MCBA. Sem2. CC3. Unit 5

ENZYME:

Characteristics of enzyme:

1. Enzymes are proteins specialized to catalyze biological reactions. They are among the most remarkable biomolecules known because of their extraordinary specificity and catalytic power, which are far greater than those of man made catalysts.
2. Enzymes are protein in nature, only exception is ribozyme which is nothing but a RNA molecule involved in RNA splicing. Although enzymes catalyze biochemical reactions, they can do so only in association with a non protein molecule called **cofactor**. Cofactor may be metal ions or organic molecules derived from vitamin (coenzyme). A coenzyme or metal ion that is tightly or even covalently bound to the enzyme protein is called **prosthetic group**. A complete catalytically active enzyme together with its bound coenzyme and/or metal ion is called **holoenzyme.** The protein part of such enzyme is called **apoenzyme** or apoprotein. Coenzymes are usually derived from vitamin. Coenzymes are usually function as intermediate carriers of functional groups, or specific atoms, or of electrons that are transferred in the overall enzymatic reaction. Coenzymes undergo alterations during the enzymatic reactions, which are later regenerated.

Vitamin	Coenzyme form	Function
Thiamin(B1)	Thiamine pyrophosphate(TPP)	Decarboxylation
Riboflavin(B2)	Flavin adenine dinucleotide(FAD)	Hydrogen atom/electron transfer
Niacin or nicotinic acid	Nicotinamide adenine dinucleotide(NAD+)	Hydrogen atom/electron transfer
Pantothenic acid	Coenzyme A	Acyl group transfer
Pyridoxin(B6)	Pyridoxal phosphate	Amino group transfer
Biotin	Biocytin	Carboxyl group transfer

Some enzymes require metal ions as cofactor. In such enzymes, the metal ions may serve as – i) the primary catalytic centre or ii) a bridging group to bind substrate and the enzyme together through the formation of coordination complex or iii) an agent stabilizing the conformation of the enzyme protein in its catalytically active form. Example – metal ions serve as cofactor – Mg^{2+} , Mn^{2+} , Zn^{2+} ,$Fe^{2+/3+}$,$Cu^{2+/1+}$, K^{+} .

3. A small portion of the enzyme molecule is directly involved in the substrate binding and catalysis. The **active site** of an enzyme represents as the small region at which the substrate binds and participates in the catalysis. The active site is made up of amino acids which are far from each other in the linear sequence of amino acids. Active sites are regarded as clefts or pockets and are not rigid in structure and shape, rather flexible to promote specific binding. The substrate binds at the active site by weak non covalent

bonds or interaction to form enzyme – substrate complex. The product is released after catalysis and the enzyme is available for reuse.

4. Catalysis is the prime function of enzymes. The nature of catalysis taking place in the biological system is similar to that of non biological catalysis. For any chemical reaction to occur, the reactants have to be in activated state or transition state. The energy required by the reactants to reach the activated state or transition state is known as **activation energy**. The reactants when heated attain the activation energy. The catalyst (or the enzyme in the biological system) reduces the activation energy and this cause the reaction to proceed at a lower temperature. The enzyme lowers the energy barrier of reaction thus making the reaction to go faster. Enzymes do not alter the equilibrium, they only enhance the velocity of the reaction.
5. The prime requisite for enzyme catalysis is that the substrate (S) must combine with the enzyme (E) at the active site to form enzyme-substrate complex (ES) which ultimately results in to the product (P) formation.

   $E + S \Leftrightarrow ES \Longrightarrow E + P$

   A few theories have been put forth to explain mechanism of enzyme – substrate complex formation.

   a) Lock and key model or Lock and key hypothesis: This hypothesis was proposed by Emil Fisher. According to this model, the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. Like a key into a lock, only the correct size and shape of the substrate (the key) would fit into the active site (the key hole) of the enzyme (the lock).

   b) According to the induced fit model, suggested by Daniel Koshland , the active site continues to change until the substrate is completely bound to the active site of the enzyme.

