

# ALMUSTAQBAL UNIVERSITY



## College of Medical and Health Techniques

### Medical Laboratories Techniques Departments

## Biochemistry Lectures for 2<sup>nd</sup> Year Students

(2 Credit Hrs. Theory + 2 Credit Hrs. Practice / Week = 3 Credit Unit

**Academic Year: 2024 - 2025**

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# **Enzymes, Properties, Functions and Enzymes**

## **Classifications**

**Date: Sep., 29<sup>th</sup> , 2024**

### **Learning Objectives:**

1. To study what are enzymes, their general properties.
2. What is meant by catalytic activity of enzymes?
3. Study the nomenclature and classification of enzyme as approved by International Union of Biochemistry (IUB).
4. To learn holoenzyme, apoenzyme, coenzyme and isoenzyme
5. To learn the mechanisms of enzyme catalyzed reactions and various factors affecting enzyme activity.
6. Study the role of metal ions in enzymes.
7. To know what is enzyme catalyzed reaction and how an enzyme functions by lowering the energy of activation.
8. Study lock-and-key theory and induced fit theory of mechanism of action of enzymes.
9. Enzyme specificity and learn different types of specificity.

### **Key Points:**

1. Enzymes are protein or conjugate protein or nucleoproteins (RNA complexes with proteins) catalysts utilized by essentially all mammalian cells in specific biochemical reactions in different organs of the body and which may also be physically located in different organelles and structures within a cell.
2. Enzymes speed up these reactions by decreasing their activation energy which are thermodynamically possible.
3. In addition to certain narrow ranges of pH, temperature, and protein and salt concentration, most enzymes require additional organic molecules and / or inorganic ions for optimal enzyme function.
4. An understanding of enzyme kinetics allows for laboratory measurement of plasma enzyme levels as well as determination of possible enzyme inhibition.
5. Damaged or dying cells within organs can release enzymes into the circulation; these plasma enzyme levels can be used clinically to develop a differential diagnosis of a patient with respect to specific organ disease and dysfunction

6. Many enzymes have isozymes, i.e., polypeptide chains that differ in amino acid sequence but have similar enzymatic activity. Some enzymes are composed of two or more different polypeptide chains giving rise to isozymes that differ in chain composition. In a number of diseases, specific isozymes become elevated in serum, facilitating diagnosis.

**Enzymes** are another important group of biomolecules synthesized by the living cells. They are **catalysts of biological systems (hence are called as biocatalysts), colloidal, thermo labile and protein in nature**. The striking characteristics of enzymes are their catalytic power and specificity. Actions of most enzymes are under strict regulation in a variety of ways. **Substances on which enzymes act to convert them into products are called substrates**.

### **Catalytic Activity of Enzymes:**

Enzymes have immense catalytic power and accelerate reactions at least a million times, by **reducing the energy of activation**. Before a chemical reaction can occur, the reacting molecules are required to gain a minimum amount of energy; this is called the **energy of activation**. It can be decreased by increasing the temperature of the reaction medium. But in human body which maintains a normal body temperature fairly constant, it is achieved by enzymes.

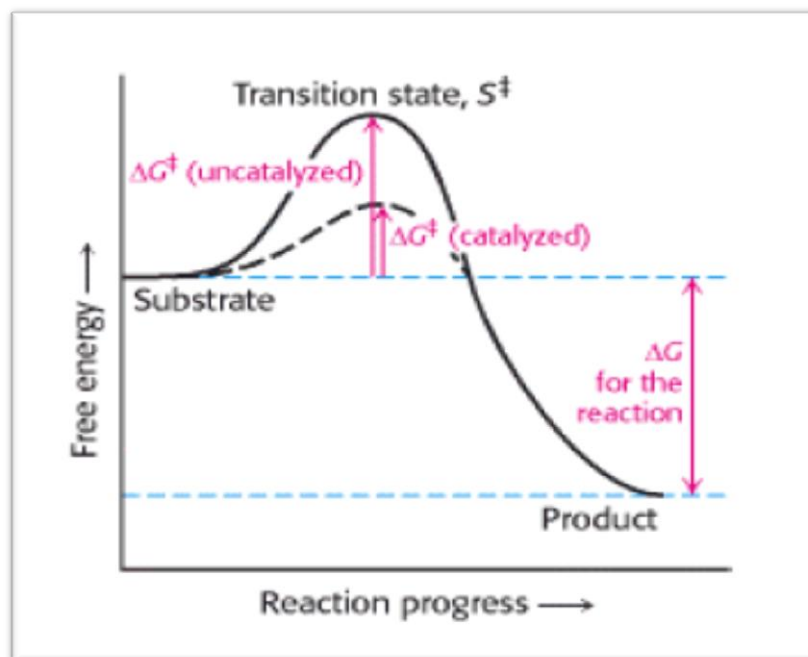
### **Enzymes and Free Energy Changes:**

