4. The active site is not rigid in structure and shape. It is rather ***flexible*** to promote the specific substrate binding.

5. Generally, the active site possesses a ***substrate binding site*** and a ***catalytic site.*** The latter is for the catalysis of the specific reaction.

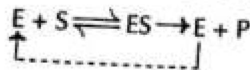
6. The coenzymes or cofactors on which some enzymes depend are present as a part of the catalytic site.

7. The substrate(s) binds at the active site by weak noncovalent bonds.

8. Enzymes are specific in their function due to the existence of active sites.

9. The commonly found amino acids at the active sites are serine, aspartate, histidine, cysteine, lysine, arginine, glutamate, tyrosine etc. Among these amino acids, ***serine*** is the most frequently found.

10. The substrate[S] binds the enzyme (E) at the active site to form enzyme-substrate complex (ES). The product (P) is released after the catalysis and the enzyme is available for reuse.



$$E + S \rightleftharpoons ES \rightarrow E + P$$

ENZYME INHIBITION

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a ***decrease in catalytic activity*** of that enzyme. The inhibitor may be organic or inorganic in nature. There are three broad categories of enzyme inhibition

1. Reversible inhibition.
2. Irreversible inhibition.
3. Allosteric inhibition.

1. Reversible inhibition

The inhibitor binds non-covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition is further sub-divided into

I. Competitive inhibition (***Fig.6.7A***)

II. Non-competitive inhibition (***Fig.6.7B***)

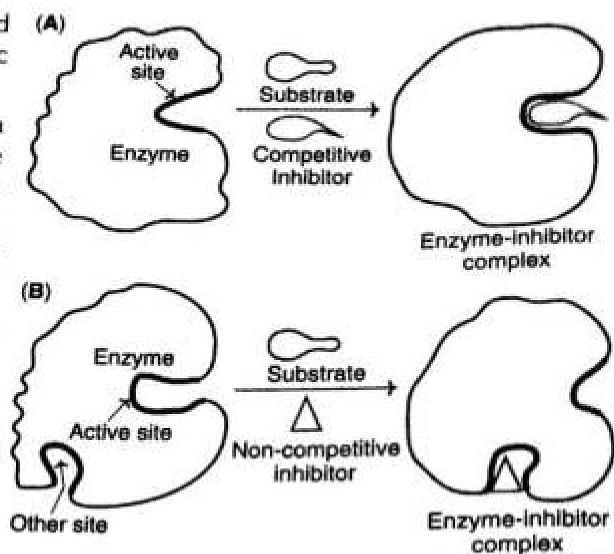
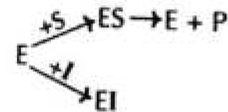


Fig. 6.7 : A diagrammatic representation of (A) Competitive and (B) Non-competitive inhibition.

I. **Competitive inhibition :** The inhibitor (I) which closely resembles the real substrate (S) is regarded as a ***substrate analogue***. The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor holds the active site, the enzyme is not available for the substrate to bind. During the reaction, ES and EI complexes are formed as shown below



The relative concentration of the substrate and inhibitor and their respective affinity with the enzyme determines the degree of competitive inhibition. The inhibition could be overcome by a high substrate concentration. In competitive inhibition, the ***K_m value increases*** whereas V_{max} remains ***unchanged*** (***Fig.6.8***).

The enzyme succinate dehydrogenase (SDH) is a classical example of competitive inhibition with succinic acid as its substrate. The compounds, namely, malonic acid, glutaric acid and oxalic acid, have structural similarity with succinic acid and compete with the substrate for binding at the active site of SDH.

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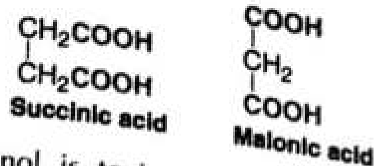


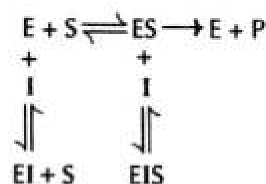
Methanol is toxic to the body when it is converted to formaldehyde by the enzyme alcohol dehydrogenase (ADH). Ethanol can compete with methanol for ADH. Thus, ethanol can be used in the treatment of methanol poisoning.