      Unlike lock and key model, the induced fit model shows that enzymes are rather flexible structure in which the active site continuously reshapes by its interaction with the substrate until the substrate is completely bound.
6. Enzymes remain same before and after the reactions they catalyze and are required in very small amount.
7. As enzymes are proteins in nature, their activity is dependent on pH and temperature of the reaction system.

   It is not surprising that pH will influence the activity of the enzyme. The active sites on enzymes are frequently composed of ionizable groups that must be in the proper ionic form in order to maintain the conformation of the active site, bind the substrate, or catalyze the reaction. Furthermore, one or more of the substrate themselves may contain ionizable groups and only one ionic form of that substrate may bind with the enzyme or undergo catalysis. For most of the enzyme, velocity vs pH graph shows a peak, indicating that at that particular pH, enzyme is maximum active. Such pH is called “optimum pH”. At optimum pH ,enzyme will achieve its biologically active form and catalyze the

reaction with maximum velocity. At very high and low pH, decline in enzymatic activity may be due to formation of improper ionic form of enzyme and/or substrate. For most of the enzyme, optimum pH lies in between 6.5-7.2. But there is exception. Optimum pH of pepsin, the digestive enzyme, is near 2. Whereas for alkaline phosphatase, it is around 9.

Most chemical reactions proceed at a faster velocity as the temperature is raised. An increase in temperature imparts more kinetic energy to the reactant molecules resulting in more productive collisions per unit time. Enzyme catalyzed reactions behave similarly, up to a point. Enzymes are complex protein molecules. The catalytic activity results from a precise, highly ordered tertiary structure that juxtaposes specific amino acid R groups in such a way, so as to form the stereo specific substrate binding sites and the catalytic centre. The tertiary structure of enzyme is maintained primarily by a large number of weak non covalent bonds/ interactions. If the molecule absorbs too much of energy, the tertiary structure will disrupt and the enzyme will be denatured, that is, lose catalytic activity. Thus, as the temperature increases, the expected increase in velocity resulting from increased enzyme and substrate collisions is offset by the increasing rate of denaturation. Consequently, a plot of velocity vs temperature usually shows a peak, referred as "optimum temperature", indicating that at a particular temperature, enzyme will be most active due to its biologically active structure. For most of the enzyme, optimum temperature is 37˚C or near to it. But there is also an exception, Taq polymerase, used in PCR (polymerase chain reaction), has optimum temperature at 94 ˚C.

8. Enzyme activity can be regulated by covalent modification, feedback inhibition and allosteric interaction.

   In covalent modification, enzymes are interconverted between active and inactive form by covalent bond formation (phosphorylation / dephosphorylation) .

   In feedback inhibition, the end product of the reaction sequence can act as an inhibitor of the first enzyme (key enzyme) involved in the reaction sequence. This inhibition is mediated by allosteric interaction.

   In allosteric interaction, modulator molecules bind with enzyme reversibly and non covalently at the site other than the active site. Modulator molecule may be (+) allosteric effector that increases the enzyme activity or (-) allosteric effector that decrease the enzyme activity.

Classification of enzyme:

Traditionally enzymes often were named by adding the suffix **–ase** to the substrate upon which they acted. Ex: urease, phosphatase, arginase,protease. But confusion arose with the name – pepsin, trypsin, catalase etc.

So a new system of nomenclature of enzyme was developed by Enzyme Commission (EC) based on the nature of reaction it catalyzes. Each enzyme is assigned by recommended name (which is

usually short and appropriate for everyday use), Systematic name (which identifies the reaction it catalyzes), Classification no.(which is used when accurate identification of enzyme is required).

ATP + Creatine $\Longleftrightarrow$ ADP + Phosphocreatine
Recommended name: Creatine kinase,
Systematic name: ATP **:** Creatine phosphotransferase.
Classification no.: EC 2.7.3.2
EC stands for Enzyme commission
First digit (2) for the Class name (transferase)
Second digit (7) for the subclass(phosphotransferase)
Third digit (3) for the sub-subclass(phosphotransferase with a nitrogeneous group as acceptor)
Fourth digit(2) is the serial no. of the enzyme in the list.

