Plant Physiology and Biochemistry

Enzymology

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Contents:

Key words: Enzyme, proteinaeous, catalytic activity, regulation of enzyme activity, factors affecting enzyme activity.

ENZYMOLOGY

What are Enzymes?

Enzymes are biocatalyst. They speed up the rate of reactions taking place in the cell without themselves undergoing any permanent change. In the absence of enzymes most of the metabolic reactions taking place in the biological system would have taken very long time period to complete.

In an enzyme-catalyzed reaction, reactants are called *substrate* and each enzyme act on a specific substrate or substrates to produce particular product or products.

$$\text{Substrate (s)} + \text{Enzyme} \rightarrow \text{Product (s)} + \text{Enzyme}$$

Substrate binds to a particular site on the enzyme called *active site* where catalysis takes place (Fig.1). Metabolic reactions taking place in the cell are catalyzed by their own particular enzymes i.e. enzymes are specific in action. In a given cell there are large number of enzymes e.g. in *E.coli* about 1700 enzymes have been characterized [1]. In eukaryote this number may be many folds. Although it is difficult to estimate precise number of enzymes in a cell but clues are emerging from the genome analysis.

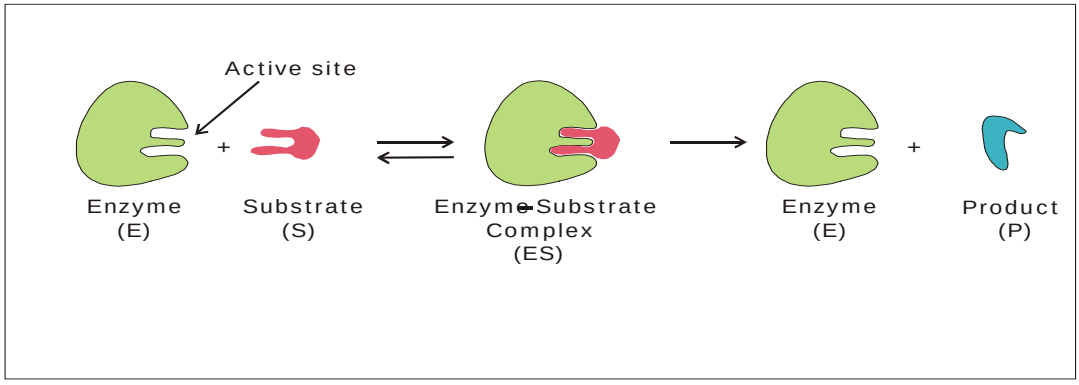

Figure: 1 Substrate (S) binds at the active site of enzyme (E) to form Enzyme-Substrate complex (ES), which breaks to form Product (P) and liberate the enzyme to complex with other substrate molecule.

Most of the enzymes are protein in nature with the exception of Ribozyme (RNA molecules), which can catalyze their own splicing of introns [2].

Concept of co-factor, holoenzyme, apoenzyme

Many enzymes require non- protein molecules for their activity. Such components are termed as *co-factors*. The co- factor may be inorganic or organic. The inorganic co-factor may comprise metal ions e.g. copper for *cytochrome C oxidase*, zinc for *carboxypeptidase*, nickel for *urease*, iron for *catalase* etc. and their binding to enzyme is essential for enzyme activity[1]. The organic co-factor comprises carbon containing organic molecules (mostly derivatives of vitamins) e.g. NAD^+ and $NADP^+$. They are generally regarded as *co-enzyme* since, they usually bind to the enzyme before the other substrate bound, participate in many reactions and may be recovered to their original form by many other enzymes. Co-factors undergo chemical transformation during reaction and may in some cases be covalently bound to the enzyme. When these co-factors are tightly bound to the enzyme they are termed as ***prosthetic group.*** A complete catalytically active enzyme together with bound co-factor and protein part is called as ***holoenzyme.*** When co-factor is removed from the enzyme, the remaining protein part of the enzyme is known as ***apoenzyme*** (Fig.2). Any small molecule or other species that can reversibly bind to an

enzyme is termed as ***ligand*** e.g. light dark regulated reversible binding of 2-carboxyarabinitol –1, phosphate (CA1P) to Ribulose 1,5,bisphosphate carboxylase/oxygenase (RUBISCO)[3].

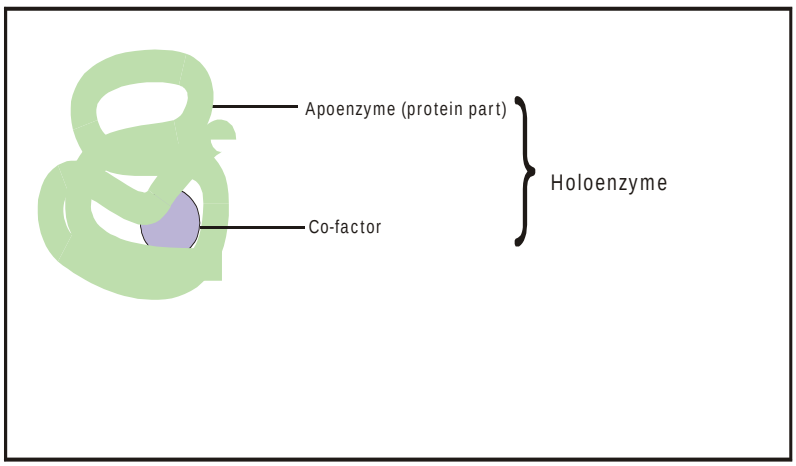

Figure:2 Some enzymes require non-protein molecules (co-factor) for their activity. Protein part of such enzymes is known as apoenzyme. Apoenzyme together with cofactor is called holoenzyme.

Discovery of Enzymes [1,4]

The practical use of catalytic activity of enzymes has been utilized by mankind for processing raw materials from plants and animals for a long time period. These early observations were merely based on observations and folklore rather than any systematic studies or appreciations of the chemical nature of the processes being used. Traditional processes such as production of alcoholic beverages and yeast-fermented doughs in baking bread are displayed in ancient wall paintings. Processes for preserving food or preserving milk by making cheeses as well practiced traditionally.

The first observation of enzymatic degradation reaction was in 1783 by Spallanzani (1729-1799) who observed the liquification of meat pieces by stomach juices of birds.

Kirchhoff (1815) observed the liquification of starch paste into sugars by barley extracts and he assumed gluten protein of barley to be responsible for this conversion. Subsequently, Payer and Persoz (1833) termed the working principle of this sachharification as ***diastase.*** The term diastase is still used in industry for *amylase.*

Pasture (1857) showed that fermentation is closely associated with live yeast. He distinguished between "organized ferments" (cellular) and "unorganized ferments" (soluble).

Kuhne (1878) used the term ***enzyme*** to distinguish this unorganized ferments. In Greek enzyme means '*in yeast'*. Concrete evidence for this assumption was provided by Buchner in 1897. He showed the production of alcohols from sugars using cell free extract of yeast.