Some more examples of the enzymes with substrates and competitive inhibitors (of clinical and pharmacological significance) are given in *Table 6.2*.

Antimetabolites : These are the chemical compounds that block the metabolic reactions by their inhibitory action on enzymes. Antimetabolites are usually structural analogues of substrates and thus are competitive inhibitors (*Table 6.2*). They are in use for cancer therapy, gout etc. The term ***antivitamins*** is used for the antimetabolites which block the biochemical actions of vitamins causing deficiencies, e.g. sulphonilamide, dicumarol.

II. **Non-competitive inhibition :** The inhibitor binds at a site other than the active site on the enzyme surface. This binding impairs the enzyme function. The inhibitor has no structural resemblance with the substrate. However, there usually exists a strong affinity for the inhibitor to bind at the second site. In fact, the inhibitor does not interfere with the enzyme-substrate binding. But the catalysis is prevented, possibly due to a distortion in the enzyme conformation.

The inhibitor generally binds with the enzyme as well as the ES complex. The overall relation in non-competitive inhibition is represented below



For non-competitive inhibition, the ***K_m value is unchanged*** while ***V_{max} is lowered*** (***Fig.6.9***).

Heavy metal ions (Ag^+, Pb^{2+}, Hg^{2+} etc.) can non-competitively inhibit the enzymes by binding with cysteinyl sulfhydryl groups. The general reaction for Hg^{2+} is shown below.

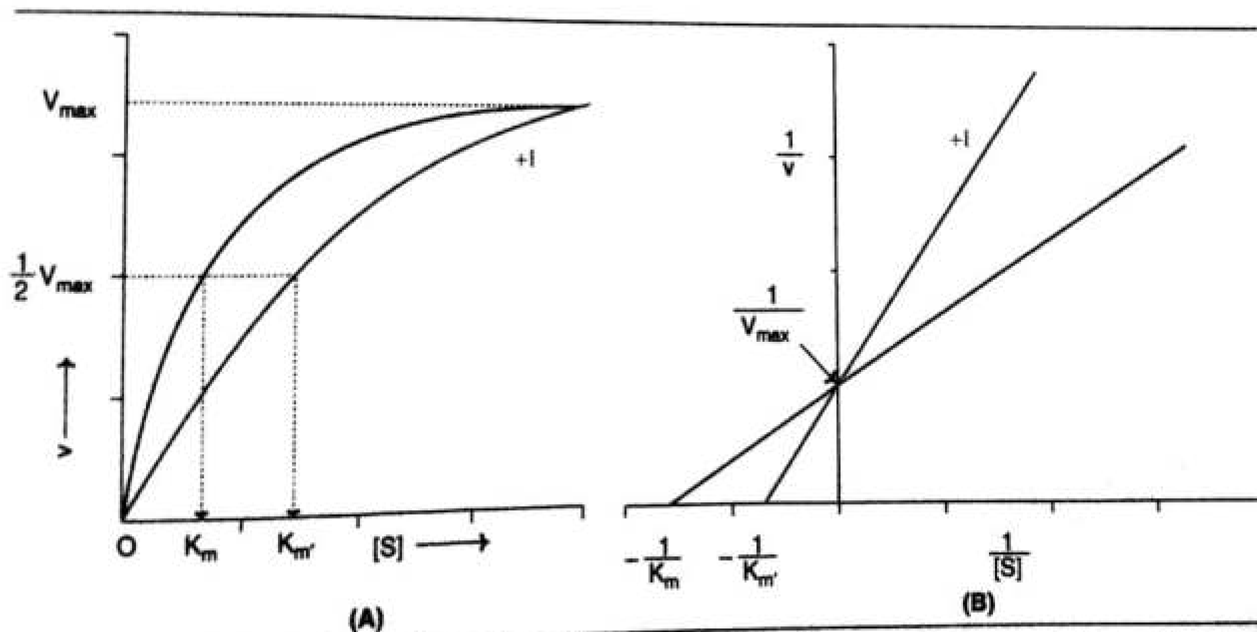


Fig. 6.8 : Effect of competitive inhibitor (I) on enzyme velocity. (A) Velocity (v) versus substrate (S) plot. (B) Lineweaver-Burk plot (Red lines with inhibitor; competitive inhibitor increases $K_{m'}$, unalters V_{max}).