EC no.	Enzyme name	Type of the reaction they catalyzed	Examples
1.	Oxidoreductase	Oxidation – reduction of the substrate	Dehydrogenase, oxidase, reductase, peroxidase, catalase, oxygenase, hydroxylase, oxidative deaminase etc.
2.	Transferase	Group transfer b/w two substrates	Transketolase, Transaldolase, kinase, amino-,acyl-,methyl-,glucosyl-(transferase) etc.
3.	Hydrolase	Hydrolytic cleavage of C-C, C-N, C-O and other bonds of the substrate	Peptidase, esterase, glycosidase, phosphatase, phospholipase, amidase, ribonuclease, thiolase, deaminase etc.
4.	Lyase	Addition of groups to double bonds or formation of double bonds by removal of group	Decarboxylase, aldolase, hydratase, synthase,lyase etc.
5.	Isomerase	Geometric or structural change with in molecule	Isomerase, epimerase, racemase, mutase.
6.	Ligase	Formation of C-C, C-S, C-O and C-N bonds by condensation reactions coupled with cleavage of high energy molecule(ATP/GTP)	Synthetase, Carboxylase etc

Velocity equation of enzyme catalyzed reaction (rapid equilibrium approach by Henri, Michaelis and Menten):

The simplest enzyme catalyzed reaction involves a single substrate(S) going to combine with an enzyme(E) to form an enzyme-substrate(ES) complex which breaks down into free enzyme and single product(P). This system is called unireactant system. The reaction system is

$$E+S \underset{k_{-1}}{\overset{k_1}{\Longleftrightarrow}} ES \underset{k_{-2}}{\overset{k_2}{\Longleftrightarrow}} E + P.$$

For simplicity, it is assumed that none of the product reverts back to initial substrate. This assumption is only valid if we concern ourselves with initial velocity(V_0) in the forward direction before significant concentration of product has accumulated. Thus the reaction under consideration can be written

$$E+S \underset{k_{-1}}{\overset{k_1}{\Longleftrightarrow}} ES \overset{k_p}{\Longrightarrow} E + P.$$

To simplify the matter, it is assumed that, an equilibrium condition arises very rapidly i.e, E,S and ES equilibriate very rapidly compared to the rate at which ES breaks down to E+P. The velocity at any time depends on the concentration of ES.

So, $V_0 = k_p [ES]$ ---------(1) where k_p is called catalytic rate constant and k_1 and k_{-1} are forward and backward rate constant. The total enzyme is distributed between E and ES. $[E]_t = [E] + [ES]$ -----------(2).

Dividing the equation (1) by equation (2), we obtain

$V_0 / [E]_t = k_p [ES]/\{[E] + [ES]\}$ --------(3)

Because of the equilibrium assumption, [ES] can be expressed in terms of [E], [S] and K_s, where K_s is dissociation constant of ES complex. $K_s = [E][S]/[ES] = k_{-1}/ k_1$. So $[ES] = [E][S]/ K_s$. Putting the value of [ES] in equation (3),

We obtain $V_0 / [E]_t = k_p \{[E][S]/ K_s\} / \{[E] + [E][S]/ K_s\}$

So we get $V_0 / k_p [E]_t = [S]/\{ K_s + [S]\}$ ------(4)

If $V_0 = k_p [ES]$, then $k_p [E]_t = Vmax$, the maximal velocity that would be observed when all the enzyme is present as ES.

Then equation (4) becomes $V_0 / Vmax = [S]/\{ K_s + [S]\}$.

But if the rate at which ES forming E+P is higher compared to the rate at which ES dissociates back to E+S, then E,S and ES will not be at equilibrium. For this reason, steady state approach is introduced by Briggs and Haldane. According to them, very shortly after mixing E and S, a steady state will be established in which the concentration of ES remains essentially constant with time. The velocity equation is very similar as described earlier.

$V_0 / [E]_t = k_p [ES]/\{[E] + [ES]\}$.

As the concentration of ES is constant, the rate of formation of ES is equal to rate of breakdown of ES. Rate of formation of ES = $k_1[E][S]$ and the rate of breakdown of ES = $k_{-1}[ES] + k_p[ES] = [ES](k_{-1} + k_p)$.