Emil Fischer (1894) showed the specificity of enzyme for their substrate. He proposed 'Lock and key' model to explain the interaction between enzyme and substrate while performing experiments on carbohydrate metabolizing enzymes.

Up to this time not much was known about the chemical nature of enzyme. The fact that enzymes were made of protein was established many years later after the crystallization of many enzymes. James Sumner (1926) published the first crystallization of an enzyme '*urease*'and showed crystals were composed of proteins and their dissolution in solution led to enzymatic activity. After Sumner report of crystallization of urease, many enzymes were purified and crystallized.

During late 1950s the protein structure begun to appear by X-ray crystallography. Kedrew (1957) was first to deduce the three dimensional structure of a protein myoglobin through X-ray crystallography. Phillips (1965) gave the three dimensional structure of first enzyme *lysozyme* using X-ray crystallography. Today the details of

3-dimensional structure of many enzymes are known based on information gained from X-ray crystallography and other techniques.

In 1957 Sanger reported the first amino acid sequence of a protein, the hormone insulin and Smith et.al. (1963) reported the first amino acid sequence an enzyme *ribonuclease* and Gutte and Merrifield(1969) have chemically synthesized it for the first time from the amino acid precursors [5].

During late 1950s and 1960s number of observations were made that suggested enzyme show flexibility. Koshland (1958) proposed a "induced fit" hypothesis to account for enzyme activity and specificity [6].

Monod and colleagues[7] (1963) put forwarded an allosteric model to explain the regulation of enzyme by binding of small molecules which helped in understanding of regulation of many enzymes in cells.

Now, recombinant DNA technology has made it possible to alter the catalytic activity and specificity of an enzyme by introducing mutations at defined positions. This has helped in understanding the mechanism of enzyme action better and designing of novel enzymes with specific properties.

Nomenclature of Enzymes [8,9]

Initially whenever a new enzyme has been discovered and characterized it is given a name depending upon the substrate and the nature of the reaction it catalyzed. Enzymes were generally given names ending with suffix-ase added to the substrate on which they act. (e.g. *urease*, catalyzes the hydrolysis of urea; *lactase*, catalyze the hydrolysis of lactose) or indicate something about the reaction catalyzed (as in the case of lactate dehydrogenase, which catalyze the dehydrogenation of lactate to yield pyruvate). Some trivial names are different indicating nothing about the substrate or the reaction catalyzed by the enzyme (e.g. *trypsin, chymotrypsin, papain, catalase* etc.). Therefore to bring consistency in the nomenclature of enzymes, need for systematic way of naming and classifying enzymes was realized.

Present day accepted nomenclature of enzyme is the recommendations of "Enzyme Commission" set by International Union of Biochemistry and Molecular Biology in 1955.

Enzymes are classified into six major classes on the basis of the reaction catalyzed by them.

1. *Oxidoreductases* catalyze oxidation –reduction reactions.
2. *Transferases* catalyze group transfer reactions.
3. *Hydrolases* catalyze hydrolytic reactions.
4. *Lyases* catalyze elimination reactions in which a double bond is formed.
5. *Isomerases* catalyze isomerization reactions.
6. *Ligases* catalyze reactions in which two molecules are joined at the expense of an energy source.

Catalytic properties of enzymes [10,11]

Enzymes are very ***efficient***. They increase the rate of a reaction by as much as 10^{17} fold. A very high enhancement in rate of a reaction have been observed when enzyme catalyzed reactions are compared with non-enzyme catalyzed reactions under similar conditions. Table I shows the rate enhancements shown by some enzymes.

Urease	10^{14}
Carboxypeptidase A	10^{11}
Triosephosphate isomerase	10^{9}
Alcohol dehydrogenase	2×10^{8}
Phosphorylase	3×10^{11}

Table: I. Rate enhancement shown by some enzymes

Generally enzymes work at moderate temperature and pH values near to neutral. However in some extremophiles enzymes function in more extreme conditions of temperature and pH.

Specificity of enzymes[1,2]

Enzymes are *specific* in their action. The specificity of enzyme results from their three dimensional structure and shape of the active site and requires the interaction between substrate and enzyme at least at three different points in the active site. The range of specificity varies between enzymes. Some enzymes show *group specificity* i.e. they catalyze a reaction involving particular chemical group e.g. *alcohol dehydrogenase*, which will catalyze the oxidation of a variety of alcohols e.g. ethanol, acetaldehyde,lactic acid etc. and *hexokinase* which catalyzes the phosphorylation of hexose sugars such as D- hexose.

Other enzymes show *absolute specificity* i.e. they act on a particular substrate e.g. *urease* will catalyze the hydrolysis of urea.

Some enzymes show *bond specificity* i.e. these enzymes can act on different substrates containing a particular bond e.g. phosphatase act on substrates containing phosphate bond, peptidase act on peptide bonds, and esterase on ester bonds.

Another type of specificity shown by the enzyme is *sequence specificity* e.g. *restriction endonuclease* which recognizes particular sequences in DNA.

Another distinct feature of enzyme-catalyzed reaction is their stereo chemical specificity. If a substrate can exit in two stereo chemical forms, chemically identical but with a different arrangements of atoms in three dimensional space, then only one of the isomer will undergo catalysis by a particular enzyme e.g. NAD^+ and $NADP^+$ requiring dehydrogenase. Enzyme catalyzes the transfer of hydrogen from substrate to a particular side of the nicotinamide ring of NAD^+.

Mechanism of Enzyme activity

In an enzyme-catalyzed reaction, formation of enzyme-substrate complex is the first step; substrate (S) binds to the enzyme (E) to form a complex (ES). Formation of ES complex leads to the formation of transition state species, which then forms the product.

Substrate binds to the enzyme at the active site usually by non-covalent interactions (hydrogen bonding, electrostatic, hydrophobic interactions and van-der waal forces). Active site is a cleft or crevices in the protein (Fig.1) and consists of certain amino acids side chains that are essential for catalysis generally situated far apart in the primary sequence of protein. Catalytic reaction takes place at active site in many steps.

Two models have been proposed to describe the binding of substrate to the enzyme.

'Lock and Key' model, proposed by Emil Fischer (1894), which assumes a high degree of similarity between the shape of the substrate and the geometry of the binding site on the enzyme (Fig.3A). Substrate binds to a site where shape complements its own, like a key in a lock. This model does not take into account the conformational flexibility of proteins.

