CELLULAR ENZYMES

7.1 Introduction and history of cellular enzymes

Enzymes (Gk. *en* = in; *zyme* = yeast) are proteinaceous substances which are capable of catalysing chemical reactions of biological origins without themselves undergoing any change. Enzymes are biocatalysts. An enzyme may be defined as "a protein that enhances the rate of biochemical reactions but does not affect the nature of final product." Like the catalyst the enzymes regulate the speed and specificity of a reaction, but unlike the catalyst they are produced by living cells only. All components of cell including cell wall and cell membrane have enzymes. Every cell produces its own enzymes because they can not move from cell to cell due to having high molecular weight. Maximum enzymes (70%) in the cell are found in mitochondrion. The study of the composition and function of the enzyme is known as **enzymology**.

The term enzyme (meaning in yeast) was used by Willy Kuhne (1878) while working on fermentation. At that time living cells of yeast were thought to be essential for fermentation of sugar. Edward Buchner (1897), a German chemist proved that extract zymase, obtained from yeast cells, has the power of fermenting sugar (alcoholic fermentation). Zymase is complex of enzymes (Buchner isolated enzyme for the first time).

Later J.B. Sumner (1926) prepared a pure crystalline form of urease enzyme from Jack Bean (*Canavalia ensiformis*) and suggested that enzymes are proteins. Northrop and Kunitz prepared crystals of pepsin, trypsin and chymotrpsin Arber and Nathans got noble prize in 1978 for the discovery of restriction endonucleases which break both strands of DNA at specific sites and produce sticky ends. These enzymes are used as microscissors in genetic engineering.

7.2 Nature of enzymes

Mostly enzymes are proteinaceous in nature. With some exception all enzymes are proteins but all proteins are not enzymes. Enzymic protein consist of 20 amino acids, which constitute other proteins. More than 100 amino acids linked to form an active enzyme. The polypeptide chain or chains of an enzyme show tertiary structure. Sequence of the amino acid in specific enzymic proteins. Their tertiary structure is very specific and important for their biological activity. Loss of tertiary structure renders the enzyme activity.

DNA is the master molecule, which contains genetic information for the synthesis of proteins. It has been found that DNA makes RNA and RNA finally makes proteins. The process of RNA formation from DNA template is known as transcription and synthesis of proteins as per information coded in mRNA is called translation. The above relation can be given by the formula given below.

$$\text{DNA} \xrightarrow{\text{transcription}} mRNA \xrightarrow{\text{translation}} \text{Protein/En zymes}$$

Some enzymes like pepsin, amylase, urease, etc., are exclusively made up of protein i.e. simple proteins. But most of the other enzymes have a protein and a non-protein component, both of which are essential for enzyme activity. The protein component of such enzymes is known as **apoenzyme** whereas the non-protein component is called **cofactor** or **prosthetic group.** The apoenzyme and prosthetic group together form a complete enzyme called **holoenzyme.**

Apoenzyme + Prosthetic group = Holoenzyme

Activity of enzyme is due to cofactor which can be separated by dialysis. After separation of cofactor the activity of holoenzyme or conjugated enzyme is lost.

Co-factor is small, heat stable and may be organic or inorganic in nature.

Three types of co-factors may be identified. Prosthetic group, coenzyme, and metal ions.

(1) **Prosthetic group :** Prosthetic groups are organic compounds distinguished from other co-factors in that they are permanently bound to the apoenzyme, e.g., in peroxisomal enzymes peroxidase and catalase which catalyzes breakdown of hydrogen peroxide to water and oxygen, heme is the prosthetic group and is a permanent part of the enzymes active site.

(2) **Coenzymes :** Coenzymes are also organic compounds but their association with the apoenzyme is transient, usually occurring only during the course of catalysis. Furthermore, the same coenzyme molecule may serve as the co-factor in a number of different enzymes catalyzed reactions. In general coenzymes not only assist enzymes in the cleavage of the substrate but also serve as temporary acceptor for one of the product of the reaction. The essential chemical component of many coenzymes are vitamins, *e.g.*, coenzyme Nicotineamide adenine dinucleotide (NAD), Nicotineamide adenine dinucleotide phosphate (NADP) contains the vitamin niacin, coenzyme A contains pantothenic acid, Flavin mononucleotide (FMN), Flavin adenine dinucleotide (FAD) contains riboflavin (Vitamin B_2), and thiamine pyrophosphate (TPP) contains thiamine (Vitamin B_1).

(3) **Metal ions :** A number of enzymes require metal ions for their activity. The metal ions form coordination bonds with specific side chains at the active site and at the same time form one or more coordination bonds with the substrate. The latter assist in the polarization of substrate bonds to be cleaved by the enzyme. The common metal ions are Zn^{++}, Cu^{++}, Mg^{++}.

Inorganic part of enzyme acts as prosthetic group in few enzyme they are called activator. These activators are generally metals. Hence these enzymes are called "Metallo enzyme" such as

S.No.	Activators	Enzymes
(1)	Iron (*Fe)*	Acotinase, Catalase and Cytochrome oxidase
(2)	Zinc (*Zn)*	Dehydrogenase, Carbonic andydrase
(3)	Copper (*Cu*)	Triosinase, Ascorbic acid oxidase
(4)	Magnesium (*Mg*)	Kinase, Phosphatase

(5)	Manganese (*Mn*)	Peptidase, Decarboxylase
(6)	Molybdenum (*Mo)*	Nitrate reductase
(7)	Nickel (*Ni*)	Urease
(8)	Boron	Enolase

Differences between apoenzyme and coenzyme.

S.No.	Characters	Apoenzyme	Coenzyme
(1)	Constitution	Protein part of holoenzyme or conjugated enzyme.	Non-protein organic part attached with apo-enzyme to form holoenzyme.
(2)	Specificity	Specific for an enzyme.	Can act as cofactors for many enzymes.
(3)	Requirement	Essential for catalytic activity.	It brings out the contact between substrate and enzyme and also helps in removing a product of chemical reaction.
(4)	Group transfer	Does not help in group transfer.	Helps in group transfer.

7.3 Nomenclature and Classification

Dauclax, (1883) introduced the nomenclature of enzyme. Usually enzyme names end in suffix-ase to the name of substrate e.g. Lactase acts on lactose, maltase act on maltose, amylase on amylose, sucrase on sucrose, protease on proteins, lipase on lipids and cellulase on cellulose. Sometimes arbitrary names are also popular e.g. Pepsin, Trypsin and Ptylin etc. Few names have been assigned as the basis of the source from which they are extracted e.g. Papain from papaya, bromelain from pineapple (family Bromeliaceae). Enzymes can also be named by adding suffix–ase to the nature of chemical reaction also e.g. oxidase, dehydrogenase, catalase, DNA polymerase.

(1) **According to order classification**

The older classification of enzymes is based on the basis of reactions which they catalyse. Many earlier authors have classified enzymes into two groups :

(i) The hydrolysing enzymes

(ii) The desmolysing enzymes.

Other classify enzymes into three groups

(i) The hydrolysing enzymes

(ii) The transferring enzymes

(iii) The desmolysing enzymes

In the first classification transferring enzymes are included in the hydrolysing enzymes since some of them are known to act as transferring as well as hydrolysing enzymes.

(i) **Hydrolysing enzyme :** The hydrolysing enzymes of hydrolases catalyse reactions in which complex organic compounds are broken into simpler compounds with the addition of water. Most of the hydrolysing (digestive) enzymes are located in lysosomes. Depending upon the substrate hydrolysing enzymes are :

(a) **Carbohydrases :** Most of the polysaccharides, disccharides or small oligosaccharides are hydrolysed to simpler compounds, e.g., hexoses or pentoses under the influence of these enzymes.

Lactase on lactose to form glucose to galactose, sucrase/invertase on surcose to form glucose and fructose, amalyse or diastase on starch to form maltose, maltase on maltose to form glucose, cellulase on cellulose to produce glucose.

(b) **Easterases :** These enzymes catalyse the hydrolysis of substances containing easter linkage, e.g., fat, pectin, etc. into an alcoholic and an acidic compound.

$$\text{Fat} \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Phosphoric acid easters} \xrightarrow{\text{phosphatase}} \text{Phosphoric acid} + \text{Other compounds}$$

(c) **Proteolytic enzymes :** The hydrolysis of proteins into peptones, polypeptides and amino acids is catalysed by these enzymes

$$\text{Protein} \xrightarrow{\text{Pepsin}} \text{Peptones}$$

$$\text{Polypeptides} \xrightarrow{\text{Peptidases}} \text{Amino acids}$$

(d) **Amidases :** They hydrolyse amides into ammonia and acids.

$$\text{Urea} \xrightarrow{\text{Urease}} \text{Ammonia} + \text{Carbon dioxide}$$

$$\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{Ammonia} + \text{Aspartic acid}$$

(ii) **Desmolysing enzymes :** Most of the desmolysing enzymes are the enzymes of respiration e.g. oxidases, dehydrogenases, (concerned with transfer of electrons), transaminases carboxylases etc.

(2) **According to IUB system to classification**

The number of enzymes is very large and there is much confusion in naming them. In 1961 the Commission on enzymes set up by the 'International Union of Biochemistry' (IUB) framed certain rules of their nomenclature and classification.

According to IUB system of classification the major points are :

- Reactions (and enzymes catalyzing them) are divided into 6 major classes each with 4-13 subclasses.

• The enzyme name has two parts-first name is of substrate. The second ending in *ase* indicates type of reaction.