The **free energy of activation** is the energy absorbed by reactant molecules before they have a chance to convert to products. The free energy of activation is a barrier to chemical reactivity. When this barrier is large, the rate of a chemical reaction is very slow. The lower the barrier, the faster is the reaction rate. The barrier exists for almost all chemical reactions because, for bonds to be broken. Enzymes *lower* the free energy of activation,  $E_{act}$ . For chemical reaction to proceed energy barrier must be overcome **(Fig.1)**



Energy is needed to transform substrate into “transition state (AB)”. Transition state has the highest “Free energy”, “Gibbs Free Energy of Activation ( $\Delta G^\ddagger$ )” of any component in the reaction pathway; “The free energy needed for a productive reaction is compared between an uncatalyzed and enzyme-catalyzed reaction. Enzymes have developed as extremely efficient catalysts because their active sites have evolved to bind transition states very

tightly. It is this tight binding, which stabilizes the transition state and lowers the free energy of activation.



**Fig. 1: Lowering of activation energy by enzymes**

### **Protein Nature of Enzymes:**

All physical and chemical properties of proteins with different techniques are applicable for enzymes because the enzymes are proteins in nature with some modification. All enzymes are proteins and may compose of non-protein compounds or cofactors. Therefore:

$$\text{Holoenzyme} = \text{Apoenzyme} + \text{Cofactors (Metal ions or Coenzyme)}$$

$$\text{Active Enzyme} = \text{Large M. wt.} + \text{Small M. wt.}$$

Some enzymes are active in the presence of apoprotein portion or apoenzyme only such as pancreatic ribonuclease. Others are composed coenzyme or metal ion (anion or cation) beside the apoprotein portion. The combination of cofactor plus the protein portion, the **apoenzyme**, forms the complete catalytic entity and is known as the **holoenzyme as indicated above equation**. In general with the exception of *ribozymes* which are few RNA molecules with enzymatic activity, **all the enzymes are protein in nature with large mol. wt.** Few enzymes are simple proteins while some are conjugated proteins. In such enzymes the **non-protein part is called prosthetic group or coenzyme** and the protein part is called as **apoenzyme**.

## **Enzymes are Biocatalysts:**

A catalyst is a substance that increase or accelerate the rate of a particular chemical reaction without itself being consumed. At the end of a catalyzed reaction, the catalyst appears unchanged in form and quantity, whereas the main reaction materials have undergoes transformation into new product. The acceleration may occur in solution and the process is called homogeneous catalysis. Catalysis on an insoluble surface is termed heterogeneous catalysis.

## **Characteristics of Enzymes:**

1. Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.
2. They are heat labile.
3. They are water-soluble.

## **Enzyme Co-factors:**

**Co-factors:** Organic cofactors are bound covalently or non-covalently to the apoenzyme include in metal ions or activated forms of water soluble vitamins. Covalently bound cofactors are sometimes referred to as **prosthetic groups**.

## **Coenzymes:**

1. Enzymes may be simple proteins, or complex enzymes, containing a non-protein part, called the **prosthetic group**. The prosthetic group is called the **co-enzyme**. It is heat stable.
2. The protein part of the enzyme is then named the **apoenzyme**. It is heat labile, these two portions combined together are called the **holo-enzyme**.
3. Co-enzymes may be divided into **two groups**
  - a. Those taking part in reactions catalyzed by **oxidoreductases** by donating or accepting hydrogen atoms or electrons.
  - b. Those co-enzymes taking part in reactions transfer groups **other than hydrogen**.

Since the involvement of coenzyme in a given reaction on a substrate is so intimate that coenzyme is often called as **co-substrate or second substrate**. Coenzymes can be **classified according to the group whose transfer they facilitate**. Based on this concept we may classify coenzymes as follows:

### **1. For transfer of groups other than hydrogen**

CoA-SH, Thiamine pyrophosphate (TPP), Pyridoxal phosphate, Folate coenzymes, Biotin and Cobamide coenzyme.

## 2. For transfer of hydrogen

$\text{NAD}^+$ ,  $\text{NADP}^+$  ; FMN, FAD ; Many coenzymes contain adenine, ribose and phosphate and are derivatives of adenosine monophosphate such as  $\text{NAD}^+$  and FAD.

