9. Biomolecules

The most abundant chemical in living organisms is water. If we perform an analysis on a plant tissue, animal tissue or a microbial paste, we obtain a list of elements like carbon, hydrogen, oxygen and several others and their respective content per unit mass of a living tissue. But the relative abundance of carbon and hydrogen with respect to other chemicals is higher in living organism than in earth's crust.

1. Introduction

Although more than 25 types of elements can be found in biomolecules, six elements are most common. These are called the CHNOPS (carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur). These elements are compatible with life because of their special properties. They are called Biological elements or bioelements.

1.1. Major Elements

The four classes of macromolecules that make life possible (protein, carbohydrates, lipids, and nucleic acids) are all made of carbon, along with the other three main organic elements. Aside from the major four macromolecules mentioned above, the next major elements would be phosphorus, sulfur, sodium, chlorine, potassium, calcium and magnesium. These make up 3.5% of living things. Phosphorus helps connect individual units of DNA into a long chain. Sulfur forms bridges between different parts of a protein, which gives the protein its 3D shape. Sodium, chlorine, potassium, and calcium are essential for nerve cells to send electrical signals to other cells. And some enzymes require magnesium to work.

1.2. Trace Elements

Trace elements are present at low levels in organisms and makeup just 0.5% of living cells. However, living things would not be able to survive without trace elements. Trace elements include iron, iodine, manganese, molybdenum, selenium, silicon, tin, vanadium, boron, chromium, cobalt, copper and fluorine. Iron is found in red blood cells and helps to carry oxygen in the bloodstream. Iodine is important for making different forms of thyroid hormone, which regulates growth and energy levels in humans. Many of the trace elements are required by enzymes in order to make chemical reactions happen.

1.3. Metabolites

If one were to make a list of biomolecules, such a list would have thousands of organic compounds including amino acids, sugars, etc. We can call these biomolecules as 'metabolites'. Metabolites are of two types :

- **Primary metabolites –** Metabolites have identifiable function & play a known role in physiological processes. They are found in animal, plant, fungus and microbes.
- **Secondary Metabolite –** Metabolites have no identifiable function & play no direct role in physiological processes. They are found in plant, fungus and microbes. However, many of secondary metabolites are useful to 'human welfare' (e.g., rubber, drugs, spices, scents and pigments). Some secondary metabolites have ecological importance.

Some Secondary Metabolites

Pigments	Carotenoids, Anthocyanins, etc.
Alkaloids	Morphine, Codeine, etc.
Terpenoides	Monoterpenes, Diterpenes etc.
Essential Oils	Lemon, grass oil, etc.
Toxins	Abrin, Ricin
Lectins	Concanavalin A
Drugs	Vinblastin, Curcumin, etc.
Polymeric Substances	Rubber, Gums, Cellulose

1.4. How to Analyze Chemical Composition?

In order to study various biomolecules found in living tissues (a vegetable or a piece of liver etc.) grind it in trichloroacetic acid (Cl_3CCOOH) with help of pestle and mortar. It is strained through cheese cloth or cotton and we obtain two fractions. The filtrate is called acid soluble pool while the retentate is an acid insoluble fraction. Chemicals present in both the fractions are further separated by various analytical techniques and identified.

Average Composition of Cells

Components	% of the total cellular mass
Water	70 – 90
Proteins	10 – 15
Carbohydrates	3
Lipids	2
Nucleic Acids	5 – 7
Ions	1

The acid soluble pool contains chemicals with small molecular mass of 18-800 daltons. They are biomicromolecules. Acid insoluble fraction contains chemicals with large molecular mass of more than 800 daltons, they are biomacromolecules. Biomacromolcules are of large size and high, molecular weight, complex molecules that are formed by condensation of biomicromolecules. Their molecular mass is in the range of ten thousand daltons and above. Biomacromolecules are of three types-proteins, nucleic acids and polysaccharides.

Depending upon the molecular weight and solubility, biomolecules are divided into two categories; The acid soluble pool represents roughly the cytoplasmic composition. The macro molecules from cytoplasm and organelles become the acid – insoluble fraction. Together they represent the entire chemical composition-of living tissues or organisms.

- **Micromolecules** small sized, have low molecular weight, simple molecular structure and high solubility, diffusible. These include, water, minerals, gases, carbohydrates, amino acids and nucleotides.
- **Macromolecules** are large sized, have larger molecular weight, complex conformation and low solubility, non-diffusible. They are generally formed by polymerization of micromolecules. These include polysaccharides, proteins and nucleic acids, Lipid.

  Inorganic compounds like sulfate, phosphate etc. are also seen in the acid soluble fraction. Therefore elemental analysis gives elemental composition of living tissues in the form of H, O, N, C etc. While analysis of compounds gives an idea of the kind of organic and inorganic constituents present in living tissues.

  From a chemistry point of view, one can identify functional groups like aldehydes, ketones, aromatic compounds etc. But from a biological point of view, we shall classify them into amino acids, Nucleotide bases, fatty acids etc. All the macromolecules except lipids are formed by the process of polymerization, a process in which repeating subunits termed monomers are bound into chains of different lengths. These chains of monomers are called as polymers. Various types of micromolecules and macromolecules are given below:

1.5. Water (elixir or liquid of life or cradle of life)

In protoplasm it is about 80-85 % but it is 95 % in free state and 5 % inbound state. It constitutes 70-80% of the body of a living being. 70% human body is water but in embryo, water content is 90-95% while in Jelly Fish it is 99%. In human body 55% of water (20-30 litres) occurs inside cells while 45% of water is present in all extracellular fluids.

2. Carbohydrates

These are compounds of C, H and O where H and O occur in the ratio of 2 : 1 and general formula is $C_n H_{2n} O_n$. Value of n is 3 or more. Exception is deoxyribose sugar, $C_5H_{10}O_4$. Also called saccharides or sugars. They form 80% of dry weight of plants but 1% of dry weight of animal. These are most abundant organic compound on earth. Carbohydrates are of two broad categories, simple and complex.

2.1. Monosaccharides

They are the sugar units that cannot be further hydrolysed into simpler units. There are two major classes of monosaccharides.

- **Aldoses:** Sugars containing an aldehyde group are known as aldoses, e.g., Glucose, galactose, mannose, ribose and glycerose.
- **Ketoses:** Sugars containing a ketonic group are known as ketoses. e.g., Dihydroxyacetone, fructose and seduloheptulose.

  Depending upon the number of carbon atoms, aldoses and ketoses are further classified as Trioses (C3) ,Tetroses (C4),Pentoses (C5) Hexoses (C6), Heptoses(C7).

(Some Common Aldoses and Ketoses)

Monosaccharide	Aldose	Ketose
1. Trioses (C3)	Glyceraldehyde	Dihydroxy acetone
2. Tetroses (C4)	Erythrose, Threose	Erythrulose
3. Pentoses (C5)	Ribose, Deoxyribose, Xylose, Arabinose	Ribulose
4. Hexoses (C6)	Glucose, Galactose, Mannose	Fructose
5. Heptoses(C7)	Glucoheptose, Galactoheptose	Sedoheptulose

Glyceraldehyde and dihydroxyacetone are trioses. Glycerol and mannitol are alcoholic sugar.

Pentose sugars (C-5) ribose and deoxyribose, occur in nucleotides and nucleic acids.

Xylose (Pentose sugars) is non-nutritive sweetener. It is available as xylitol.

Fructose (Sweetest sugars) is fruit sugar present mostly in fruits.

Glucose (=dextrose) is grape sugar as well as blood sugar, cellular fuel, most preferred and favored respiratory fuel hence called immediate (instant) source of energy.

Galactose (brain sugar, most rapidly transported sugar) is a part of amino sugars, agar-agar, glycolipid, glycoprotein, lactose are never present in free stage. Ascorbic acid (vitamin C) is sugar acid.

- **Structure of monosaccharide: of two types :**

  (i) **Open chain structure:** It is present in all types of monosaccharides. Aldehyde group is terminal but ketone group is sub terminal in chain.

(ii) **Ring structure:** It is present in monosaccharides having more than 4 carbons. It is of two types.

(a) Furanose – 4 carbon (or 5 atom) in ring (less stable)

(b)Pyranose— 5 carbon(or 6atom) in ring (more stable)

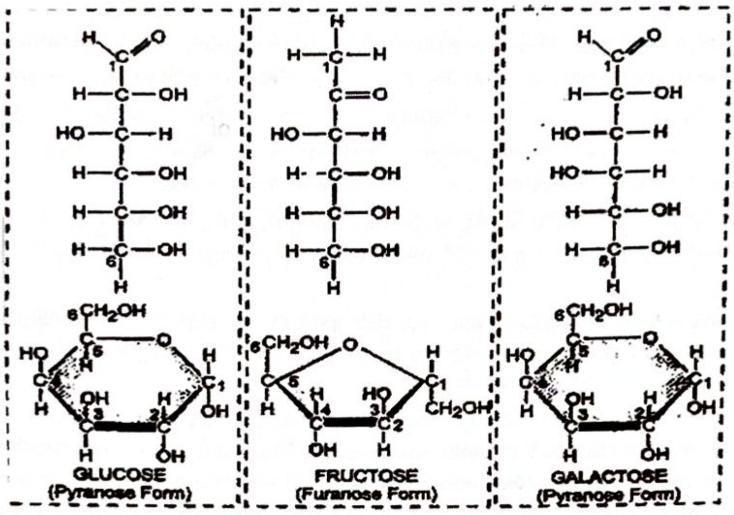


Fig. : Open chain and ring forms of three hexoses

- **Properties of monosaccharide :**

(i) All monosaccharides have an asymmetric carbon (chiral) and can rotate polarized light to right (dextrorotatory or D-) or left (laevorotatory or L-). Fructose (levulose) is present in honey is levorotatory. Most sugars are dextrorotatory. (Di-hydroxy acetone phosphate is optically inactive).

(ii) Some carbohydrates having free aldose or ketose group are called reducing sugars because they can reduce cupric ion to cuprous state (Fehling's or Benedict's solution). e.g. Monosaccharides, maltose and lactose. Some carbohydrates do not have free aldehyde or ketone group, so cannot reduce Fehling solution called non reducing sugar e.g. Polysaccharides, Oligosaccharides except maltose and lactose.

- **Function of Monosaccharide :**

(i) Most organisms create energy by breaking down the monosaccharide glucose, and harvesting the energy released from the bonds.

(ii) Monosaccharides are used to form long fibers, which can be used as a form of cellular structure.

(iii) Some bacteria can produce a similar cell wall from slightly different polysaccharides. Even animal cells surround themselves with a complex matrix of polysaccharides, all made from smaller monosaccharides.

(iv) Carbon skeleton of monosaccharides is also used in the formation of fatty acids, chitin and amino acids.

(v) The monosaccharides serve as the building blocks or monomers for the formation of disaccharides such as lactose and sucrose, polysaccharides, such as cellulose, glycogen, starch etc.

2.2. Oligosaccharide

Formed by condensation of 2-9 monosaccharides (join by glycosidic bond). Glycosidic bond is formed between aldose or ketose group of carbohydrate with alcoholic or nitrogen group of another organic compound (C-O-C or C-N-C).

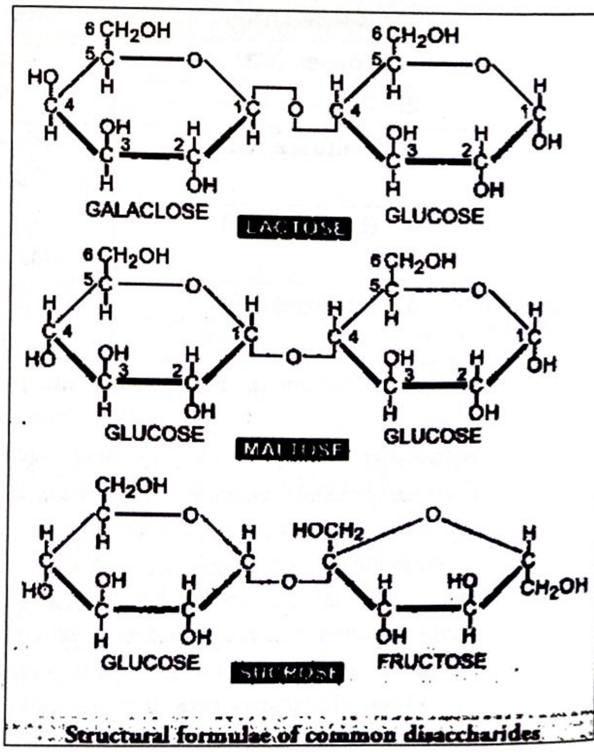
Bond is commonly formed during condensation of monosaccharides to form oligosaccharides and polysaccharides. A molecule of water is released at each condensation, so called dehydration synthesis. On the basis of number of monosaccharide units given by them on hydrolysis. These can be of following types

Disaccharides : Formed of two monosaccharides. Hexose disaccharides are common.

Sucrose is commercial or table sugar (glucose + fructose). It is non reducing. Sucrose is technically Glc (α 1→2) Fru or Fru (β 2→1) Glc.

Maltose/malt sugar is reducing sugar. It has α 1 → 4 glycosidic bond b/w two glucose molecules.

Lactose or milk sugar is reducing sugar formed by β 1 → 4 condensation b/w galactose and glucose. It is max. in elephant milk (8.8%), human milk (7%) and minimum in porpoise (1.3%).



Structural formulae of common disaccharides

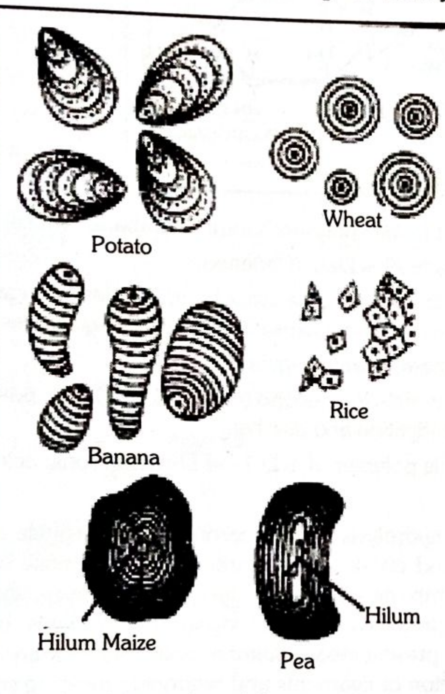
- **Trisaccharide** -Raffinose (non reducing trisaccharide) made of galactose(α 1→6)glucose(α 1→β2)fructose residues. It is found in sugarcane, plant of higher altitude.
- **Tetrasaccharide** - Trachyose is a non reducing tetrasaccharide, act as transporting car
- bohydrate and formed of glu (α 1→6) fru (α1 → 6) gal (α 1→2) gal. Stachyose is found in phloem and Chinese artichoke.

2.3. Polysaccharide

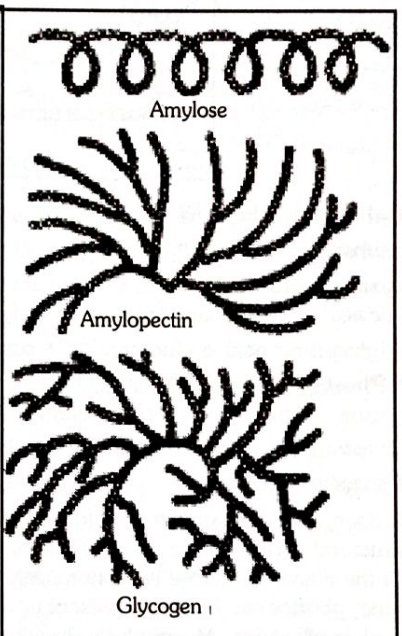
These are complex carbohydrate or glycans or non-sugars, which are usually insoluble, non diffusible, tasteless and without osmotic effect. Formed by condensation of more than 10 monosaccharide, so they are polymers. Depending upon the chain composition they can be classified as

- **Homopolysaccharide-** Formed by the polymerisation of only one type of monosaccharide monomers e.g., (a) Pentosans like xylans, arabans. (b) Hexosans like glycosans (starch, dextrin, glycogen and cellulose), fructosans (e.g., inulin), galactans and mannans.

(i) **Starch-** It is reserve branched homopolysaccharide of plant. Each starch grain has a central point or hilum around which starch is laid as concentric (e.g., Wheat) or eccentric (e.g., Potato) shells. Largest starch grain is found in Canna but smallest in Rice. Shape of starch grain is rounded in Wheat, oval in Potato, spindle in Banana, dumb-bell in Euphorbia. Starch has straight chain or amylose of 1→4 α-D glucose and side chains or amylopectin of glucose that are attached to straight chains by 1→6 α-D glycosidic linkages. It gives blue colour with I_2 due to amylose fraction. Amylopectin gives purple colour with I_2.



Starch grains from different sources



Form of common homopolysaccharide molecules

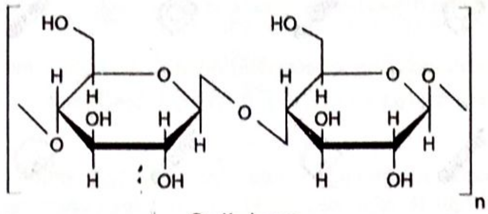
(ii) **Glycogen-** It is reserve branched homopolysaccharide (glucosan) of animal and fungal cells (called animal starch.) It occurs as ellipsoidal, flattened granules, which give reddish (red-violet) colour with iodine. It found in contact with SER, where they are formed in situ from glucose through condensation process called glycogenesis. It is stored in liver, muscles. Main or 'straight' chain has α-D 1→4 glycosidic linkages. It is helically coiled. After every 8-12 glucose units it bears a side chain through α-D 1→6 glycosidic linkages.

(iii) **Cellulose-** It is fibrous unbranched homo-polysaccharide (glucosan) that forms the structural component (as microfibrils) of plant cell wall, some primitive fungi and tunic of ascidians. Cellulose content is high (95%) in fibers of Hemp and Jute. Cotton fiber is 90% cellulose.

(iv) **Cellulose** is the most abundant organic substance on earth. Cellulose does not stain with I_2. It has a linear chain of 8000 glucose linked by β-1→4 glycosidic bond. Adjacent glucose lie at 180° to each other. Cellulose can be digested by only a few microbes, present in the gut of ruminants and white ants. In human diet, it provides roughage that stimulates peristalsis and bowel movements. It produces cellulose xanthate (in rayon and cellophane), cellulose acetate (photographic films, terricot, shatter-proof glass), cellulose nitrate (nitrocellulose, gun cotton, propellant explosive).

(v) ***Cellulose*** is the substance that makes up most of a plant's cell walls such as algae and the oomycetes, major constituent of paper, paperboard, and card stock.