• The enzyme has a systematic code No. (E.C.). The first digit denotes the class, the second sub-class, the third sub-sub-class and the fourth one is for the particular enzyme name. Thus, E.C. 2.7.1.1 denotes class 2 (Transferases)-subclass 7 (transfer of phosphate) sub-sub-class 1 (an alcohol functions as phosphate acceptor). The 4^{th} digit indicates hexokinase.

Major classes of enzymes are as follows :

(i) **Oxidoreductases :** These enzyme catalyse **oxidation reduction** reactions, usually involving the transfer of hydrogen atoms or ions from one molecule to another. There are three main types of these enzymes :

(a) **Oxidases :** Where the hydrogen is transferred from a molecule to oxygen, *e.g.,* cytochrome oxidase. They play very important role in E.T.S. in photosynthesis as well as respiration,

(b) **Dehydrogenases :** Where the hydrogen is transferred to a coenzyme such as NAD^+, *e.g*, Succinic dehydrogenase. They help in oxidation of organic molecules during aerobic respiration.

(c) **Reductase :** It is cause addition of hydrogen or an electron and remove oxygen. e.g., Nitrate reductase requires NAD (coenzyme I) as coenzyme for the reaction.

(ii) **Transferases :** These enzyme catalyse the transfer of a specific group (e.g. amino, methyl, acyl, phosphate) from one kind of molecule to another e.g. transphosphorylases, transaminases, transpeptidases, transmethylases, kinases, etc.

(iii) **Hydrolases :** These enzyme catalyse the hydrolysis of organic foods i.e. the breakdown of large molecules by addition of water e.g.all digestive enzymes such as lipases (digest the stored food material of caster seeds) amylases, esterases, phosphatases, carbohydrases, proteases.

(iv) **Lyases :** These enzymes catalyses the breakage of specific covalent bonds and removal of groups without hydrolysis e.g. fumerases, carboxylases, aminases, histidine decarboxylase that splits *C–C*-bond of histidine, forming CO_2 and histamine.

(v) **Isomerases :** These enzymes catalyses the rearrangement molecular structure to form isomers. e.g. phosphohexose isomerase (phosphoglucomutase) act on glucose 6-phosphate to form fructose 6-phosphate (both C_6 compounds); epimerase.

(vi) **Ligases or synthetases :** These enzymes form bonds join two molecules together, using energy supplied from the breakdown of ATP,e.g., DNA ligase is used to repair breaks in DNA molecules. Amino-Acyl synthetase is used to activate t-RNA by attaching amino acid at 3^1 end. Tryptophase synthetase is used to convert tryptophase amino acid to IAA, etc.

7.4 Site of enzyme action

All enzymes are produced in the living cells. About 3,000 enzymes have recorded. These are of two types with regard to the site where they act : intracellular and extracellular.

(1) **Intracellular enzymes :** Most of the enzymes remain and function inside the cells, They are called the intracellular enzymes or endoenzymes. Some of these enzymes are found in cytoplasmic matrix. Certain enzymes are bound to ribosomes, mitochondria and chloroplast etc.

(2) **Extracellular enzymes :** Certain enzymes leave the cells and function outside them. They are called the extracellular enzymes or exoenzymes. They mainly include the digestive enzymes. e.g. salivary amylase, gastric pepsin, pancreatic lipase secreted by the cells of salivary glands, gastric glands and pancreas respectively, lysozyme present in tears and nasal secretion.

Rennet tablets with enzyme renin from calf's stomach are widely used to coagulate protein caseinogen for cheese (casein) formation.

7.5 Mechanism of enzyme action

Chemical reaction takes place between molecules when they are activated. An activated molecule is at a higher energy level than other molecules. Increase in the number of activated molecules increases the speed of the chemical reaction. Energy is required to bring the inert molecules into the activated state. The amount of energy required to raise the energy of molecules at which chemical reaction can occur is called **activation energy.** Enzymes act by decreasing the activation energy so that the number of activated molecules is increased at lower energy levels. If the activation energy required for the formation of the enzyme-substrate complex is low, many more molecules can participate in the reaction than would be the case if the enzyme were absent.

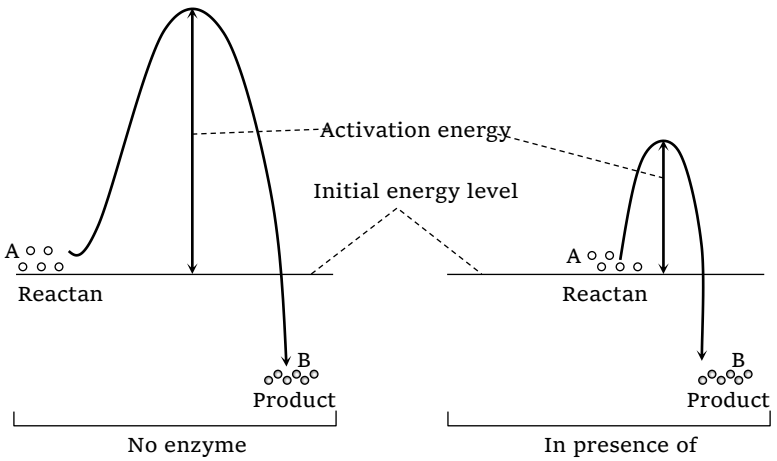


Fig : Graphic representation showing that activation energy of an enzyme catalysed reaction is lower than that of an

For example, activation energy, without adding the enzyme for the conversion of H_2O_2 into H_2O and O_2 is 18,000 calories per mole. But after addition of enzyme (catalase) the value is reduced to only 5,500 calories.

$$H_2O_2 \xrightarrow[\text{(an enzyme)}]{catalase} 2H_2O + O_2$$

7.6 Mode of enzyme action

In 1913 Michaelis and Menten proposed that for a catalylic reaction to occur it is necessary that enzyme and substrate bind together to form an enzyme substrate complex.

It is, however, difficult to demonstrate such complexes experimentally. Subsequently, the complex breaks up releasing the product and regenerating the original enzyme molecules for reuse.

$$\underset{\text{(Enzyme)}}{E} + \underset{\text{(Substrate)}}{S} \rightarrow \underset{\text{(Enzyme-substrate Complex)}}{E\text{-}S\ Complex}$$

$$E\text{-}S\ Complex \rightarrow \underset{\text{(Enzyme)}}{E} + \underset{\text{(Product)}}{P}$$

It is amazing that the enzyme-substrate complex breaks up into chemical products different from those which participated in its formation (i.e., substrates).

On the surface of each enzyme there are many specific sites for binding substrate molecules called **active sites** or catalytic sites. Structurally, each active site is an indentation on enzyme surface. It is lined by approximately 20 amino acids. During the course of reaction the substrate molecules occupy these sites. The active sites are located close to each other, hence, the substrate molecules also come close and react with one another. It is thought that when enzyme and substrates bind together, the shape of the enzyme molecule undergoes slight change. This produces strain in critical bonds in the substrate molecules and as a result these bonds break and new bonds are formed. The new chemical compound thus formed has little affinity for the enzyme and moves away from it.

There are two views regarding the mode of enzyme action :

(1) Lock and key hypothesis

(2) Induced fit hypothesis

(1) **Lock and key hypothesis :** The hypothesis was put forward by Emil Fisher (1894) . According to this hypothesis the enzyme and its substrate have a complementary shape. The specific substrate molecules are bound to a specific site of the enzyme molecule.

The theory can be explained easily by the fact that a particular lock can be opened by a particular key specially designed to open it. Similarly enzymes have specific sites where a particular substrate can only be attached. The lock and key model accounts for enzyme specificity.

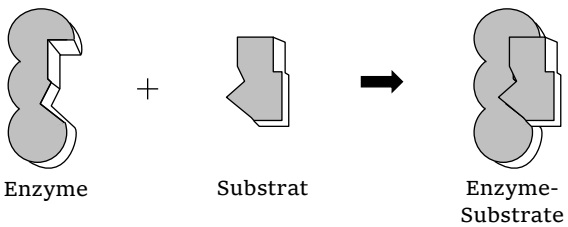


Fig : Lock and key model of enzyme

(2) **Induced fit hypothesis :** This hypothesis was proposed by Daniel, E. Koshland (1959).

According to this view, the active sites of an enzyme are not rigid. When the substrate binds to enzyme, it may induce a change in shape of the enzyme molecule in such a way that it is fit for the

substrate-enzyme interaction. The change in shape of the enzyme molecules can put strain on the substrate. This stress may help bonds to break, thus promoting the reaction. In other words :

According to this theory active site of the enzyme contains two group-**buttressing** and **catalytic.** The buttressing group is meant for supporting the substrate. The catalytic group is able to weaken the bonds of reactants by electrophilic and nucleophilic forces. Both buttressing and catalytic groups are normally at a distance. When substrate comes in contact with the buttressing group, the active site of enzyme undergoes conformational changes to bring the catalytic group opposite the substrate bonds to be broken.

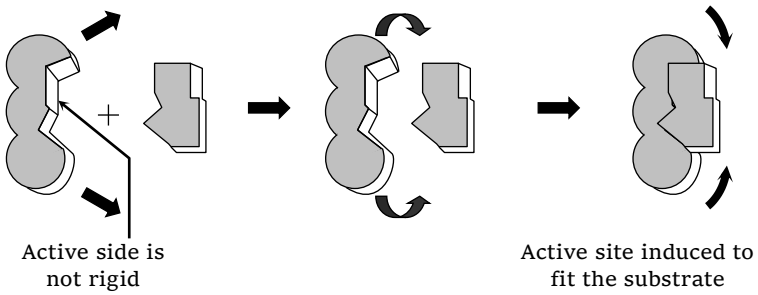


Fig : Reduced fit model of enzyme

7.7 PROPERTIES OF ENZYMES

The common properties of enzymes are listed below :

(1) **Molecular weight :** Enzymatic proteins are substances of high molecular weight. **Peroxidase** one of the smaller enzymes has molecular weight of 40,000, where as **catalase** one of the largest-has a molecular weight of 250,000 (urease 483,000). Enzyme molecules are therefore larger than those of usual simple organic substances but are nevertheless small enough to dissolve completely in aqueous media to yield clear nonturbid solution.