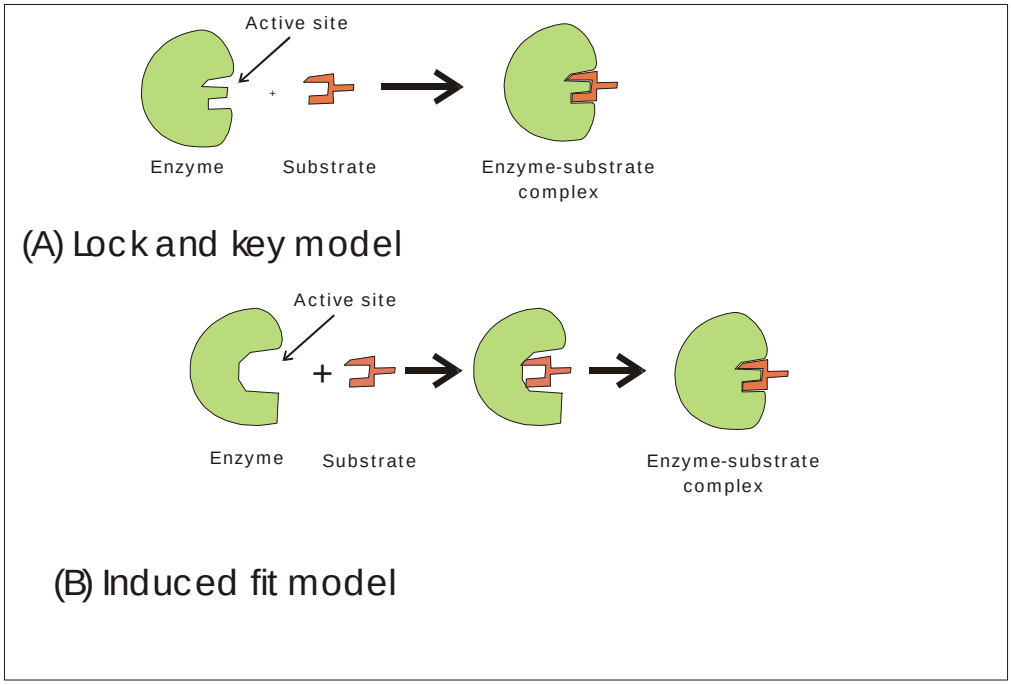

Figure:3 Models for binding of substrate to enzyme.

(A) *In Lock and Key model, shape of the substrate and conformation of the active site of enzyme are complementary to each other like lock and key.*

(B) In induced fit model, enzyme undergoes a conformational change upon binding of the substrate. The shape of the active site becomes complementary to the shape of the substrate only after the substrate binds to the enzyme.

According to ' ***Induced fit '*** model proposed by Koshland (1958), binding of substrate induces a conformational change in the enzyme that results in a complementary fit, once the substrate is bound (Fig.3B). The binding site has different three-dimensional shape before substrate is bound.

Energy profile of a typical chemical reaction (Fig.4) shows that in order to proceed from reactant to products an energy barrier ($\Delta G^{\ddagger}$) must be over come. The highest point of energy profile is designated as *transition state* of the reaction and is the main energetic barrier to the product formation [13,14,15]

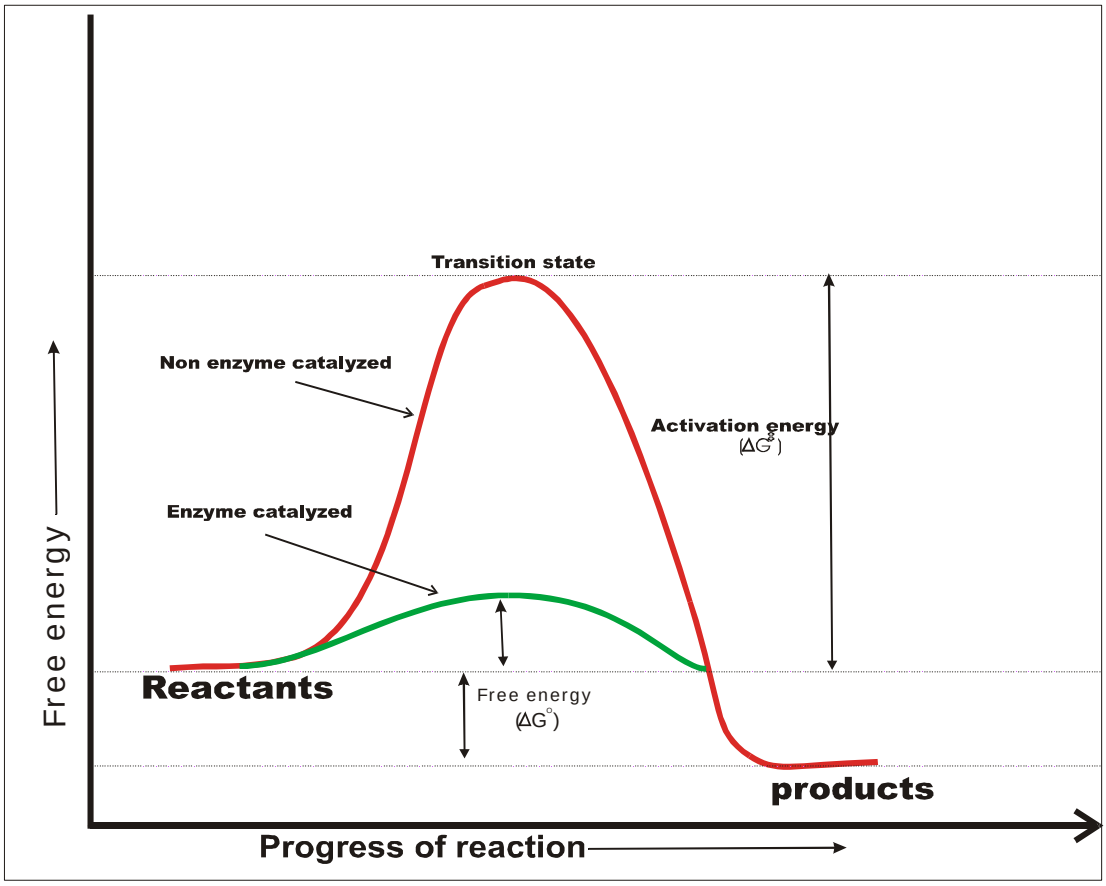

Figure: 4 **Effect of enzyme on free energy changes for an energetically favorable reaction.**

In order to speed up the reaction at constant temperature, this transition state energy barrier must be overcome. Once this barrier is overcome, the reaction is much more likely to proceed down hill to the formation of product.

In enzyme-catalyzed reaction, enzyme lowers this activation energy by providing alternative reaction pathways. As enzyme is recovered unchanged after the reaction, the free energy change (ΔG°) depends on the initial and final stages of the reaction and not on various intermediate stages formed during the reaction. This implies that enzyme does not alter the magnitude of the free energy change (ΔG°) and therefore does not cause a shift in the equilibrium between the reactants and products; it merely shifts the rate at which that equilibrium is attained. Enzymes speed up the rate at which this equilibrium is attained in a reaction using one or more of the following factors to achieve transition state stabilization.

Proximity and orientation of substrate:

In enzyme catalyzed reactions involving more than one substrate, enzyme can increase the rate of a reaction by bringing the substrates into close proximity to each other by binding them at adjacent sites. The reaction between the substrates will be faster than depending on the chance collision between the substrates. Substrates in solution may not also have proper orientation to interact with each other when they colloid. Enzyme facilitates their interaction by holding them in right orientation after binding to them.