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Table 6.2 Selected examples of enzymes with their respective substrates and competitive inhibitors

Enzyme	Substrate	Inhibitor(s)	Significance of inhibitor(s)
Xanthine oxidase	Hypoxanthine xanthine	Allopurinol	Used in the control of gout to reduce excess production of uric acid from hypoxanthine
Monoamine oxidase	Catecholamines (epinephrine, norepinephrine)	Ephidrene, amphetamine	Useful for elevating catecholamine levels.
Dihydrofolate reductase	Dihydrofolic acid	Aminopterin, amethopterin, methotrexate	Employed in the treatment of leukemia and other cancers
Acetylcholine esterase	Acetylcholine	Succinyl choline	Used in surgery for muscle relaxation, in anaesthetised patients.
Dihydropteroate synthase	Para aminobenzoic acid (PABA)	Sulfonilamide	Prevents bacterial synthesis of folic acid.
Vitamin K epoxide reductase	Vitamin K	Dicumarol	Acts as an anticoagulant.
HMG CoA reductase	HMG CoA	Lovastatin, compactin	Inhibit cholesterol biosynthesis

Heavy metals also lead to the formation of covalent bonds with carboxyl groups and histidine, often resulting in irreversible inhibition.

2. Irreversible inhibition

The inhibitors bind covalently with the enzymes and inactivate them, which is irreversible. These inhibitors are usually toxic poisonous substances.

Iodoacetate is an irreversible inhibitor of the enzymes like papain and glyceraldehyde 3-phosphate dehydrogenase. Iodoacetate combines with sulfhydryl (–SH) groups at the active site of these enzymes and makes them inactive.