(vi) **Cellulose** is the main ingredient of textiles made from cotton, linen, and other plant fibers. It can be turned into rayon, an important fiber that has been used for textiles since the beginning of the 20th century. In human nutrition, cellulose is a non-digestible constituent of insoluble dietary fiber, acting as a hydrophilic bulking agent for feces and potentially aiding in defecation.

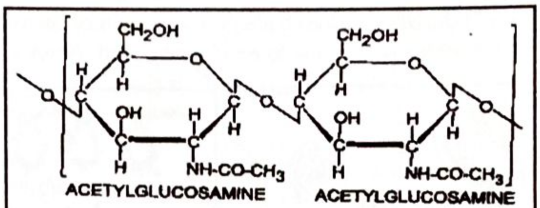


Cellulose

- **Heteropolysaccharide-** Formed by condensation of more than one type of monosaccharides or monosaccharide derivatives, e.g., chitin, pectin, hemicellulose, mucopolysaccharides.

  (i) **Chitin**

  Linear unbranched structural heteropolysaccharide formed of β1→4 linked chains of acetyl glucosamine. Adjacent monomers lie at an angle of 180°. It is second most abundant organic molecule.



Structure of Chitin

  (ii) **Hemicellulose-**It is a branched chain homo- and hetero-polysaccharide of arabans, xylans, mannans, galactans. It is binding substance of cell wall but also present as food is Date (Phoenix).

  (iii) **Mucopolysaccharides -**They are slimy, acidic or aminated heteropolysaccharides of sugar derivatives. e.g. Gum. Agar - agar, alginic acid, carrageenan. Agar-agar is polymer of D-galactose 3-6 anhydro L-galactose with sulfate esterification.

  (iv) **Heparin-** (glucuronic acid + glucosamine + sulphate) is anticoagulant.

  (v) **Husk of Plantago ovate-** (Psyllium, Isbagol) is rich in mucopolysaccharides. Husk powder is not degraded in human alimentary canal. So taken orally for relief during constipation and diarrhea.

  (vi) **Pectins -**It is found in plant cell walls. Pectic acid is polymer of 100 1→4 D-galacturonic acids.

- **Functions of polysaccharides**

  Starch and glycogen are the major storage food. On hydrolysis storage carbohydrates provide both energy and carbon chains. Chitin is the structural carbohydrate of fungal walls and exoskeleton of arthropods. Cellulose is the structural substance of cell walls in most of the plants. Cellulose is economically important in the production of furniture, shelter, fuel, paper, textiles, ropes, rayon, cellophane, plastics etc. Mucilage present as a protective coating around aquatic plants, bacteria, blue-green algae etc., is derived from polysaccharides. Mucopolysaccharides present inside human body are keratin sulfate, chondroitin sulfate and hyaluronic acid. They have several functions - lubrication of ligaments and tendons, providing strength and flexibility to skin etc. Heparin prevents blood clotting inside blood vessels of animals. Some mucopolysaccharides have medicinal and other commercial importance e.g., husk of Plantago ovata, mucilage of Aloe, and alginic acid. Pectins are commercial jellying agents.

3. Amino Acids

Colourless crystalline, water soluble and aminated organic acids formed of C, H, O, N. Plant, fungus and some microbes can synthesize amino acid from inorganic N_2 or alfa keto acid by reductive amination but animal depend on plants. Amino acids are organic compounds containing an amino group and an acidic group as substituents on the same carbon i.e., the $\alpha-$ carbon. Hence, they are called $\alpha-$amino acids. They are substituted methanes. There are four substituent groups occupying the four valency positions. These are hydrogen, carboxyl group, amino group and a variable group designated as R group. Based on the nature of R group there are many amino acids(200). However, those which occur in proteins are only of twenty types. The R group in these proteinaceous amino acids could be a hydrogen (the amino acid is called glycine), a methyl group (alanine), hydroxyl methyl (serine), etc. The chemical and physical properties of amino acids are essentially of the amino, carboxyl and the R functional groups.

$$H_2N-\overset{\displaystyle H}{\underset{\displaystyle R}{\overset{|}{\underset{|}{C}}}}-COOH$$

Based on number of amino and carboxyl groups, there are acidic (e.g., glutamic acid), basic (lysine) and neutral (valine) amino acids. Similarly, there are aromatic amino acids (tyrosine, phenylalanine, tryptophan). A particular property of amino acids is the ionizable nature of $-NH_2$ and $-COOH$ groups. Hence in solutions of different pHs, the structure of amino acids changes.

$$\underset{(A)}{H_3^+N-\overset{R}{\overset{|}{C}}H-COOH} \rightleftharpoons \underset{(B)}{H_3^+N-\overset{R}{\overset{|}{C}}N-COO^-} \rightleftharpoons \underset{(C)}{H_2N-\overset{R}{\overset{|}{C}}H-COO^-}$$

B is called zwitterionic form.

There are more than 200 amino acids found in a cell, out of which 20 occur in proteins called protein amino acid or magic 20 because these can be arranged in innumerable sequences (20^n, n= No. of AA in Protein) e.g. Protein of 20 AA may be of 20^{20} types.

3.1. Protein Amino acids (NH_2.CHR.COOH.)

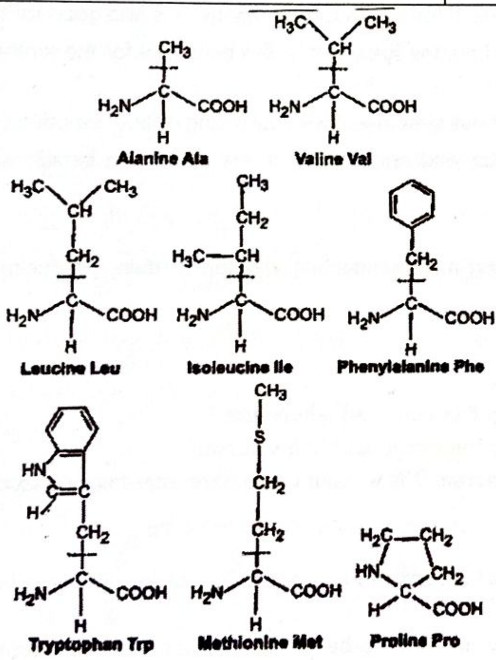
Protein amino acids besides having a carboxyl group, have an amino group attached to α-amino acids. α-carbon also has one hydrogen and a hydrocarbon or alkyl group. Glycine is simplest, smallest but tryptophan is most complex. Glutamic acid is first formed amino acid. Proline and hydroxyproline have –NH (imino group) instead of – NH_2. So these are imino acids instead of amino acids. (Except these all are α-amino acids). All amino acids have chiral carbon, so optically active except glycine (optically inactive). All protein amino acids are levorotatory but mostly non-protein amino acids are dextrorotatory.

3.2. Non-protein Amino acids-

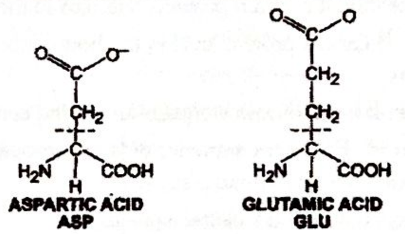
Non protein amino acids do not help in protein synthesis but occur freely or in combined state in non-protein substances. Their number is more than 180. e.g. Ornithine, Citrulline.

Amino acid may be

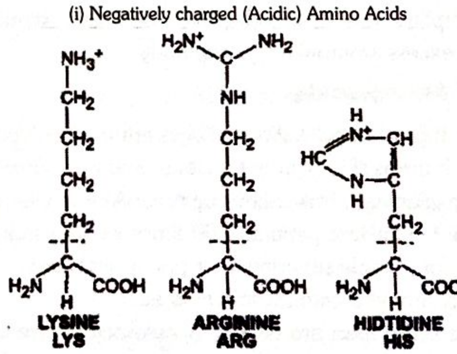
Group	Amino Acid With one letter Symbol
Neutral (mono amino mono carboxylic acid)	Glycine (G), Alanine(A), Valine(V), Leucine(L), Isoleucine(I)
Acidic(mono amino di carboxylic acid)	Aspartic Acid(D), Glutamic Acid(E).
Basic(di amino mono carboxylic acid)	Lysine (K), Arginine (R)
Sulphur Containing	Cysteine(C), Methionine(M)
Alcoholic	Serine(S), Threonine (T)
Aromatic-- Cyclic	Phenylalanine(F), Tyrosine(Y)
Aromatic--Heterocyclic	Histidine(G), Tryptophan(W)
Imino acid	Proline(P)= Has imino group (-NH) at place of amino group



Polar uncharged amino acid



(i) Negatively charged (Acidic) Amino Acids



(ii) Positively charged (Basic) Amino Acids

Polar charged amino acid

Amino Acids are classified into Two General Types: Essential and Non-Essential Amino Acids.

3.3. Essential amino acids

Essential amino acids are those that cannot be synthesized by the body on its own and thus need to be acquired through your diet. These are the nine amino acids that your body cannot create on its own, and that you must obtain by eating various foods. Adults need to eat foods that contain the following eight amino acids: methionine, valine, tryptophan, isoleucine, leucine, lysine, threonine and phenylalanine. Histidine, the ninth amino acid, is only necessary for babies.

- **Functions of essential amino acids**
  - **Tryptophan**: Necessary for the synthesis of neurotransmitter serotonin. It helps relieve migraine and depression.
  - **Tyrosine**: Is precursor of dopamine, norepinephrine and adrenaline. It enhances positive mood. It is also antioxidant.
  - **Valine**: Essential for muscle development. Side effects of high levels of valine in the body include hallucinations.
  - **Isoleucine**: Necessary for the synthesis of hemoglobin, major constituent of red blood cells.
  - **Leucine**: Beneficial for skin, bone and tissue wound healing. It promotes growth hormone synthesis.

- **Lysine**: Component of muscle protein, and is needed in the synthesis of enzymes and hormones. It is also a precursor for L-carnitine which is essential for healthy nervous system function.
- **Methionine**: Is antioxidant. It helps in breakdown of fats and aids in reducing muscle degeneration. It is also good for healthy skin and nail.
- **Phenylalanine**: Beneficial for healthy nervous system. It boosts memory and learning. It may be useful against depression and suppressing appetite.

3.4. Non essential amino acids

Amino acids that can be produced in our body. Their uses and functions in our body are equally as important as the limiting amino acids. The difference is that those kind of amino acids can be found in our food.

Functions of non-essential amino acids

There are 12 non-essential amino acids which are as follows:

- **Alanine**: Removes toxic substances released from breakdown of muscle protein during intensive exercise. Side effects: Excessive alanine level in the body is associated with chronic fatigue.
- **Cysteine**: Component of protein type abundant in nails, skin and hair. It acts as antioxidant (free radical scavenger), and has synergetic effect when taken with other antioxidants such as vitamin E and selenium.
- **Cystine**: The same as cysteine, it aids in removal of toxins and formation of skin.
- **Glutamine**: Promotes healthy brain function. It is also necessary for the synthesis of RNA and DNA molecules.
- **Glutathione**: Is antioxidant and has anti-aging effect. It is useful in removal of toxins.
- **Glycine**: Component of skin and is beneficial for wound healing. It acts as neurotransmitter. The side effect of high level glycine in the body is that it may cause fatigue.
- **Histidine**: Important for the synthesis of red and white blood cells. It is a precursor for histamine which is good for sexual arousal. Improve blood flow. Side effects of high dosage of histidine include stress and anxiety.
- **Serine**: Constituent of brain proteins and aids in the synthesis of immune system proteins. It is also good for muscle growth.
- **Threonine**: Balances protein level in the body. It promotes immune system. It is also beneficial for the synthesis of tooth enamel and collagen.
- **Asparagine**: It helps promote equilibrium in the central nervous system—aids in balancing state of emotion.
- **Aspartic acid**: Enhances stamina, aids in removal of toxins and ammonia from the body, and beneficial in the synthesis of proteins involved in the immune system.
- **Proline**: plays role in intracellular signaling.
- **L-arginine:**plays role in blood vessel relaxation, stimulating and maintaining erection in men, production of ejaculate, and removal of excess ammonia from the body.

3.5. Functions of Amino acids

(1) Cysteine : It produces disulfide linkages amongst polypeptides.

(2) Tyrosine : It forms skin pigment melanin and two hormones thyroxine and adrenaline.

(3) Alanine : α-alanine forms coenzyme A (CoA) and vitamin pantothenic acid (a β-vitamin).

(4) Aspartame : Synthetic peptide, 200 times sweeter than sucrose. It is without unpleasant after-taste of saccharin. It is however, not suitable for people suffering from phenylketonuria.

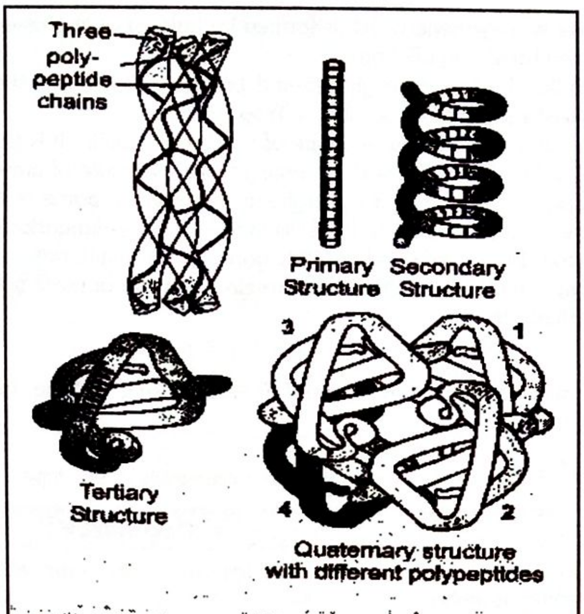
(5) Cystine is di amino dicarboxylic amino acid.

(6) Amino acids other than aromatic and heterocyclic are called aliphatic amino acids. Except for glycine, all other protein amino acids are laevorotatory.

Arginine and histidine are semi-indispensable because they are slow to be formed in humans. Two amino acid can join by amino group of one and carboxylic group of other forming a amide linkage or peptide bond (– CO – NH –). Term peptide is used for a short chain of amino acids, e.g., dipeptide (two amino acids), tripeptide (three amino acids) etc. When chain has more than 20 amino acids, it is called polypeptide. Protein has more than 50 amino acids. It may have one or more polypeptides.

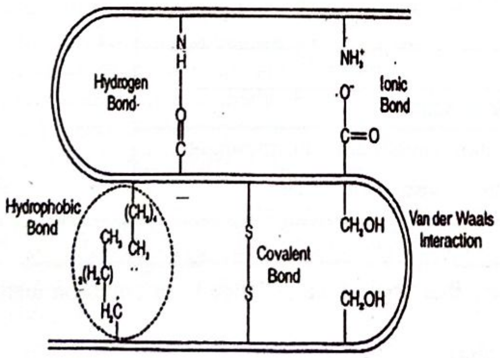
4. Proteins

Proteins (Mulder, Berzelius) are polymers of amino acid. These are insoluble in water and most abundant organic compound of animal cell, which forms 50% of total dry mass. It is most diverse organic compound of earth. Protein having all essential and semi- essential amino acids called first class or complete protein e.g. animal proteins and others are incomplete or second class protein e.g, protein of plants. But only soybean protein is nearly complete. A protein has one polypeptide is called monomeric, e.g. myoglobin, lysozyme and protein have two or more poly-peptides c/d multimeric/oligomeric, e.g., Haemoglobin.The individual polypeptides of a multimeric protein are called subunits or protomers. The maximum number of protomers described for a protein 72 in pyruvate dehydrogenase complex. Minimum molecular weight of a protein is 4500 (adrenocorticotrophin hormone-ACTH with 39 amino acids). Highest molecular weight is of Pyruvate dehydrogenase (mol wt. 4,600,000, polypeptides 72).

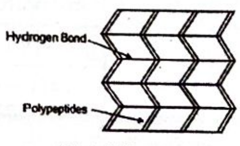


Levels of structure in a protein molecule

	Protein	Characteristic	Occurrence
(a)	Nucleoproteins	(i) Deoxyribonucleoproteins (ii) Ribonucleoproteins	Chromatin (histones + DNA)
(b)	Mucoproteins	Mucoid carbohydrates plus proteins	Ribosomes (Globular proteins + RNA)
(c)	Glycoproteins	Oligosaccharides plus proteins	Mucin, Ovomucoid (egg white).
(d)	Lipoprotein	Complex lipoproteins.	Membrane surface.
(e)	Phosphoproteins	Simple lipoproteins.	Chylomicrons, HDL, LDL, VLDL
(f)	Metalloproteins	Proteins with phosphorus	Membranes.
(g)	Chromoproteins (Protein with pigment)	Protein with metals, ions.	Caseinogen(milk), Ovovitellin (egg yolk) Ferritin (Fe), Siderophilin (Fe), Ceruloplasmin (Cu).
		Hemoglobin (Fe)	- Erythrocytes
		Myoglobin (Fe)	- Muscles
		Haemocyanin (Cu)	- Blood of some invertebrates.
		Cytochromes (Fe)	- Electron Carriers
		Flavoproteins-FMN, FAD	- Enzymes
		Phytochrome	Plant Photomorphogenetic Reactions.




Collagen Helix



β-Pleated (Diagrammatic)

4.1. Structure of Protein

- **Primary structure (1°) :** First formed structure, which is made up of a linear row of amino acids joined by only peptide bonds and genetically controlled by DNA. It is final structure of globular protein e.g. globulin.
- **Secondary structure (2°):** Stabilized by H- bonds. It is of three main types:
  (a) α-helix: It is right handed helix in which 3.5 amino acids are found per helix and hydrogen bond forms between amide group (-NH-) of first and carbonyl group (-CO-) fourth amino acid. It is final structure of fibrous and globular protein e.g. keratin. Glycine and Proline are unable to form 'H' bond so called helix breaker (breaks the spiral and forms random coils).

(b) β-pleated: It is a sheet like arrangement, which is formed by holding of two or more polypeptide chains in parallel or anti parallel manner by hydrogen bonds. eg silk fibroin.

(c) Collagen Helix: Polypeptide having more glycine and proline amino acids, then three parallel polypeptide forms right super helix, which is stabilized by hydrogen bond, e.g. Tropocollagen.

- **Tertiary structure (3°):** It is 3-dimensional final structure of functional protein. It is also called native state of enzyme. It is final structure of enzyme Five types of bonds are involved in forming tertiary structure of proteins. They are van der Waals interactions, Ionic bonds, hydrogen bonds, disulfide bonds and hydrophobic interactions. Some of these bonds can easily be broken by high temperature, radiations, detergents, acids, alcohols. It results in loss of three-dimensional conformation of the protein molecules. This process is called denaturation. But peptide and disulfide bonds are strong bonds.
- **Quaternary structure:** Present in multimeric /oligomeric proteins. In it two or more polypeptide chains interact and get oriented with respect to each other in tertiary structure.