(2) **Amphoteric nature :** Each molecule of enzyme possess numerous groups which yield H^+ in slightly alkaline solutions and groups which yield OH^- ions in slightly acidic solutions. Unlike many other substances, therefore, the enzymatic protein is amphoteric, *i.e.*, capable of ionizing either as an acid or as a base depending upon the acidity of the external solution.

(3) **Colloidal nature :** All enzymes are colloidal in nature and thus provide large surface area for reaction to take place. They posses extremely low rates of diffusion and form colloidal system in water.

(4) **Specificity of enzyme :** Most of the enzymes are highly specific in their action. A single enzyme will generally catalyse only a single substrate or a group of closely related substrates. e.g. the enzyme lactase catalyzes the hydrolysis of lactose and no other disaccharide and the enzyme malic dehydrogenase removes hydrogen atom from malic acid and not from other keto acids. The enzymes posses active sites which are highly specific centres composed of varying number and sequence of amino acids. The active site possess a particular binding site which complexes only with specific substrate. Thus, only a suitable substrate fulfils the requirements of active site and closely fixes with it.

(5) **Heat specificity :** The enzymes are thermolabile i.e. heat sensitive. They function best at an optimum temperature (20°*C*-40°*C*). Their activity decrease with decrease as well as increase in temperature and stops at 0°C and above 80°*C*.

(6) **Catalytic properties :** Enzymes are active in extremely small amounts, e.g. on molecule of invertase can effectively hydrolyze 1,000,000 times its own weight of sucrose. One molecule of catalase is able to catalyze conversion of 5,000,000 molecules of hydrogen peroxide. The enzyme remains unchanged, qualitatively or quantitatively after the reaction.

(7) **Reversibility of reaction :** The enzyme-controlled reactions are reversible. The enzymes affect only the rate of biochemical reactions, not the direction. They can accelerate the reaction in either direction, i.e. onwards and backwards depending upon the availability of suitable energy sources e.g. Lipase can catalyase splitting of fat into fatty acids and glycerol as well as synthesis of fatty acids and glycerol into fats.

$$\text{Fat} \xrightleftharpoons{\text{Lipase}} \text{Glycerol+ Fatty acid}$$

(8) **pH sensitivity :** The enzymes show maximum activity at an optimum pH (6-7.5). Their activity slows with decrease and increase in pH till it stops. Each enzyme has its own different favourable pH value.

(9) **High efficiency :** The effectiveness of an enzymic reaction is expressed in terms of its turn over number or catalytic centre activity means number of substrate molecules on which one enzymes molecules acts in one minute.

Turn over number depends on the number of active sites of an enzyme. An active site is an area of the enzyme which is capable of attracting and holding particular substrate molecules by its specific charge, size and shape so as to allow the chemical change, Enzymes show 3-D structure. R (alkyl) groups of amino acids from active sites during folding polypeptide chains. Usually 3-12 amino acids form an active site. More the member of active sites, more is the turnover number of enzymes. Enzyme react with substrate only at these active sites. The whole surface of enzyme is not reactive. Enzymes have high turn over number (Catalytic number).

Highest turn over number is of **carbonic anhydrase** (36 million/min or 600000 per second) and lowest is of lysozymes (30/min or 0.5 per second). So carbonic anhydrase is fastest enzyme. It has zinc as activator. It hydrates 36 million CO_2 molecules per minute in RBC into H_2CO_3.

Turn over number depends upon number of active sites, rapidity of reaction and separation of end product.

(10) **Team work :** The enzymes generally work in teams in the cell, the product of one enzyme controlled reaction serving as the substrate for the next. In germinating seeds, starch is changed into glucose by two enzymes : amylase and maltase. Amylase splits the starch into the double sugar maltose, which is then broken by maltase into the single sugar glucose. Eleven different enzymes work sequentially to convert glucose to lactic acid in animal as well as plant cells.

(11) **Destruction by poisons :** Enzymatic activity can be retarded or inhibited by the use of toxic substances like cyanide and iodoacetic acid, cyanide destroys the respiratory enzyme cytochrome oxidase.

7.8 ENZYME INHIBITION

Interaction of an enzyme with substances other than the normal substrate changes the structure of enzyme. If this change occurs, there is loss in catalytic efficiency or complete in activation of enzyme. Inhibition may be of following types :

(1) **Competitive inhibition :** Substances (inhibitors) which are structurally similar to the substrates and complete for the active site of the enzyme are known as competitive inhibitors. Usually such inhibitors show a close structural resemblance to the substrates to the enzyme they inhibit. In such a case, inspite of enzyme substrate complex, enzyme inhibitor complex is formed and enzyme activity is inhibited.

$$\underset{\text{Enzyme}}{E} + \underset{\text{inhibitor}}{I} \rightarrow \underset{\text{Enzyme-inhibitorcomplex(EI)}}{EI}$$

The concentration of EI complex depends on the concentration of free inhibitor. Because EI complex readily dissociates, the empty active sites are then available for substrate binding. The effect of a competitive inhibitor on activity is reversed by increasing the concentration of substrate.

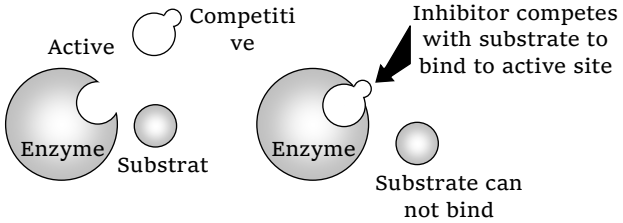


Fig : Competitive inhibition : the enzyme can not function (inhibited) as long as inhibitor remains bound. However, should the inhibitor becomes free (unfound), a substrate

A classic example of competitive inhibition is succinic acid dehydrogenase which oxidises succinic acid to fumaric acid. If concentration of malonic acid, is added, the activity of succinic dehydrogenase decreases rapidly. Hence malonic acid acts as a competitive inhibitor since it has structural resemblance to succinc acid.

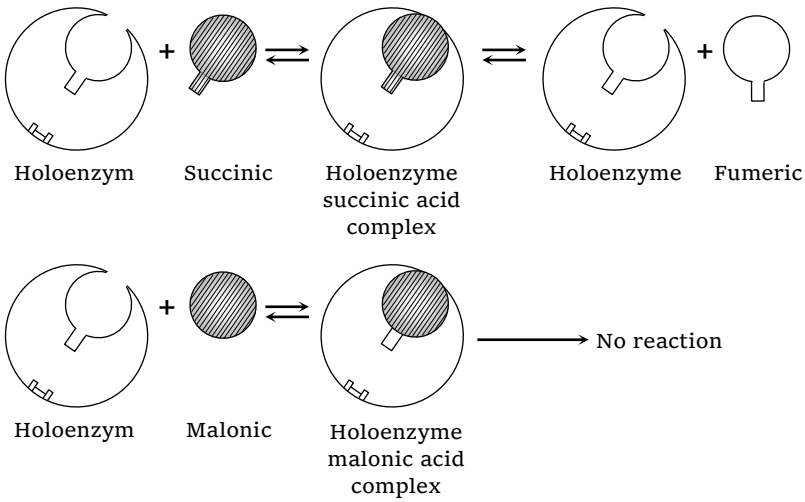


Fig : Upper : Mechanism of enzyme action showing formation of enzyme substrate complex and products
Lower : Representation of inhibition of enzyme activity by a complex inhibitor

The competitive inhibition can be reversed by increasing the concentration of the substrate. Competitive inhibitors are used in control of bacterial pathogens. Sulpha drugs is similar to PABA (para aminobenzoic acid) act as competitive inhibitors in the synthesis of folic acid in the bacterial cells because they compete with p-amino benzoic acid for the active sites of the enzyme and check the synthesis.

(2) **Non-competitive inhibition :** These substances (poisons) do not combine with active sites but attach somewhere else and destroy the activity of enzyme.

Both EI and ES complexes are formed. Inhibitor binding alters the three dimensional configuration of the enzyme and thus blocks the reaction. Non competitive inhibitor do not compete directly with the substrate for binding to the enzyme.

The non-competitive inhibition can not be reversed by increasing the concentration of the substrate i.e. irreversible. e.g. cyanide inhibits the mitochondrial enzyme cytochrome oxidase which is essential for cellular respiration. This kills the animals. Cyanide (inhibitor) does not compete for active sites of enzyme with substrate because it has no similarity with substrate (cytochrome) but it acts at some other site of enzyme.

More AMP is a non competitive inhibitor of fructose biphosphate phosphatase, the enzyme that catalyses the conversion of fructose 1, 6 biphosphate to fructose 6 phosphate. Toxic metal ions destroy essential sulphydryl groups of certain enzymes.