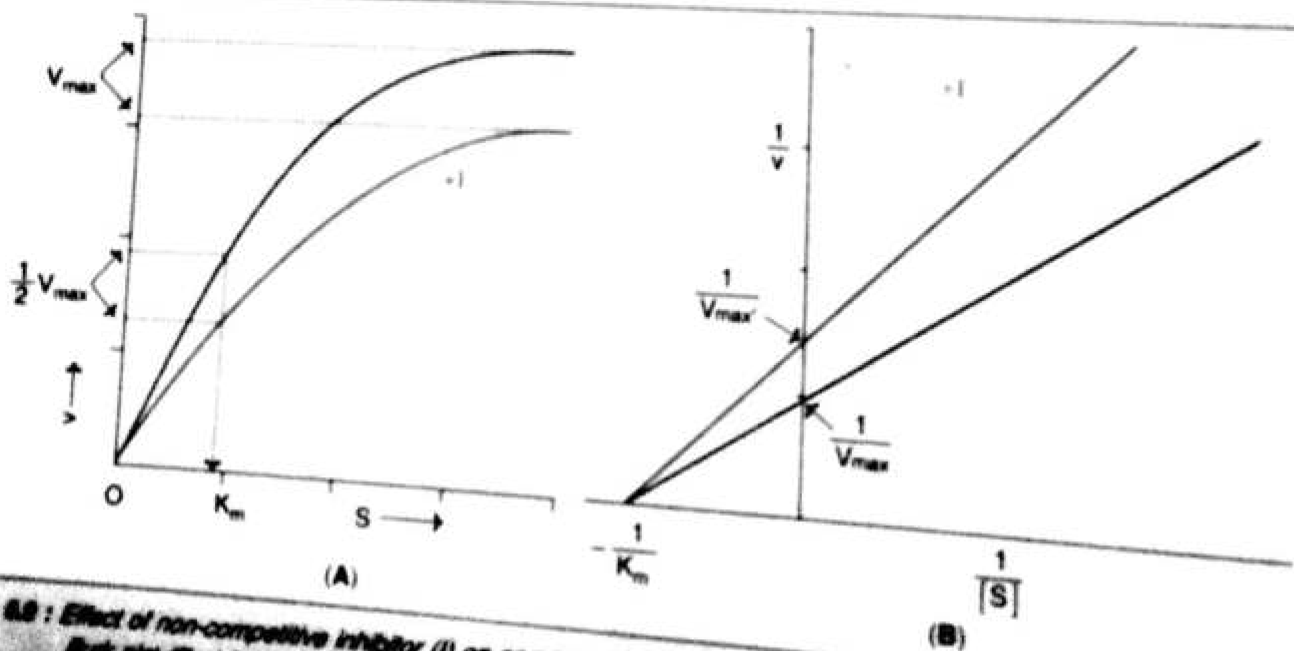

Fig. 6.8 : Effect of non-competitive inhibitor (I) on enzyme velocity (A) Velocity (v) versus substrate (S) (B) Lineweaver-Burk plot (Red lines with inhibitor, non-competitive inhibitor does not change K_m but decreases V_{max}).

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Diisopropyl fluorophosphate (DFP) is a ***nerve gas*** developed by the Germans during Second World War. DFP irreversibly binds with enzymes containing serine at the active site, e.g. ***serine proteases, acetylcholine esterase***.

Many organophosphorus insecticides like melathion are toxic to animals (including man) as they block the activity of acetylcholine esterase (essential for nerve conduction), resulting in paralysis of vital body functions.

Disulfiram (Antabuse®) is a drug used in the treatment of alcoholism. It irreversibly inhibits the enzyme aldehyde dehydrogenase. Alcohol addicts, when treated with disulfiram become sick due to the accumulation of acetaldehyde, leading to alcohol avoidance. (***Note :*** Alcohol is metabolized by two enzymes. It is first acted upon by alcohol dehydrogenase to yield acetaldehyde. The enzyme aldehyde dehydrogenase converts acetaldehyde to acetic acid.)

The penicillin antibiotics act as irreversible inhibitors of serine – containing enzymes, and block the bacterial cell wall synthesis.

Irreversible inhibitors are frequently used to identify amino acid residues at the active site of the enzymes, and also to understand the mechanism of enzyme action.

Suicide inhibition

Suicide inhibition is a specialized form of irreversible inhibition. In this case, the original inhibitor (the structural analogue/competitive inhibitor) is converted to a more potent form by the same enzyme that ought to be inhibited. The so formed inhibitor binds irreversibly with the enzyme. This is in contrast to the original inhibitor which binds reversibly.

A good example of suicide inhibition is ***allopurinol*** (used in the treatment of gout, ***Refer Chapter 17***). Allopurinol, an inhibitor of xanthine oxidase, gets converted to alloxanthine, a more effective inhibitor of this enzyme.

The use of certain purine and pyrimidine analogues in cancer therapy is also explained on the basis suicide inhibition. For instance, ***5-fluorouracil*** gets converted to fluorodeoxyuridylate which inhibits the enzyme thymidylate synthase, and thus nucleotide synthesis.

3. Allosteric Inhibition

The details of this type of inhibition are given under allosteric regulation as a part of the regulation of enzyme activity in the living system.

ENZYME SPECIFICITY

Enzymes are highly specific in their action when compared with the chemical catalysts. The occurrence of thousands of enzymes in the biological system might be due to the specific nature of enzymes. Three types of enzyme specificity are well-recognised

1. Stereospecificity,
2. Reaction specificity,
3. Substrate specificity.

Specificity is a characteristic property of the active site.

1. **Stereospecificity or optical specificity :** Stereoisomers are the compounds which have the same molecular formula, but differ in their structural configuration.

The ***enzymes act only on one isomer*** and, therefore, exhibit stereospecificity.

e.g. L-amino acid oxidase and D-amino acid oxidase act on L- and D-amino acids respectively.

Hexokinase acts on D-hexoses;

Glucokinase on D-glucose;

Amylase acts on α-glycosidic linkages;

Cellulase cleaves β-glycosidic bonds.