Thus at steady state, $k_1[E][S] = [ES](k_{-1} + k_p)$, so $[ES] = k_1[E][S]/ (k_{-1} + k_p)$. Then $[ES] = [E][S]/Km$ where $Km =(k_{-1} + k_p)/ k_1$ (Km is Michaelis constant).

Putting the value of [ES] in velocity equation,

$V_0 / [E]_t = k_p [E][S]/Km /\{[E] + [E][S]/Km \}$, $V_0 / k_p [E]_t = [S]/\{ Km + [S]\}$,

$V_0 / Vmax = [S]/\{ Km + [S]\}$.

Now when the velocity is exactly half of the maximum velocity i.e., $V_0 = Vmax/2$, then Km = [S]. Thus Michaelis constant is equal to substrate concentration when the reaction velocity is half maximal. Its unit is moles/lit and is independent of enzyme concentration.

Again we know that, $Km=(k_{-1} + k_p)/ k_1$. If the rate of decomposition of ES into E+P is much lower than that of E+S, i.e., $k_{-1} >> k_p$ then $Km = k_{-1} / k_1 = Ks$. So Km is equal to the dissociation constant of ES complex, when $k_{-1} >> k_p$. At this condition, Km is a measure of substrate binding affinity of the enzyme. A high Km value indicates weak binding and low Km value indicates strong binding of enzyme with the substrate.

Effect of [S] on the velocity of the reaction:

At low [S], rate of the reaction(V_0) is proportional to [S] and follow first order kinetics. As [S] increases, rate of reaction increases in lesser extent. With further increase in [S], the reaction rate become essentially independent of [S]and reaches a constant value Vmax. At that position the enzyme is said to be fully saturated with substrate and the reaction follows zero order kinetics. A plot of V_0 vs [S] shows a hyperbolic graph.

Michaelis Menten equation is $V_0 / Vmax = [S]/\{ Km + [S]\}$.

$V_0 = Vmax. [S]/\{ Km + [S]\}$,

$1/V_0 = \{ Km + [S]\}/Vmax. [S]$,

$1/V_0 = (Km/Vmax)1/[S] + 1/Vmax$ (this reaction is similar to y=mx+c, where $y=1/ V_0$, m= Km/Vmax, x=1/[S], c=1/Vmax)

Plotting $1/ V_0$ along Y axis and 1/[S] along X axis, we obtain a straight line that makes an intercept in Y axis. By extending the straight line beyond the intersect we obtain another intersect on X axis. Intersect on X axis represent 1/Km and intersect on Y axis represent 1/Vmax. This plot is known as Lineweaver Burk plot or double reciprocal plot as Intersect on X axis gives reciprocal of Km and intersect on Y axis gives reciprocal of Vmax.

Enzyme unit:

The enzyme unit or international unit for enzyme (IU) is a unit of enzyme's catalytic activity. 1 IU is defined as the amount of enzyme that catalyses the conversion of one micromole (µmole) of substrate into product per minute under specified condition of the assay method (25˚ C). The enzyme unit was adopted by the International Union of Biochemistry (IUB) in 1964. Enzyme unit can be expressed as Katal also. 1 Katal is the enzyme activity that converts one mole of substrate per second under specified assay condition.

So 1 IU = 1 µmole/min = 1/60 µmole/sec = 0.01667 µmole/sec = 16.67 nmole/sec = 16.67 nKatal.

Specific activity: It is defined as amount of enzyme unit present per mg of protein. It is a parameter of enzyme purity. Specific activity of an enzyme increases with sequential purification procedure.

Turnover Number: It is the number of substrate molecules converted into product per mole of enzyme molecule in unit time when the enzyme is fully saturated with the substrate.

Multienzyme complex: A multienzyme complex contains several copies of one or several enzymes packed into one assembly. Example – Pyruvate dehydrogenase $_{complex}$ that carries out the linking reaction between glycolysis and TCA, converting pyruvate into acetyl CoA.