4.2. Protein Classification

In shape: Can be fibrous (e.g., elastin, collagen, actin, keratin) or globular (e.g. histone, hemoglobin, egg albumin, serum globulin) or intermediate (myosin, fibrinogen)

In composition: Proteins can be

- **Simple Proteins:** Without additional groups. Mostly storage proteins are of this type.

	Protein	Occurrence
(a)	Albumins	Leucosin (cereal grains), legumelin (legume seeds), serum albumin (blood), ovalbumin (egg), lactalbumin (milk).
(b)	Globulins	Legumin (legume seeds), tuberin (Potato), serum globulin (blood), vitellin (egg yolk).
(c)	Prolamines	Zein (Maize grains), Hordein (Barley grains), Gliadin (Wheat grains)
(d)	Glutelins	Glutelin (maize grains), Glutenin (Wheat grains), oryzenin (Rice grains).
(e)	Histones	In nucleoproteins.
(f)	Protamines	In nucleoproteins of fish sperms.
(g)	Scleroproteins	Keratin, Elastin, Collagen.

- Conjugate Proteins: With additional groups
- Derived Proteins : Formed by digestion of proteins.

	Name	Characteristics	Occurrence
(a)	Proteins	Early derivative of reaction on protein	Fibrin (from Fibrinogen)
(b)	Metaproteins	Hydrolysis of protein	First fraction
(c)	Proteoses	Hydrolysis of protein	Intermediate fraction
(d)	Peptones	Hydrolysis of protein	Polypeptides
(e)	Peptides	Hydrolysis of protein	Fraction with few amino acid residues.

Some Proteins and their Functions

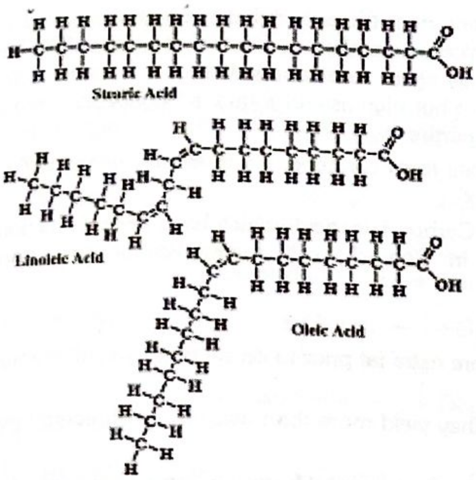
Protein	Functions
Collagen	Intercellular ground substance
Trypsin	Enzyme
Insulin	Hormone
Antibody	Figure infectious agents
Receptor	Sensory reception (smell, taste, hormone, etc.)
GLUT – 4	Enables glucose transport into cells

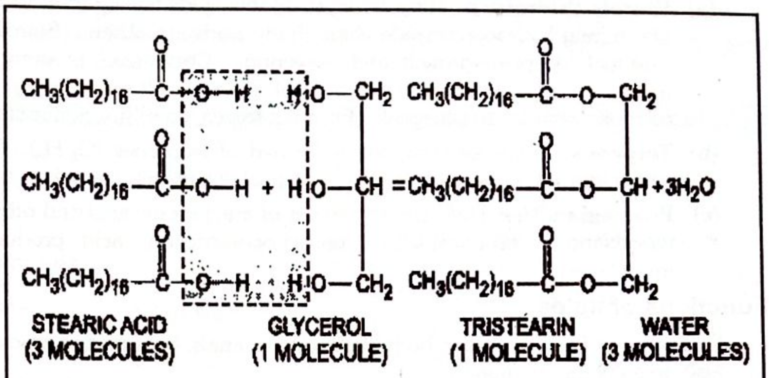
5. Lipids

They are compounds of C, H and O where ratio of H and O is less than that of water. They form emulsion with water but are soluble in organic solvents.

5.1. Fatty acids (monocarboxylic organic acid- R.COOH)

These are chains of hydrocarbons (4-30 carbons), which have carboxylic group in end. Head of fatty acid is polar hydrophilic while tail is nonpolar and hydrophobic so they are amphipathic. On aggregation forms micelle and form monomolecular layer with water. Animal cannot form 3 fatty acids (linoleic, linolenic, arachidonic acids) called essential fatty acids/EFA (Evans & Burr). EFA present in sunflower, safflower, coconut, cotton , sesame, rapeseed, mustard groundnut. Linoleic acid is most essential fatty acid because rest of the fatty acid can be formed from it. Coconut oil has no EFA but Cotton, Sun-flower, Groundnut oil has all EFA. Milk has all essential fatty acids. Saturated fatty acids have only single bonds in hydrocarbon chain. They have formula $C_nH_{2n}O_2$. e.g. Butyric acid (C_4), Caprilyc acid (C_8), Capric acid (C_{10}), palmitic acid (C_{16}), stearic acid (C_{18}), arachidonic acid (C_{20}).





Structural formula of tristearin

Palmitic acid is the most common saturated fatty acid. Saturated fatty acid has high melting point which increases with the length of hydrocarbon chain. Unsaturated fatty acids have both double bonds and single bonds in hydrocarbon chain e.g. oleic (monoenoic-1DB), linoleic (dienoic-2DB), linolenic (trienoic-3DB), arachidonic acid (tetraenoic-4DB). Oleic acid is most common. Has low melting point which decreases on increasing the number of double bonds Unsaturated fatty acid with one double bond is called monounsaturated fatty acid or MUFA. Fatty acid with more than one double bonds is called PUFA or polyunsaturated fatty acid. All the three essential fatty acids (EFA) are also polyunsaturated or PUFA or Vit-F. PUFA retards the atherosclerosis by emulsifying cholesterol into lipoprotein which metabolises in liver so recommended in hypertension.

5.2. Types of lipids: Lipids are generally divided into three categories (Bloor, 1943)

- **Simple**—Made up of fatty acids + alcohol only. (e.g. true fats, waxes, cutin, suberin),
  - **(a) Triglycerides or Neutral or True Fats:** Esters of three fatty acids and one molecule of trihydric alcohol glycerol. Oils are liquid at room temp. They have either small or unsaturated fatty acids. Oils with PUFA are called polyunsaturates. e.g. Sunflower. They can be converted into saturates (vegetable ghee) by hydrogenation. It improves taste & colour of oils. But, it destroys EFA. Unsaturated fatty acids have a tendency to combine with O_2 so tend to solidify, so called drying oils. Hard fats are solid at room temp., due to saturated fatty acids. e.g. animal fat. (except of cold region) Butter and ghee are soft because it has short chain fatty acids in good quantity. Butyric acid (C_4) is shortest fatty acid present in butter along with caprylic (C_8) acid and capric acid (C_{10}).
  - **(b) Waxes :** Inert esters of long chain fatty acids with long chain monohydric higher alcohols like ceryl, cetyl, mericyl alcohol, e.g., bee's wax, sebum, spermaceti (from sperm Whale), lanolin (wool fat), plant waxes. Bee wax is mericyl and hexacosyl palmitate while spermaceti is cetyl palmitate (hardest wax). Candles are prepared from paraffin wax and stearic acid. Paraffin wax is obtained from crude petroleum. It is used in boot polishes, wax paper etc.
  - **(c) Cutin:** It is a complex lipid produced by cross-esterification and polymerisation of hydroxy fatty acids, as well as other fatty acids with or without esterification by alcohols other than glycerol. Cutin occurs in the aerial epidermal cell walls as well as a separate layer of cuticle on the outside of these epidermal cells. Cuticle has 50-90% cutin. Cutin reduces the rate of transpiration. It also binds epidermal cells.
  - **(d) Suberin:** It is a mixture of fatty material having condensation products of glycerol and phellonic acid or its derivatives. Suberin makes the cell wall strong and impermeable. It occurs in the walls of cork cells and endodermal cells.
- **Conjugated**—Has fatty acid + alcohol + additional group e.g. phospholipid, glycolipid, sphingolipid.
  - **(a) Phospholipids:** It is major structural components of cell membrane.so called membrane lipids. These are esters of fatty acids with glycerol where one fatty acid is replaced by a phosphoric acid esterified with non-nitrogenous or nitrogenous base like choline and ethanolamine. Lecithin is choline having phospholipid (liver, yolk) but cephalin has ethanolamine (RBC, Brain). Phosphoric acid and N_2 having base functions as polar hydrophilic head while two fatty acids behave as hydrophobic nonpolar tails. Due to both polar and nonpolar groups is called amphipathic or amphiatic. Fatty acids form a monolayer in contact with water while phospholipids produce a double layer.
  - **(b) Lipoprotein:** Complex of triglycerides, phospholipid, cholesterol, protein. They are four types: High density lipoprotein (HDL/α protein—20%), low density lipoprotein (LDL/ β protein—46%), very low density lipoprotein (VLDL/pre-β protein – 12%), Chylomicrons. LDL & VLDL causes atherosclerosis and coronary thrombosis.
  - **(c) Sphingolipids:** They are lipids having amino alcohol sphingosine. Sphingomyelins contain an additional phosphate attached to choline like phospholipids. They occur in myelin sheath of nerves. Cerebrosides possess sugar residue galactose. They occur in nerve membranes. Ganglio- sides possess sugar residues glucose, galactose, sialic acid and acetyl glucosamine. They influence ion transport through the membrane as well as function as receptors of viral particles. Gangliosides occur in grey matter. Excessive accumulation of gangliosides produces disorders like Tay-Sachs disease. Since cerebrosides and gangliosides contain sugar residues, they are also called glycolipids.
  - **(d) Chromolipids:**These contains pigments such as carotenoids.e.g. carotene, vitamin A.
- **Derived**—Derivatives of lipid or lipid like substances e.g., sterols, terpenes, prostaglandins).
  Derived lipids do not undergo Saponification (hydrolysation) with alkali (class II lipids) while others can undergo Saponification or soap formation with alkalies (class I lipids).

(a) **Sterols (Steroids):** They are crystallisable lipids having four hydrocarbon rings (three cyclohexane and one cyclopentane) and a long hydrocarbon side chain. Fatty acids are absent. Sterols are precursors of steroid hormones and vit-D. They are essential for plant growth and flowering. Cholesterol is strengthening agent in the membranes of animal cells and mycoplasmas. Ergasterol is plant sterol (Phytosterol) found in fungus also but stigmasterol found in Soyabean. Steroids are anabolic stimulants. Diosgenin (from Dioscorea/Yam) has antifertility properties.

(b) **Terpenes:** Lipid-like compounds formed of isoprenes ($C_5 H_8$). Terpenes form carotene, xanthophylls, vitamins A, E ,K, gibberellins, abscisic acid, Rubber, essential volatile oils (camphor, menthol).

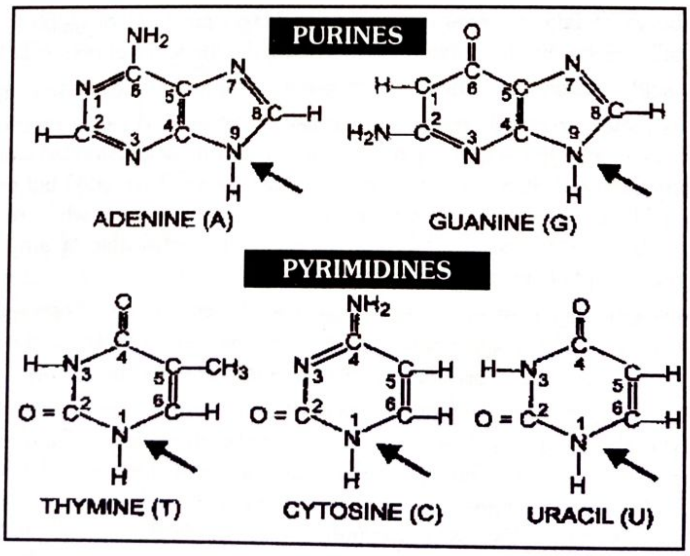
(c) **Prostaglandins:** They are derivatives of arachidonic acid and other 20 Carbon fatty acids which have several functions like vasodilation, vasoconstriction, bronchoconstriction, acid production in stomach, cell communication and hormone modulation.

5.3. Functions of lipids

- Fats serve as food reserve in both plants and animals. Hibernating animals store extra fat prior to on set of winter. Migratory birds also do so before migration.
- They function as concentrated food because as compared to carbohydrates they yield more than twice as much energy per unit weight (9.3 kcal/gm: 4.5 kcal/gm).
- Fats can be converted to carbohydrates. Therefore, fats stored in oil seeds (e.g., Groundnut, Mustard, Castor, Sunflower, Cotton, and Coconut) not only provide energy but also raw materials for growth of embryo.
- In seeds and spores lipids help in thermal insulation, protection from ultraviolet radiations and loss of water.
- Vitamin A, D, E and K are soluble in fats. The latter not only act as their carriers but also protect them from oxidation.
- In animals fat occurs as droplets inside cells called adipocytes. Adipocytes of cold blooded or poikilothermic animals have higher amount of unsaturated fatty acids as compared to warm blooded or homoeothermic animals.
- Subcutaneous fat rounds off the body contours of animals and human beings. In animals the fats produce a shock absorbing cushion around eye balls, gonads, kidneys and other vital organs.
- Drying oils having unsaturated fatty acids are used in paint industry.
- Waxes form a protective layer over the animal fur. They protect the floating leaves of aquatic plants against wetting. In land plants they reduce the rate of transpiration.
- Myelin sheath around nerve fibres takes part in insulation.
- Phospholipids, glycolipids and sterols are components of cell membranes.
- Fragrance of many plant products is due to fat-like substances called terpenes.
- In birds, oil from preen gland is used to lubricate feathers and protect them from wetting. Hair are similarly lubricated in mammalian skin. It prevents their felting. The skin is also protected from drying up.
- Desert animals employ fat as source of metabolic water, e.g., Kangaroo, Rat and Camel. Kangaroo or Desert Rat does not drink water. Camel uses fat stored in its hump for obtaining metabolic water during extreme desiccating conditions.

6. Nucleotides

Basic unit of nucleic acid. Small, complex biomolecules, formed by condensation of a pentose sugar, a N base and at least one phosphoric acid. These are basic unit of nucleic acid. The nucleotides form about 2% of the cell contents.



Nitrogen Bases : They are of two types--- Purines and Pyrimidines.

Purines : 9-membered double ring N bases, which has N_2 at 1, 3, 7 & 9 positions, e.g., adenine (A), guanine (G).

Pyrimidines : These are a 6-membered nitrogen base, which has nitrogen at 1 and 3 positions, e.g., cytosine(C), thymine (T), uracil (U). Uracil is found in RNA only but Thymine in DNA only.

Made of a nitrogen base, a pentose sugar and phosphate. Phosphate joins with sugar at its 5th carbon, sugar with nitrogen base at carbon 1st while nitrogen base is usually attached to sugar at its 9th (purine) or 1st (pyrimidine) atom.

No. Base	Sugar	Nucleoside	Phosphoric acid Nucleotide
Purines :			
1. Adenine	*+ Ribose* ⟶ *Adenosine*	$+H_3PO_4$ ⟶	*Adenylic Acid or Adenosine Monophosphate (AMP)*
	or Deoxyribose ⟶ Deoxyadenosine	"	*Deoxyadenylic acid or Deoxyadenosine monophosphate (dAMP)*
2. Guanine	*+ Ribose* ⟶ *Guanosine*	$+H_3PO_4$ ⟶	*Guanylic Acid or Guanosine Monophosphate (GMP)*
	or Deoxyribose ⟶ Deoxyguanosine	"	*Deoxyguanylic acid or Deoxyguanosine monophosphate (d GMP)*
Pyrimidines :			
1. Cytosine	*+ Ribose* ⟶ *Cytidine*	$+H_3PO_4$ ⟶	*Cytidylic acid or Cytidine Monophosphate (CMP)*
	or Deoxyribose ⟶ Deoxycytidine	"	*Deoxycytidylic acid or Deoxycytidine monophosphate (dCMP)*
2.Thymine	*+ Deoxyribose* ⟶ *Deoxythymidine only*	$+H_3PO_4$ ⟶	*Deoxythymidylic Acid or Deoxythymidine Monophosphate (dTMP)*
3. Uracil	*+ Ribose only* ⟶ *Uridine*	$+H_3PO_4$ ⟶	*Uridylic acid or Uridine Monophosphate (UMP)*

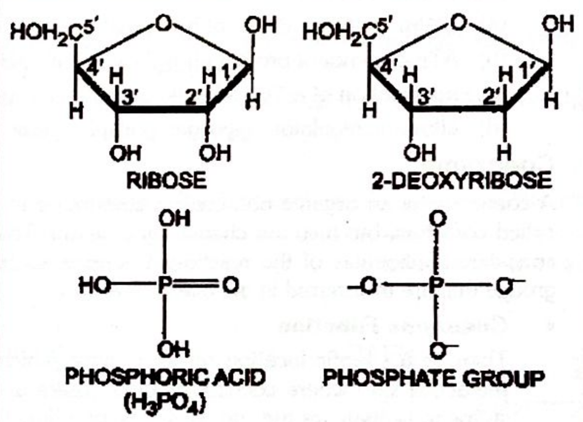
6.1. Nucleosides

Formed by a N base and pentose sugar. N base combines with pentose sugar molecule at its carbon atom 1st in a N-glycosidic bond (C-N-C) by one of it's N atoms (1st in pyrimidines and 9th in purines).

Common nucleotides are AMP (adenosine mono-phosphate, adenylic acid), CMP (cytidine mono-phosphate, cytidylic acid), GMP (guanosine mono-phosphate, guanylic acid), UMP (uridine mono-phosphate, uridylic acid), de AMP (deoxyadenosine monophosphate, deoxy-adenylic acid), de GMP (deoxyguanosine mono-phosphate, deoxyguanylic acid), deCMP (deoxycytidine monophosphate, deoxy-cytidylic acid) and deTMP (deoxythymidine mono-phosphate, deoxythymidylic acid).

Cyclic AMP (c-AMP) act as second chemical messenger in many hormone controlled reactions.

Cyclic GMP (c-GMP) is functional in $Ca^{2}+$ or calmodulin mediated chemical reactions.