Stereospecificity is explained by considering three distinct regions of substrate molecule specifically binding with three complementary regions on the surface of the enzyme (***Fig.6.10***). The class of enzymes belonging to ***isomerases do not exhibit stereospecificity***, since they are specialized in the interconversion of isomers.

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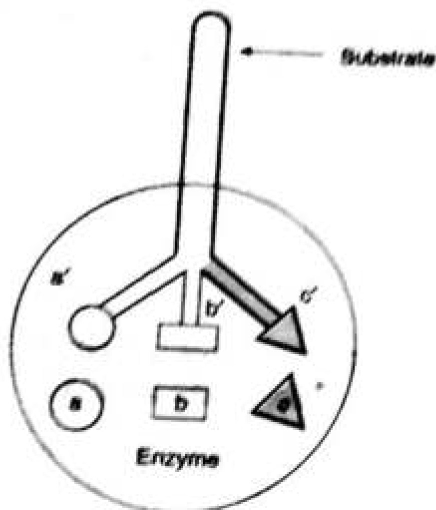

Fig. 6.10 : Diagrammatic representation of stereospecificity (a', b', c')—three point attachment of substrate to the enzyme (a, b, c).

2. **Reaction specificity :** The same substrate can undergo different types of reactions, each catalysed by a separate enzyme and this is referred to as reaction specificity. An amino acid can undergo transamination, oxidative deamination, decarboxylation, racemization etc. The enzymes however, are different for each of these reactions (For details, ***refer Chapter 15***).

3. **Substrate specificity :** The substrate specificity varies from enzyme to enzyme. It may be either absolute, relative or broad.

- **Absolute substrate specificity :** Certain enzymes act only on one substrate e.g. glucokinase acts on glucose to give glucose 6-phosphate, urease cleaves urea to ammonia and carbon dioxide.
- **Relative substrate specificity :** Some enzymes act on structurally related substances. This, in turn, may be dependent on the specific group or a bond present. The action of trypsin is a good example for ***group specificity*** (***Refer Fig.8.7***). Trypsin hydrolyses peptide linkage involving arginine or lysine. Chymotrypsin cleaves peptide bonds attached to aromatic amino acids (phenylalanine, tyrosine and tryptophan). Examples of ***bond specificity***- glycosidases acting on glycosidic bonds of carbohydrates, lipases cleaving ester bonds of lipids etc.
- **Broad specificity :** Some enzymes act on closely related substrates which is commonly known as broad substrate specificity, e.g. hexokinase acts on glucose, fructose, mannose and glucosamine and not on galactose. It is possible that some structural similarity among the first four compounds makes them a common substrate for the enzyme hexokinase.

COENZYMES

The protein part of the enzyme, on its own, is not always adequate to bring about the catalytic activity. Many enzymes require certain non-protein small additional factors, collectively referred to as cofactors for catalysis. The cofactors may be organic or inorganic in nature.

The non-protein, organic, low molecular weight and dialysable substance associated with enzyme function is known as coenzyme.

The functional enzyme is referred to as ***holoenzyme*** which is made up of a protein part ***(apoenzyme)*** and a non-protein part ***(coenzyme)***. The term prosthetic group is used when a non-protein moiety is tightly bound to the enzyme which is not easily separable by dialysis. The term ***activator*** is referred to the inorganic cofactor (like Ca^{2+}, Mg^{2+}, Mn^{2+} etc.) necessary to enhance enzyme activity. It may, however, be noted that some authors make no distinction between the terms cofactor, coenzyme and prosthetic group and use them interchangeably.

Coenzymes are second substrates : Coenzymes are often regarded as the second substrates or ***co-substrates***, since they have affinity with the enzyme comparable with that of the substrates. Coenzymes undergo alterations during the enzymatic reactions, which are later regenerated. This is in contrast to the substrate which is converted to the product.