Donating and accepting a proton

Side chains of amino acids forming the active site may directly participate in making the substrate more reactive by donating or accepting proton *(acid- base catalysis*).Groups such as imidazole, hydroxyl, carboxyl, sulfhydryl, amino and phenolic can act as acids or bases. Hydrolysis of an ester for example may be faster in the presence of an acid or base (Fig. 5)

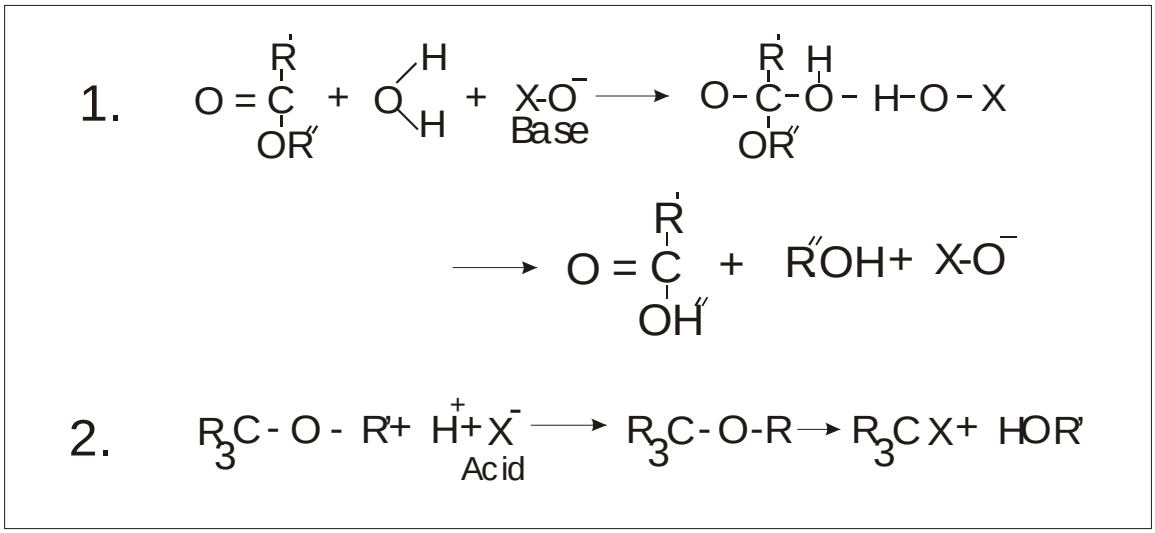

Figure: 5 **Acid-base catalysis**: hydrolysis is faster in the presence of acid or base.

By forming covalent interactions

In covalent catalysis, functional groups present in side chains of amino acids constituting the active site forms the temporary covalent bond with a portion of the substrate and generate a covalent intermediate that shifts the reaction towards the transition state, thus helping to overcome the activation energy barrier. Enzymes that utilize the covalent catalysis to lower the activation energy barrier achieve this by rapidly forming and breaking the intermediates.

Metal ions such as copper, zinc, iron and manganese, which are firmly bound to the side chains of a protein, can lose or gain electrons without altering the bonds that hold them to the protein. This ability makes them important in oxidation-reduction reactions, which involves loss or gain of electron (*metal ion catalysis*). For example involvement of Zn^{+2} in the enzymatic activity of *Carboxypeptidase A*. The enzyme catalyzes the hydrolysis of C-terminal peptide bonds of proteins. A zinc ion is complexed to three side chains of Carboxypeptidase and to a carbonyl group on the substrate making it susceptible to attack by water and allowing the hydrolysis to proceed more rapidly (Fig.6).

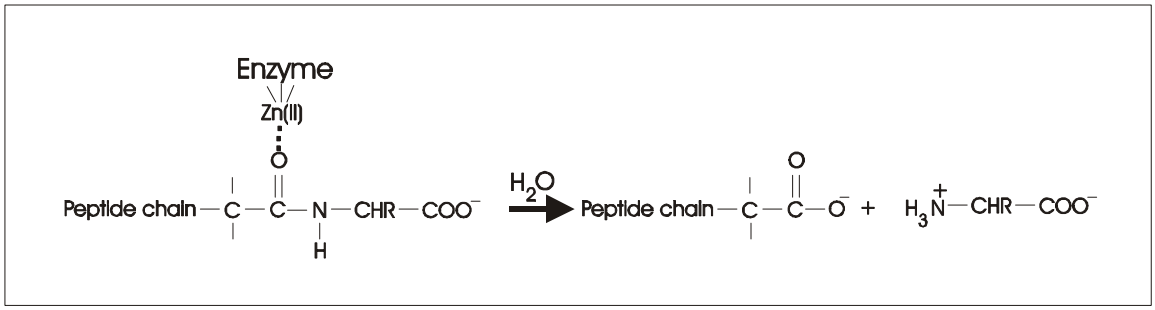

Figure:6 Binding of Zn ion to substrate makes it more susceptible to attack by water and thus allowing hydrolysis of peptide bond more rapidly.

By causing conformation distortion

Once a substrate binds to an enzyme, it can cause a distortion of the bonds in the substrate. This would speed up the rate of a reaction if the distortion changes the geometry and electronic structure of the substrate more closely resemble to the transition state.

Enzyme may also cause stretching of bonds in substrate, making them less stable and more reactive to the other substrate.

Enzyme Kinetics

Mechanism of an enzyme-catalyzed reaction can be studied by determining the rate of the reaction and how it changes in response to various experimental factors.

A key factor affecting the rate of an enzyme-catalyzed reaction is the substrate concentration [S]. The rate of catalysis (Vo) varies with the substrate concentration [S] in a manner as shown in the fig. (7). At relatively low concentration of substrate [S], Vo showed linear increase with an increase in [S].