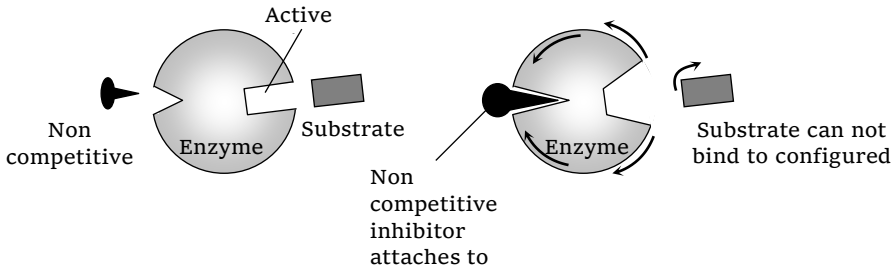
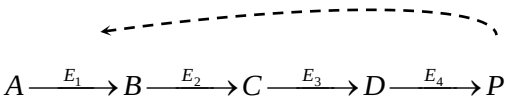


Fig : Non competitive inhibition : An inhibitor may bind to a site away from the active site thus not competing the substrate, yet changing the enzymes conformation so that the substrates no

S.No	Competitive inhibition		Non competitive inhibition
(1)	The structure of the inhibitor molecule is similar to the substrate.	(1)	The structure of the inhibitor is different from the substrate.
(2)	The inhibitor gets attached to the enzyme's active site.	(2)	The inhibitor forms a complex at a site other than the active site on the enzyme.
(3)	The reaction can be reversed at any stage by increasing the substrate concentration.	(3)	The reaction will keep on decreasing till there is saturation of inhibitor.
(4)	The substrate competes with the inhibitor for the position of the active site.	(4)	The substrate does not compete with the inhibitor as the name indicates.

(5)	The inhibitor does not alter the structure of the enzyme.	(5)	The inhibitor alters the structure of the enzyme in such a way that even if the substrate gets attached, the end products will not be formed.
(6)	The competition of pesticides with the neurotransmitter chemicals while binding to chemoreceptor sites on dendrites.	(6)	Cyanide and azides combines with the prosthestic groups of cytochrome oxidase and inhibits the electron transport chain.

(3) **Feedback inhibition :** In number of cases, accumulation of the final product of the reaction is capable of inhibiting the first step of reaction.



The product P checks the activity of enzyme which converts A into B. It is quite useful mechanism because it checks the accumulation of products.

The phenomenon in which the end product of a metabolic pathway can regulate its own production by inhibition of the sort is called **feed back inhibition** or negative feed back inhibition. This type of inhibition can be shown in *Escherichia coli* bacterium which synthesises the amino acid isoleucine from a substrate threonine by a series of intermediate reactions (i.e. α ketobutyrate threonine deaminase, α Aceto hydroxy butyrate, α keto β methyl valerate etc).

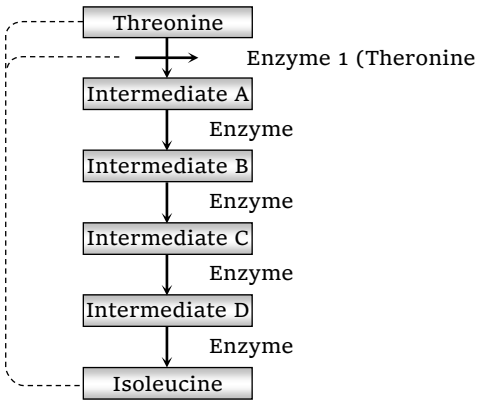


Fig : Feed back

When isoleucine accumulates in amounts more than required, it stops its own production by inhibiting the activity of the enzyme. Threonine deaminase which catalyzes the first reaction of the series. This type of metabolic control in which the first enzyme of a series is inhibited by the end product, is known as end product inhibition.

(4) **Allosteric inhibition (modulation) :** Allosteric literally means ' another place'. Still other inhibitors join an enzyme at a specific site and change the form of the active site meant for the substrate. These inhibitors are known as modifiers

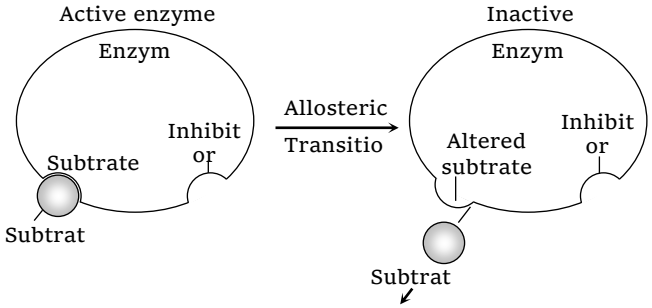
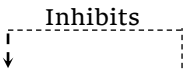


Fig : Diagram showing the active allosteric enzyme becoming inactive due to allosteric transition brought about by the attachment of the end product (inhibitor) on the allosteric (inhibitor) site

or modulators and the sites where they fit in are called allosteric sites. Modulators are of two types- positive (activators) and negative (inhibitors). Allosteric enzyme phosphofructokinase is activated by ADP and inhibited by ATP. Diphosphofructose, phosphate is activated by ATP and inhibited by AMP. Change of active site form prevent the binding of substrate to the enzyme and stops the reaction. The process is called allostery or allosteric inhibition, The enzyme with allosteric sites are called allosteric enzymes. Jacob and Monod have termed this phenomenon as allosteric transition.

An example of allosteric enzyme inhibition is hexokinase that converts glucose to glucose 6-phosphate. Glucose 6-phosphate causes allosteric inhibition of hexokinase. This is called feedback allosteric inhibition.



$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose 6 - phosphate} + \text{ADP}$$

7.9 Some terms regarding Enzymes

(1) **Zymogen :** Certain enzymes are produced by the living cells in an inactive (non-functional) form. They are called the zymogens or proenzymes. It is then converted, usually by proteolysis (hydrolysis of the protein), to the active form when it has reached the site of its activity. Pepsinogen and trypsinogen are zymogens produced by gastric glands and pancreas respectively. They are necessary to life because they degrade dietary proteins into amino acids that are used by the cell.

Pepsinogen is changed to active pepsin by hydrogen ions in the stomach. Trypsinogen is activated to trypsin by an enzyme enterokinase in the small intestine. Once small amount of pepsin or trypsin is formed, it itself catalyzes the activation of remaining proenzyme. This process is called **autocatalytic reaction,** or **autocatalysis**.

(2) **Isoenzyme** (Isozymes) **:** There are certain enzymes which have slightly different molecular structure but performing the same catalytic function. Such enzymes are called isoenzymes or simply isozymes. Isoenzyme of an enzyme differ from one another in their amino acid sequence, molecular weight, immunological and electrophoretic behaviours. Hence, they can be separated by electrophoresis. When the variants of an enzyme are within the same species of an organism they are called intraspecific or **ontogenic variants**; when they are from different species they are called interspecific or **phylogenetic variants**. Isoenzymes provide a clue to the genetic relationships of organism. Similarity in isoenzymes is corrected with similarity in genotype.

The isoenzyme differ in optimum activity and enable the organism adapt to varied environmental conditions. It is held that the isoenzymes are produced by genetic changes during evolution.

Isoenzymes may be homologous or analogous. Homologous isoenzymes have essentially similar molecular structure and catalytic properties. Analogous isoenzymes, though catalyse similar reaction, have different molecular structure and catalytic properties.

More than 100 enzymes are known to have isoenzyme. A good example of isoenzyme is lactic dehydrogenase (LDH). It catalyzes change of pyruvate to lactate. There are five LDH isoenzymes in

muscles of heart have been dilineated designated as LDH_1-LDH_5. The different LDH isoenzymes differs significantly in maximum activities (V_{max}) and in Michaelis constant (K_m) for their substrates especially for pyruvate. Alcohol dehydrogenase has four isoenzyme in maize. α-amylase (wheat endosperm) has 16 isoenzymes.

(3) **Inducible enzyme :** An enzyme which is synthesized only in the presence of its substrate (inducer) is called inducible enzyme e.g., β-galactosidase.

(4) **Constitutive enzymes (House keeping enzyme) :** The enzyme which are found in constant amounts under different growth conditions (regardless of its metabolic states) are called constitutive enzyme e.g. enzymes of sugar break down i.e. glycolysis.

(5) **Repressible enzyme :** The presence of a specific substance may inhibit continued production of specific enzyme (enzyme repressor) e.g. glucokinase.

(6) **Ribozymes :** Study of post transcriptional processing of RNA molecules has led to the most exciting discovery of the existence of some catalytic RNA molecules which have been called as RNA enzymes or ribozymes. All enzymes are not proteins as confirmed by Cech (1981) and Altman (1983). Ribozyme and RNAase-P are two non protein enzyme where RNA acts as catalyst. Ribozyme was reported from Tetrahymens (a protozoans) by Cech. The substrate for ribozyme is usually an RNA molecule. RNAase-P (Ribonuclease) discovered by Altman.

(7) **Michaelis constant :** Michaelis and Menten (1913) introduced a constant K_m (Michaelis constant).

It is a mathematical derivative or constant which indicates the substrate concentration at which the chemical reaction catalysed by an enzyme attains half its maximum velocity (V_{max}).

K_m indicates affinity of the enzyme for its substrate.

$$K_m = \frac{1}{2} V_{max}$$

K_m value differs from substrate to substrate because different enzymes differ in their affinity towards different substrate. A high K_m indicates low affinity while a low K_m shows strong affinity. Protease acts on different proteins. So it K_m value will differ from protein to protein.

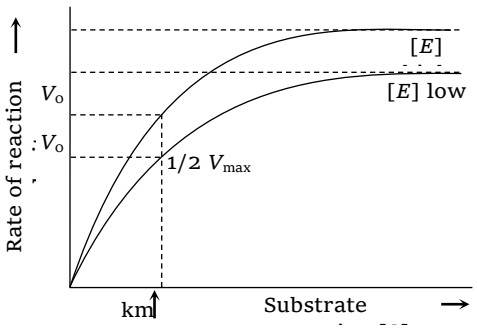


Fig : Reaction velocity '*V*' and substance concentration (*S*) for a typical enzyme catalysed reaction

They propose general theory of enzyme action and kinetics. According to them it accounts for most of the features of enzyme catalyzed reactions. Enzyme combine with substrate to form enzyme-substrate (ES) complex and subsequently breaks down to product, regenerating the free enzyme.