Structure of ribose, deoxyribose sugars and a phosphate

High energy nucleotides

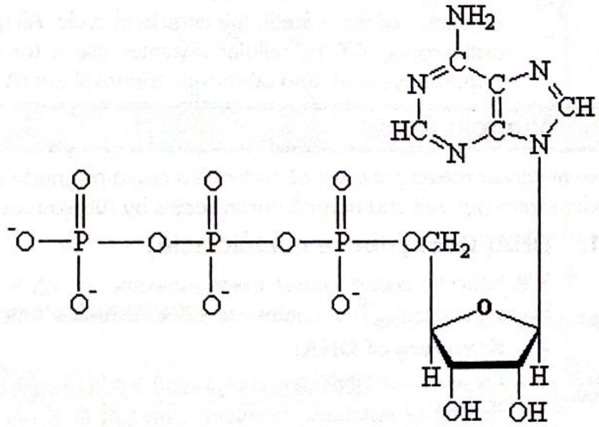
Nucleotides can have more than one phosphate. Nucleotides with three phosphates hold their last two phosphates with high energy bonds. ATP (ADP ~ P) is energy currency of cell (quickest source of stored energy). ATP and other nucleotide tri phosphates are called higher nucleotides. Only nucleotides tri phosphate polymerize to form nucleic acid.

ATP (Adenosine triphosphate)

It has an adenine, a ribose sugar and a row of three phosphates out of which the last two are attached by high energy bonds (~ P ~ P). ATP was discovered by Lohmann in muscles. Lipmann (father of ATP cycle) discovered its energy carrying nature.

ADP + Pi + energy ↔ATP reaction can occur manner of build up and breakdown process. It is also called universal energy carrier or energy currency of a cell.

The enzyme carrying its build up and breakdown reactions is called ATPase. The last and second last phosphate bond yields an energy equivalent of 8.15 kcal per mole (7.3 kcal and 8.9 k cal). First bond is never broken (contains 3k.cal energy). App. 2 kilo ATP formed and hydrolyzed per day in human.



Dinucleotides

Vitamin			Nucleotide	Components
1. Nicotinamide	(i) NAD^+	or	Nicotinamide Adenine Dinucleotide	Nicotinamide–Ribose–Phosphate –Phosphate–Ribose–Adenine
	(ii) $NADP^+$	or	Nicotinamide Adenine Dinucleotide Phosphate	Nicotinamide–Ribose–Phosphate –Phosphate – Phosphoribose – Adenine
2. Riboflavin	(i) FMN	or	Flavin Mononucleotide	Riboflavin – Phosphate
	(ii) FAD	or	Flavin Adenine Dinucleotide	Riboflavin–Phosphate –Phosphate –Ribose – Adenine
3. Pantothenic Acid	CoA	or	Coenzyme A	Pantothenic Acid – Mercaptoethytamine –Phosphate - Phosphate– Phosphorbose – Adenine

6.2. Functions of Nucleotides

- **Nucleic Acid :** Nucleotides are building blocks of nucleic acids. In this function they are reactive in triphosphate state. Ribonucleotides from RNA while deoxyribonucleotides produce DNA.
- **Energy Carriers :** Higher nucleotide act as energy carrier e.g. CTP, UTP, GTP, ATP (most common).
- **Carbohydrate Synthesis :** UDP and ADP activate glucose e.g., UDP-Glucose, ADP-Glucose.
- **Phospholipid Synthesis :** CDP and CTP take part in phospholipid synthesis.
- **Cyclic Nucleotides :** Cyclic AMP function as second messenger and controls a number of reactions in response to specific stimulations or first messenger. Cyclic GMP has antagonistic response to some calmodulin controlled reactions, e.g., heart beat (stimulated by camp in response to adrenaline and decelerated by cGMP in response to acetylcholine).
- Metabolic regulators:
  (a) c-AMP is the mediator of hormonal actions;
  (b) ATP-dependent protein phosphorylation - activates phosphorylase and inactivates glycogen synthase;
  (c) adenylation of a Tyr of bacterial glutamine synthetase - more sensitive to feedback inhibition and less active;
  (d) allosteric regulator - glycogen phosphorylase activated by ATP and inactivated by AMP.

6.3. Coenzyme

A coenzyme is an organic non-protein compound that binds with an enzyme to catalyze a reaction. Coenzymes are often broadly called cofactors, but they are chemically different. They bind to the active site of the enzyme and participate in catalysis but are not considered substrates of the reaction. Coenzymes often function as intermediate carriers of electrons, specific atoms or functional groups that are transferred in the overall reaction.

- **Coenzyme Function**

  There is a specific location on an enzyme which binds to substrates and helps turn them into products. This location, called the active site, where coenzymes bind. There are several ways coenzymes assist in enzyme function, including changing their shape to activate, or turn on, enzymes, or aiding in chemical reactions by acting as carriers of energy or molecular groups.

  NAD^+, $NADP^+$, FMN and FAD are coenzymes of dehydrogenases and other enzymes involved in oxidation-reduction. The coenzymes are useful in removing and transferring hydrogen.

  (a) $FAD/FMN + 2H.A \longrightarrow FADH_2 / FMNH_2 + A$

  (b) $FADH_2/FMNH_2 + B \longrightarrow FAD / FMN + 2H.B$

  (c) $NADP^+/NAD^+ + 2H.A \longrightarrow NADPH / NADH + H^+ + A$

  (d) $NADPH/NADH + H^+ + B \longrightarrow NADP^+/ NAD^+ + 2H.B$

  Coenzyme A (CoA, CoASH, or HSCoA) is a coenzyme, notable for its role in the synthesis and oxidation of fatty acids, and the oxidation of pyruvate in the citric acid cycle. All genomessequenced to date encode enzymes that use coenzyme A as a substrate, and around 4% of cellular enzymes use it (or a thioester, such as acetyl-CoA) as a substrate. In humans, CoA biosynthesis requires cysteine, and adenosine triphosphate (ATP).

7. Nucleic Acids

They are linear mixed polymers of nucleotides called polynucleotides. They have C, H, O, N, P. Nucleic acids were first discovered by Mishear as nuclein from pus cell and named nucleic acids by Altmann due to their acidic property. Nucleic acids are of two types, DNA and RNA.

7.1. DNA(Deoxyribose nucleic acid)

It is helically coiled largest macromolecule, which is made up of two antiparallel polydeoxyribonucleotide chains held together by hydrogen bonds. Two chains are plectonemically (interlocked) coiled around a common axis.

- **Structure of DNA:**

  Structure of DNA was deciphered by 3D, right handed double helical model of Watson and Crick (1953). This was based on finding of Astebery, Franklin, Chargaff & X-ray diffraction pictures (Wilkins). Wilkins, Watson and Crick were awarded Nobel Prize for this in 1962.

Chargaff had discovered certain structural peculiarities called Chargaff's rules –

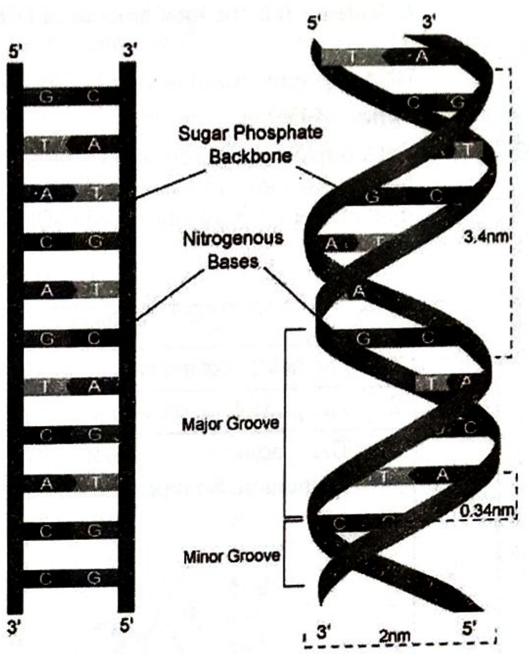
(i) Purines & pyrimidines occur in equal amount.

(ii) Phosphate and sugar occur in equal number.

(iii) Molar amount of adenine is equal to that of thymine and cytosine equal to guanine.

(iv) Base ratio A + T/C + G is specific for a species. It is rarely one 3D, right handed double helical model

It was given for B-DNA. According to it DNA is made of two anti parallel strands which are coiled around a common axis like twisted hanging ladder.



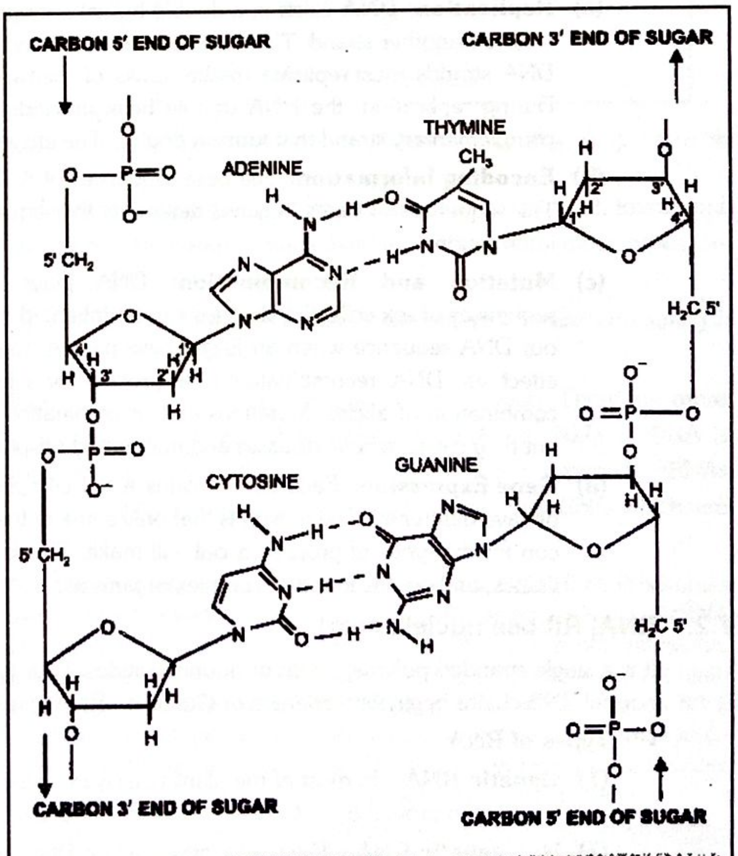
Coiling forms alternate major (22 Å long) and minor (12 Å long) grooves. Histone protein present at less than 30^0 in Major groove and at 45^0 in minor groove. DNA has diameter of 20 Å. One turn of spiral has a distance of 34Å. One spiral contains 10 nucleotides in each chain so that the distance between adjacent nucleotides is 3.4 Å. Each DNA chain has a backbone of alternate deoxyribose sugar ($C_5H_{10}O_4$) and phosphate. Nitrogen bases are attached to sugars molecules at right angles to main strand. Phosphate is connected to sugar at 5' carbon, nitrogen base attached to sugar at 1' carbon. Phosphate is attached to next sugar by its 3'carbon. It forms a phosphodiester linkage between adjacent nucleotides. Direction is 3' → 5' in one chain and 5' →3' in other depending upon the free carbon atom of deoxyribose sugar present at the polynucleotide ends. So it is antiparallel to each other. N base are of 4 types-adenine, guanine , cytosine , thymine.

Adenine of one chain lies opposite to thymine and attached by two hydrogen bonds But, cytosine opposite to guanine of the other strand by three hydrogen bonds, so the two chains of DNA are complementary.

They are held together by hydrophobic interactions of N_2 bases and hydrogen bonds. One helix of DNA twisted by 360° but two adjacent nucleotide pair are twisted by 36°.

- **Properties of DNA:**

DNA duplex is actually made of two strands, out of which only one is functional. The functional one is called template strand or antisense strand. Other strand is meant for protecting it and is complementary to the antisense strand, called sense strand. Maximum amount of DNA found in chromatin of nucleus in eukaryotes and nucleoid of prokaryotes. A small amount (1 – 5%) occurs in plastid and mitochondria. DNA having free ends is called linear DNA. In circular DNA, two ends are linked covalently. It is found in nucleoid of prokaryotes as well as inside cell organelles (plastids, mitochondria). DNA can form its duplicate in the process of replication (autocatalytic function). DNA also produces RNA called transcription (heterocatalytic function).



A small segment of DNA molecule

In eukaryotes, a DNA often has inactive area called introns or IVS (intraveining sequences). Active areas are then called exons. DNA having introns are called split genes. H_2 bonds are broken at high temp. (80-90°C) called denaturation or melting and renaturation/annealing occurs on cooling.

Palindromic DNA: DNA helix has nucleotide in a serial arrangement but opposite in two strands.

-T-T-A-A-C-G-T-T-A-A........

-A-A-T-T-G-C-A-A-T-T........

Repetitive DNA: This type of arrangement is found near centromere of chromosome and is inert in RNA synthesis. The sequence of nitrogenous bases is repeated several times. In contrast to eukaryotes, the DNA of prokaryotes does not contain repeated base sequences. Function of repetitive DNA is unknown. This can replicate but cannot transcribe.

Satellite DNA: It may have repetitive base pairs up to 1-60 bp. Microsatellite has 1-6 bp and minisatellite has 11-60 bp. They are used in DNA matching or finger printing (Jefferey). They are also called variable number of tandem repeat (VNTR).

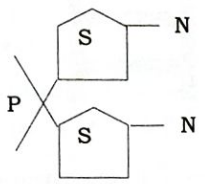
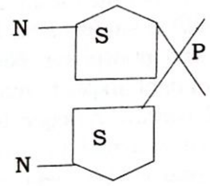
C Value : It is the total amount of DNA in a genome or haploid set of chromosomes. One picogram (10^{-12}gm) DNA is roughly 31 cm long.

DNA was synthesized in vitro by Kornberg in 1959.

- **Types of DNA:**

DNA duplex of Watson and Crick is right handed and is called B-DNA. The plane of base pairs is perpendicular to axis of the helix. Five more models have been proposed. In A-DNA a single turn of right-handed helix contains 11 base pairs, with a diameter of 23 Å. Right-handed C-DNA has 9 base pairs with a diameter of 20Å. Right-handed D-DNA has 8 base pairs per turn of spiral.

Difference between B and Z DNA

B DNA	Z DNA
1. The helical coiling is right handed.	1. The helical coiling is left handed.
2. The phosphate backbone is regular.	2. The phosphate backbone is zigzag.
3. The adjacent sugar residues have same orientation. So repeating unit is mononucleotide. 	3. The adjacent sugar residues have opposite orientation. So repeating unit is dinucleotide. 
4. Length of one turn (pitch) is 34Å (03.4 nm) which has 10 base pairs or 10 repeating units.	4. Length of one turn (pitch) is 45Å which has 12 base pairs or six repeating nucleotide units.
5. The angle of twist per repeating unit is 36°	5. The angle of twist per repeating unit is 60°.
6. Diameter is 20 Å.	6. Diameter is 18 Å.
7. Distance b/w two base pair is 3.4Å (0.34 nm)	7. Distance b/w two base pairs is 3.75Å (0.375nm)

- **Function of DNA**

(a) Replication: DNA exists in a double-helical arrangement, in which each base along one strand binds to a complementary base on another strand. T's can only bind to A's and C's only to G's. When a cell divides, the chromosomes containing the DNA strands must replicate (make copies of themselves) so that both daughter cells receive full set of genetic material. During replication, the DNA double helix unwinds, allowing each strand to act as a template for a newly synthesized complementary strand that forms a double. The enzyme DNA polymerase assists in the process.

(b) Encoding Information: The base sequences of A, T, C and G along a DNA strand are organized into units called genes. The sequences of bases in genes determine the sequence of amino acids in proteins. This process is known as encoding of information.

(c) Mutation and Recombination: DNA plays a role in the evolution of species. If changes occur to the DNA sequences of sex cells, the changes can be inherited by next generation. So mutation is nothing but a change that occurs in our DNA sequence when an illegal base pairing occurs or as a result of environmental factors such as UV light, smoking effect etc. DNA recombination is a process by which pieces of DNA are broken and recombined to produce new combination of alleles. Mutations and recombination are beneficial since they create genetic diversity at the level of genes but they cause genetic diseases and malformed offspring.

(d) Gene Expression: Each cell contains a full complement of genes, yet cells from different tissues and organs look and behave differently. The reason is that only some of the DNA of each cell is used to make proteins. DNA plays a role of traffic cop for the types of proteins a cell will make. This is how a single fertilized egg cell differentiates into many types of cells, tissues, and organs found in complex organisms.

7.2. RNA(Ribose nucleic acid)

It is a single stranded polymer chain of ribonucleotides. Four types of ribonucleotides are involved – AMP, GMP, CMP and UMP. All normal RNA chains begin with adenine or Guanine. RNA was synthesized in vitro by Ochoa.

- **Types of RNA**

(1) Genetic RNA : In most of the plant viruses the genetic material consists of RNA, such genetic RNA act as genetic material. It has been proved by F. Conrat in tobacco mosaic virus.

(2) Non genetic RNA : They are synthesized on DNA template by transcription and are of following types –

(a) Messenger RNA (mRNA) : Long RNA which constitutes only 5% of total. It brings coded information from DNA to cytoplasm (ribosome) for protein synthesis. The sedimentation coefficient of m-RNA is 8S. Their adjacent nitrogen bases specify a particular amino acid.

The term mRNA was given by Jacob and Monod. It carries genetic information (base sequence of nucleotides), which is called as genetic code.

Molecular weight varies from 5,00,000 to 20,00,000. It has methylated region at 5' end, which acts as a cap for attachment with ribosome.

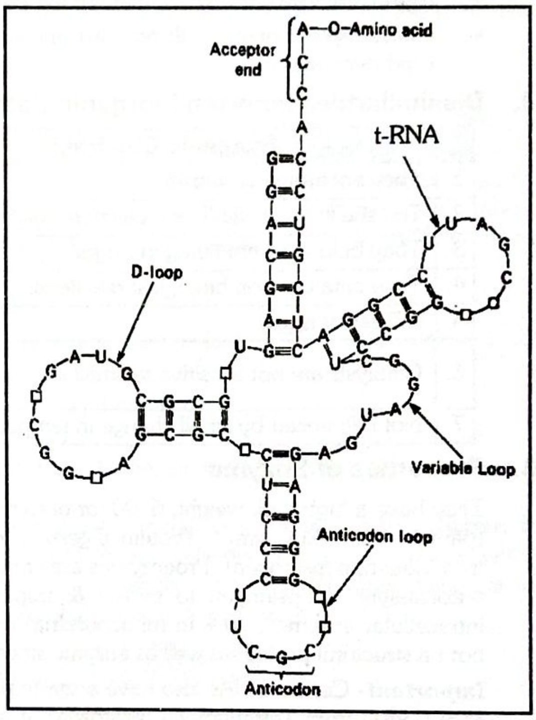
The cap region (7me-G^5) is followed by an initiation codon AUG, either immediately or after a small noncoding region which is followed by coding region and termination codon (UAA, UA and UAG) is followed by poly A, either immediately or after a small noncoding region at 3' ends.