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Table 6.3 Coenzymes of B-complex vitamins

Coenzyme (abbreviation)	*Derived from vitamin*	*Atom or group transferred*	*Dependent enzyme (example)*
Thiamine pyrophosphate (TPP)	Thiamine	Aldehyde or keto	Transketolase
Flavin mononucleotide (FMN)	Riboflavin	Hydrogen and electron	L - Amino acid oxidase
Flavin adenine dinucleotide (FAD)	Riboflavin	"	D - Amino acid oxidase
Nicotinamide adenine dinucleotide(NAD^+)	Niacin	"	Lactate dehydrogenase
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	"	"	Glucose 6-phosphate dehydrogenase
Lipoic acid	Lipoic acid	"	Pyruvate dehydrogenase complex
Pyridoxal phosphate (PLP)	Pyridoxine	Amino or keto	Alanine transaminase
Coenzyme A (CoA)	Pantothenic acid	Acyl	Thiokinase
Tetrahydrofolate (FH_4)	Folic acid	One carbon (formyl, methenyl etc.)	Formyl transferase
Biocytin	Biotin	CO_2	Pyruvate carboxylase
Methylcobalamin; Deoxyadenosyl cobalamin	Cobalamin	Methyl/isomerisation	Methylmalonyl CoA mutase

*Details for each coenzyme are given in Chapter 7 on vitamins

Coenzymes participate in various reactions involving transfer of atoms or groups like hydrogen, aldehyde, keto, amino, acyl, methyl, carbon dioxide etc. Coenzymes play a decisive role in enzyme function.

Coenzymes from B-complex vitamins : Most of the coenzymes are the derivatives of water soluble B-complex vitamins. In fact, the biochemical functions of B-complex vitamins are exerted through their respective coenzymes. The chapter on vitamins gives the details of structure and function of the coenzymes (***Chapter 7***). In ***Table. 6.3***, a summary of the vitamin related coenzymes with their functions is given.

Non-vitamin coenzymes : Not all coenzymes are vitamin derivatives. There are some other organic substances, which have no relation with vitamins but function as coenzymes. They may be considered as non-vitamin coenzymes e.g. ATP, CDP, UDP etc. The important non-vitamin coenzymes along with their functions are given in ***Table 6.4***.

Nucleotide coenzymes : Some of the coenzymes possess nitrogenous base, sugar and

Table 6.4 Coenzymes not related to B-complex vitamins

Coenzyme	*Abbreviation*	*Biochemical functions*
Adenosine triphosphate	ATP	Donates phosphate, adenosine and adenosine monophosphate (AMP) moieties.
Cytidine diphosphate	CDP	Required in phospholipid synthesis as carrier of choline and ethanolamine.
Uridine diphosphate	UDP	Carrier of monosaccharides (glucose, galactose), required for glycogen synthesis.
S - Adenosylmethionine (active methionine)	SAM	Donates methyl group in biosynthetic reactions.
Phosphoadenosine phosphosulfate (active sulfate)	PAPS	Donates sulfate for the synthesis of mucopolysaccharides.

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phosphate. Such coenzymes are, therefore, regarded as nucleotides e.g. NAD^+, $NADP^+$, FMN, FAD, coenzyme A, UDPG, etc.

Coenzymes do not decide enzyme specificity : A particular coenzyme may participate in catalytic reactions along with different enzymes. For instance, NAD^+ acts as a coenzyme for lactate dehydrogenase and alcohol dehydrogenase. In both the enzymatic reactions, NAD^+ is involved in hydrogen transfer. The ***specificity of the enzyme is mostly dependent on the apoenzyme and not on the coenzyme.***

MECHANISM OF ENZYME ACTION

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 an activated state or transition state.

Enzymes lower activation energy : The energy required by the reactants to undergo the reaction 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 causes the reaction to proceed at a lower temperature. Enzymes do not alter the equilibrium constants, they only enhance the velocity of the reaction.

The role of catalyst or enzyme is comparable with a tunnel made in a mountain to reduce the barrier as illustrated in ***Fig.6.11***. The enzyme lowers energy barrier of reactants, thereby making the reaction go faster. The enzymes reduce the activation energy of the reactants in such a way that all the biological systems occur at body temperature (below 40°C).

