	Triterpene	squalene (shark liver oil), cholesterol, lanosterol (both precursors of steroid hormones)
7	40	8	Tetraterpene	β – carotene (carrot and tomato), lycopene (tomato)
8	Many	Many	Polyterpene	rubber, gutta

Functions of terpenes in plants

1. As Plant growth regulators – Important among them are abscisic acid, gibberellins and brassinolide which is a sterol.
2. As electron carriers e.g. ubiguinone in electron transport system.
3. As photosynthetic pigments e.g. β – carotene
4. As constituents of cell membranes e.g. steroids.
5. Plants defence: e.g. damaged plants exude resinous materials. The role of such resins (mostly terpenes) is to seal to the wound, so that the infecting and attacking microbes fail to enter inside. Arbutin, a terpenoid, is an allellopathic chemical inhibiting the growth and germination of other plants nearby. Even, the major insecticidal principles of neem are terpenoids e.g. Azadirachtin.
6. Insect repellents e.g. Citronellal in citronella oil.
7. Pollination helpers e.g. perfumes present in many flowers help attract insects and result in pollination.
8. Fungal antibiotics e.g. β -Phellandrenes.

Functions of terpenes in animals

1. Carries of sugars e.g. Dolichol.
2. Constituents of cell membranes e.g. Steroids.
3. Ants and termites use terpenoid trail pheromones to mark a path. This explains why ants are often seen marching in a single line over long distances.
4. As Juvenile Hormones in insects.
5. As sex attractants in a number of insects.

Uses of terpenes

1. Natural rubber (a polyterpene - major source- *Hevea brasiliensis*) is used in the manufacture of vulcanized and high strength less friction rubber.
2. As flavoring agents in foods, drinks, ice creams, and even cigarettes. A widely used such material is Vanillin present in vanilla beans.
3. Perfumer e.g. Geraniol is widely used as a perfumery material.
4. Oil of turpentine is used as a solvent in paints.
5. As fragrances in soaps, talcum powders and cosmetics.
6. Insects' repellents e.g. oil of citronella.
7. Insect control agents e.g. as juvenile hormones which prevent metamorphosis in insects and neem based insecticidal preparations.
8. In medicines: Ex.
    a) Santonin has been used in India since long for the control of intestinal worms.
    b) Digitoxin is used in heart beat regularization.
    c) Cineole is used as a nasal decongestant.

ALKALOIDS

The term 'alkaloid' was originally used to refer to naturally occurring substances of plant origin that are alkaline in reaction, optically active, contain nitrogen as part of a heterocyclic and do have varying degrees of psychological responses in man and animals.

Nomenclature of alkaloids

Alkaloids are normally named by adding the suffix 'ine' to various distinguishable features such as sources, physiological action in animals, their properties etc. Thus,

1. The name of the genera of the plants containing them e.g. Atropine (*Atropa*), nicotine (*Nicotiana*) etc.
2. The names of the species of the plants producing them e.g. cocaine (*Erythroxolon coca*).
3. The common name of the organism producing them e.g. ergotamine (ergot fungus).
4. The specific physiological activity observed in animals e.g. emetine (emetic – causing vomiting), narcotine (narcotic-alters mind).

Classiciation of alkaloids

Various criteria are used for classifying alkaloids. Thus, those alkaloids for which the precursors are the amino acids are grouped as True Alkaloids, while others are grouped as Pseudo alkaloids. Further, those alkaloids which contain nitrogen atom present in a side chain are called Proto alkaloids.

However, the most widely used classification system of alkaloids is based on their skeleton rings. Accordingly, the alkaloids are classified as follows.