Water-soluble vitamin	Coenzymes	Typical reaction type	Consequences of deficiency
<b>Thiamine, (B1)</b>	Thiamine pyrophosphate, TPP	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
<b>Riboflavine, (B2)</b>	FAD, FMN	Oxidation-reduction	Cheliosis and angular stomatitis (lesions of the mouth), dermatitis
<b>Nicotinamide, (B3)</b>	$\text{NAD}^+$ / $\text{NADH}$ $\text{NADP}^+$ / $\text{NADPH}$	Oxidation-reduction	Pellagra (dermatitis, depression, diarrhea)
<b>Pantothenic acid, (B5)</b>	Acetyl-CoA	Acyl-group transfer	Hypertension
<b>Pyridoxal, (B6)</b>	Pyridoxal-5-phosphate	Group transfer to or from amino acids	Depression, confusion, Convulsions
<b>Biotin</b>	Biotin-lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
<b>Folic acid</b>	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development, megaloblastic anemia
<b>Cyano-cobalamin, (B12)</b>	5-Deoxy-adenosyl cobalamin	Transfer of methyl groups; intra-molecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis

## **Metal Ions:**

Inorganic cofactors that include mainly metal ions are listed below:

$\text{Mg}^{2+}$	$\text{Ca}^{2+}$
$\text{Fe}^{2+}/\text{Fe}^{3+}$	$\text{Mn}^{2+}$
$\text{Zn}^{2+}$	$\text{Co}^{2+}$
$\text{Cu}^+/\text{Cu}^{2+}$	$\text{Cl}^-$

Metal ions often used for one or more of the following:

1. Binding substrates in the proper orientation
2. Mediating oxidation-reduction reactions
3. Electrostatically stabilizing or shielding negative charges (electrostatic catalysis).

Types of metal ion binding include:

- a. Metalloenzymes** contain tightly bound metal ions: (usually  $\text{Fe}^{+2}$ ,  $\text{Fe}^{+3}$ ,  $\text{Cu}^{+2}$ ,  $\text{Zn}^{+2}$ , or  $\text{Mn}^{+2}$ )
- b. Metal-activated enzymes** contain loosely bound metal ions: (usually  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{+2}$ , or  $\text{Ca}^{+2}$ )
- c.** Some prosthetic groups are **metallo-organic** compounds, e.g. heme

## **Role of Metal Ions in Enzymes**

The activity of many enzymes depends on the presence of certain metal ions such as  $\text{K}^+$ ,  $\text{Mg}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Cu}^{++}$ .

- 1. Metal activated enzymes:** In certain enzymes the metals **form a loose and easily dissociable complex**. Such enzymes are called **metal-activated enzymes**. The metal ions can be removed by dialysis or any other such method from the enzyme without causing any denaturation of apoenzyme.
- 2. Metalloenzymes:** The second category of metal enzymes is called as **metallo-enzymes**. In this case **metal ion is bound tightly to the enzyme and is not dissociated** even after several extensive steps of purification.



### **Metals play variety of roles such as:**

1. They help in either maintaining or producing (or both), active structural conformation of the enzyme,
2. Formation of enzyme-substrate complex,
3. Making structural changes in substrate molecule,
4. Accept or donate electrons.

### **Nomenclature and Enzyme Classification:**

Enzymes are generally named after adding the suffix '**ase**' to the name of the substrate, sometimes the name also includes a designation of the type of reaction catalyzed. **Examples** (Ribonucleic acid (RNA) is hydrolyzed by an enzyme called *ribonuclease*) and (Lactic acid is oxidized to pyruvic acid by an enzyme called *lactate dehydrogenase*, LD or LDH). **e.g.** enzymes acting on nucleic acids are known as *nucleases*, enzymes hydrolyzing dipeptides are called *dipeptidases*, lactase acts on the substrate lactose, and the products glucose and galactose are formed. Enzymes that hydrolyze starch (amylose) are termed as amylases; those that dehydrogenate the substrates are called dehydrogenases. These are known as the **trivial names** of the enzymes. Few exceptions such as trypsin, pepsin, and chymotrypsin are still in use. Further, **few enzymes exist in their inactive forms** and are called as **proenzymes or zymogens**, e.g. *pepsin* has *pepsinogen* as its zymogen. The zymogens **become active after undergoing some prior modification** in its structure by certain agents. **Many times the active form of enzyme acts on zymogen and catalyzes its conversion into active form and this process is called as autocatalysis.**

International Union of Biochemistry and Molecular Biology (IUBMB) in 1964, (modified in 1972 and 1978), suggested the IUBMB system of nomenclature of enzymes. In order to have a uniformity and unambiguity in identification of enzymes, **International Union of Biochemistry (IUB)** adopted a nomenclature system **based on chemical reaction type and reaction mechanism**. According to this system, enzymes are grouped in **six major classes**. Each enzyme is characterized by a code number (enzyme code number or EC No.) comprising four figures (digits) separated by points, As per this system, the name starts with the EC No followed by 4 digits.

1. **The first digit** represents the major class.
2. **The second digit** indicates the type of group involved in the reaction.



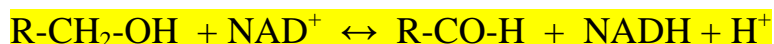
3. **The third** digit is the sub-sub class or subgroup which denotes the reaction more precisely indicating substrate on which the group acts.
4. **The fourth** digit gives the number of the particular enzyme or the serial number of the enzyme in the list.