(i) Monocistronic m-RNA: Carries information for one complete protein only. e.g., eukaryotes.

(ii) Polycistronic m-RNA: Carries information for more than one polypeptide. e.g. prokaryotes.

Life span of mRNA is roughly of two minutes in prokaryotes and 1-4 hrs in eukaryotes (in liver). This type of RNA is rather short lived (ephemeral) and so has a rapid turnover.

(b) Transfer RNA (t-RNA) or soluble RNA (s-RNA) or adaptor Molecule : It is about 10-20% of total RNA. t-RNA are smallest (having 75 to 80 ribonucleotides) and have 4S sedimentation coefficient. It is responsible for carrying amino acid molecules to site of protein synthesis.



Due to modification of some bases e.g. pseudouridine (ψ), dihydrouridine (DHU), inosine (I), single-stranded tRNA gets coiled into hairpin like or clover leaf-like form (two dimensional by Holley) or L-shaped from (three dimensional by Klug.) Molecular model of t-RNA is popularly known as cloverleaf model (Two dimensional) which depicts the following structural specialities –

(i) About half of the nucleotides are base-paired to produce double helix segments. Five regions are single-stranded – AA binding site, T ψ C loop, DHU loop, extra arm and anticodon loop.

(ii) All tRNA have guanine residue (G) at 5' terminal end and unpaired CCA sequence at 3' end which is called amino acid binding site because the amino acid becomes attached to adenylic acid (A of CCA sequence) during polypeptide synthesis.

(iii) Amino acid stem or helix has 7 paired bases.

(iv) **T loop (T ψ C loop/ pseudouridine loop)** has seven unpaired bases and is involved in the binding of tRNA molecules to ribosomes (Ribosomal binding site). Stem of this loop has five paired bases.

(v) The anticodon stem includes five paired bases. The anticodon loop has seven unpaired bases, the third, fourth and fifth of which form anticodon. This anticodon permits temporary complementary pairing with three bases on mRNA (codon).

(vi) D-stem is made up of four base pairs (depending on species of tRNA). DHU loop is also variable in size, containing 8 to 12 unpaired bases. DHU loop helps in binding of aminoacyl synthetase.

(vii) Extra arm is variable and lacking in some tRNA. This lies between T loop and anticodon loop. There are more than 20 types of tRNAs. (more than 20 types of amino acid). Molecular weight is 25,000 to 30,000. tRNA is adaptor molecule which is meant for transferring amino acids to ribosomes for synthesis of polypeptide. tRNAs carry specific amino acids at particular site during protein synthesis according to codons of mRNA which are recognized by anticodons of tRNAs.

(c) Ribosomal RNA (r-RNA) : It is 80% of total RNA. It is most stable type. Molecular weight is 12,00,000. Ribosomes has 65-80% r-RNA. In ribosomes, 60% RNA is double stranded or helical. The eukaryotic cells have four kinds of rRNA molecules namely 28S rRNA, 18S rRNA, 5.8S and 5S rRNA. The 28 S, 5.8 S and 5S rRNA occur in 60S ribosomal subunit-while 18 S rRNA occurs in 40S ribosome subunits of 80S ribosome of eukaryotes. The prokaryotic cells contain three kind of rRNA molecules namely 23 S rRNA 16 S rRNA and 5S rRNA. 23S rRNA and 5S rRNA occur in 50 S ribosomal subunit, while 16 S rRNA occurs in 30 S ribosomal subunit of 70 S ribosomes or prokaryotes. Small sized RNA occurring free in the cytoplasm is small cytoplasmic RNA (sc RNA) eg 75 scRNA with 6 proteins produce signal recognition particles (SRP) which helps in taking and binding a ribosome to ER for producing secretory proteins.

8. Enzymes (Biocatalysts)

Enzymes or middleman of cell are globular proteinaceous substances that are capable of catalyzing chemical reaction of cell without any change in themselves. Termed by Kuhne. Buchner isolated the first enzyme. (actually discover the enzyme and awarded Nobel Prize).

Buchner found that yeast extract could cause fermentation of sugar. This was complex of enzymes, which takes part in alcoholic fermentation. Buchner popularized name of enzymes Protein, nature of enzyme was first found out by Sumner. He also purified the enzyme urease and obtained it in crystalline form from Jack bean(canavalia). (awarded as noble Prize).Northrop confirmed the protein nature of enzymes by crystallized pepsin and trypsin. Although, enzymes are produced by living cells only, yet they are able to catalyze reactions even in extracted form. Every cell produces its own enzymes. They cannot transfer from one cell to other. Till now more than 2000 enzymes are discovered.

8.1. Similarities between Inorganic Catalysts and Enzymes

- **Minute Quantity:** Both enzymes and catalysts are required in minute quantity.
- **Initiation of Reaction:** They have no role in starting the reaction.
- **Equilibrium:** Both do not change equilibria, as equally affects both side of reversible reaction.
- **Lowering of Activation Energy:** They lower amount of activation energy required to start reaction.
- **Reaction Velocity:** Both increase the reaction velocity.
- **Unchanged Form:** Both remains unchanged qualitatively as well as quantitatively at the end of reaction, so that they can be used over again.

8.2. Dissimilarities between Inorganic Catalysts and Enzymes

	Inorganic Catalysts		Enzymes
1.	They are inorganic substances.	1.	All enzymes except few are protein (organic)
2.	The size is small with low molecular weight.	2.	Size is larger with high molecular weight.
3.	They belong to nonliving in origin.	3.	Enzymes originate in living cells.
4.	They catalyse non biological reactions.	4.	Enzymes catalyse biochemical reactions.
5.	Efficiency is low.	5.	Efficiency is high.
6.	Catalysts are not sensitive to small changes in pH.	6.	Enzyme operate within narrow pH range and are highly sensitive to pH changes.
7.	Not influenced by small change in temperature.	7.	Inactivated near 0^0C & denatured above 45^0C.

8.3. Properties of Enzyme

They have a high mol. weight, 6000 for bacterial ferredoxin and 46×10^5 for pyruvate dehydrogenase. They occur in the colloidal state (particle size 1-200 nm). Protein digesting enzymes are often produced in inactive form called proenzymes or zymogens (e.g., trypsinogen, pepsinogen). Proenzymes are converted into active enzymes under specific conditions by a inducer. Pepsin & trypsin are autocatalytic, (pepsinogen to pepsin & trypsinogen to trypsin respectively). They may be active inside cells (endoenzymes or intracellular enzymes, 70% in mitochondria) or outside cells (exoenzymes, intercellular enzymes e.g., digestive enzymes). Myosin is both a structural protein as well as enzyme since its head has ATP-ase property

Important- Certain RNAs also have enzyme property. e.g.Riboenzymes (RNA enzymes): The first ribozyme was discovered by Cach et al (1981) from Tetrahymena (protozoa). It removes introns from newly synthesised RNA. RNA-ase P (ribonuclease P; Altman): It separates tRNAs from hnRNAs at their 5' ends. Peptidyl transferase (Noller): 23S r RNA of ribosome act as enzyme (in prokaryotes).

8.4. Characteristics of Enzymes

- **Proteinaceous nature :** All enzymes are chemically made up of proteins (except ribozyme and ribonuclease-P). They however may have additional inorganic or organic substances for their activity.
- **Amphoteric nature:** The enzymes are capable of ionizing either as an acid or as a base depending upon the acidity of the external solution. Hence their nature is amphoteric i.e., they can act as acid as well as base.
- **Colloidal nature :** They are colloidal in nature due to which they present a large surface area for reaction to take place. They are hydrophilic and form hydrosol in the cell.
- **Reversibility :** Like a true catalyst enzymes have been found to accelerate the chemical reaction in either direction i.e., forward and backward depending upon the availability of suitable energy requirement, pH, concentration of end products and availability of reactants.

$$\underset{\text{Ethyl alcohol}}{CH_3CH_2OH} + NAD^+ \longrightarrow \underset{\text{Acetaldehyde}}{CH_3CHO} + NADH + H^+$$

- **Molecular weight :** Enzymatic proteins are substances of high molecular weight. Peroxidase, which is one of the smallest enzymes, has a molecular weight of 40,000 whereas catalase, one of the largest enzymes, has a molecular weight of 250,000 (Urease 483,000).
- **Specificity of enzyme :** Enzymes are highly specific in nature i.e., a particular enzyme can catalyze only particular type of reaction e.g., the enzyme malic dehydrogenase removes hydrogen atom from malic acid and not from other keto acids. The specificity of enzyme is determined by sequence of amino acids in the active sites. The active site possesses a particular binding site which complexes only with specific substrate Thus a suitable substrate fulfills the requirements of active site and closely fixes with it.
- **Unchanged form:** Enzymes are in no way transformed or used up in the chemical reaction but come out unchanged at the end of reaction.

- **Chemical reaction :** Enzymes do not start a chemical reaction but increase the rate of chemical reaction. They do not change the equilibrium as well. However, they increase the rate of chemical reaction and bring about equilibrium very soon. Carbonic anhydrase is the fastest acting enzyme.

$$CO_2 + H_2O \underset{}{\overset{\text{Carbonic anhydrase}}{\rightleftharpoons}} \underset{\text{(Carbonic acid)}}{H_2CO_3}$$

- In absence of any enzyme this reaction is very slow, with about 200 molecules of H_2CO_3 being formed in an hour. However, by using the enzyme present within the cytoplasm called carbonic anhydrase the reaction speeds dramatically with about 600,000 molecules being formed every second. The enzyme has accelerated the reaction rate by about 10 million times.
- **Efficiency :** Efficiency of an enzyme is judged by its 'turn over number' i.e., number of substrate molecules changed per minute by a molecule of enzyme. It depends upon the number of active sites present over an enzyme, precise collisions between reactants and the rate of removal of end products. The optimum turn-over number for enzyme carbonic anhydrase is 36 million, catalase 5 million, enzyme sucrase or invertase 10,000 and flavoprotein 50.

8.5. Structure of Enzyme

Number of polypeptides in enzyme may be one in monomeric, (e.g. ribonuclease, chymotrypsin) and many in oligomeric, (e.g. Rubisco). Tertiary structure produces active site and makes enzyme functional. Each enzyme has active sites formed by R-groups of 3-12 non-adjacent amino acids for catalyzing activity. An active site has a depression (crevice or pit) where substrate molecules get fit for undergoing change. Enzymes are specific due to specific sequence of amino acid in active site. Histidine is most common amino acid of active site. e.g. Pepsin (Tyrosine only). Structure and specificity are most important properties of enzyme. These are determined by sequence of amino acid particularly in active site (primary structure)

Simple enzyme: They are made of only protein (e.g., pepsin, amylase, urease).

Conjugate enzymes: These have protein part (apoenzyme) an additional non-protein part (cofactor). Complete enzyme is called holoenzyme.

Cofactor: Three kinds of cofactors may be identified: prosthetic groups, co-enzymes and metal ions.

(a) Loosely attached organic cofactor is coenzyme (e.g., NAD, FAD, FMN, TPP. CoA).

(b) Firmly attached organic cofactor is prosthetic group (e.g., pyridoxal phosphate, heme, biotin).FMN, FAD are considered both coenzymes and prosthetic groups. Mostly vit- B act as coenzyme.

(c) Inorganic factors (minerals) are called as activators.A number of enzymes require metal ions for their activity which form coordination bonds with side chains at the active site and at the same time form one or more coordination bonds with the substrate, e.g., zinc is a cofactor for the proteolytic enzyme carboxypeptidase. Catalytic activity is lost when the co-factor is removed from the enzyme which testifies that they play a crucial role in the catalytic activity of the enzyme.

Activator	Metalloenzymes
Na	ATPase
Zn	Carbonic anhydrase, alcohol dehydrogenase, LDH, carboxypeptidase
Mn	Peptidases, decarboxylase
Mo	Nitrate reductase, dinitrogenase
Fe	Catalases, aconitase, peroxidase, cytochrome oxidase
Cu	Ascorbic acid oxidase cytochrome oxidase
Mg	Hexokinase, DNA polymerase, Pyruvate kinase, phosphatases
Ca	Lipase, Succinic dehydrogenase
Co	Peptidase
Ni	Urease
Cl	Salivary amylase

8.6. Isoenzymes or Isozymes

These are molecular forms of same enzyme with slight difference in molecular weight but have same substrate, e.g, 3 for creatine phosphokinase, 4 for alcoholic dehydrogenase, 16 for α-amylase and 5 for lactate dehydrogenase (LDH). They differ in their substrate affinity, maximum activity and regulatory properties.

8.7. Allosteric enzyme

These have allosteric sites that control conformation of active sites with the help of modulators. e.g., glucokinase, phosphofructokinase. Modulators are of two types—positive (activator) and negative (inhibitors). Allosteric enzyme phosphofructokinase is activated by ADP and inhibited by ATP and Diphosphofructosephosphatase is activated by ATP and inhibited by AMP.

8.8. Classification of enzyme

Previously enzymes were divided into two categories, hydrolyzing and desmolysing.

(a) Hydrolyzing: Mostly hydrolyzing or digestive enzymes are found in lysosome and break down substrate with help of water. e.g. Carbohydrases, Lipases, Proteases, Esterases, Nucleases,

(b) **Desmolysing enzymes :** Break without help of water e.g. oxidases, dehydrogenases, transaminases, carboxylases, etc.
IUB or International Union of Biochemistry: It has classified the enzyme in six class.

(a) **Oxido-reductase:** Helps in oxidation/reduction by transfer of electron or hydrogen. e.g. dehydrogenase, cytochrome oxidase/reductase, catalase, peroxidase.

(b) **Transferases:** Helps in group transfer (e.g. Kinases, transaminase).

(c) **Hydrolases:** Helps in hydrolysis, e.g., amylase, sucrase, lactase, maltase.

(d) **Lyases:** Helps in cleavage without hydrolysis, addition to double bond or breaking e.g., aldolase, fumarase, decarboxylase, carbonic anhydrase, aconitase.

(e) **Isomerases:** Helps in isomerization, e.g. isomerases (aldose to ketose or vice versa), epimerases (change position of main constituent) and mutases (position change of a side group).

(f) **Ligases/Synthetase:** Joins with ATP, e.g., Pyruvate carboxylase, Thiokinase, RUBP carboxylase.
IUB introduced the enzyme commission number to represent the properties of enzyme. Which has four digit – I digit- Class of enzyme, II digit- Sub-class of enzyme, III digit- Sub- sub-class of enzyme, IV digit- No. of enzyme. e.g., 1.1.1.1. for alcoholic dehydrogenase, 2.7.1.1. for hexokinase.

Nomenclature of enzyme

Earlier it was based on activity or characteristic of enzyme. e.g. Pepsin (pepsis = digestion), Papain (from papaya), Bromaline (from pineapple = Bromeliaceae).Nowadays enzymes are named by adding suffix-ase (Duclaux) after the substrate (e.g., lipase, maltase, amylase) or chemical reaction (e.g., succinate dehydrogenase). Some old names also persist, e.g., pepsin, trypsin. Modern names are more systematic, informative, but longer. In it first part indicates substrate and second part indicates type of reaction. e.g. ATP + D-Glucose-------------- D-Glucose6-phosphate + ADP (ATP: D-Glucose phosphotransferase). But now a days short names are used called Trivial names e.g. hexokinase = glucokinase.

8.9. Nature of Enzyme Action

Each enzyme (E) has a substrate (S) binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced. This complex is short-lived and dissociates into its product (s) P and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP).

The formation of the ES complex is essential for catalysis.

$$E+S \rightleftharpoons ES \longrightarrow EP \longrightarrow E+P$$

The catalytic cycle of an enzyme action can be described in the following steps :

(1) First, the substrate binds to the active site of the enzyme, fitting into the active site.

(2) The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.

(3) The active site of the enzyme, now in close proximity of the substrate breaks the chemical bonds of the substrate and the new enzyme. product complex is formed.

(4) The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

8.10. Mechanism of Enzyme Action

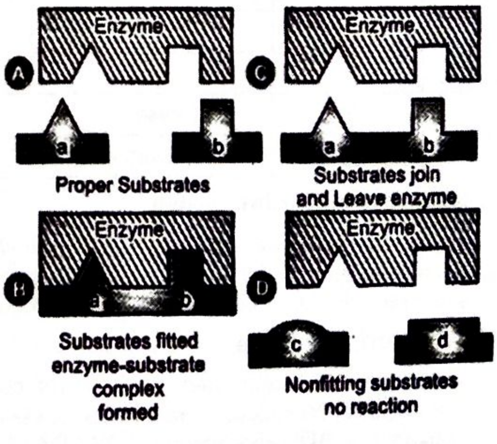
Reactants do not undergo chemical change automatically. They do so in transition state. This state has more free energy than reactants or products. Higher free energy helps in collision of reactants in transition state. Inability of reactants to react due to requirement of extra energy for converting them to transition state is called energy barrier. Energy required to overcome energy barrier is called activation energy. Enzymes bring reactants together over their active sites with help of weak non-covalent bonds. Enzyme accelerates rate of reaction 10^7 to 10^{20} (average 10^{10}). They work in milliseconds and ratio of enzyme to substrate is as high as 1:1000000 and as low as 1:100000. They lower the amount of activation energy.

There are two theories about mode of enzyme action.

- **Lock and Key Theory (Template Theory, Emil Fischer):** The static active sites of the enzymes have a specific geometric shape wherein the substrate molecules fit in just like a key in a particular lock. Temporary enzyme-substrate complex (ES complex) is formed. Substrate molecules undergo change, forming products. A temporary enzyme-product complex (EP complex) is formed. Products are released and the active sites of enzyme become free to pick up more substrate molecules.

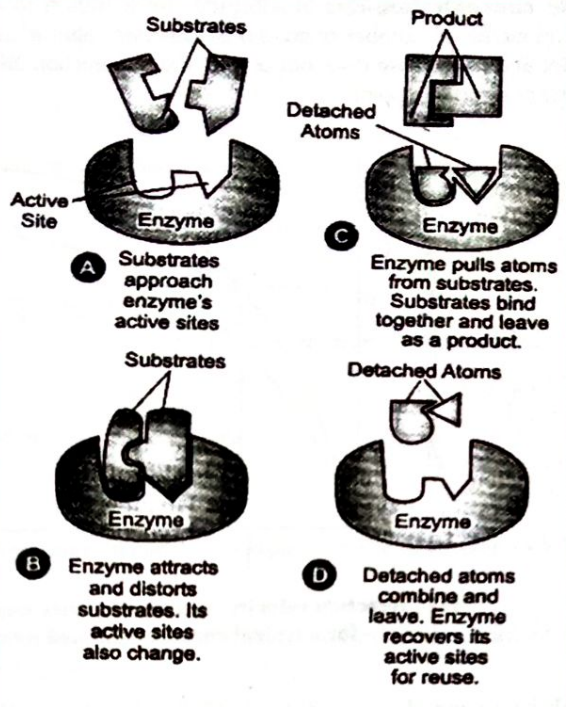
  Evidences

  Hypothesis explains how only a small quantity of enzyme (1: 100,000) is required. Even this enzyme remains unaffected and can be re-used and competitive inhibitor inhibits rate of reaction. A complex formed by enzyme and substrate has been observed with help of crystallography and confirmed through changes in absorption spectra. It explains competitive inhibition.