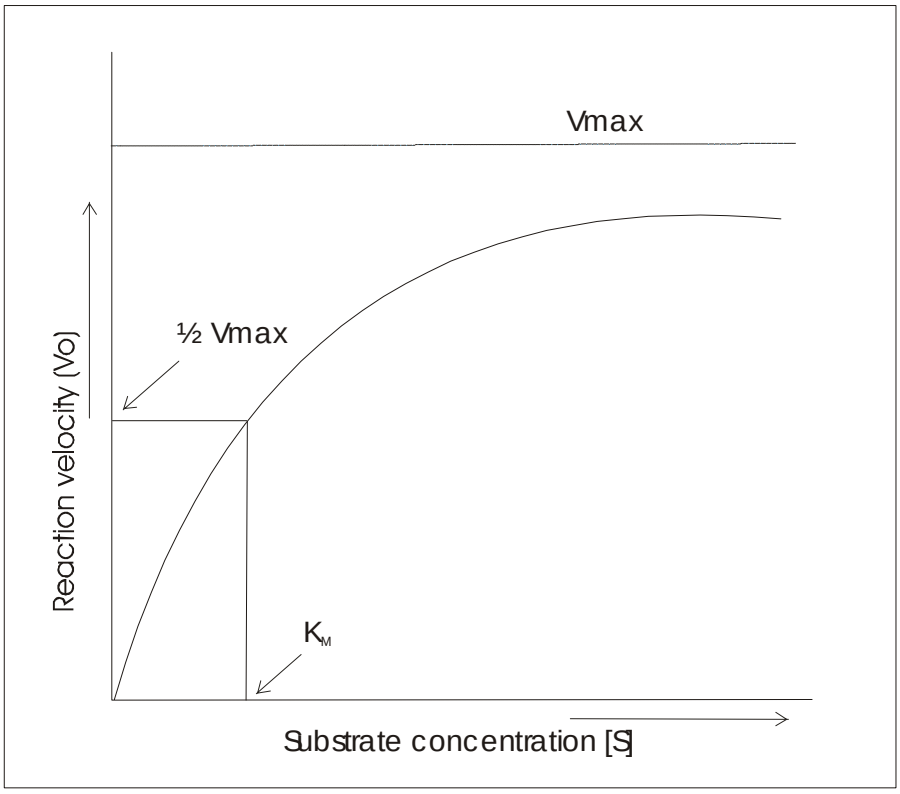

Figure:**7** Reaction velocity as a function of substrate concentration.

At high [S], increase in Vo is smaller in response to [S] increase, finally reaching to a point where there is no further increase in Vo with increase in [S]. This point is called Vmax (maximum velocity).

Leonor Michaelis and Maud Menten (1913) proposed a equation to express algebraically the kinetic behaviors of a unimolecular enzyme-substrate interactions.

The equation assumes the formation of ES complex as a necessary step in an enzyme catalyzed reaction and enzyme binds to its substrate to form enzyme-substrate complex in a reversible manner.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E \quad \textbf{.....(1)}$$

Where E is free enzyme, S is substrate, ES-enzyme-substrate complex and k_1, k_{-1} and k_2 are the rate constants for the formation of ES, release of S and release of P respectively.

At any given instant in an enzyme-catalyzed reaction, the enzyme exists in two forms, the free or uncombined form E and the combined form ES.

During initial phase, at low [S], most of the enzyme is in uncombined form E and the rate is proportional to [S].

Next is the phase of steady state in which [ES] reaches a dynamic equilibrium. Enzyme is saturated with substrate so that further increases in [S] have no effect on the rate. At this point Vmax is achieved. This condition exists when [S] is high and all the free enzyme is converted to ES form.

The saturation effect is characteristic feature of enzyme-catalyzed reaction and is responsible for the plateau formation in the fig. (7).

The rate-limiting step in the enzyme-catalyzed reaction is the breakdown of ES complex to product and free enzyme.

$$\mathbf{Vo = K_2\,[ES]\ldots\ldots\ldots\ldots\ldots\ldots..(2)}$$

The rates of formation and breakdown of ES can be given by:

$$\mathbf{\text{Rate of formation of ES} = K_1\,[E]\,[S]\ldots\ldots\ldots\ldots\ldots\ldots(3)}$$

$$\mathbf{\text{Rate of breakdown of ES} = (k_{-1} + K_2)\,[ES]\ldots\ldots\ldots\ldots..(4)}$$

Under steady state assumption, the rate of formation of [ES] is equal to rate of breakdown of [ES].

$$\mathbf{\text{Therefore, } k_1\,[E][S] = (K_{-1} + K_2)\,[ES] = K_{-1} + K_2 / K_1 = [E]\,[S]/\,[ES]\ldots\ldots\ldots\ldots\ldots\ldots(5)}$$

New constant K_M called Michaelis-Menten constant can be defined for

$$\mathbf{K_{-1} + K_2 / K_1 = K_M \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)}$$

Inserting equation (6) into (5)

$$\mathbf{[E][S]/\,[ES] = K_M}$$

$$\mathbf{or\ \ [ES] = [E]\,[S]/\,K_M\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(7)}$$

The concentration of uncombined enzyme [E] is equal to total enzyme concentration $[E]_T$ minus the concentration of ES complex.

$$\mathbf{[E] = [E]_T - [ES]\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(8)}$$

Substituting for [E] in equation (7)

$$\mathbf{[ES] = ([E]_T - [ES])\,[S] / K_M \ldots\ldots\ldots(9)}$$

$$[ES] = \frac{[E]_T}{1 + [S]/K_M} \quad \text{……..(10)}$$

$$[ES\} = \frac{E_T [S]}{[S] + K_M} \quad \text{…….(11)}$$

By substituting this expression for [ES] into equation (1)

$$V_0 = k_2 [E]_T \frac{[S]}{[S] + K_M} \quad \text{……..(12)}$$

Maximum rate, Vmax is attained when

$$[ES] == [E]_T$$

$$\text{Thus } V_{max} = k_2 [E]_T \quad \text{……..(13)}$$

Substituting equation (13) into (12)

$$V_o = V_{max} \frac{[S]}{[S] + K_M} \quad \text{……(13) \{Michaelis-Menten equation\}}$$

This is the Michaelis- Menten equation that gives quantitative relationship between the initial velocity (Vo), the maximum velocity (V max) and the substrate concentration [S].

If Vo is half to Vmax, then equation (13) can be rewritten as

$$\frac{V_{max}}{2} = V_{max} \frac{[S]}{[S] + K_M} \quad \text{…….(14)}$$

$$\frac{1}{2} = \frac{[S]}{[S] + K_M} \quad \text{……(15)}$$

Solving for K_M

$$K_M = 2[S] - [S] = [S] \quad \text{…….(16)}$$

Therefore K_M is the substrate concentration at which initial velocity is half of the maximum velocity.

The hyperbolic curve in Michaelis- Menten equation graph is not accurate for detecting Vmax and K_M . The graph needs to be linearize to permit reliable extrapolation of the data. These problems can be overcome by plotting reciprocals of Vo and [S]—Lineweaver-Burk Plot.

The Michaelis- Menten equation can be converted to its reciprocal form.

$$V_o = \frac{V_{max} [S]}{[S] + K_M}$$

$$\frac{1}{V} = \frac{[S] + K_M}{V_{max} [S]}$$

$$= [S] / Vmax [S] + K_M / Vmax [S]$$

$$1/V = K_M / Vmax [S] + 1 / Vmax$$

A plot of 1 / V vs 1 /[S] gives a straight line as shown in the figure (8).