It consists of three enzyme: i) Pyruvate dehydrogenase ii) Dihydrolipoyl transacetylase iii) Dihydrolipoyl dehydrogenase and

five coenzyme: i) TPP ii) Lipoic acid iii) CoASH iv) FAD v) NAD^+

Isozyme:

Isoenzymes or Isozymes are the group of enzymes those are very similar in their catalytic properties but they differ in their physical properties such as pI or electrophoretic mobility. They also differ in their quaternary structure and work in different tissues, i.e., Lactate dehydrogenase, which catalyzes the reaction, Lactate + $NAD^+ \Longleftrightarrow$ Pyruvate + NADH +H^+ occurs as five different isoenzymes forms. All the five isozymes are tetramer with same molecular weight about 140kd, and all contain four polypeptide chains, each of molecular weight 35kd. The five isozymes consist of five different combinations of two different polypeptide chains designated as M and H. The isoenzyme predominating in skeletal muscle has four identical M chains and is designated as M_4. Another, which predominates in heart, has four identical H chains and is designated H_4. The other three isozymes have the composition M_3H, M_2H_2, MH_3. Single M and H chains have been isolated and found to differ significantly in amino acid content and sequence. Each subunit coming from either of the polypeptide chain is catalytically inactive, but when combines with others of the same or different type, produce active tetrameric enzyme. Kinetic study of lactate dehydrogenase isozymes has revealed that although they all catalyze the same

reaction, their differ significantly in their Km values for their substrate, particularly for pyruvate, as well as their Vmax values. The isozyme M_4, predominating in skeletal muscle and embryonic tissue has relatively low Km value for pyruvate and relatively high rate at which it can reduce pyruvate. Whereas H_4 isozyme, predominating in heart muscle, has relatively high Km value for pyruvate and reduce it at relatively low rate. The other lactate dehydrogenase isozymes have kinetic properties intermediate between those M_4 and H_4 in proportion to their relative content of M and H chain.

Enzyme inhibition:

Enzyme inhibition is done by a substance which when binds with the enzyme, decreases its catalytic activity.

Inhibition is of three types: 1. Reversible, 2. Irreversible, 3.Allosteric.

In reversible inhibition, inhibitor binds with the enzyme noncovalently and the inhibition is reversed if the inhibitor is removed. Reversible inhibition is of three types: i) Competitive inhibition ii) Non competitive inhibition iii) Uncompetitive inhibition.

Competitive inhibition: There are some inhibitors which have structural similarities with that of the substrate. This type of inhibitors competes with the substrate for substrate binding site. Such inhibitors are known as Competitive inhibitors and inhibition is called Competitive inhibition. Addition of excess substrate can withdraw the inhibitor from the substrate binding site of the enzyme and thereby effect of inhibition can be withdrawn. Example: in bacteria, antibacterial sulfonamides act as competitive inhibitor of the enzyme dihydropteroate synthetase , an enzyme involved in folate biosynthesis. In human, malonate acts as competitive inhibitor of TCA enzyme succinate dehydrogenase. In presence of competitive inhibitor, Vmax value remains unchanged but Km is decreased by a factor.

In absence of inhibitor, velocity equation : $V_0 = Vmax. [S]/\{ Km + [S]\}$

In presence of competitive inhibitor: $V_0 = Vmax. [S]/\{ Km(1+ I/Ki) + [S]\}$

Noncompetitive inhibition: In this inhibition, inhibitor binds at a site other than the substrate binding site. This binding impairs the enzyme function. In this type of inhibition, inhibitor has no structural resemblance with the substrate. So inhibitor can bind with free enzyme as well as with enzyme-substrate complex. Example: heavy metals(Ag^+, Pb^{2+}, Hg^{2+}) can noncompetitively inhibit the enzyme by binding with cysteinyl sulfhydryl group. For noncompetitive inhibitor, Km remains unchanged but Vmax is lowered.

In absence of inhibitor, velocity equation : $V_0 = Vmax. [S]/\{ Km + [S]\}$

In presence of noncompetitive inhibitor: $V_0 = Vmax/(1+ I/Ki) . [S]/\{ Km+ [S]\}$