Briefly, the four digits characterize class, sub-class, sub-sub-class and serial number of a particular enzyme.

### **The six classes of enzyme classification are:**

1. **Oxidoreductase:** Enzymes involved in oxidations and reductions of their substrates, e.g. *alcohol dehydrogenase, lactate dehydrogenase, xanthine oxidase, glutathione reductase, glucose-6-phosphate dehydrogenase*.
2. **Transferases:** Enzymes that catalyze transfer of a particular group from one substrate to another, e.g. *aspartate and alanine transaminase (AST/ALT), hexokinase, phosphoglucomutase, hexose-1-phosphate uridyltransferase, ornithine carbamoyl transferase*, etc.
3. **Hydrolases:** Enzymes that bring about hydrolysis, e.g. *glucose-6-phosphatase, pepsin, trypsin, esterases, glycoside hydrolases*, etc.
4. **Lyases:** Enzymes that facilitate removal of small molecule from a large substrate, e.g. *fumarase, arginosuccinase, histidine decarboxylase*.
5. **Isomerases:** Enzymes involved in isomerisation of substrate, e.g. *UDP-glucose, epimerase, retinal isomerase, racemases, triosephosphate isomerase*.
6. **Ligases:** Enzymes involved in joining together two substrates, e.g. *alanyl-t. RNA synthetase, glutamine synthetase, DNA ligases*.

**Example:** Alcohol Dehydrogenase: EC 1.1.1.1



Structure of the alcohol dehydrogenase protein (E.C.1.1.1.1) complexed with nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and zinc.

### **Six Classes of Enzymes – Enzyme Classification**

- EC 1. Oxidoreductases
- EC 2. Transferases
- EC 3. Hydrolases
- EC 4. Lyases
- EC 5. Isomerases
- EC 6. Ligases

The major six classes of enzyme classification were indicated below:

**Class 1: Oxidoreductases:** Transfer of hydrogen or addition of oxygen; e.g. Lactate dehydrogenase (NAD); Glucose-6-phosphate dehydrogenase (NADP); Succinate dehydrogenase (FAD); dioxygenases.

**Class 2: Transferases:** Transfer of groups other than hydrogen. Example, Aminotransferase. (Subclass: Kinase, transfer of phosphoryl group from ATP; e.g. Hexokinase).

**Class 3: Hydrolases:** Cleave bond and add water; e.g. Acetylcholine esterase; Trypsin.

**Class 4: Lyases:** Cleave without adding water, e.g. Aldolase; HMG-CoA lyase; ATP Citrate lyase. (Subclass: Hydratase; add water to a double bond).

**Class 5: Isomerases:** Intramolecular transfers. They include racemases and epimerases. Example, Triose phosphate isomerase.

**Class 6: Ligases:** ATP dependent condensation of two molecules, e.g. Acetyl-CoA carboxylase; Glutamine synthetase; PRPP synthetase.

Class	Reaction type	Important subclasses
1 Oxidoreductases	<p>Reaction: <math>A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}</math></p>	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	<p>Reaction: <math>A-B + C \rightleftharpoons A + B-C</math></p>	C <sub>1</sub> -Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	<p>Reaction: <math>A-B + H_2O \rightleftharpoons A-H + B-OH</math></p>	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	<p>Reaction: <math>A + B \rightleftharpoons A-B</math></p>	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	<p>Reaction: <math>A \rightleftharpoons \text{Iso-A}</math></p>	Epimerases <i>cis trans</i> Isomerases Intramolecular transferases
6 Ligases ("synthetases")	<p>Reaction: <math>B + A + XTP \rightleftharpoons A-B + XDP</math></p>	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

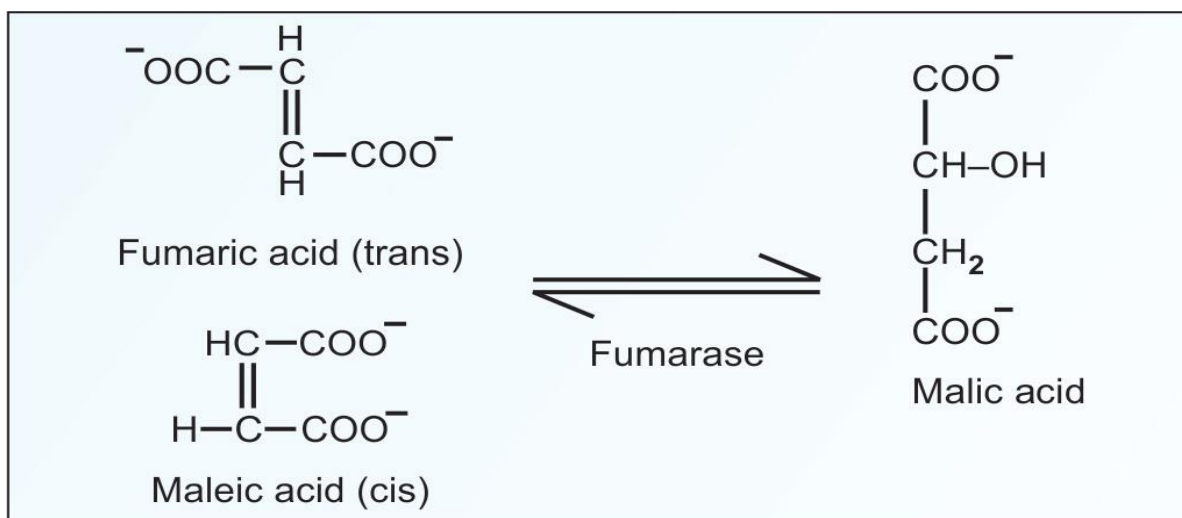
## Enzyme Specificity:

Enzyme specificity defines the capacity of protein catalysts to recognize and bind only one or a few molecules, the **substrate [S]**, excluding all others, a process referred to as **binding specificity**. Binding specificity permits many biochemical reactions to occur simultaneously within the same biological space. The specificity is of **three different types** namely:

1. **Stereochemical specificity,**
2. **Reaction specificity**
3. **Substrate specificity.**

## Stereo-specificity

**Optical Specificity.** *There can be many optical isomers of a substrate. However, it is only one of the isomers which acts as a substrate for an enzyme action*, e.g. for the oxidation of *D*- and *L*-amino acids, there are two types of enzyme which will act on *D*- and *L*-isomers of amino acids. Secondly there can be a product of enzyme action which can have isomers. However, it is only one kind of isomer which will be produced as a product, e.g. *Succinic dehydrogenase* while acting on succinic acid will give only fumaric acid and not malic acid which is its isomer.



## Example of stereo-specificity

Enzymes exhibit geometric specificity exemplified by the fumarase reaction, in which the Krebs cycle intermediate, fumarate (the *trans* isomer), but not malate (the *cis* isomer) undergoes hydration. This type of enzyme specificity is called **stereo-chemical specificity**. Lactate dehydrogenase, acting on pyruvate will form only L-lactate, but not the D-lactate,

## Reaction Specificity:

A substrate can undergo many reactions but in reaction specificity **one enzyme can catalyze only one of the various reactions**. For example, oxaloacetic acid can undergo several reactions but each reaction is catalyzed

by its own separate enzyme which catalyzes only that reaction and none of the others.

### **Substrate Specificity:**

The extent of substrate specificity varies from enzyme to enzyme. There are two types of substrate specificity viz, absolute specificity and relative specificity.

1. **Absolute specificity** is comparatively rare such as *urease* which catalyses hydrolysis of urea. Some enzymes are absolutely specific. For example, hydrolysis of urea to ammonia and carbon dioxide is catalyzed by urease. Urea is the only substrate for **urease**. Thiourea, though structurally similar to urea, will not act as the substrate for urease. Thus, these enzymes show absolute specificity.
2. **Relative substrate specificity** is further divided as:
  - a. **Group Dependent.** Examples of group specificity are trypsin, chymotrypsin. *Trypsin* hydrolyses the residues of only *lysine* and *arginine*, while *chymotrypsin* hydrolyses residues of only aromatic amino acids.
  - b. **Bond Specificity:** Bond specificity is observed in case of proteolytic enzymes, *glycosidases* and *lipases* which act on peptide bonds, glycosidic bonds and ester bonds respectively. Most of the proteolytic enzymes are showing group (bond) specificity. For example, trypsin can hydrolyze peptide bonds formed by carboxyl groups of arginine or lysine residues in any protein.

### **Enzyme Active Site:**

#### **What is the active site or catalytic site of an enzyme?**

Active site or catalytic site of an enzyme is:

- The region that binds substrate(s) and converts it into product(s);
- Relatively small part of the whole enzyme molecule;
- Three-dimensional entity formed by amino acid residues that can lie far apart in the linear polypeptide chain;
- Substrate binds in active site by multiple weak forces such as (Electrostatic interactions, Hydrogen bonds, Van der Waals bonds, Hydrophobic interactions, Reversible covalent bonds).
- Binding of substrate to active site gives the Enzyme-Substrate complex (ES) as shown in figure below;
- Catalytically active residues within the active site acts on substrate, forming "Transition state" and then products, which are released to product and the enzyme was liberated unchanged.