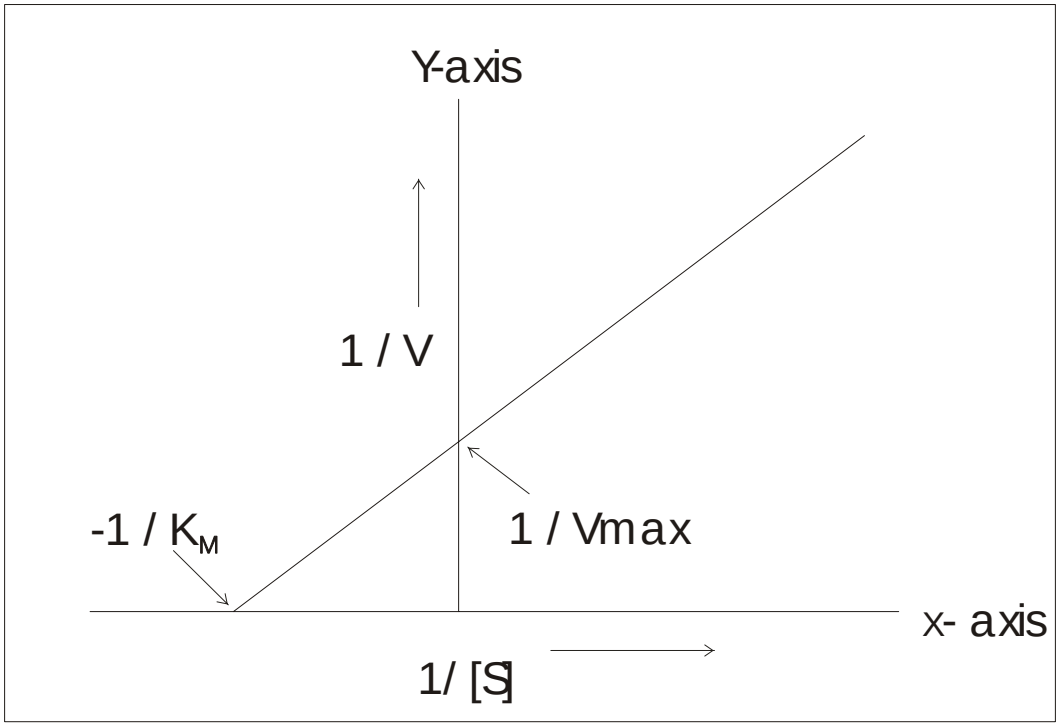

Figure:8 Lineweaver-Burk Plot

It is also known as double reciprocal plot. K_M and Vmax can be easily determined from the graph from the intercepts on X and Y- axis respectively.

Regulation of enzyme activity[16]

In cell, metabolic pathways consist of sequence of specific enzyme catalyzed reactions. Some metabolic pathways involve synthesis of important molecules of cells whereas other break down the molecules. A cell must regulate all its metabolic pathways constantly so that each type of molecule is produced in a quantity appropriate to the requirements of that cell. In cell, regulation of metabolic pathways is mainly achieved by regulating the activity of enzymes involved, in response to increased or diminished need for a metabolic product. Catalytic activity of these enzymes may be regulated by small ions or other molecules (substrate, inhibitor etc) to regulate the quantity of metabolite in a cell / metabolic pathways.

Regulation of enzyme activity by inhibitors:

Presence of enzyme does not ensure its biological activity. Various inhibitors can bind to the enzyme and slow down the rate of enzyme catalyzed reaction. Inhibitors can be naturally occurring in the cell or artificial.

Inhibition of enzyme activity by inhibitor can be *irreversible* or *reversible.*

Irreversible inhibition

In this type of inhibition, inhibitor covalently binds to the active site of the enzyme and irreversibly inactivates them. Inhibitor binds to some functional group in the active site and block the site for substrate or leave it catalytically inactive.

Reversible Inhibition

Binding of inhibitor to the enzyme is reversible. Inhibitor can be removed by dialysis or simple dilutions to restore the full enzyme activity.

It can be of two types: competitive and non-competitive inhibition.

In *competitive inhibition*, the inhibitor resembles the true substrate in structure and binds to the active site of the enzyme so that substrate cannot bind to the active site of the enzyme and prevent the catalytic action (Fig.9) Inhibitor binds to the same site on the enzyme where substrate binds but it does not undergo catalytic steps. In order to regain enzyme activity, the inhibitor must be dissociated from the enzyme and replaced by a substrate molecule. Effect of a competitive inhibitor depends upon the relative concentration of inhibitor, substrate and their affinity for the enzyme. Competitive inhibition can be overcome by increasing the substrate concentrations. At very high substrate concentration, molecules of substrate will greatly outnumber the molecules of inhibitor and effect of inhibitor will be negligible.

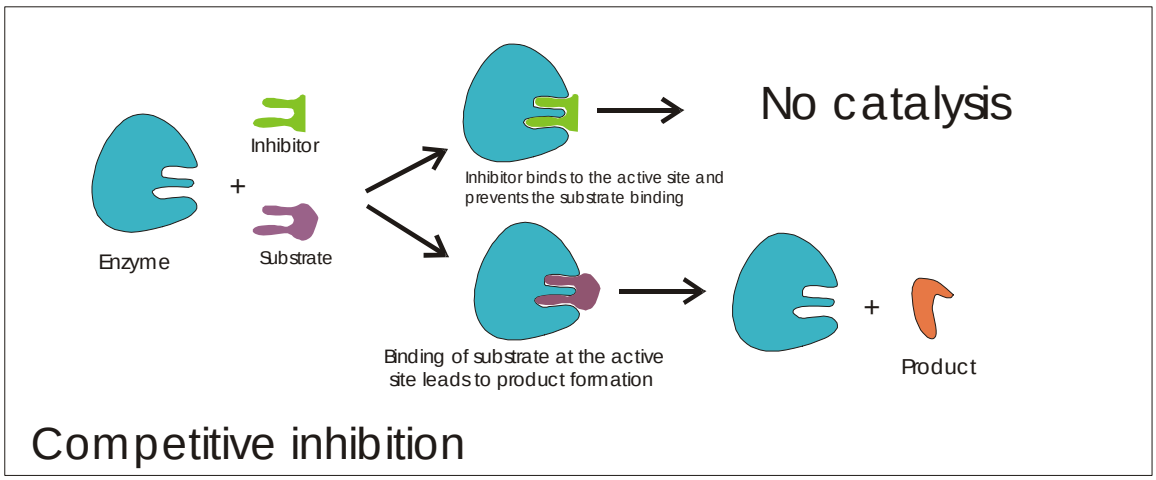

Figure:9 **Competitive inhibition :** Inhibitor resembles the substrate in structure and can bind to the active site of the enzyme. Binding of inhibitor cannot undergo catalysis whereas the enzyme can catalyze substrate.

In non-competitive inhibition, the inhibitor does not necessarily resembles the natural substrate and do not bind to the active site of the enzyme. Inhibitor binds to the enzyme at a site other than the active site. The binding of inhibitor may cause a conformational change in the enzyme active site thereby preventing the binding of the substrate to the enzyme (Fig 10). In this type of inhibition inhibitor does not compete with the substrate for binding to the enzyme hence inhibition cannot be overcome by increasing the substrate concentration.

