

# Enzymes

## Enzymes: basics

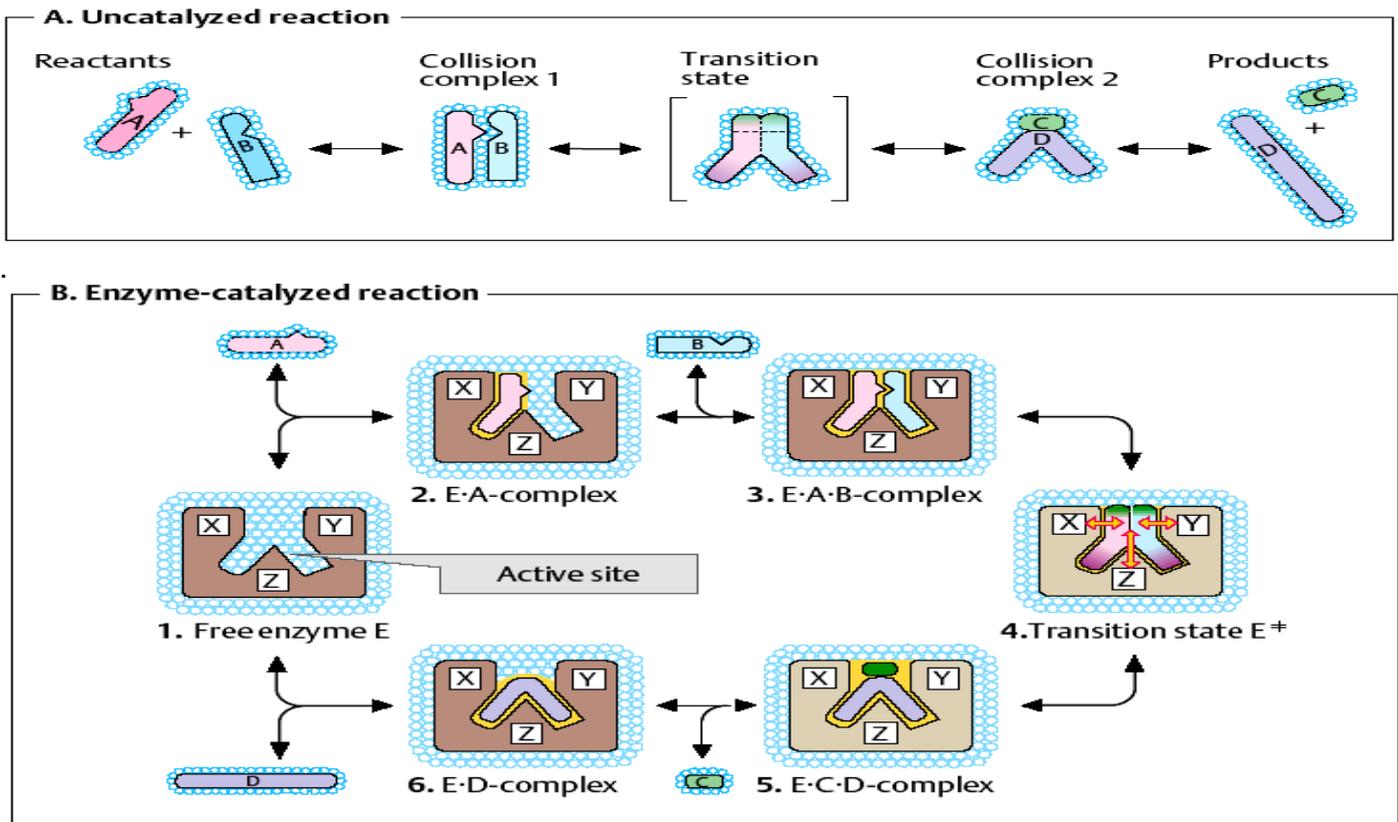
- More than 2000 different enzymes are currently known.
- The function of enzymes and other catalysts is to lower the activation energy,  $\Delta G^\ddagger$ , for a reaction and thereby enhance the reaction rate. The equilibrium of a reaction is unaffected by the enzyme.