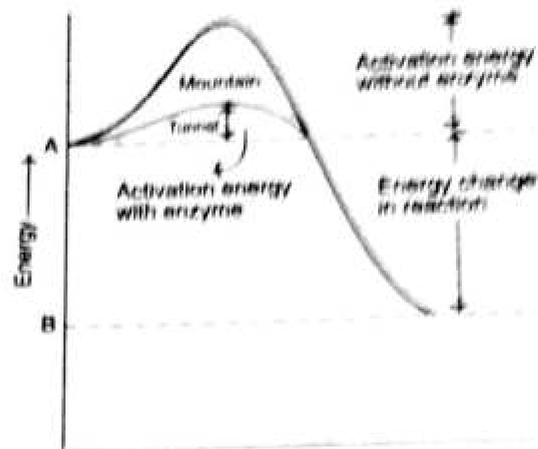

Fig. 6.11 : Effect of enzyme on activation energy of a reaction (A is the substrate and B is the product. Enzyme decreases activation energy).

Enzyme-substrate complex formation

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 the product formation (P).

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

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

Lock and key model or Fischer's template theory

This theory was proposed by a German biochemist, Emil Fischer. This is in fact the very first model proposed to explain an enzyme catalysed reaction.

According to this model, the structure or conformation of the enzyme is rigid. The substrate fits to the binding site (now active site) just as a key fits into the proper lock or a hand into the proper glove. Thus the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind. This model does not give any scope for the flexible nature of enzymes, hence the model totally fails to explain many facts of enzymatic reactions, the most important being the effect of allosteric modulators.

Induced fit theory or Koshland's model

Koshland, in 1958, proposed a more acceptable and realistic model for enzyme-substrate complex formation. As per this model,

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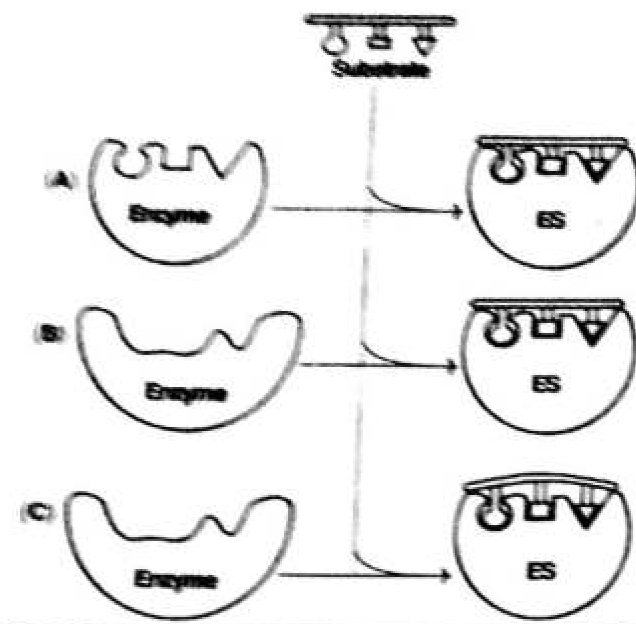

Fig. 6.12 : Mechanism of enzyme-substrate (ES) complex formation (A) Lock and key model (B) Induced fit theory (C) Substrate strain theory.

the active site is not rigid and pre-shaped. The essential features of the substrate binding site are present at the nascent active site. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate binding site. Further, due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis (**Fig.6.12**).

Induced fit model has sufficient experimental evidence from the X-ray diffraction studies. Koshland's model also explains the action of allosteric modulators and competitive inhibition on enzymes.

Substrate strain theory

In this model, the substrate is strained due to the induced conformation change in the enzyme. It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product.

In fact, a combination of the induced fit model with the substrate strain is considered to be operative in the enzymatic action.