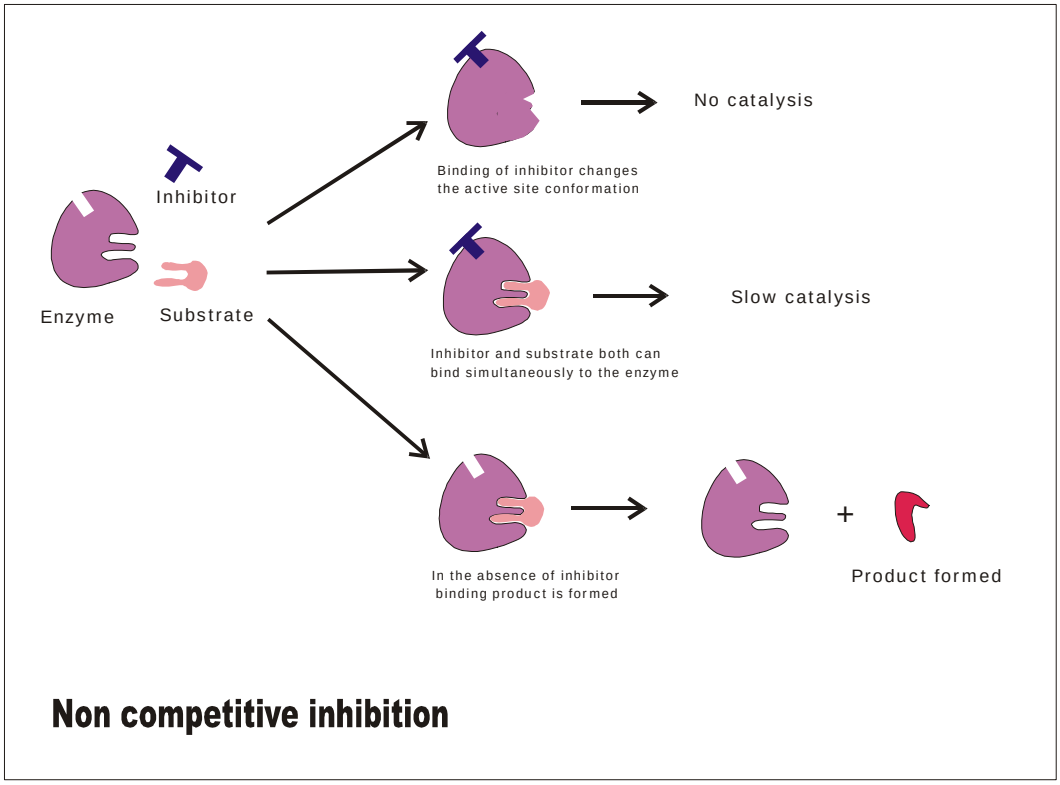

Figure:10 Non-competitive inhibitions. Inhibitor binds to the enzyme at a site different from the active site. Binding of inhibitor destroys the active site of the enzyme and inhibits the catalytic activity of the enzyme. As inhibitor does not resemble the substrate in structure it does not interfere with the substrate for binding to the active site of the enzyme.

Allosteric regulation of enzymes

Many important enzymes of metabolism have quaternary structure consisting of more than one polypeptide subunits joined together by various weak interactions. Binding of subunits influence the change in the shape and properties of each others. Multi-subunit enzymes that undergo such changes are called *allosteric enzyme* (there are single chain enzymes e.g. a ribonucleotide reductase that are allosterically regulated [17]).

An allosteric enzyme usually consists of two or more catalytic subunits and one or more regulatory subunits. The existence of two or more linked catalytic subunits allow for co-operativity in which one subunit activity influence the activity of its neighbors. When a molecule of substrate binds to the active site of one catalytic subunit of an allosteric enzyme it causes a change in the other catalytic subunits making it easier for substrate to bind to them. Conversely when a inhibitor bind to an allosteric site of a regulatory subunit, it causes a different change in the catalytic subunit making it harder for the substrate to bind to them.

In a multi- step metabolic reaction, when the end product inhibits the first step in the series is called ***feed-back inhibition*** [18]**.** This type of regulation is well illustrated by the biosynthesis of isoleucine from threonine in bacteria. *Threonine dehydratase*, the first enzyme in the conversion of threonine to isoleucine is inhibited by isoleucine when its concentration reaches a high level (Fig. 11). Isoleucine inhibits by binding the enzyme at regulatory site other than the active site. This inhibition is reversible and allosteric in nature.[19]

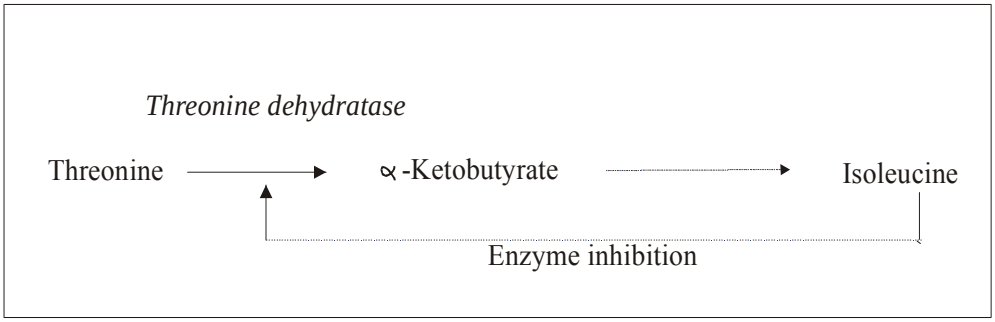

Figure:11 Inhibition of first enzyme *Threonine dehydratase* by isoleucine in the biosynthesis pathway of isoleucine .

Activity of allosteric enzymes may be controlled by small molecules called *effectors* which may have no structural similarity with the substrate or product. Effectors bind on enzyme to a site that is other than the active site (regulation site). Binding of effector molecule induces a reversible conformational change in the enzyme that causes an alternation of the structure of the active site and consequently effecting the binding of the substrate (Fig.12). Their binding may enhance or decrease the activity of the enzyme. Thus the effectors may be *activators* or *inhibitors.*

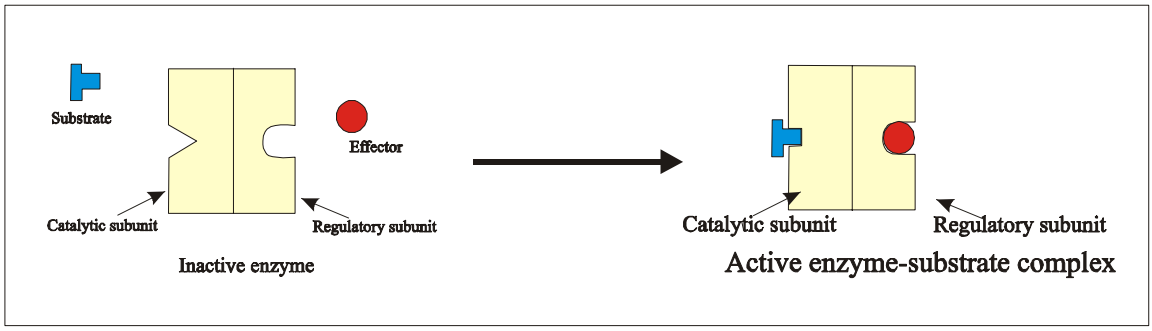

Figure: 12 Binding of effector to the regulatory site induces a reversible conformation change in active site of the enzyme to effect binding of substrate.