### Michaelis-Menten Theory:

1. In 1913, Michaelis and Menten put forward the **Enzyme-Substrate complex theory**. Accordingly, the enzyme (E) combines with the substrate (S), to form an enzyme-substrate (ES) complex, which immediately breaks down to the enzyme and the product (P) as in figure below

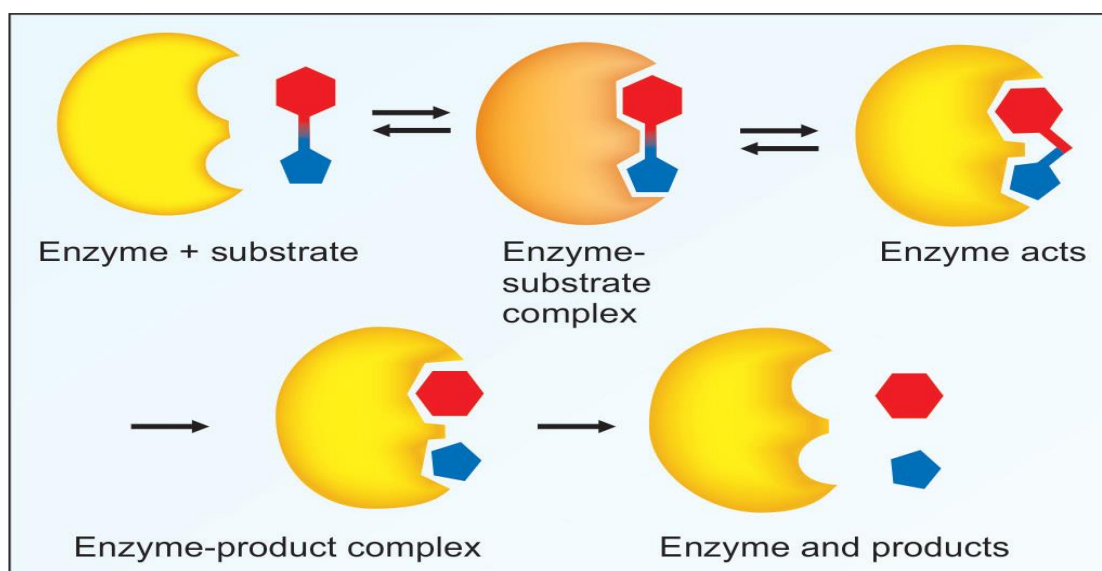


2. Alkaline phosphatase hydrolyzes a number of phosphate esters including glucose-6-phosphate. The active center of this enzyme contains a Serine residue, and the reaction is taking place in the following two steps:



Thus, the overall reaction is  $\text{Glucose-6-P} \longrightarrow \text{Glucose} + \text{Pi}$

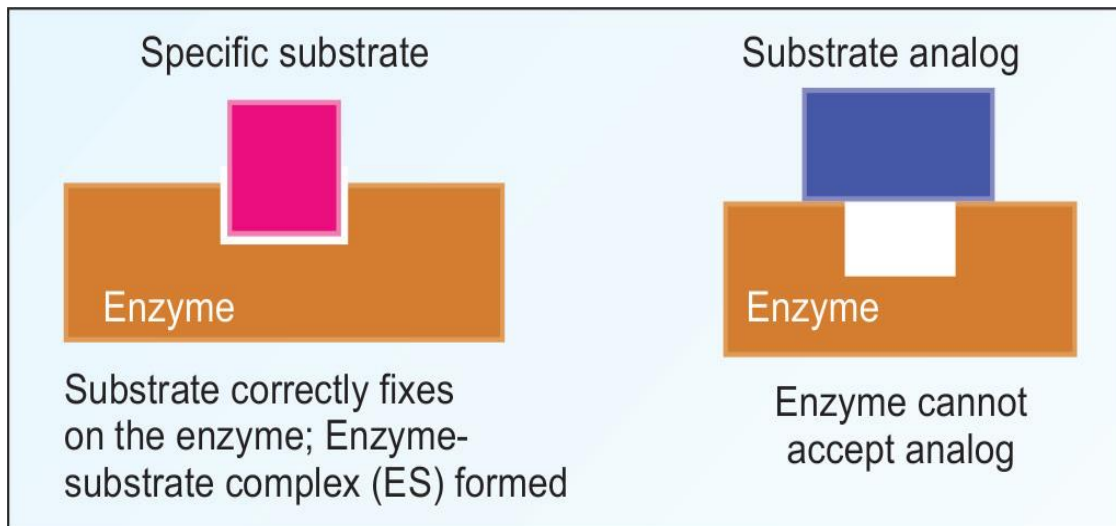
In this reaction mixture, the enzyme substrate complex, E-Serine-O-P, has been isolated.