S. No.	Class	Examples
1	Pyridine and piperidine	Conine, nicotine
2	Tropane	Atropine (*Atropa belladonna*), Cocaine
3	Quinoline	Quinine (*Cinchona officinalis*)
4	Isoquinoline	Papaverine (*Papaver somniferum*)
5	Phenanthr-ene	Morphine (*Opium poppy*)
6	Indole	Codeine, strychrine and ergotamine
7	Purine	Caffeine (tea and coffee)
8	Pyrolidine	Hygrine (Aswagandha)
9	Imidazole	Pilocarpine
10	Phenylalkylamine	Ephedrine
11	Pyrolizidine	Retronecine
12	Quinolizidine	Lupinine
13	Steroidal	Solanidine
14	Terpenes	Aconitine

Based on the precursor amino acids also, the alkaloids can be classified which is given below

S. No.	Precursor amino acids	Alkaloids
1	Tryptophan	Indoles, Quinolines
2	Lysine	Piperidines
3	Ornithine	Tropanes, Pyrrolidines
4	Tyrosine	Isoquinolines, Phenylalkylamines
5	Histidine	Imidazoles
6	Asparagine	Pyridines

Functions of alkaloids in plants

1. Act as protective agents against herbivorous animals due to their bitter taste and toxicity. The earlier adaptations like thorns and stinging hairs proved inadequate and then over the years, the plants learnt to produce such phytochemicals for the said purpose. Among such phytochemicals, the alkaloids rank the number one. Possessing alkaloids thus became an evolutionary development.
2. Some alkaloids act as the source of nitrogen in case of deficiency of nitrogen. It is suggested that plants which can not store excess nitrogen

and can not afford to throw the element either temporarily store the excess nitrogen in the form of alkaloids, especially in vacuoles, and redraw the nutrient element later when necessary.

3. Some alkaloids act as plant growth regulators.
4. Some alkaloids act as the final products of detoxification of various Nitrogen containing substances.

Uses of alkaloids

1. In medications: Alkaloids are particularly known for their therapeutic value. Accordingly, alkaloids find use in treating various health disorders. Some such uses are furnished below.

S. No.	Alkaloid	Use
1	Aconitine	Antipyretic
2	Cocaine	Vasoconstrictor, local anesthesia
3	Codeine	Cough suppressants
4	Reserpine	Anti high blood pressures
5	Emetine	Antiamoebic, emetic
6	Morphine	Analgesic, narcotic
7	Quinine	Antimalarial
8	Ajmalicine	Antihistamine
9	Atropine	Boosting nerve systems

2. As flavor and taste enhancing agents: Capsaicin is the active ingredient of red pepper, and is used as flavor and taste enhancing agent.
3. In Plant Biotechnology: Colchicine is used to produce polyploidy in plants along with swollen and sterile seeds. Polyploid fruits and vegetables are now very common in super markets.

4. As psychoactive agents:

    a. Stimulants e.g. nicotine and caffeine
    b. Hallucinogens e.g. ergot alkaloids (LSD, lysergic acid and heroin)
    c. Tranquilizers e.g. morphine and codeine

5. As pain killers e.g. aconitine and morphine
6. As poisons e.g. conine and stachydrine (*Medicago sativa*).

PHENOLICS

The term Phenolic refers to a substance which possesses an aromatic ring containing one or more OH groups.

Phenolics constitute one of the most widespread classes of secondary metabolites. Commonly talked about class of compounds like anthocyanins, flavonoids, lignins, tannins, coumarins and chalcones are all phenolic substances.

Phenolics are water insoluble substances. However, the water solubility increases with the increase in the number of OH groups. Phenolics absorb electromagnetic radiation strongly - many of them in the visible part of the spectrum (anthocyanins) and are coloured.