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

$$ES \xrightarrow{k_3} E + P$$

Where S is the substrate; E is the enzyme; ES is the enzyme-substrate; K_1, K_2, K_3 are rate constant.

The Michaelis Menten equation description how reaction relatively varies with substrate concentration as given

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Where V_0 is the initial reaction; V_{max} is the maximum relative or the reaction rate with excess substrate; K_m is the Michaelis constant=K_2+K_3/K_1 ; [S] is the substrate concentration.

The above reaction show that the greater the affinity between an enzyme and its substrate, the lower the K_m (in units moles per litre) of the enzyme substrate reaction. Stated inversely, $1/K_m$ $1/K_m$ is the measure of affinity of the enzyme for its substrate.

Allosteric enzymes do not show typical Michaelis Menten constant or allosteric enzymes do not obey K_m constant. K_m mostly lies between 10^{-1} to 10^{-6} M.

7.10 Factors affecting the Enzyme activity

Like all chemical reactions, enzymatic reactions are sensitive to environmental conditions. Thus, the substrate concentration, enzyme concentration, pH. temperature and inhibitors all affect the rate of enzymatic reaction. These factors affect the active site of the enzyme and formation of the enzyme-substrate complex.

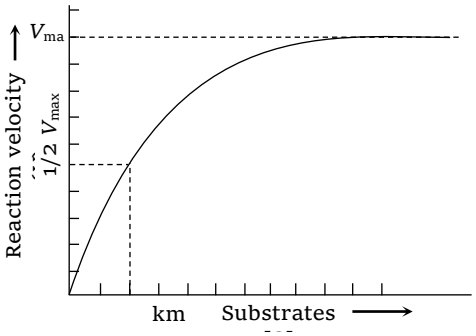


Fig : Effect of substrate concentration on the velocity of enzyme action

(1) **Substrate concentration :** If there are more enzyme molecules than substrate molecules, a progressive increase in the substrate molecules increases the velocity of their conversion to products. However, eventually the rate of reaction reaches the maximum. At this stage the active sites of all the available enzyme molecules are occupied by the substrate molecules. Therefore, the substrate molecules occupy the active sites vacated by the products and cannot increase the rate of reaction further.

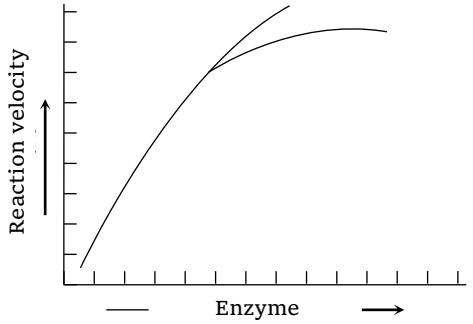


Fig : Effect of enzyme concentration on the velocity of

(2) **Enzyme concentration :** The rate of reaction is directly proportional to enzyme concentration. An increase in enzyme concentration will cause a rise in the rate of reaction up to a point and them the rate of reaction will be constant. Increasing the enzyme concentration increases the number of available active sites.

(3) **Product concentration :** Accumulation of the product of enzyme reaction lowers the enzyme activity. Enzyme molecules must be freed to combine with more substrate molecules. Normally the product are quickly removed from the site of formation and the reaction does not suffer.

(4) **Hydrogen ion concentration (pH) :** Some enzyme act best in an acid medium, other in an alkline medium, for every enzyme there is an optimum pH where its action is maximum e.g. 2 for pepsin, 6.8 for salivary amylase, 8.5 for trypsin. Most enzyme show maximum activity in a pH range of about 6.0 to 7.5 i.e.,near neutral pH (endoenzymes). A shift to the alkaline or acid side rapidly decreases the enzyme activity and finally stops it altogether. This is due to denaturation of enzyme molecule i.e. change in its physical structure. The H^+ ions combines with negatively charged R groups on the enzyme. This electrically neutralizes the R groups and distrupt ionic bonds in the enzyme's folding pattern, thus changing its shape.

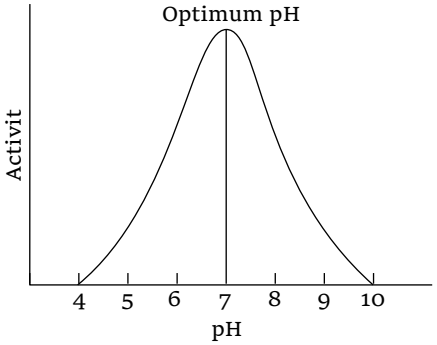


Fig : Activity/pH curve for

(5) **Temperature :** Within certain limits (5-40°*C*) the rate of an enzyme catalysed reaction increases as the temperature increases. The Q_{10} of most enzymatic reactions is 2, i.e., every 10°*C* rise in temperature doubles the rate of reaction. Most enzymes show maximum activity in a temperature range of 25 to 40°*C*. Beyond this temperature, there is sharp fall in the rate of reaction.

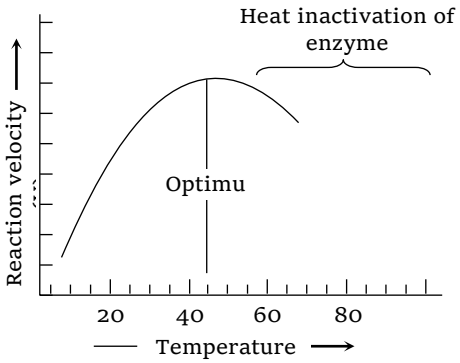


Fig : Effect of temperature on the velocity of enzyme action

Rise in temperature increases the kinetic energy of the molecules. Therefore, at higher temperature an increasing number of molecules have the required activation energy and can take part in chemical reactions. At higher temperatures, the kinetic activity of molecules in an enzyme becomes strong enough to break the weak hydrogen bonds that maintain the tertiary structure of the enzyme. Modification in the physical form of the enzyme results in the loss of its catalytic activity. This change in structure is called **denaturation** of protein. This is the permanent change, and the denatured enzyme protein remains inactive even if the temperature is then brought down. The enzymes are not destroyed by freezing, and regain their lost activity if the temperature is raised to normal.

Dry seeds can tolerate higher temperatures as compared to germinating seeds because dry seeds have dehydrated enzymes.

Deep freezing of food for preserving them for long periods is done not only to prevent the growth and multiplication of microorganisms but also to inactivate enzymes. It makes impossible for the microorganisms to digest the food.

(6) **Enzyme inhibitors :** Certain chemical compounds inhibit activity of enzyme molecules either permanently or temporarily. Thus, di-isopropyl flurophosphate (DFP) inhibits the action of various enzymes catalysing hydrolysis of ester linkage. Inhibition is permanent or irreversible. On the other hand, some antibacterial drugs and poisons do not cause permanent damage to the functional groups of the enzyme and therefore, if these (inhibitiors) are removed, the enzyme becomes fully functional.

(7) **Poisons and radiation :** Poisons such as cyanide and radiation destroy the tertiary structure of the enzymes, making them ineffective.

Important Tips

- Enzyme is called 'biological middle man'.
- Most of the vitamins of B complex group act as coenzyme.
- Smallest enzyme is peroxidase and largest being catalase found in perxisome.
- Myosin a structural component of muscle. It has ATPase activity also.
- 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.
- Enzymes show reversible reactions and act by lowering energy of activation by more than 50%. They work in milliseconds and rate of enzyme to substrate is as high as 1:1000000.
- Synthesis of enzymes occur in polysome (aggregation of ribosomes).
- The structure of allosteric enzyme was studied by Monod et al (1965).
- Competitive inhibitor increase Michaelis constant (K_m) but it has no effect on $V_{max.}$
- Deficiency of an enzyme is called enzymophenia.
- cAMP mediated cascade model of enzyme regulation was proposed by **Sutherland.**
- All coenzymes are cofactors but all cofactors are not coenzymes.
- Allozyme are similar enzymes formed by different genes.
- Non competitive inhibitors decrease the V_{max} of enzyme but they have no effect on K_m .
- Peptidases enzyme digests other enzymes.
- Viruses completely lack enzymes.
- Tertiory structure of protein component of enzyme is destroyed by a number of factors like heat, high energy radiation and salts of heavy metals (e.g. Ag^+, Hg^{2+}, As^+.)
- Markers are used for identification, Mitochondrial markers include succinate dehydrogenase, glutamate dehydrogenase and cytochrome oxidase. Glucose 6-phosphate is marker of E.R., RNA for ribosome, acid phosphatase for lysozyme, etc.
- Regulators of metabolism are enzymes, vitamins and hormones.
- RNA polymerase enzyme form RNA from DNA and DNA polymerase is responsible for synthesis of DNA from DNA.
- Enzyme that catalyses the conversion of soluble proteins into insoluble ones, process is called enzyme coagulation.
- PEP carboxylase catalyses the C_4 cycle of photosynthesis.
- Fritz Lipmann discovered coenzymes.
- It is more difficult to denaturate the enzyme substrate complex than isolated enzyme.
- Albinism is caused by the deficiency of tyrosinase.
- Iron porphyrin coenzyme or cofactor is cytochrome.
- Nitrogenase enzyme is inactivated by oxygen.
- Nitrogenase enzyme is responsible for the reduction of molecular nitrogen to the level of ammonia in legumenous root nodule.
- Nitrate reductase enzyme is responsible for the formation of NO_2.
- Amylopsin acts upon polysaccharide in alkaline medium.
- Due to enzymatic transformations huge amount of starch is deposited in potato tubers.