**Enzymes:** are mostly soluble colloid protein compound acts as biological catalysts—i. e., substances of biological origin that accelerate chemical reactions. Each cell is equipped with its own genetically determined set of enzymes.

**Q/**What are the most important properties of enzymes?

1. Almost all enzymes are **proteins** (With the exception of a few catalytically active RNAs, the “*ribozymes*”).
2. Water soluble-nondialyzable-amphoteric compounds
3. Enzymes are active outside the cells (enzymes are active outside the cells)
4. Life depends on the existence of powerful and specific catalysts (the enzymes). Almost every biochemical reaction is catalyzed by an enzyme.
5. Enzymes are involved in **metabolic pathways &** many regulatory mechanisms that allow the metabolism to adapt to changing conditions.
6. Enzymes are highly effective **biological** catalysts, commonly enhancing reaction rates by a factor of  $10^5$  to  $10^{17}$ . E.g. each *Catalase* enzyme molecule can break 40 millions molecules of  $H_2O_2$ .

**Lysozyme:** Globular proteins with deep cleft across part of its surface. It present in tears & degrade polysaccharides of bacterial cells.



## Purification of Enzymes:

The intracellular enzymes should be liberated from the cell by:

- 1-Grinding the tissue with sand
- 2-Using homogenizer
- 3-By rupturing the cell wall by dehydration with acetone and then filtration to dissolve lipid membrane.
- 4-protein extraction by: 1- dialysis 2-suitable buffer 3-Adsorption on different surface active substances 4-By glycerol.

5-Recently the most important methods are electrophoresis & chromatography.

**Q/What are the types of enzymes?**

**A/** 1-digestive enzymes: Secreted by stomach, pancreas, & salivary glands

2-Food enzymes: exist in food

3-Exist in all cells &organs. Act to maintain normal function of cells.

**Q/Why all enzymes molecules have large size?**

**A/** A significant part of the energy used for enzymatic rate enhancements is derived from weak interactions (hydrogen bonds and hydrophobic and ionic interactions) between substrate and enzyme. The enzyme active site is structured so that some of these weak interactions occur preferentially in the reaction transition state, thus stabilizing the transition state. The need for multiple interactions is one reason for the large size of enzymes.

**A. Enzymatic activity:**

The catalytic action of an enzyme, its **activity**, is measured by determining the **increase in the reaction rate** under precisely defined conditions.

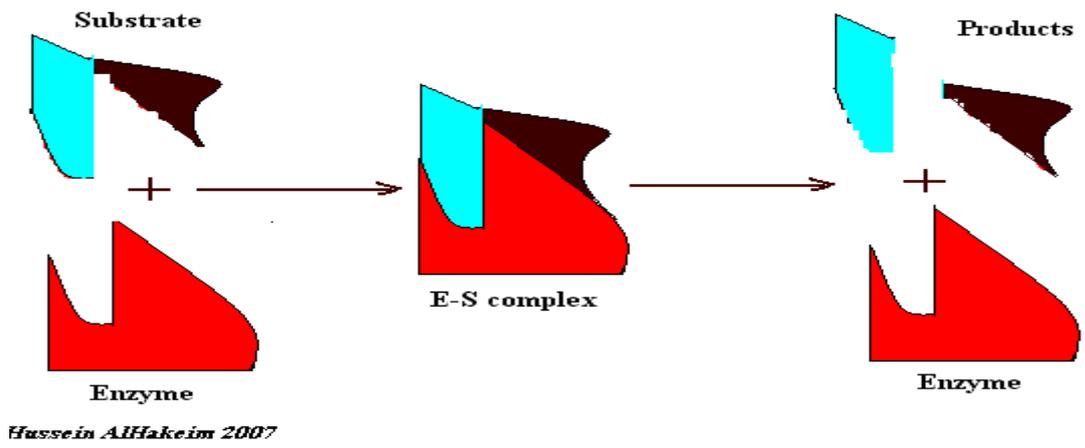
- International unit (IU) for enzymes activity: can be defined as: (The amount of enzymes which, under given assay conditions, will catalyze the conversion of (1 mmol of substrate per minute).