MECHANISM OF ENZYME CATALYSIS

The formation of an enzyme-substrate complex (ES) is very crucial for the catalysis to occur, and for the product formation. It is estimated that an enzyme catalysed reaction proceeds 10^6 to 10^{12} times faster than a non-catalysed reaction. The enhancement in the rate of the reaction is mainly due to four processes :

1. Acid-base catalysis;
2. Substrate strain;
3. Covalent catalysis;
4. Entropy effects.

1. **Acid-base catalysis** : Role of acids and bases is quite important in enzymology. At the physiological pH, histidine is the most important amino acid, the protonated form of which functions as an acid and its corresponding conjugate as a base. The other acids are $-OH$ group of tyrosine, $-SH$ group of cysteine, and ε-amino group of lysine. The conjugates of these acids and carboxyl ions (COO^-) function as bases.

Ribonuclease which cleaves phosphodiester bonds in a pyrimidine loci in RNA is a classical example of the role of acid and base in the catalysis.

2. **Substrate strain** : Induction of a strain on the substrate for ES formation is discussed above. During the course of strain induction, the energy level of the substrate is raised, leading to a transition state.

The mechanism of lysozyme (an enzyme of tears, that cleaves β-1,4 glycosidic bonds) action is believed to be due to a combination of substrate strain and acid-base catalysis.

3. **Covalent catalysis** : In the covalent catalysis, the negatively charged (nucleophilic) or positively charged (electrophilic) group is present at the active site of the enzyme. This group attacks the substrate that results in the covalent binding of the substrate to the enzyme. In the serine proteases (so named due to the presence of serine at active site), covalent catalysis along with acid-base catalysis occur, e.g. chymotrypsin, trypsin, thrombin etc.

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4. **Entropy effect** : Entropy is a term used in thermodynamics. It is defined as the extent of disorder in a system. The enzymes bring about a decrease in the entropy of the reactants. This enables the reactants to come closer to the enzyme and thus increase the rate of reaction.

In the actual catalysis of the enzymes, more than one of the processes—acid base catalysis, substrate strain, covalent catalysis and entropy are simultaneously operative. This will help the substrate(s) to attain a transition state leading to the formation of products.

THERMODYNAMICS OF ENZYMATIC REACTIONS

The enzyme catalysed reactions may be broadly grouped into three types based on thermodynamic (energy) considerations.

1. **Isothermic reactions** : The energy exchange between reactants and products is negligible e.g. glycogen phosphorylase

Glycogen + Pi ⟶ Glucose 1-phosphate

2. **Exothermic (exergonic) reactions** : Energy is liberated in these reactions e.g. urease

Urea ⟶ $NH_3 + CO_2$ + energy

3. **Endothermic (endergonic) reactions** : Energy is consumed in these reactions e.g. glucokinase

Glucose + ATP ⟶ Glucose 6-phosphate + ADP

REGULATION OF ENZYME ACTIVITY IN THE LIVING SYSTEM

In biological system, regulation of enzyme activities occurs at different stages in one or more of the following ways to achieve cellular economy.

1. Allosteric regulation
2. Activation of latent enzymes
3. Compartmentation of metabolic pathways
4. Control of enzyme synthesis
5. Enzyme degradation
6. Isoenzymes

1. Allosteric regulation and allosteric inhibition

Some of the enzymes possess additional sites, known as allosteric sites (*Greek* : allo–other),

BIOMEDICAL / CLINICAL CONCEPTS

- ***The existence of life is unimaginable without the presence of enzymes—the biocatalysts.***
- *Majority of the coenzymes (TPP, NAD⁺, FAD, CoA) are derived from B-complex vitamins in which form the latter exert their biochemical functions.*
- *Competitive inhibitors of certain enzymes are of great biological significance. Allopurinol, employed in the treatment of gout, inhibits xanthine oxidase to reduce the formation of uric acid. The other competitive inhibitors include aminopterin used in the treatment of cancers, sulfanilamide as antibactericidal agent and dicumarol as an anticoagulant.*
- *The nerve gas (diisopropyl fluorophosphate), first developed by Germans during Second World War, inhibits acetylcholine esterase, the enzyme essential for nerve conduction and paralyses the vital body functions. Many organophosphorus insecticides (e.g. melathion) also block the activity of acetylcholine esterase.*
- *Penicillin antibiotics irreversibly inhibit serine containing enzymes of bacterial cell wall synthesis.*

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