ASSIGNMENT

GENERAL INTRODUCTION AND PROPERTIES OF ENZYMES

Basic Level

1. Who first used the term "Enzyme"

(a) J.B. Sumner (b) Kuhne (c) Thompson (d) Garnier

2. Biological catalysts are called

(a) Auxins (b) Gibberellins (c) Enzymes (d) All the above

3. Who was able to crystallise *urease* enzyme into pure crystalline protein form

(a) J.B. Sumner (b) Garnier (c) Thomas (d) Wilkins

4. The protein part of enzyme is known as

(a) Holoenzyme (b) Apoenzyme (c) Isoenzyme (d) All of the above

5. An average cell contains about

(a) 1000 enzymes (b) 2000 enzymes

(c) 3000 enzymes (d) More than 5000 enzymes

6. Which one of the following enzyme is composed of simple protein

(a) Peroxidase (b) Phosphoglyceromutase (c)Proteinase (d) Amylase

7. Which one of the following enzyme is not composed of simple protein

(a) Amylase (b) Pepsin (c) Urease (d) None of the above

8. Molecular weight of enzyme is

(a) Less than 5000 (b) 5000 to 10000 (c) 10000 to 20000 (d) More than 40000

9. Enterokinase converts

(a) Trypsinogen to trypsin (b) Pepsinogen to pepsin

(c)Both (a) and(b) are correct (d) None of these

10. Most of the biochemical reactions differ from those occurring in the non living world in

(a) Requiring energy (b) Releasing energy (c) Being enzymatic (d) Being spontaneous

11. Enzymes are useful of plants because

(a) They are building block of chlorophyll

(b) They are essential for the metabolic processes

(c) They enhance absorption of water and salts

(d) They are responsible for paratonic movements

12. Non-competitive inhibitors alter

(a) Structure of enzyme (b)Structure of active site

(c)Velocity of enzymatic reaction (d)All the above

13. Enzyme inhibition caused by a substrate analog is

(a) Competitive (b) None-competitive (c) In-competitive (d) Semi-competitive

14. Competitive inhibition could be reversed by increasing the concentration of
(a) Substrate (b) Product (c) Enzyme (d) Coenzyme

15. Competitive inhibitors (like malonate) alter
(a) Velocity of reaction (b) Value of Km (Michelis constant)
(c) Active site (d) Structure of enzyme

16. Enzyme can be made functionless by
(a) Removing its product as fast as fast it is formed
(b)Doubling its concentration
(c) Decreasing its concentration (d)Blocking its active site

17. When coenzyme is combined with apoenzyme, it is called
(a) Cofactor (b) Holoenzyme
(c) Substrate enzyme complex (d) Vitamin A

18. 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

19. Blocking of the active site of an enzyme inhibition
(a) Non-competitive (b) Competitive (c) Allosteric (d) Feed back

20. Enzymes were discovered for the first time in
(a) Yeast (b) Maize (c) Bacteria (d) Algae

21. Enzymes, vitamins, hormones have one thing in common
(a) All are synthesized organisms (b)All are proteins
(c) All aid in regulating metabolism (d)All enhance oxidative metabolism

22. In the cell digestive enzymes are mostly in
(a) Lysosome (b) Cell wall (c) Ribosome (d) Chromosomes

23. In seeds the digestion is made possible at relatively low temperature by certain
(a) Proteins (b) Hormones
(c) Vitamins (d) Nitrogenous complex substances

24. Enzyme zymase converts
(a) Sugar into starch (b)Starch into sugar
(c) Fructose into glucose (d)Hexose into ethyl alcohol

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

26. Which of the following enzyme digests other enzymes
(a) Dehydrogenases (b) Lipases (c) Peptidases (d) Aldolases

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

28. Alloenzymes are

(a) Enzyme precursors

(b) Similar enzymes formed from different genes

(c) Different enzymes of an enzymes system (d)Antienzymes.

29. Lactic dehydrogenase (LDH) that takes part in catalysis of pyruvate lactate, is an example of

(a) Isoenzyme (b) Zymogen (c) Coenzyme (d) Apoenzyme

30. Spoilage of food material is prevented in cold storage due to

(a) Reduced respiration at low temperature

(b) Reduced enzyme activity in food articles

(c) Reduced enzyme activity in microbes as well as food articles (

d) Purified nature of air

31. Enzymes used in breaking DNA at specific sites are

(a) DNA-ases (b) Endonucleases

(c) Restriction endonucleases (d) Exonucleases

32. Enzymes exist in the cells as

(a) Solid (b) Crystals (c) Solution (d) Colloid

33. Nitrate reductase enzymes is responsible for the formation of

(a) N_2 (b) NO_2 (c) NO_3 (d) Ammonia

34. Which enzyme digests the stored food material of castor seeds

(a) Lipase (b) Amylase (c) Diastase (d) Protease

35. DNA polymerase enzyme is responsible for the Synthesis of

(a) DNA from RNA (b) DNA from DNA (c) RNA from DNA (d) RNA from DNA

36. Enzyme concerned with transfer of electrons are

(a) Hydrolase (b) Dehydrogenase (c) Transaminase (d) Desmolase

37. The enzyme which converts glucose into ethyl alcohol is

(a) Diastase (b) Maltase (c) Zymase (d) Invertase

38. The enzyme which converts proteins into peptones is

(a) Erypsin (b) Pepsin (c) Trypsin (d) Lipase

39. Which of the following is not a part of enzyme but is activates the enzyme

(a) *K* (b) *Zn* (c) *Mg* (d) *Mn*

40. The enzyme responsible for the reduction of molecular nitrogen to the level of ammonia in leguminous root nodule is

(a) Nitrogenase (b) NItrate reductase (c) Nitrite reductase (d) All the above

41. Which of the following is iron porphyrin coenzyme or cofactor

(a) Cytochrome (b) FAD (c) CoA (d) NAD

42. FAD or FMN is a coenzyme. Which vitamin is incorporated into its structure

(a) Vitamin *C* (b) Vitamin B_1

(c) Vitamin B_6 (d) Vitamin B_2 (Riboflavin)

43. Enzymes capable of changing their form are called

(a) Apoenzyme (b) Holoenzyme (c) Isoenzyme (d) Allosteric enzymes

44. Which one of the following enzyme contains *Mo* as prosthetic group

(a) Phosphatase (b) Dehydrogenase (c) Isomerase (d) Nitrate reductase

45. Which one of the following enzyme contains *Mn* metallic ion as the prosthetic group

(a) Phosphatase (b) Dehydrogenase (c) Pepsidase (d) Catalase

46. Which one of the following metallic ion does not occur as prosthetic group in any enzyme

(a) *Mg* (b) *Cu* (c) *Zn* (d) *Ag*

47. Nickel is the component of enzyme

(a) Nitrogenase (b) Nitrate reductase (c) Urease (d) None of these

48. Which mineral element is essential for the activity of aconitase enzyme

(a) Magnesium (b) Manganese (c) Calcium (d) Iron

49. Which mineral element is essential for the activity of enolase enzyme

(a) Copper (b) Cobalt (c) Zinc (d) Boron

50. Synthesis of enzymes take place by

(a) Transamination (b) Deamination (c) Translation (d) None of the above

51. Who got the Nobel prize working on enzymes in the year 1978

(a) W. Arber and D. Nathans (b) Nass and Nass

(c) R. Misra (d) H.G. Khorana

52. During glycolysis enzyme hexokinase changes glucose to glucose–6-phosphate. Glucose-6-phosphate is inhibited by

(a) Feedback inhibition (b) Positive feedback

(c) Competitive inhibition (d) Non-competitive inhibition

53. Which of the following is a loosely bound coenzyme

(a) *Cu* (b) *Mn* (c) *Zn* (d) Vitamin B_{12}

54. Which of the following coenzyme is a derivative of pantothenic acid (Vitamin B-complex)

(a) *NAD* (b) *NADP* (c) *FAD* (d) *CoA*

55. Which of the following is not an attribute of enzymes

(a) They are proteinaceous in nature

(b) They speed up the rate of biochemical reactions

(c) They are specific in nature (d) They are used up in reactions

56. At the time of cotton seeds germination, the stored food is digested by

(a) Diastase (b) Maltase (c) Lipase (d) Amylase

57. Enzyme are basically **or** All enzymes contain

(a) Sugars (b) Proteins (c) Fats (d) Vitamins

58. The enzyme *lipase* converts

(a) Proteins into amino acids (b) Proteins into peptones

(c) Peptones into amino acids (d) Fats into fatty acids and glycerol

59. Template/ lock and key theory of enzyme action is supported by

(a) Enzymes speed up reaction

(b) Enzymes occur in living beings and speed up certain reactions

(c) Enzymes determine the direction of reaction

(d) Compounds similar to substrate inhibit enzyme activity

60. In certain metabolic pathways, a number of enzymes are required. These multienzyme complexes occur enclosed in

(a) Membrane (b) Area with in ATP (c) Microbodies (d) Endoplasmic reticulum.

61. Inorganic cofactor is often called

(a) Coenzyme (b) Prosthetic group (c) Modulator (d) Activator

62. Active site of an enzyme is formed of

(a) Amino groups of some amino acids (b) Carboxyl groups of some amino acids

(c) -HS bonds of amino acids (d) R-groups of selected amino acids.