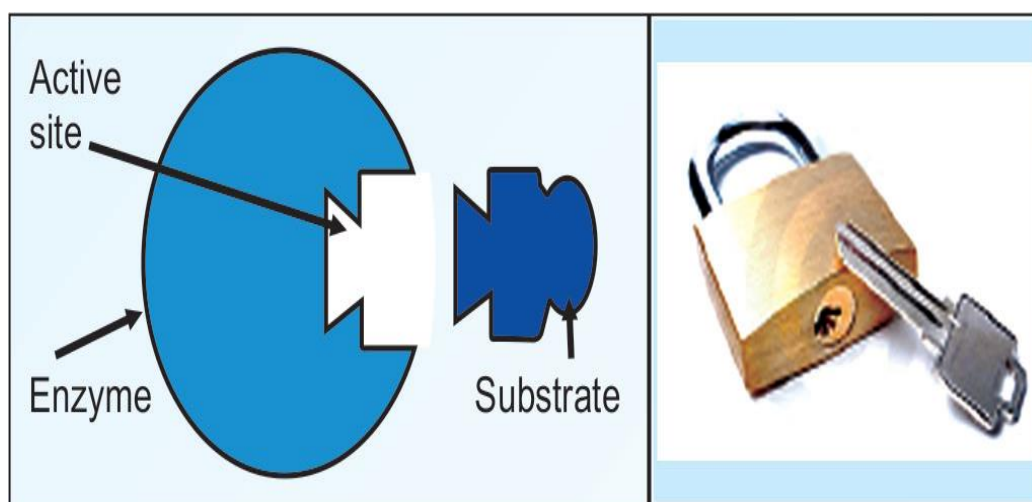
### Fischer's Template Theory

1. It states that the three-dimensional structure of the active site of the enzyme is complementary to the substrate.
2. Thus **enzyme and substrate fit each other**. Substrate fits on the enzyme, similar to **lock and key**. The lock can be opened by its own key only (Fig 1 and 2).
3. However, Fischer envisaged a rigid structure for enzymes, which could not explain the flexibility shown by enzymes.