Schematic representation of enzyme and substrate interaction according to 'look-and-key' hypothesis.

- **Induced-Fit Theory (Koshland):**
  According to it dynamic active site has two groups buttressing or supporting & catalytic. As substrate gets joins with buttressing region and configuration of active site changes so that catalytic group comes to opposite to substrate and the catalytic group weakens bonds of reactants by electrophilic and nucleophilic changes.



Working of an enzyme according to the induced fit hypothesis

Conformational changes during formation of enzyme-substrate complex have been observed through X-ray diffraction and optical rotation analysis.

8.11. Factors Influencing Enzyme Activity

- **pH-**Optimum pH varies for enzyme to enzyme, e.g., 2 for pepsin, 6.8 for salivary amylase, 8.5 (trypsin). Most intracellular enzymes function at near neutral pH.
- **Temperature-**Enzymes are thermolabile. High temp. denatures enzymes. Lower temperature inactivates them. Optimum temperature for enzyme activity is $30^0 - 40^0$C for most animals. It is $20^0 - 30^0$C in case of plants. Within limits, Q_{10} is 2 – 3, Time factor (initial rise followed by fall) also operates. Freezing of food prevents its spoilage and keeps it preserved for a long time due to non-activity of enzymes contained in food as well as microbial enzymes. Due to denaturation of enzymes at high temp., very few cells can tolerate temperature above 45^0 C. Exceptions are microorganisms living in hot spring (100^0C). They have heat resistant enzymes. The enzyme active at high temperature called extremozyme eg; Tag polymerase (Used in PCR)

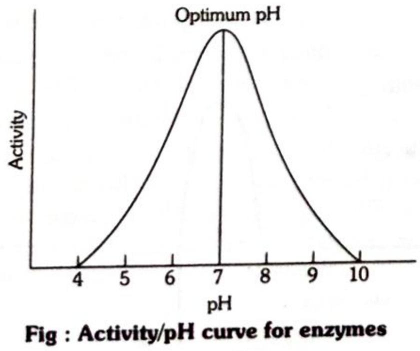


Fig : Activity/pH curve for enzymes

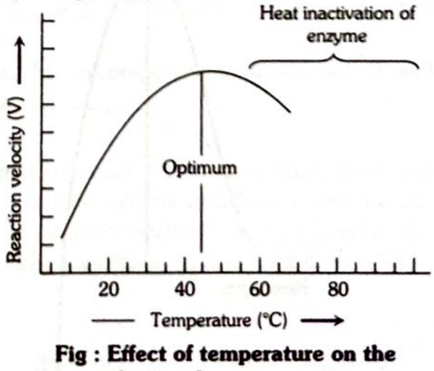


Fig : Effect of temperature on the velocity of enzyme action

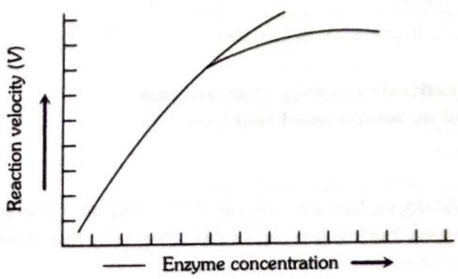


Fig : Effect of enzyme concentration on the velocity of enzyme action

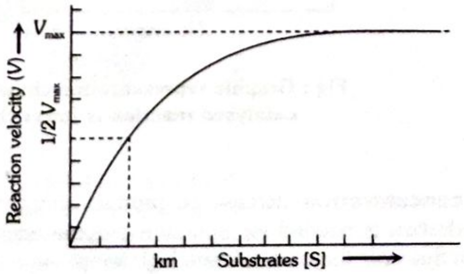


Fig : Effect of substrate concentration on the velocity of enzyme action

- **Enzyme and Substrate concentration**-Rise in substrate concentration increases rate of reaction due to higher number of active sites being used and increased number of collisions. However, after a certain substrate concentration, velocity or reaction reached a saturation point and further rise does not occur. Rate of reaction also increases with increase in enzyme concentration up to a point called limiting or saturation point.

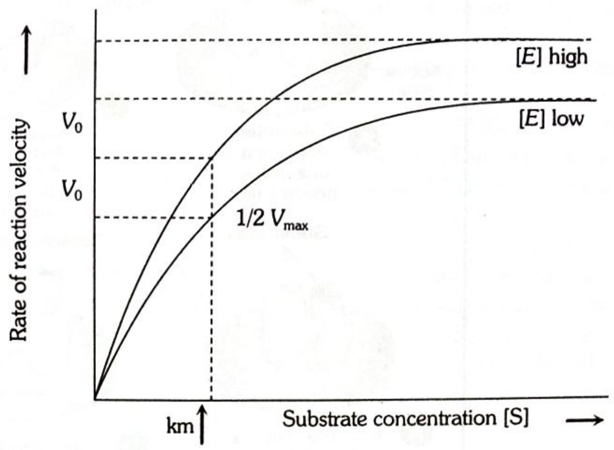


Fig : Reaction velocity 'V' and substance concentration (S) for a typical enzyme catalysed reaction

- **Michaelis constant** (Michaelis and Menten): Michaelis Menton Constant or K_m is a mathematical derivative or constant, which indicates the substrate concentration at which chemical reaction catalyzed by an enzyme attains half its maximum velocity (V_{max}). K_m indicates affinity of enzyme for its substrate. This value differs, from substrate to substrate because different enzymes differ in their affinity towards different substrate. High Km indicates low affinity while a low K_m shows strong affinity. Protease acts on different proteins. So its K_m value will differ from protein to protein. K_m value = 10^{-1} to 10^{-6}. Allosteric enzyme do not show typical Michaelis Menten constant, so they do not obey K_m constant. The structure of allosteric enzymes was studied by Monod.
- **Turn over Number / Catalytic Number /Kcat**
  Substrate molecules changed into product per minutes, by one molecule of enzyme is called turn-over number or efficiency. The fastest enzyme is carbonic anhydrase. Its turnover is 36 million, (10 million times faster than non catalyzed reaction) and 30 for lysozyme and 24 for ATPase (slowest). T.O.N. depends upon number of active sites, rapidity of reaction and separation of end products.
  Enzymes are highly specific in their substrate, temperature and pH.

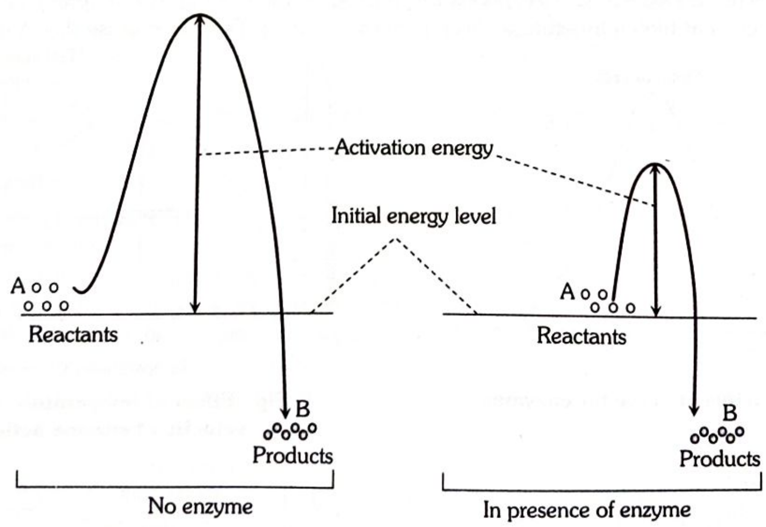
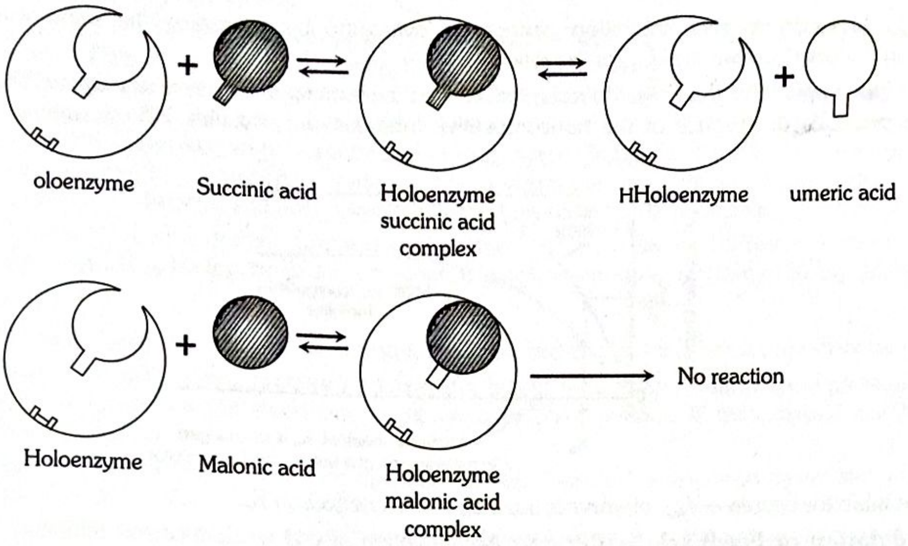


Fig : Graphic representation showing that activation energy of an enzyme catalysed reaction is lower than that of an uncatalysed reaction

- **Product concentration**-Increase on product concentration decreases the rate of reaction. Rather reverse reaction starts. A proper hydration is needed for optimum enzyme activity. Reduced hydration (10–20%) in seeds and spores makes enzymes inactive. In this state they can tolerate high temp.
- **Protein poisons** -Enzyme inhibition can be due to protein poisons (e.g., cyanide, lead, nickel), end products (allosteric, feed back or retro-inhibition, e.g., excess of glucose phosphate inhibits hexokinase or glucokinase activity; excess of isoleucine inhibits threonine deaminase by Umbarger).

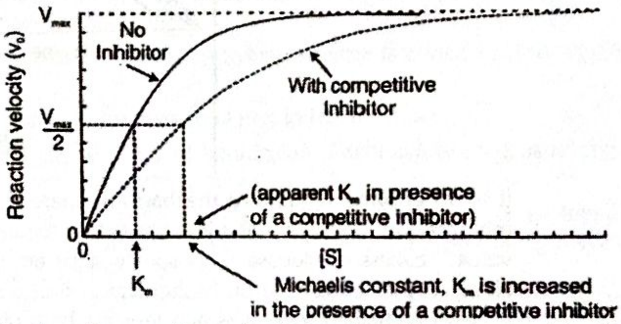


Lower : Representation of inhibition of enzyme activity by a complex inhibitor

8.12. Enzyme Inhibition

Reduction or stoppage of enzyme activity due to presence of inhibitor and adverse conditions It is of four types -- denaturation, noncompetitive inhibition, competitive inhibition and allosteric inhibition.

- **Competitive Inhibition** : The inhibitor or substrate analogue (structure is similar to enzyme) combines reversibly to the free enzyme at the active site. e.g., malonate or oxaloacetate inhibits succinate dehydrogenase. The effect is reduced or nullified by increase substrate concentration. Sulphonamides compete with PABA (para-amino benzoic acid) required for synthesis of folic acid in microbes. They were used to cure microbial infection during second world war. Humans and animals are not affected because they take pre-formed folic acid.



- **Effect on V_{max}** : The effect of a competitive inhibitor is reversed by increasing [S]. At a sufficiently high substrate concentration, the reaction velocity reaches V_{max} observed in the absence of inhibitor.
- **Effect on K_m** : A competitive inhibitor increases the apparent k_m for a given substrate. This means that in the presence of a competitive inhibitor more substrate is needed to achieve ½ V_{max} e.g., sulpha drugs for folic acid synthesis in bacteria and inhibition of succinic dehydrogenase by Malonate.
- K_i is dissociation constant of enzyme inhibitor complex. It is applicable to competitive inhibitors only. Low K_i is essential for enzyme activity while a high K_i decreases it.
- **Noncompetitive Inhibition:**

  This inhibitor (not similar to substrate) binds to enzyme (other than active site) and destruct enzyme activity. Substrate may combine with this inhibited enzyme but product is not formed. Cyanide inhibits cytochrome oxidase by combining irreversibly with Cu. Di-isopropyl fluorophosphates or DIFP (nerve gas) reacts with hydroxyl group of serine of acetylcholine-esterase. Malathion, parathion and some other pesticides act similarly. Ido-acetamide inhibits enzymes, which have sulphahydryl or imidazole group.

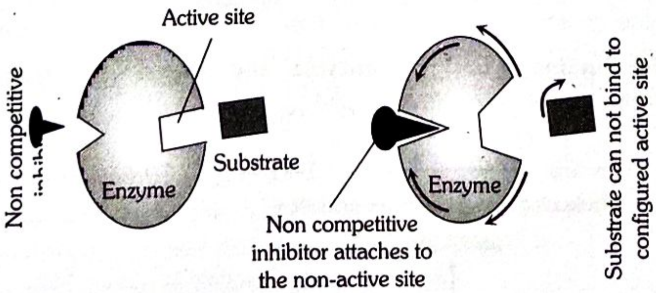
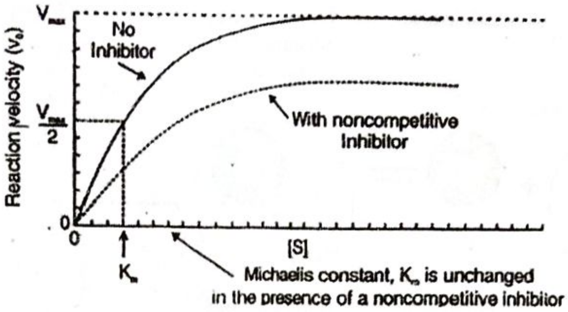


Fig : Non competitive inhibition : An inhibitor may bind to a site away from the active site thus not competing the substrate, yet changing the enzymes conformation so that the substrates no longer fits.

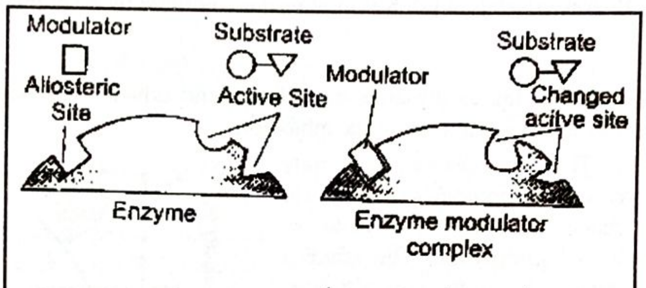
Some non-competitive inhibitors combine reversibly with enzyme-substrate complex to form EIS. The effect is not reversed by increased substrate concentration. These are not released by dilution.

- **Effect on V_{max}** : Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate. Thus noncompetitive inhibitors decrease the V_{max} of the reaction.
- **Effect on K_m** : Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Thus; the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor. e.g., cyanide kills an animal by inhibiting cytochrome oxidase.



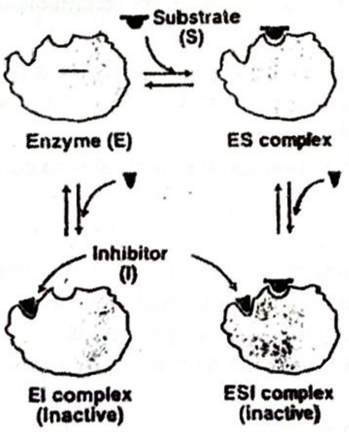
Non-competitive inhibitor decrease V_{max} of enzyme but they have no effect on K_m.

- **Allosteric Modulation or Feedback Inhibition** : Accumulation of end product causes inhibition in the activity of the first enzyme of the series.



Allosteric inhibition

It is an enzyme regulatory mechanism where a product of a simple or chain reaction can function as a temporary allosteric inhibitor (an inhibitor that combines with a regulatory or allosteric site, other than active site) if concentration crosses a threshold value. Excess of glucose 6-phosphate stops activity of hexokinase that helps in its formation from glucose & ATP. Threonine is changed into isoleucine by Escherichia coli in 5 step. Availability of excess isoleucine inhibits threonine metabolism because in excess isoleucine function as allosteric inhibitor of threonine deaminase.



- **A noncompetitive inhibitor binding to both free enzyme and enzyme - substrate complex** Effect of a noncompetitive inhibitor on the reaction velocity (V_0) versus substrate [S] plot.

Threonine

Threonine ⟶ 1→ 2 → 3 → 4 Substances →

Isoleucine (end product accumulation)

deaminase

Inhibits I enzyme

Hexokinase

Glucose + ATP ⟶ Glucose 6 phosphate

(accumulation inhibit the activity of I enzyme)

- **Enzyme Denaturation** : Tertiary structure of protein component of enzyme is destroyed by a number of factors like heat, high energy radiations and salts of heavy metals (Ag^+, Hg^{2+}, As^+).

8.13. Enzyme Related Hereditary Diseases

They are caused by deficient production of enzymes due to defective heredity, e.g., albinism (deficiency tyrosinase), galactosemia (defective galactose 1-Puridyl transferase), phenylketonuria (deficient phenylalanine hydroxylase or monooxygenase), methaemoglobinemia (defective methaemoglobin reductase), fructosuria (defective fructokinase). Antibodies that behave as enzymes are called abzymes. Lysozyme is antibacterial enzyme. It was discovered by A. Flemming (1922) from drops of nasal mucus. It is actually glycosidase, which causes lysis of bacteria by hydrolyzing glycosidic bonds in cell wall. Tears are rich is lysozyme. Diagnosis of some diseases can be done by measuring certain enzymes in serum because level of enzymes increases is serum during diseases e.g., Lipase level decreased in diabetes and liver disorders. Acid phosphatase level increases in obstructive jaundice and rickets.

Enzymopenia is deficiency of an enzyme. Enzymuria is presence of enzymes in the urine. Earliest known enzyme is diastase. Enzymes and antibody are called Molecular Radar. Smallest enzyme is peroxidase and largest being catalase found in peroxisome.