63. A protein having both structural and enzymatic traits is

(a) Myosin (b) Collagen (c) Trypsin (d) Actin

64. The prosthetic group (metallic cofactor) of various respiratory enzymes is

(a) *Ca* (b) *Fe* (c) *Mg* (d) *Mo*

65. A dialysable non-protein organic substance which combines with apoenzyme to make a functional enzyme is

(a) Hormone (b) Coenzyme or vitamin (c) Proenzyme (d) Prosthetic group

66. Who discovered 'co-enzymes'

(a) James Sumner (b) Fritz Lipmann (c) Mayerhoff (d) Edward Buchner

67. Amylopsin acts upon

(a) Polysaccharide in any medium (b) Polysaccharide in acidic medium

(c) Polysaccharide in alkaline medium (d) Polysaccharide in neutral medium

68. The enzyme which catalyzes the photosynthetic C_4 cycle is

(a) RuDP carboxylase (b) PEP carboxylase (c) Carbonic anhydrase (d) None of these

69. Enzymes are absent in

(a) Algae (b) Fungi (c) Bacteria (d) Virus

70. Which of the following is called "*biological middle man"*

(a) Hormone (b) Vitamin (c) Enzyme (d) All the above

71. Cytochrome oxidase enzyme contains

(a) Magnesium (b) Manganese (c) Iron (d) Cobalt

72. Metabolic poisons which alter the structure of an apoenzyme are
(a) Competitive inhibitors (b)Substrate analogs
(c) Product inhibitors (d)Non-competitive inhibitors

73. Synthesis of enzymes occurs in
(a) Lysosome (b) Nucleolus (c) Polysome (d) Spherosome

74. cAMP mediated 'Cascade model' of enzyme regulation was proposed by
(a) Fischer (b) Sutherland (c) Sumner (d) Koshland

75. Which of the following enzyme can from RNA from DNA
(a) Restriction enzyme (b) DNA polymerase
(c) RNA polymerase (d) Reverse transcriptase

76. Co-enzyme is
(a) Always a protein (b) Often a vitamin
(c) Always an inorganic compound (d) Often a metal

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

78. "*Enzymes are proteins".* it was suggested by
(a) Miller (b) Sumner (c) Pasteur (d) Leeuwenhock

79. NADP is
(a) A coenzyme (b) A part of tRNA (c) An enzyme (d) A part of rRNA

80. Enzymes as they exist inside the cell are
(a) In solid form (b) In crystalline form (c) In colloidal form (d) In solution form

81. In seeds, digestion is made possible at relatively low temperature by
(a) Proteins (b) Enzymes
(c) Auxins (d) Nitrogenous complex substances

82. Match List I and List II and select the correct answer using the code given below in the lists :

List I (Enzyme)	**List II (Enzyme function)**
(1) Ligase	Joins short of segments of DNA together
(2)DNA polymerase	Cuts DNA at specific DNA sequence
(3) Helicase	Breaks the hydrogen bonds between complementary pairs during DNA replication

Code:
(a) 1,2 and 3 are correct (b) 1 and 2 are correct,3 is false
(c) 1 is correct, 2 and 3 are false (d) 1 and 3 are correct ,2 is false

83. Which of the following is a correct sequence for the appearance of compounds in a cell

(a) Amino acids → protein → enzyme (b) Protein→ enzyme→ amino acids

(c) Disaccharides→ monosacchrides→ polysaccharides

(d) Monosaccharides→ starch→ sucrose

84. Example of *amide* enzyme is

(a) Arginase (b) Lactase (c) Zymase (d) Lipase

85. Who demonstrated that alcoholic fermentation was an enzymatic process

(a) Louis Pasteur (b) Justus Liebeg (c) Edward Buchner (d) James Sumner

86. On the total enzymes present in the cell approximately how many occur in mitochondrion

(a) 10% (b) 30% (c) 50% (d) 70%

87. Cofactor for alcohol dehydrogenase is

(a) K^+ (b) Fe^{++} (c) Zn^{++} (d) Na^{++}

88. The nucleic acids are broken into nucleotides by.....enzymes

(a) Amylases (b) Nucleases (c) Lipases (d) Proteases

89. To explain the mechanism of enzymatic action who proposed "*Lock and key hypothesis"*

(a) Fischer (b) Jacob (c) Koshland (d) Sumner

90. Which of the following is the best evidence for the template theory of enzyme action

(a) Compounds similar in structure to the substrate inhibit the reaction

(b) Enzymes speed up reactions by definite amounts

(c) Enzymes are founds in living organisms and increase the rate of certain reactions

(d) Enzymes determine the direction of a reaction

91. An enzyme acts by

(a) Reducing the energy of activation (b) Increasing the energy of activation

(c) Decreasing the pH (d) Increasing the pH

92. Who proposed the principal of "*induced fit"*

(a) Jacob (b) Fischer (c) Koshland (d) Laderberg

93. The most important property of an enzyme is its

(a) Composition (b) Thermal denaturation (c) Specificity (d) Solubility

94. An enzyme can be synthesized by chemically bonding together molecules of

(a) Carbohydrates (b) Amino acids (c) Lipases (d) CO_2

95. A coenzyme is

(a) Same enzyme that occurs in different tissues such as heart and muscle

(b) One that shares the function of other enzyme

(c) Organic or inorganic in nature and helps activate metabolic enzymes

(d) Organic non protein in nature and helps to activate metabolic enzymes

96. During enzyme activity, the coenzyme acts as
(a) Activator of oxidation reduction reactions
(b) A donor or acceptor of atoms which are added to or removed from the substrates
(c) Both (a) and (b)
(d) None of the above

97. Amount of enzyme transforming one mole, of substrate per minute at 25^0 C under optimal condition of measurement is called
(a) Enzyme purity (b) Specific activity
(c) Unit of enzyme activity (d) Catalytic centre activity

98. The enzyme which catalyzes the photosynthetic C_4 cycle is
(a) RuDP carboxylase (b) PEP carboxylase (c) Carbonic anhydrase (d) None of these

99. Rice or bread taste sweet on prologed chewing because of the breakdown of starch in them. The enzyme in the saliva which takes part in this reaction is
(a) Pepsin (b) Renin (c) Amylase (d) Invertase

100. The best example of extracellular enzymes (exoenzyme) is
(a) Succinic dehydrogenase (b) Digestive enzymes
(c) Nucleases (d) Rubisco

101. The conversion of insoluble and complex food substances into soluble and simpler ones by the action of enzymes is known as
(a) Translocation (b) Ingestion (c) Digestion (d) Assimilation

102. Which of the following is correct in an enzyme controlled reaction (E= enzyme, S= substrate, P= product)
(a) E+S=ES=E (b) E+S=ES=E+P (c) E+S=E+P (d) E+S=P=E+P

103. Why dry seeds can tolerate higher temperatures as compared to germinating seeds because dry seeds have
(a) Dormant embryo (b) Insulated seed coats
(c) Dehydrated enzymes (d) Temperature resistant food

104. The enzyme is said to be working at maximum efficiency
(a) When substrate coming in contact with active site are negligible
(b) When substrate concentration is increased to point of saturation
(c) When substrate concentration is low (d) None of the above

105. Cytochrome oxidase is a marker enzyme for
(a) Circular DNA of mitochondria (b) Outer membrane of mitochondria
(c) Inner membrane of mitochondria (d) Matrix of mitochondria

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

107. Enzymes which convert starch into maltose is
(a) Maltase (b) Diastase (c) Invertase (d) Hydrogenase

108. In plants enzymes are present in
(a) Only in leaves (b) Only in flowers
(c) Only in storage organs (d) All living cells of plant body

109. A high fever is dangerous to halman because
(a) BMR is lowered (b) Fats are oxidised
(c) Proteins are used up quickly (d) Enzymes are denatured

110. Vitamin B_1 is a constituent of a coenzyme-
(a) TPP (b) CoA (c) NAD (d) FMN

111. Which one of the following is a coenzyme
(a) Mg^{++} (b) A part of s-RNA (c) A part of t-RNA (d) FMN

112. Enzymes which act normally with in cells are called
(a) Apoenzymes (b) Exoenzymes (c) Endoenzymes (d) Ferments

113. Some respiratory enzymes are found in bacteria in
(a) Cell wall (b) Cell membrane (c) Mesosomes (d) Nucleoid

114. UbQ (ubiquinone) is
(a) Activator (b) Protein cofactor
(c) Non protein coenzyme (d) Protein coenzyme

115. Who prepared pure crystals of enzyme pepsin and trypsin-
(a) Northrop (b) Sumner (c) Pasteur (d) Buchner

116. Which one of the following enzymes is inactivated by oxygen-
(a) Dehydrogenase (b) Nitrogenase (c) Phosphate (d) Urease

117. Ribozyme is
(a) RNA without sugar (b) RNA without phosphate
(c) RNA having enzyme activity (d) RNA with extra phosphate

118. No cell could live without
(a) Phytochrome (b) Enzymes (c) Chloroplasts (d) Proteins

Advance Level

119. Which level of protein structure is affected by DNA
(a) Primary structure (b) Secondary structure (c) Tertiary structure (d) Quaternary structure

120. Most enzymes involved in respiration and photosynthesis are made up of carbon, hydrogen and the following
(a) O_2, *N* and *S* (b) *P* and *S* (c) *Fe* and *Mn* (d) *Cu* and *Mo*

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

122. An enzyme extract when subjected to electric field separates into two fractions, each catalysing the same reaction. The fractions are
(a) Coenzyme (b) Allosteric enzyme (c) Isoenzyme (d) Inducible enzyme

123. The ratio of the enzyme to substrate molecule can be as high as
(a) 1:1000 (b) 1:1,00,000 (c) 1:10,00,000 (d) 1:50,000