Biogenesis

Phenolics are produced in plants by

1. Shikimate pathway through phenylalanine ($C_6.C_3$)
2. Acetate/ malonate pathway

Classification of phenolics

The phenolics are classified based on their carbon skeletons

S. No	No. of carbon atoms	Carbon skeleton	class	example
1	6	C_6	Simple phenols	Hydroquinone, catechol
2	7	$C_6.C_1$	Hydroxybenzoates	4-Hydroxybenzoate
3	8	$C_6.C_2$	Phenylacetates	4-Hydroxyphenyl acetate
4	9	$C_6.C_3$	1. Hydroxy cinnamates 2. Phenylpropenes 3. Coumarins 4. Chromones	1. Caffeate 2. Eugenol 3. Esculetin 4. Eugenin
5.	10	$C_6.C_4$	Naphthoquinones	Juglone
6.	13	$C_6.C_1.C_6$	Xanthones	Mangiferin
7	14	$C_6.C_2.C_6$	Stilbenes	Resveratrol (eucalyptus)
8	15	$C_6.C_3.C_6$	Flavonoids	Rutin
9	18	$(C_6.C_3)_2$	Lignans	Pinoresinol
10	30	$(C_6.C_3.C_6)_2$	Biflavonoids	Amentoflavone
11	N	$(C_6)_n$	Melanins	
12	N	$(C_6.C_3)_n$	Lignins	
13	N	$(C_6.C_3.C_6)_n$	Condensed tannins	

Some important phenolics

Lignin

Lignin, a cell wall strengthening material, is a $C_6.C_3$ polymer. It is more abundant in wood, corky tissues and straw. Lignin imparts resistance to degradation by water and microorganisms. In plant cell walls where lignin is more common, lignin remains embedded in cellulose microfibrils in a dense cross connected network.Lignin is made up of three aromatic (phenolic) alcohols of which the most important is *p*- coumaryl alcohol

Flavonoid

: Flavonoids are 15 C phenolics with $C_6.C_3.C_6$ carbon skeleton. Flavonoids are widely distributed in the plant kingdom. They absorb radiation very strongly in the visible part of the electromagnetic spectrum and are differently coloured. Anthocyanins which impart color to flowers, leaves, and fruits of many plants are flavonoids. In flowers, they help attract the pollinating insects. A specific example of flavonoid is delphinidin, the substance that gives blue color to grapes. Flavonols and flavones are also important flavonoids. Flavonoids occur as glycosides attached to sugars, normally glucose and occasionally galactose.

Tannins:

Tannins are $C_6.C_3.C_6$ ploymeric phenolics. They are astringent phenols and tan animal skin to leather. Tannins cross link proteins, thus denaturing them. They thus keep the microorganisms away and impart a protective function.

Coumarins

Coumarins form the starting metabolite of a number of important phenolics. Coumarin imparts the aroma of freshly cut grass.

Functions of phenolics

1. Pigmenatation: Pigmentation of the outer surface and inner parts of plants and animals is one of the important functions of phenolics. Thus, melanin is responsible for the darkening of the skin in human beings. Chlorogenic acid imparts darkening of the potato tuber. Browning of the cut tissues of many fruits is also due to the presence of phenolic substances.
2. Regulation of plant growth: Important phenolics regulating plant growth are ferulic acid and phytoalexins.
3. Disease resistence: Protocatechuric acid imparts resistance to smudge caused by *Colletotrichum circinam* in onions.
4. Alleollopathy: Gallotannins obtained from gallic acid is an allellochemical in many plants.
5. Insects control: A number of coumarins act as juvenile hormones in insects by preventing metamorphosis and hold promise in future insects control.
6. Electron carrier: Some phenolics act as electron carriers e.g. ubiquinone / ubiquinol in Electron Transport System.
7. Uv protection: Phenolics help protect animals / plants against damage by Ultra Violet radiation.

Industrial uses of phenolics

1. In manufacture of medicines: eg. Aspirin, a widely used chemical as antipyretic and heart beat regulator is manufactured from simple phenolic compounds.
2. Phenolics (polymers) are used for making adhesive resins.
3. Pehnolics (polymers) are used as electrical insulating materials.
4. Phenolics are used in the manufacture of pesticides like 2,4-D (weedicide), and carbaryl (insecticide).
5. Phenolics are widely used ingredients in cosmetics, particularly sun creams.
6. Phenolics are used in the manufacture of plastics like Bakelite.
7. Phenolics are extensively used in the making of coloring materials.