8.14. Enzyme Technology

Enzymes are being used increasing in number of industry, medicine and diagnostics. Some important uses are

- **Brewing Industry :** Amylases and proteases are used for hydrolysing polysaccharides and proteins during preparation of malt. Proteases are used for preventing cloudiness in stored beer. Fermentation is now carried out by immobilized enzymes or immobilized Yeast cells.
- **Dairy Industry :** Lipase is used to enhance ripening in some cheeses. Lactase is commercially available to convert milk sugar lactose into glucose and galactose. It prevents crystal formation in ice-creams. Sucrase is used in producing soft centered candies.Rennet tablets have enzyme rennin from calf's stomach. It is used for preparation of cheese due to their ability to coagulate milk protein into casein. It was formed by Hensen.
- **Baking Industry:** Protease is used by biscuit manufacturers to lower protein content of flour. α -amylase is used to soften and sweeten bread. Yeast is employed to leaven the bread.
- **Fruit Juices :** Pectinases and cellulases are added to increase clarity.
- **Baby Foods :** Trypsin is added to pre-digest baby foods.
- **Medical Use :** Deficiency of digestive enzymes is compensated by their supply, e.g., taka diastase, digestin, polyzyme, oryzyme. The enzyme streptokinase is used for dissolving blood clots inside blood vessels.
- **Detergents :** Protease is added to detergents for removing protein stains on clothes. Amylase enzyme is added to dish-washing soaps for removing starch related stains.
- **Foam Rubber :** Enzyme catalase is used to generate oxygen from peroxide for chaining latex to foam rubber.
- **Other Industrial Uses :** Protease is used for degumming silk and manufacture of liquid glue. Pectinase is used in retting of fibres while amylase is employed to degrade starch to lower viscosity product for sizing paper.
- **Genetic Engineering :** Restriction endonucleases are used in breaking DNA segments. Cellulase and pectinase are employed to dissolve cell walls and produce naked protoplasts for somatic hybridization. DNA polymerase is employed for producing copies of same DNA. Reverse transcriptase is used for informing cistrons or genes from mRNAs.

9. Metabolism

Metabolism is a collection of biochemical reactions taking place in a cell.Metabolites are converted into each other in a series of linked reactions called metabolic pathways. Important feature of metabolic reactions is that every chemical reaction is a catalysed reaction. There is no uncatalysed metabolic conversion in living systems. Even CO_2 dissolving in water, a physical process, is a catalysed reaction in living systems. The catalysts which hasten the rate of a given metabolic conversation are also proteins. These proteins with catalytic power are named enzymes.

9.1. Metabolic Basis for Living

Metabolic pathways can lead to a more complex structure from a simpler structure (for example, acetic acid becomes cholesterol) or lead to a simpler structure from a complex structure (for example, glucose becomes lactic acid in our skeletal muscle). The former cases are called biosynthetic pathways or anabolic pathways. The latter constitute degradation and hence are called catabolic pathways. Anabolic pathways, as expected, consume energy.

important form of energy currency in living systems is the bond energy in a chemical called adenosine triphosphate (ATP).

9.2. The Living State

The most important fact of biological systems is that all living organisms exist in a steady-state characterized by concentrations of each of the biomolecules. These biomolecules are in a metabolic flux. Any chemical or physical process moves spontaneously to equilibrium. The steady state is a non-equilibrium state. Hence the living state is a non-equilibrium steady-state to be able to perform work, living process is a constant effort to prevent falling into equilibrium. This is achieved by energy input. Without metabolism there cannot be a living state.

Important

- Antiageing enzyme is catalase, which metabolises H_2O_2 (iron-porphyrin enzyme).
- Enzymopathy is pathology related to enzyme deficiency.
- Allozymes are enzymes (similar) formed by different genes.
- Maximum enzymes are found is omnivores.
- In cell 10% of total enzymes (70 in number) are found is mitochondria alone.
- Diosgenin (from Dioscorea= Yam plant) is steroid used to make anti fertility pills so called Indian contraceptive.
- Digitalin (from Digitalis= foxglove) is cardiac glycoside and used as cardiac stimulant.
- Enzymes may be constitutive, Inducible, Repressible.

9. Biomolecules – Multiple Choice Questions

1. Carbohydrates, Starch and Protein

1. Corn is immersed in the boiling water. It is then cooled, the solution becomes sweet. It is due to
(a) Enzymes are inactivated in boiling water
(b) Disaccharides are converted to monosaccharides
(c) Monosaccharides are converted to disaccharides
(d) None of these

2. Which one of the following is the sweetest sugar
(a) Fructose (b) Glucose
(c) Galactose (d) Sucrose

3. Pentoses and hexoses are the most common
(a) Disaccharides (b) Monosaccharides
(c) Oligosaccharides (d) Polysaccharides

4. A complex polysaccharide produced from sucrose by the bacterium *Leuconostoc mesenteroides* is
(a) Chitin (b) Starch
(c) Cellulose (d) Dextran

5. In which form does the food transport in plants
(a) Sucrose (b) Fructose
(c) Glucose (d) Lactose

6. Which of the following amino acids is not optically active
(a) Glycine (b) Valine
(c) Leucine (d) Isoleucine

7. Sugars are technically called carbohydrates, referring to the fact that their formulae are only multiple of $C(H_2O)$. Hexoses, therefore, have six carbons, twelve hydrogens, and six oxygen atoms. Glucose is a hexose. Choose from among the following another hexose
(a) Fructose (b) Erythrose
(c) Ribulose (d) Ribose

8. Aminoacids, as the name suggests, have both an amino group and a carboxyl group in their structure. In addition, all naturally occurring amino acids (those which are found in proteins) are called L-aminoacids. From this, can you guess from which compound can the simplest amino acid be made
(a) Formic acid (b) Methane
(c) Phenol (d) Glycine

9. It is said that elemental composition of living organisms and that of inanimate objects (like earth's crust) are similar in the sense that all the major elements are present in both. Then what would be the difference between these two groups choose a correct answer from among the following
(a) Living organisms have more gold in them than inanimate objects
(b) Living organisms have more water in their body than inanimate objects
(c) Living organisms have more carbon, oxygen, and hydrogen per unit mass than inanimate objects
(d) Living organisms have more calcium in them than inanimate objects

10. When we homogenize any tissue in an acid the acid soluble pool represents
(a) Cytoplasm (b) Cell membrane
(c) Nucleus (d) Mitochondria

11. A homopolymer has only one type of building block called monomer repeated 'n' number of times. A heteropolymer has more than one type of monomer. Proteins are heteropolymers made of amino acids. While a nucleic acid like DNA or RNA is made of only 4 types of nucleotide monomers, proteins are made of
(a) 20 types of monomers
(b) 40 types of monomers
(c) 3 types of monomers
(d) Only one type of monomer

12. A primary protein should normally have
(a) Two ends (b) One end
(c) Three ends (d) No ends

13. α- helical model of protein was discovered by
(a) Pauling and Correy (b) Watson
(c) Morgan (d) Berzelius

14. During strenuous exercise, glucose is converted into
(a) Glycogen (b) Pyruvic acid
(c) Starch (d) Lactic acid

15. Which of the following carbon is anomeric in glucose
(a) C_1 (b) C_2
(c) C_4 (d) None of these

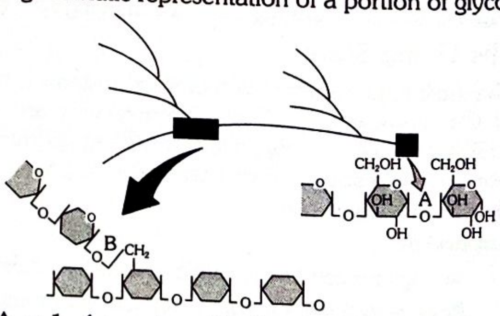
16. Which of the following fatty acids is liquid at room temperature
(a) Palmitic acid (b) Stearic acid
(c) Oleic acid (d) Linoleic acid

17. Paraffin wax is
(a) Ester (b) Acid
(c) Monohydric alcohol (d) Cholesterol

18. Which is an organic compound found in most cells
(a) Glucose (b) Water
(c) Sodium chloride (d) Oxygen

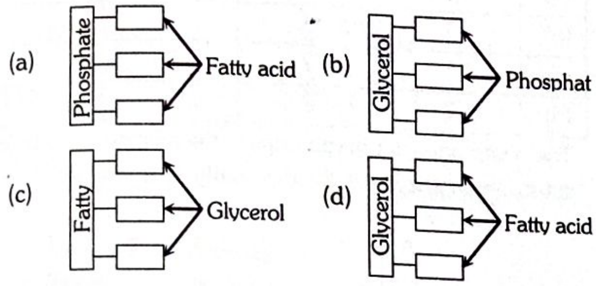
19. In a polysaccharide, the individual monosaccharides are linked by an
(a) Glycosidic bond (b) Peptide bond
(c) Ester bond (d) Phosphodiester bond
(e) Hydrogen bond

20. Observe the following figure and identify A and B bonds in the diagrammatic representation of a portion of glycogen

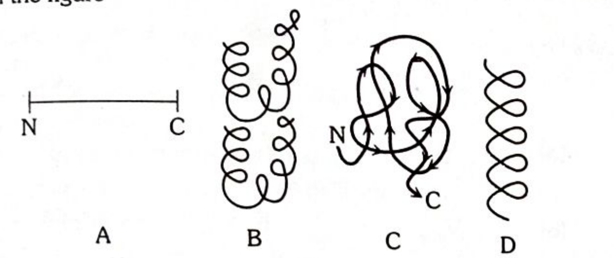


(a) A = 1- 4 α - glycosidic bonds, B = 1- 4 α - glycosidic bonds
(b) A = 1-1 α - glycosidic bonds, B = 1 - 1 α - glycosidic bonds
(c) A = 1- 6 α - glycosidic bonds, B = 1 - 4 α - glycosidic bonds
(d) A = 1- 4 α - glycosidic bonds, B = 1 - 6 α - glycosidic bonds

21. Which one of the following diagrams show a molecule of simple lipid



22. See the following figure and identify the structure of proteins in the figure



(a) A = 4° structure, B = 3° structure, C = 2° structure, D = 1° structure

(b) A = 1° structure, B = 4° structure, C = 3° structure, D =2° structure

(c) A = 4° structure, B = 2° structure, C = 3° structure, D = 1° structure

(d) A=1° structure, B = 2° structure, C = 3° structure, D = 4° structure

23. Many elements are found in living organisms either free or in the forms of compounds. One of the following is not, found in living organisms

(a) Silicon (b) Magnesium

(c) Iron (d) Sodium

24. Many organic substances are negatively charged e.g., acetic acid, while others are positively charged e.g., ammonium ion. An amino acid under certain conditions would have both positive and negative charges simultaneously in the same molecule. Such a form of amino acid is called

(a) Positively charged form (b) Negatively charged form

(c) Neutral form (d) Zwitterionic form

25. The most abundant chemical in living organisms could be

(a) Protein (b) Water

(c) Sugar (d) Nucleic acid

26. The number of 'ends' in a glycogen molecule would be

(a) Equal to the number of branches plus one

(b) Equal to the number of branch points

(c) One

(d) Two, one on the left side and another on the right side

27. Lysine and tryptophan are

(a) Proteins

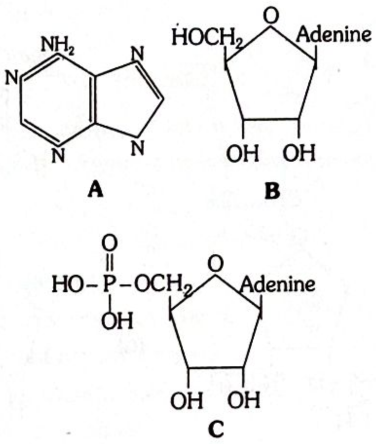
(b) Non-essential amino acids

(c) Essential amino acids

(d) Aromatic amino acids

2. Nucleotides and Nucleic acid

1. See the following figure and identify the correct combination



	A	B	C
(a)	Uracil	Adenosine (Nucleoside)	Adenylic acid (Nucleotide)
(b)	Adenosine (Nucleoside)	Adenylic acid (Nucleotide)	Adenine (N – base)
(c)	Adenine (N – base)	Adenosine (Nucleoside)	Adenylic acid (Nucleotide)
(d)	Adenine (N – base)	Adenylic acid (Nucleoside)	Adenosine (Nucleotide)

2. How many nucleotides are present in one turn of DNA helix

(a) 4 pairs (b) 8 pairs

(c) 10 pairs (d) 9 pairs

3. Which of the following bases is present in RNA in place of thymine

(a) Uracil (b) Adenine

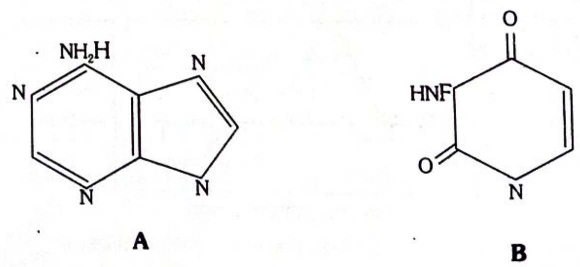
(c) Guanine (d) Water

4. The base pairs of DNA are correctly shown as

(a) $A \equiv T$ and $C = G$ (b) $A = T$ and $C = G$

(c) $A = T$ and $C \equiv G$ (d) $A \equiv T$ and $C \equiv G$

5. The given diagram shows the nitrogenous bases. Identify the correct combination



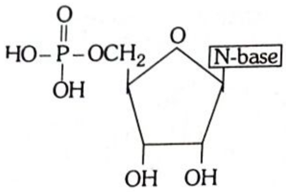
(a) A = Guanine; B = Uracil

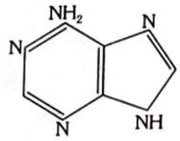
(b) A= Adenine; B = Uracil

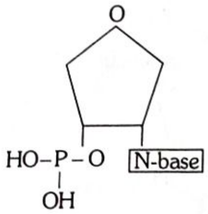
(c) A = Guanine; B = Thymine

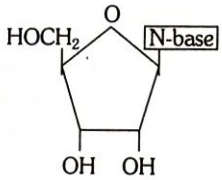
(d) A = Adenine; B = Thymine

6. Examine the following figures and select the right answer in which diagrammatic representation of a nucleotide is correctly shown

(a) 

(b) 

(c) 

(d) 

7. When you take cells or tissue pieces and grind them with an acid in a mortar and pestle, all the small biomolecules dissolve in the acid. Proteins, polysaccharides and nucleic acids are insoluble in mineral acid and get precipitated. The acid soluble compounds include amino acids, nucleosides, small sugars etc. When one adds a phosphate group to a nucleoside one gets another acid soluble biomolecule called

(a) Nitrogen base (b) Adenine
(c) Sugar phosphate (d) Nucleotide

3. Introduction, Properties, Action and Inhibition of The Enzyme

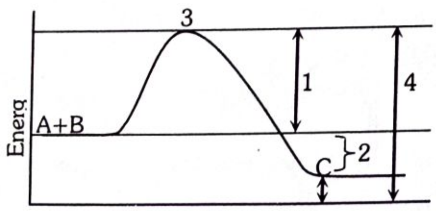
1. Who coined the term zymase for enzymes in yeast
(a) Kuhne (b) Sumner
(c) Louis Pasteur (d) Edward Buchner

2. Non-protein part of an enzyme is known as
(a) Holoenzyme (b) Apoenzyme
(c) Coenzyme (d) All the above

3. To explain the mechanism of enzymatic action, who proposed "*Lock and key hypothesis*"
(a) Fischer (b) Jacob
(c) Koshland (d) Sumner

4. Which one value is required for better enzymatic action
(a) High Ki (b) Low Ki
(c) Low Km (d) High Km

5. See the following figure and identify 1, 2, 3 and 4 from the list I to IV

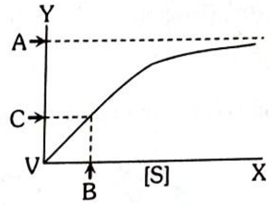


Progress of the reaction

I. Segment representing the energy of activation
II. Segment representing the amount of free energy released by the reaction
III. Transition state
IV. The segment would be the same regardless of whether the reaction was uncatalyzed or catalyzed which one is correct

	I	II	III	IV
(a)	1	2	4	3
(b)	1	3	2	4
(c)	1	2	3	2
(d)	1	2	3	4

6. The given adjacent graph depicts the change in conc. of the substrate on enzyme activity. Identify A, B, and C



	A	B	C
(a)	K_m	V_{max}	$\frac{V_{max}}{2}$
(b)	V_{max}	K_m	$\frac{V_{max}}{2}$
(c)	$\frac{V_{max}}{2}$	K_m	Ki
(d)	Ki	K_m	V_{max}

7. The enzymes required to obtain protoplasts are
(a) Cellulase and proteinase (b) Cellulase and amylase
(c) Cellulase and pectinase (d) Amylase and pectinase

8. Pepsin is inactivated at *pH*
(a) Below 3 (b) Below 2
(c) Above 5 (d) Above 3

9. The molecules that are well recognized as biocatalysts in addition to enzymes are
(a) Polysaccharides (b) Fatty acids
(c) RNAs (d) None of these

10. Which of the following statements is not correct
(a) All enzymes are proteins
(b) All enzymes are biocatalysts
(c) All proteins are enzymes
(d) All enzymes are thermolabile

11. A non-proteinaceous enzyme that acts as a catalyst for the formation of the peptide bond is

Or

"All enzymes are proteins." This statement is now modified because an apparent exception to this biological truth is
(a) Spliceosome (b) Ribozyme
(c) RNA poly I (d) RNA poly III

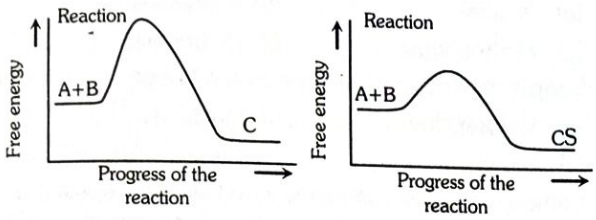
12. Inhibition of acetylcholine by DEP (Diisopropyl-fluorophosphate) is an example of
(a) Competitive inhibition
(b) Non-competitive inhibition
(c) Non-competitive irreversible inhibition
(d) Allosteric inhibition

13. Which one of the following enzyme contains *Mn* metallic ion as the prosthetic group
(a) Phosphatase (b) Dehydrogenase
(c) Peptidase (d) Catalase