**Fig. 1: Fischer's template theory**

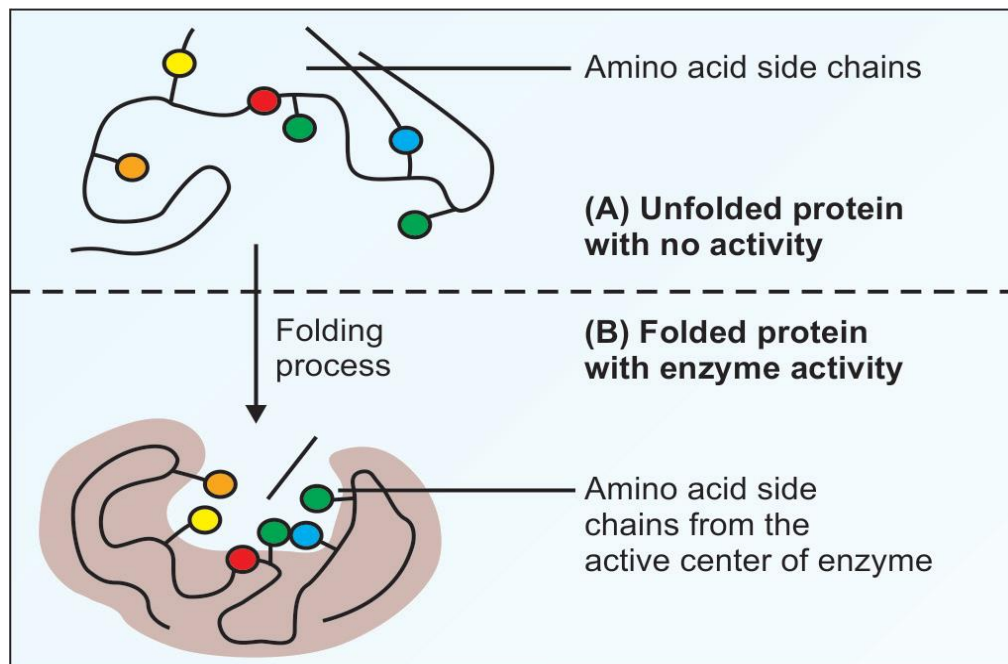


**Fig. 2: Enzyme and substrate are specific to each other. This is similar to key and lock (Fischer's theory)**

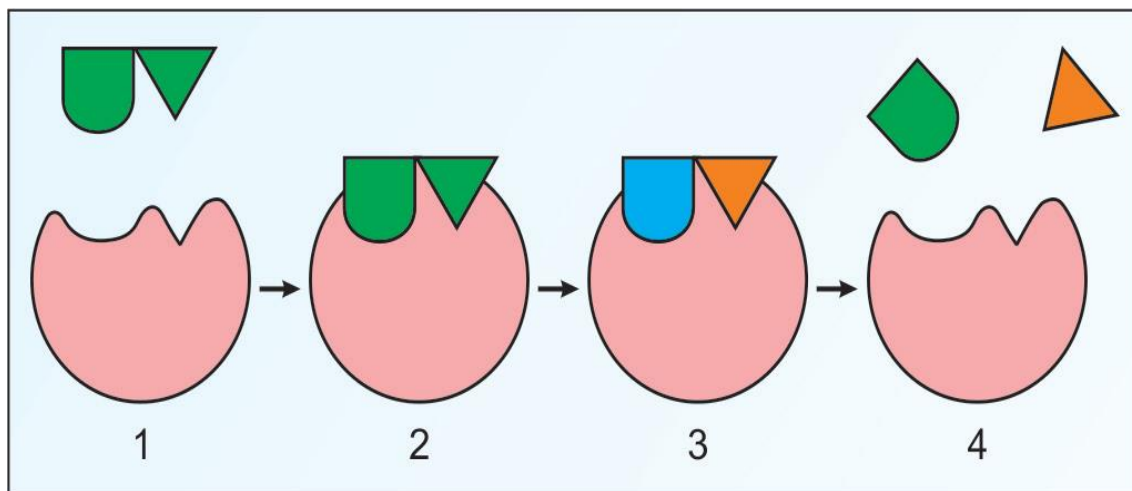
### **Koshland's Induced-Fit Theory**

1. Conformational changes are occurring at the active site of enzymes concomitant with the combination of enzyme with the substrate. At first, substrate binds to a specific part of the enzyme.
2. This leads to more secondary binding and conformational changes. The **substrate induces conformational changes in the enzyme**, such that precise orientation of catalytic groups is effected (Fig. 3 and 4). A simplified explanation is that a glove is put on a hand. At first, the glove is in a partially folded position, but hand can enter into it. When the hand is introduced, the glove is further opened. Similarly, conformational changes occur in the enzyme when the substrate is fixed.

3. When substrate analog is fixed to the enzyme, some structural alteration may occur; but reaction does not take place due to lack of proper alignment (Fig. 9). Allosteric inhibition can also be explained by the hypothesis of Koshland.



**Fig. 3: Correct alignment of amino acids in the**

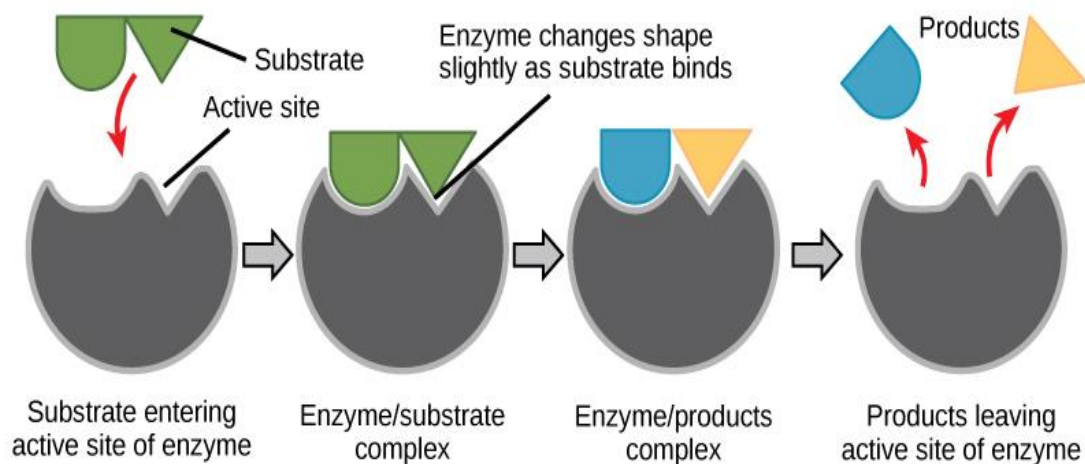


**Fig. 4 : Koshland's induced fit theory**

1. Enzyme has shallow grooves; substrate alignment is not correct.
2. Fixing of substrate induces structural changes in enzyme
3. Now substrate correctly fits into the active site of enzyme.
4. Substrate is cleaved into two products



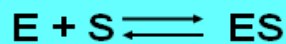
Enzymes contain a surface region referred to as the **active site** where binding and catalysis occur. It is a cleft or crevice in which are embedded specific groups, suitably oriented, which carry out the roles of binding, and bond-making or bond cleavage. The three-dimensional shape of the active site is a vital determinant in the recognition and specificity process. The **enzyme-substrate complex (ES)** is the adduct formed by the physical adsorption of the substrate to the active site. Enzyme-substrate complex formation requires specific alignment of atoms in the active site with atoms in the substrate molecule, Fig. 5 and 6



**Fig. 5 : Lock and key model**

## Enzyme Catalyzed Reaction

- The proper fit of a substrate (S) in an active site forms an enzyme-substrate (ES) complex.



- Within the ES complex, the reaction occurs to convert substrate to product (P).



- The products, which are no longer attracted to the active site, are released.
- Overall, substrate is converted to product.



**Fig. 6: Enzymatic reaction**