Covalent modification of enzyme

A number of kinds of covalent modifications (phosphorylation, adenylation, methylation, glycosylation, uridylyation) of enzymes are commonly used to regulate enzyme activity. These modifications of the enzyme and their reversal are carried out by separate different enzymes [16,20].

Phosphorylation of the enzyme is most common modification of enzymes. More than 30% of the total protein in a cell is phosphorylated. Level of phosphorylation of protein varies from one phosphorylated residue to several phosphorylated residue among phosphorylated enzymes. Most common residues that under go phosphorylation

are serine, threonine and tyrosine. Phosphorylation can have drastic effect on the conformation of enzyme and their catalytic activity. [16,20,21]

Activation of enzyme by proteolytic cleavages of precursor form

Another important example of covalent regulation of enzyme is provided by the enzyme proteases. A number of proteases are synthesized in the form of an inactive precursor called *zymogens.* These zymogens must be cleaved proteolytically to yield active enzyme. Some examples of enzymes that are activated in this way are given in the table (Table: II).

Table: II. Some enzymes and their inactive precursors.

Enzyme	Precursor
Trypsin	**Trypsinogen**
Chymotrypsin	**Chymotrypsinogen**
Carboxypeptidase	**Procarboxypeptidase**
Elastase	**Proelastase**

Other factors affecting enzyme activity [22]

Main other factors which affect the enzyme activity are temperature and pH.

Temperature

Enzymes are sensitive to temperature. At zero degree centigrade, enzymes show practically no activity. The enzyme activity increases with rise in temperature and reaches to a maximum (optimum temperature), after which it decreases with further increase in temperature (Fig .13).

The initial increase in rate of an enzyme catalyzed reaction with rise in temperature is mainly due to : increase in kinetic energy of the substrate and enzyme; and thereby increasing the chance collision between enzyme and substrate molecules. At higher temperature enzyme undergo denaturation as the conformation of enzyme get disturbed due to breaking of bonds responsible for maintaining the 3-dimensional conformation of enzyme. The optimum temperature of activity shown by enzymes vary among enzyme but generally lies between 25^{o} C to 37^{o}C.

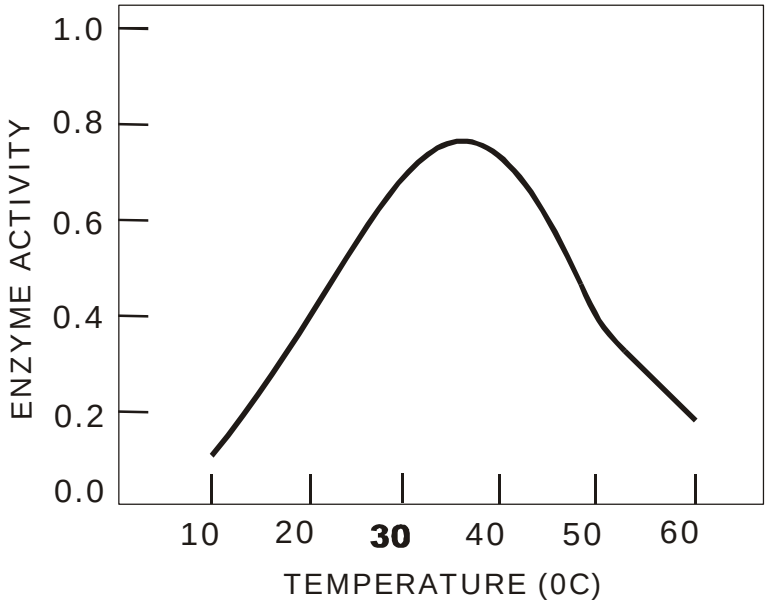

Figure: 13 Activity profile of a typical enzyme as a function of temperature

pH

Every enzyme requires a specific pH value or range of pH values for their activity. At extremely low and high pH values, enzymes generally get denatured. The pH value or range over which enzyme show maximum activity varies from enzyme to enzyme. Most enzymes are stable and show maximum activity near physiological pH (pH 7.0 to pH 7.5). Fig.14 depicts the typical profile of velocity of an enzymatic reaction as a function of pH. The pH value at which enzyme show maximum activity is known as optimum pH of enzyme activity. A decrease in enzyme activity on either side of the optimum pH is due to decrease in affinity of the substrate with enzyme and instability of the enzyme.

Enzymes being protein in nature contain many ionizable groups present in various states of ionization. Among the various ionizable state of the enzyme, only one of the ionic form of the enzyme is catalytically active. This catalytically active ionic form is maintained at a specific pH or a range of pH values.

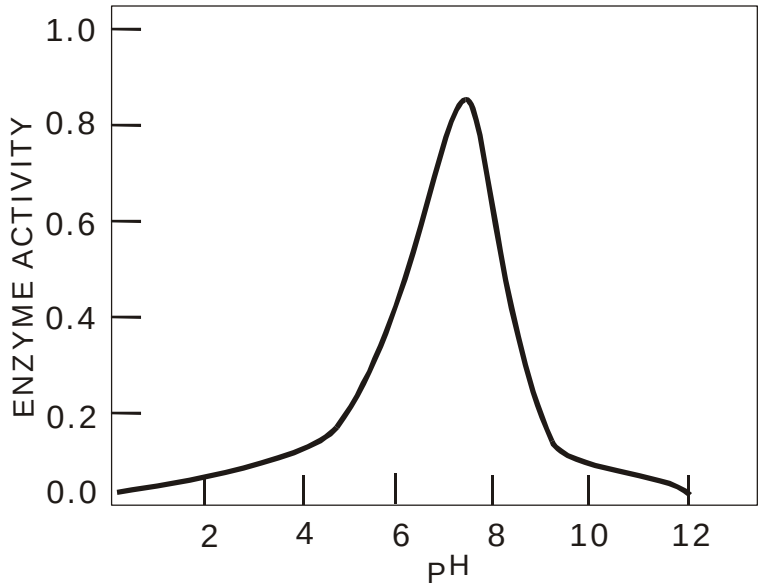

Figure: 14 Activity profile of a typical enzyme as a function of pH**.**

Determination of enzyme specific activity

Activity of an enzyme is determined by a suitable assay procedure in which the rate of disappearance of substrate or the rate of product formation is determined under specified conditions of temperature, pH, substrate etc. The unit for expressing enzyme activity was defined by enzyme commission of IUB in 1961 as Enzyme Unit (EU), later to be known as International Unit (IU). It is defined as the amount of enzyme causing loss of 1 µ mol substrate per minute under specified conditions.

Later in 1973, the commission on Biochemical Nomenclature introduced the **Katal (Kat**) as the Systeme International (SI) unit of enzyme activity: this is defined as the amount of enzyme causing loss of 1 µ mol substrate per second under specified conditions. Both the units are in current usage to express enzyme specific activity.

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Suggested readings:

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