124. Inhibitory effect of melonic acid on succinic dehydrogenase enzyme is
(a) Competitive inhibition (b) Non-competitive inhibition
(c) Feedback inhibition (d) Inhibition due to end product

125. Huge amount of starch is deposited in potato tubers due to

(a) Chemical condensation of sugars (b)Enzymatic transformations

(c) Synthesis of starch in tubers (d)Absorption of nutrients

126. Enzymes which are slightly different in molecular structure but can perform identical activity are called

(a) Isoenzymes (b) Holoenzymes (c) Apoenzymes (d) Coenzymes

127. The plant proteinases or endopeptidases enzyme is

(a) Urease (b) Papain (c) Pepsin (d) Trypsin

128. Which one of the following is a coenzyme

(a) Nicotinamide (b) Riboflavin (c) Pantothenic acid (d) All the above

129. Isoenzymes are structural variants of same enzyme LDH (Lactic dehydrogenase) has 5 isomers in liver, α-(alpha) aylase in wheat endosperm has how many isoenzymes

(a) 4 (b) 5 (c) 16 (d) None of these

130. Name of the enzyme that acts both as corboxylase at one time and oxygenase at another time

(a) Carbonic anhydrase (b) PEP carboxylase (c) RUBP carboxylase (d) None of these

131. All enzyme are not proteins which of the following enzyme is not protein-

(a) RNase-P(Ribonuclease) discovered by Alman 1983

(b) RNA enzyme (Ribozyme) discovered by Cech (1981)

(c) Both correct (d) DNA/RNA polymerase

132. Carbonic anhydrase enzyme has maximum turn over number (36 million) minimum turn over number for enzyme is 30 per minute. Which is that enzyme

(a) Penicillase (b) Lysozyme (c) DNA polymerase (d) Lactic dehydrogenase

133. Modulators are substances which are

(a) Inhibitors(-) of enzyme activity (b) Activators (+)of enzyme activity

(c) Both (a) and (b) (d) Coenzymes

134. The release of adenyl cyclase from the cell membrane changes

(a) ATP into ADP (b) ADP into ATP (c) cAMP into ATP (d) ATP into cAMP

135. Which one value is required for enzyme action

(a) High Ki (b) Low Ki (c) Low Km (d) High Km

136. Albinism is a congentital disorder resulting from the lack of the enzyme

(a) Catalase (b) Fructokinase (c) Tryosinase (d) Xenthine oxidise

CLASSIFICATION AND FACTORS AFFECTING ENZYMES

Basic Level

137. Basically how many types of enzymes have been recognised by international Union of Biochemistry 1961

(a) 4 (b) 5 (c) 6 (d) 8

138. In the modern system of nomenclature which one of the following enzyme occupies Ist position

(a) Oxidoreductase (b) Transferase (c) Hydrolase (d) Ligase

139. In the modern system of nomenclature which one of the following enzyme occupies 6th position

(a) Ligase (b) Isomerase (c) Lyase (d) Hydrolase

140. In the modern system of enzyme nomenclature each class is divided into

(a) 2 to 4 sub classes (b) 4 to 13 sub classes (c) 6 to 15 sub classes (d) 8 to 20 sub classes

141. Term-*ase* is used for

(a) Classification of enzymes (b) Enzymes with special function (c) Nomenclature of enzymes (d) All the above

142. Systematic approach of naming enzymes has been recommended by the Commission on Enzymes of the

(a) International Union of Physiology (b) International Union of Biochemistry

(c) International Union of Biotechnology (d) International Union of Genetic Engineering

143. Hydrogen is removed from a substrate with the help of enzyme

(a) Lipase (b) Decarboxylase (c) Protease (d) Dehydrogenase

144. Epimerase belongs to the class of enzymes

(a) Hydrolases (b) Ligases (c) Isomerases (d) Oxidoreductases.

145. Enzymes catalysing bonding of two components with the help of ATP are

(a) Transferases (b) Ligases (c) Lyases (d) Phosphorylases

146. Most of the digestive enzymes belong to the class of

(a) Lyases (b) Hydrolases (c) Oxidoreductases (d) Transferases.

147. An enzyme that catalyses the rearrangement of molecular structure and forms a compound of same molecular weight is called

(a) Hydrolase (b) Isomerase (c) Oxidoreductase (d) Ligase

148. As temperature changes from $3^{o}C$ to $45^{0}C$ the rate of enzyme activity will

(a) Decrease and then increase (b) Increase and then decrease

(c) Increase only (d) Decrease only

149. Dry seeds can endure higher temperature that the germinating seeds because

(a) Dry seeds have more reserve food

(b) Hydration makes the enzymes more sensitive to temperature

(c) Dry seeds are hard (d) The seedlings are tender

150. Enzymes generally have

(a) Same pH and temperature optima (b) Same pH but different temperature optima

(c) Different pH but same temperature optima

(d) Different pH and different temperature optima

151. In most plant cells most enzyme have their optimum activity at

(a) 20°*C* (b) 25°*C* (c) 30°*C* (d) 35°*C*

152. At boiling temperature an enzyme is

(a) Denatured (b) Inactivated (c) Killed (d) Unaffected

153. Enzymes are sensitive to

(a) Cold (b) Cell wall (c) Heat (d) Pressure

154. At temperature just below the freezing point, the enzyme

(a) Is slightly activated (b) Is destroyed (c) Is inactivated (d) Is uneffected

155. Which ions are toxic in enzymatic activity

(a) Mn^{++} (b) K^{+} (c) Na^{++} (d) Hg^{++}

156. Which one of the following element inhibits the activity of sulphydryl enzyme

(a) *Fe* (b) *Cu* (c) *Zn* (d) *Pb*

157. Enzymes have a very narrow optima for

(a) Light (b) Temperature (c) *pH* (d) Humidity

158. Turn over number of enzyme depends upon

(a) Size of enzyme molecule (b) Molecular weight of enzyme

(c) Active sites of enzyme molecule (d) Concentration of substrate molecule

159. How is the rate of enzyme catalysed reactions affected by every 10^0 C rise of temperature-

(a) Halves (b) Becomes four times (c) Doubles (d) Remains unchanged

160. Km value of enzyme is the substrate concentration-

(a) ½ Vmax (b) 2 Vmax (c) 4 Vmax (d) 1/4 Vmax

161. The substrate combines more rapidly with enzyme when

(a) K_m is high (b) K_m is low (c) K_i is high (d) K_i is low

162. K_m value is related to

(a) Chromatography (b) ES complex (c) ABO complex (d) Morphometry

163. Optimum pH for enzyme trypsin is

(a) 5.9 (b) 4.6 (c) 8.5 (d) 7

164. Endoenzymes generally act at

(a) Acidic pH (b) Alkaline pH (c) Neutral pH (d) Any pH

165. Nomenclature of enzyme consists of

(a) First product name and then reaction name (b) First reaction name and then product name

(c) Only product name (d) Only reaction name

Advance Level

166. Enzyme *phosphoglucomutase* belongs to

(a) Oxidase (b) Lipase (c) Carboxylase (d) Isomerase

167. Which one of the following belongs to *transferase group*

(a) Amylase (b) Transaminase (c) Citrate synthetase (d) Enolase

168. *Fumerase* enzyme belongs to which class

(a) Oxidase (b) Carboxylase (c) Transferase (d) Lyases

169. Which one belongs to hydrolase group

(a) Amylase (b) Transaminase (c) Citrate synthetase (d) Enolase

170. Esterase enzyme belongs to which of the following class

(a) Oxidoreductase (b) Carboxylase (c) Hydrolases (d) Transferases

171. Acetyl CoA enzyme belongs to

(a) Synthetase (b) Isomerase (c) Desmolase (d) Hydrolase

172. Enzyme aldolase which helps in combining dihydroxy acetone phosphate with glyceraldehyde phosphate belongs to the category of

(a) Ligases (b) Hydrolases (c) Transferases (d) Lyases.

173. Enzyme taking part in converting dihydroxyacetone phosphate to glyceraldehyde phosphate belongs to the class of

(a) Isomerases (b) Hydrolases (c) Ligases (d) Transferases.

174. Which enzyme acts efficiently at pH 2

(a) Salivary amylase (b) Pepsin (c) Trypsin (d) Lipase

175. The enzyme code of enzyme 2.7.1.1 refers to the following main groups

(a) Ligase (b) Hydrolase (c) Transferase (d) Lyase

176. Ligases are involved in the synthesis of

(a) C-O bonds (b) C-N bonds (c) C-C bonds (d) All the above

177. An enzymes can accelerate a reaction on up to

(a) 10 times (b) 10^{10} times (c) 10^{1} times (d) 10^{100}times

ANSWER

ASSIGNMENT (BASIC & ADVANCE LEVEL)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
b	c	a	b	c	c	d	d	a	c	b	d	a	a	b	d	b	c	b	a
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
c	a	a	d	b	c	b	b	a	c	c	d	b	a	b	b	c	b	a	a
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
a	d	d	d	c	d	d	d	d	c	a	a	d	d	d	d	b	d	d	a
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
d	d	a	c	d	b	c	b	d	c	c	d	c	b	c	b	c	b	a	c
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
b	d	a	a	c	d	c	b	a	a	a	c	c	b	d	c	c	b	c	b
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
c	b	c	b	c	c	b	d	d	a	d	c	b	c	a	b	c	d	a	a
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140
c	c	c	a	b	a	b	d	c	c	c	b	a	d	b	c	c	a	a	b
141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
c	b	d	c	b	b	b	b	b	c	b	a	c	c	d	d	c	c	c	a
161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177			
b	b	c	c	a	d	b	d	a	c	a	d	a	b	c	d	b			

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