14. Who proposed the principle of "*Induced fit*"
(a) Jacob (b) Fischer
(c) Koshland (d) Lederberg

15. Many of the hydrolytic reactions are
(a) Reversible (b) Irreversible
(c) Endothermic (d) Exothermic

16. The two chemical reactions are showing in the following figure. Which statement is correct for reaction 1



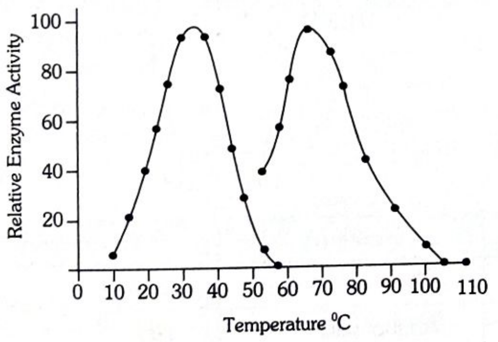
(a) Slower and more exergonic than 2
(b) Slower and more endergonic than 2
(c) Faster and more exergonic than 2
(d) Faster and more endergonic than 2

17. Proteins perform many physiological functions. For example, some functions as enzymes. One of the following represents an additional function that some proteins discharge
(a) Antibiotics
(b) Pigment conferring color to the skin
(c) Pigments making colours of flowers
(d) Hormones

18. Enzymes are biocatalysts. They catalyze biochemical reactions. In general, they reduce the activation energy of reactions. Many physicochemical processes are enzyme mediated. Some examples of enzyme-mediated reactions are given below. Tick the wrong entry
(a) Dissolving CO_2 in water
(b) Unwinding the two strands of DNA
(c) Hydrolysis of sucrose
(d) Formation of a peptide bond

19. Which of the following enzyme was first isolated and purified in the form of crystals
(a) Amylase (b) Urease
(c) Ribonuclease (d) Pepsin

20. The given graph depicts the effect of temperature on the activity of the two enzymes A and B that catalyze the same reaction. Select the correct statement (s) for these results



A. The rate of reaction in each case increases with increase in temperature and declines at higher temperatures due to denaturation of the enzyme
B. Both the enzymes A and B are thermolabile
C. At higher temperature the reactants become highly energized and fail to interact with the active site, thus decreasing the rate of reaction
D. The enzyme A is from a mesophilic organism, whereas the enzyme B is from a thermophilic organism
(a) A, B, D (b) C and D
(c) B and C (d) A and B

21. Amylopsin acts upon
(a) Polysaccharide in any medium
(b) Polysaccharide in acidic medium
(c) Polysaccharide in alkaline medium
(d) Polysaccharide in neutral medium

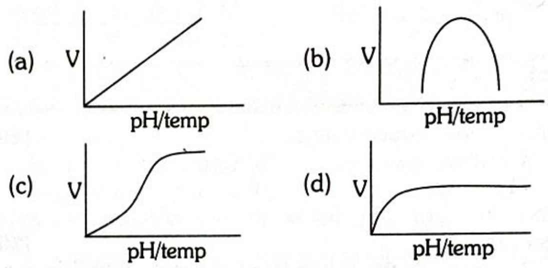
4. Classification and factors affecting enzyme

1. The enzyme which converts glucose into ethyl alcohol (C_2H_5OH) is
(a) Diastase (b) Maltase
(c) Zymase (d) Invertase

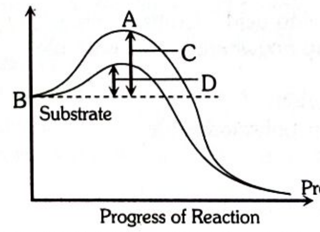
2. The enzymes ribulose biphosphate carboxylase-oxygenase and phosphoenolpyruvate carboxylase are activated by
(a) *Mg* (b) *Zn*
(c) *Mo* (d) *Mn*

3. Zymogens are
(a) Enzyme acting upon starch
(b) Group of zymase enzymes
(c) Inactive enzyme precursors
(d) None of the above

4. Which graph represents the effect of pH/temp on the velocity of a typical enzymatic reaction (V)

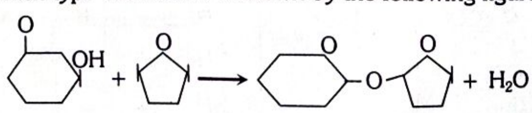


5. The given figure shows the conversion of a substrate into the product by an enzyme. Select the right option in which the components of reaction labeled as A, B, C, and D are identified correctly



	A	B	C	D
(a)	Activation energy with enzyme	Transition state	Activation energy without enzyme	Potential energy
(b)	Potential energy	Transition state	Activation energy with enzyme	Activation energy without enzyme
(c)	Transition state	Potential energy	Activation energy without enzyme	Activation energy with enzyme
(d)	Transition state	Potential energy	Activation energy without enzyme	Activation energy with enzyme

6. Which type of reaction is shown by the following figure



(a) Hydration (b) Denaturation
(c) Dehydration (d) Hydrolysis

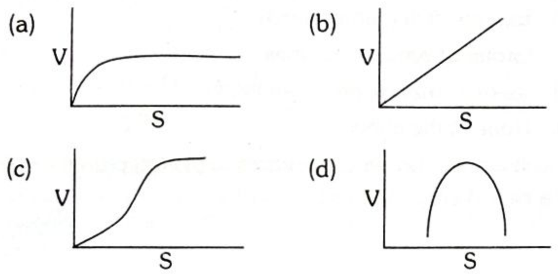
7. Enzyme concerned with the transfer of electrons is
(a) Hydrolase (b) Dehydrogenase
(c) Transaminase (d) Desmolase

8. Enterokinase converts
(a) Trypsinogen to trypsin
(b) Pepsinogen to pepsin
(c) Both (a) and (b) are correct
(d) None of these

9. Which enzyme helps in removing oil stains from clothes
(a) Streptokinase (b) Trypsin
(c) Lipase (d) Amylase

10. Enzymes which are slightly different in molecular structure but can perform an identical activity are called
(a) Isoenzymes (b) Holoenzymes
(c) Apoenzymes (d) Coenzymes

11. Which graph shows the relationship between the rate of an enzymatic activity and substrate conc.(S)



5. NEET

1. Which is non-reducing sugar [2014]
(a) Glucose (b) Galactose
(c) Mannose (d) Sucrose

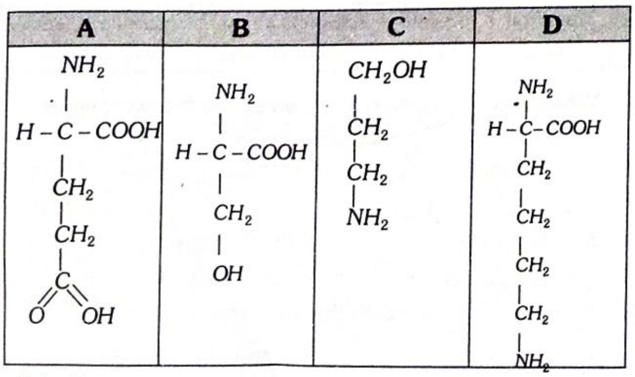
2. Which one of the following biomolecules is correctly characterized [2012]
(a) Lecithin - a phosphorylated glyceride found in the cell membrane
(b) Palmitic acid - an unsaturated fatty acid with 18 carbon atoms
(c) Adenylic acid - adenosine with a glucose phosphate molecule
(d) Alanine amino acid - Contains an amino group and an acidic group anywhere in the molecule

3. Chitin is a [2013]
(a) Polysaccharide
(b) Nitrogenous polysaccharide
(c) Lipoprotein
(d) Protein

4. The two polypeptides of human insulin are linked together by [2016]
(a) Hydrogen bonds (b) Phosphodiester bond
(c) Covalent bond (d) Disulphide bond

5. Which one of the following statements is wrong [2016]
(a) Sucrose is a disaccharide
(b) Cellulose is a polysaccharide
(c) Uracil is a pyrimidine
(d) Glycine is a sulfur-containing amino acid

6. Which one out of A-D given below correctly represents the structural formula of the basic amino acid [2012]



(a) C (b) D
(c) A (d) B

7. Lipids are insoluble in water because lipids molecules are [2002]
(a) Neutral (b) Zwitterions
(c) Hydrophobic (d) Hydrophilic

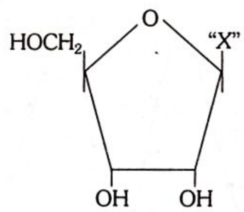
8. Largest physical and chemical molecules are [1996]
(a) Carbohydrates (b) Lipids
(c) Proteins (d) Nucleic acids

9. Carbohydrates are commonly found as starch in plant storage organs. Which of the following five properties of starch (A-E) make it useful as a storage material
(A) Easily translocated
(B) Chemically non-reactive
(C) Easily digested by animals
(D) Osmotically inactive
(E) Synthesized during photosynthesis
The useful properties are: [2008]
(a) (A), (C) and (E) (b) (A) and (E)
(c) (B) and (C) (d) (B) and (D)

10. The two functional groups characteristic of sugars are [2018]
(a) Carbonyl and hydroxyl (b) Carbonyl and phosphate
(c) Carbonyl and methyl (d) Hydroxyl and methyl

11. Which of the following biomolecules does have phosphodiester bond [2015]
(a) Monosaccharides in a polysaccharide
(b) Amino acids in a polypeptide
(c) Nucleic acids in a nucleotide
(d) Fatty acids in a diglyceride

12. Given below is the diagrammatic representation of one of the categories of small molecular weight organic compounds in the living tissues. Identify the category showed and the one blank component "X" in it [2012]



	Category	Component
(a)	Cholesterol	Guanin
(b)	Amino acid	NH_2
(c)	Nucleotide	Adenine
(d)	Nucleoside	Uracil

13. A phosphoglyceride is always made up of [2013]
(a) A saturated or unsaturated fatty acid esterified to a phosphate group which is also attached to a glycerol molecule
(b) Only a saturated fatty acid esterified to a glycerol molecule to which a phosphate group is also attached
(c) Only an unsaturated fatty acid esterified to a glycerol molecule to which a phosphate group is also attached
(d) A saturated or unsaturated fatty acid esterified to a glycerol molecule to which a phosphate group is also attached

14. Enzymes are absent in [2000]
(a) Algae (b) Fungi
(c) Bacteria (d) Virus

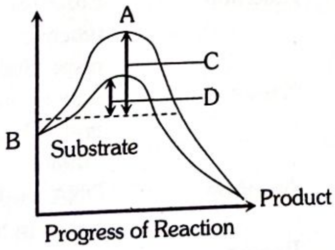
15. Three of the following statements about enzymes is correct and one is wrong. Which one is wrong [2010; 2013]
(a) Enzymes require optimum pH for maximal activity
(b) Enzymes are denatured at high temperature but in certain exceptional organisms, they are effective even at temperatures 80°-90°C
(c) Enzymes are highly specific
(d) Most enzymes are proteins but some are lipids

16. Which of the following is not a part of the enzyme but it activates the enzyme [1989]
(a) *K* (b) *Zn*
(c) *Mg* (d) *Mn*

17. Select the option which is not correct with respect to enzyme action [2014]
(a) A non-competitive inhibitor binds the enzyme at a site distinct from that which binds the substrate
(b) Malonate is a competitive inhibitor of succinic dehydrogenase
(c) The substrate binds with the enzyme at its active site
(d) Addition of a lot of succinates does not reverse the inhibition of succinic dehydrogenase by malonate

18. Which one of the following statements is incorrect [2015]
(a) In competitive inhibition, the inhibitor molecule is not chemically changed by the enzyme
(b) The competitive inhibitor does not affect the rate of breakdown of the enzyme-substrate complex
(c) The presence of the competitive inhibitor decreases the K_m of the enzyme for the substrate
(d) A competitive inhibitor reacts reversibly with the enzyme to form an enzyme-inhibitor complex

19. The figure given below shows the conversion of a substrate into the product by an enzyme. In which one of the four options (a-d) the components of reaction labeled as A, B, C, and D are identified correctly [2010]



Options:

	A	B	C	D
(a)	Potential energy	Transition state	Activation energy with enzyme	Activation energy without enzyme
(b)	Transition state	Potential energy	Activation energy without enzyme	Activation energy with enzyme
(c)	Potential energy	Transition state	Activation energy without enzyme	Activation energy with enzyme
(d)	Activation energy with enzyme	Transition state	Activation energy without enzyme	Potential energy

20. A competitive inhibitor of succinic dehydrogenase is [2008]
(a) α-ketoglutarate (b) Malate
(c) Malonate (d) Oxaloacetate

21. The catalytic efficiency of two different enzymes can be compared by the [2005]
(a) Formation of the product
(b) The *pH* of the optimum value
(c) The K_m value
(d) The molecular size of the enzyme

22. Cofactor (prosthetic group) is a part of the holoenzyme. It is [1997]
(a) Loosely attached inorganic part
(b) Accessory non-protein substance attached firmly
(c) Loosely attached organic part
(d) None of these

23. During glycolysis, enzyme hexokinase changes glucose to glucose–6– phosphate. Glucose–6–phosphate is inhibited by [1996]
(a) Feedback inhibition (b) Positive feedback
(c) Competitive inhibition (d) Non-competitive inhibition

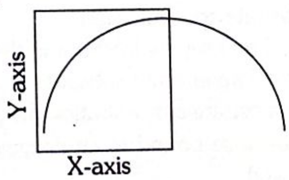
24. In which one of the following enzymes, is copper necessarily associated as an activator [2004]
(a) Lactic dehydrogenase (b) Tyrosinase
(c) Carbonic anhydrase (d) Tryptophanase

25. Which one of the following statements regarding enzyme inhibition is correct [2005]
(a) Competitive inhibition is seen when a substrate competes with an enzyme for binding to an inhibitor protein
(b) Competitive inhibition is seen when the substrate and the inhibitor compete for the active site on the enzyme
(c) Non-competitive inhibition of an enzyme can be overcome by adding a large amount of substrate
(d) Non-competitive inhibitors often bind to the enzyme irreversibly

26. Transition state structure of the substrate formed during an enzymatic reaction is [2013]
(a) Permanent and stable (b) Transient but Stable
(c) Permanent but unstable (d) Transient and Unstable

27. Modern detergents contain enzyme preparations of [2008]
(a) Thermoacidophiles (b) Thermophiles
(c) Acidophiles (d) Alkaliphiles

28. The curve is given below show enzymatic activity in relation to three conditions (pH, temperature and substrate concentration)



What do the two axes (x and y) represent [2011]

	X-axis	**Y-axis**
(a)	Enzymatic activity	Temperature
(b)	Enzymatic activity	pH
(c)	Temperature	Enzyme Activity
(d)	Substrate concentration	Enzymatic Activity

29. Fat is hydrolyzed by enzyme lipase to yield [2004]
(a) Fatty acid and amino acids
(b) Glycerol and fatty acids
(c) Glycerine and water
(d) Glycerol and amino acids

30. Which one of the following pairs is wrongly matched **[2009]**
(a) Detergents - lipase (b) Alcohol – nitrogenase
(c) Fruit juice - pectinase (d) Textile – amylase

6. AIIMS

1. Which of the following is a homopolysaccharide **[2012]**
(a) Heparin (b) Inulin
(c) Pectin (d) Hyaluronic acid

2. Which level of protein structure is affected by DNA **[1986]**
(a) Primary structure (b) Secondary structure
(c) Tertiary structure (d) Quaternary structure

3. Strands of DNA are bonded by **[1989; 1992]**
(a) Hydrogen (b) Carbon
(c) Oxygen (d) Nitrogen

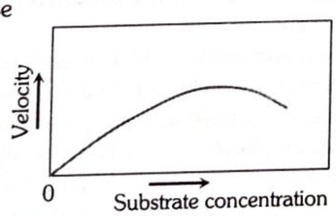
4. DNA is present in **[2004]**
Or
Which one of the following has its own DNA **[2010]**
(a) Nucleus only (b) Mitochondrion only
(c) Chloroplast only (d) All the above

5. FAD or FMN is a coenzyme. Which vitamin is incorporated into its structure **[2009]**
(a) Vitamin C (b) Vitamin B_1
(c) Vitamin B_6 (d) Vitamin B_2 (Riboflavin)

6. Inhibitory effect of malonic acid on succinic dehydrogenase enzyme is **[2003]**
(a) Competitive inhibition
(b) Non-competitive inhibition
(c) Feedback inhibition
(d) Inhibition due to the end product

7. Telomerase is an enzyme which is an **[2005]**
(a) Simple protein (b) RNA
(c) Ribonucleoprotein (d) Repetitive DNA

8. The given graph shows the effect of substrate concentration on the rate of reaction of the enzyme green gram-phosphatase



What does the graph indicate **[2005, 08]**
(a) The rate of the enzyme reaction is directly proportional to the substrate concentration
(b) Presence of an enzyme inhibitor in the reaction mixture
(c) Formation of an enzyme-substrate complex
(d) At higher substrate concentration the *pH* increase

9. An organic substance bound to an enzyme and essential for its activity is called **[2009]**
(a) Apoenzyme (b) Isoenzyme
(c) Coenzyme (d) Holoenzyme

10. When coenzyme is combined with apoenzyme, it is called **[1990]**
(a) Cofactor
(b) Holoenzyme
(c) Substrate enzyme complex
(d) Vitamin A

11. The protein part of the enzyme is known as **[2000]**
(a) Holoenzyme (b) Apoenzyme
(c) Isoenzyme (d) All of the above

12. The enzyme responsible for atmospheric nitrogen fixation is **[2000]**
(a) Nitrogenase (b) Hydrogenase
(c) Oxygenase (d) Carboxylase

13. cAMP-mediated 'Cascade model' of enzyme regulation was proposed by **[1986]**
(a) Fischer (b) Sutherland
(c) Sumner (d) Koshland

7. Assertion & Reason

Read the assertion and reason carefully to mark the correct option out of the options given below:
(a) If both the assertion and the reason are true and the reason is a correct explanation of the assertion
(b) If both the assertion and reason are true but the reason is not a correct explanation of the assertion
(c) If the assertion is true but the reason is false
(d) If both the assertion and reason are false
(e) If the assertion is false but reason is true

1. Assertion : Enzymes are defined as biological proteins.
Reason : Chemically all enzymes are globular proteins.

2. Assertion : DNA is associated with proteins.
Reason : DNA binds around histone proteins that form a pool and the entire structure is called a nucleosome.

3. Assertion : Enzymes have active sites and substrates reactive sites, on their surfaces respectively.
Reason : Active and reactive sites push the enzyme and substrate molecules away from each other.

4. Assertion : DNA molecules and RNA molecules are found in the nucleus of the cell.
Reason : On heating, enzymes do not lose their specific activity.

5. Assertion : Enzymes lower the activation energy.
Reason : A substrate molecule can be acted upon by a particular enzyme.