**Q/What are the mechanisms for the binding between active site of an enzyme & substrate?**

**A/**There are two main theories for the explanation of binding between active site of an enzyme & substrate:

**1-Lock & Key theory:**

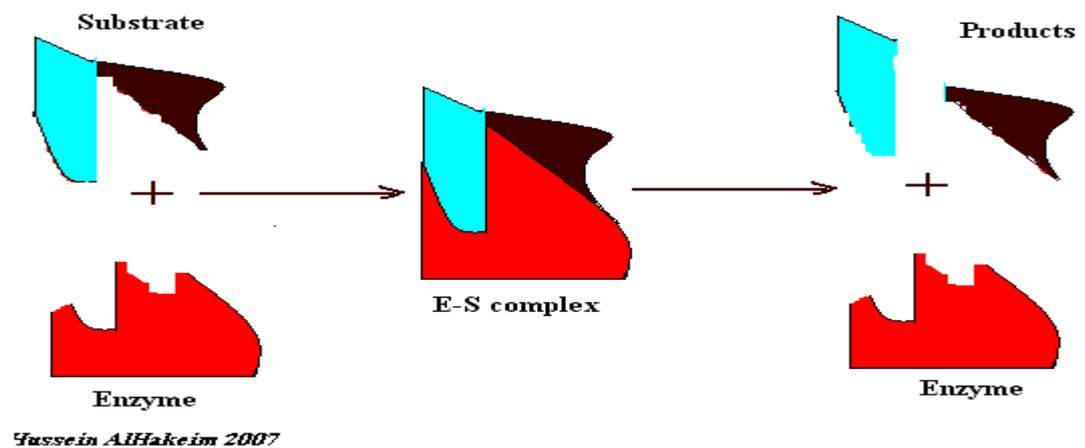
To fit the interaction between substrate & enzyme, the enzyme has amatching shpe for substrate at the active site:



*Figure: The interaction between substrate & enzyme according to key & lock theory*

**2-Induced Fit Theory (Hand &Glove):**

The active site of the enzyme changing to be complementary to that of the substrate only after the substrate is bound.



*Figure: The interaction between substrate & enzyme according to induced fit theory*

## C. Classification & Nomenclature of Enzymes:

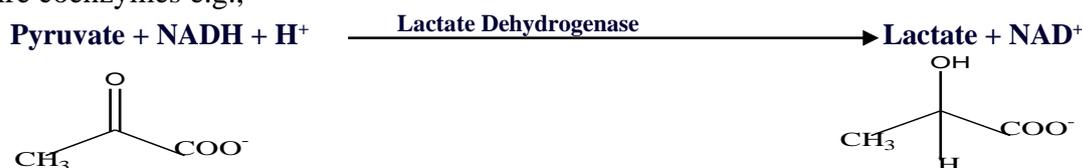
1. By using the suffix (-ase) after the reactant name. e.g. Urease, Arginase....etc.
2. Arbitrary (traditional) names. e.g. trypsin, & chemotrypsin...etc.
3. Enzymes are classified according to the type of reaction they catalyze (six types). All enzymes have the *Enzyme Catalogue* with a four-digit Enzyme Commission number (**EC number**). The first digit indicates membership of one of the six **major classes**. The next two indicate subclasses and subclasses. The last digit indicates where the enzyme belongs in the subclass.

For example:-lactate dehydrogenase has the EC number *1.1.1.27*(class 1, oxidoreductases; subclass 1.1, CH-OH group as electron *donor*; sub-subclass 1.1.1, NAD(P)<sup>+</sup> as electron *acceptor*).

#A system of *classification* has been developed that takes into account both their:1- *reaction specificity* and their 2-*substrate specificity*.

Enzymes with similar reaction specificities are grouped into each of the six major classes:

(Class 1)=**Oxidoreductases** catalyze the transfer of reducing equivalents from one redox system to another. They require coenzymes e.g.,



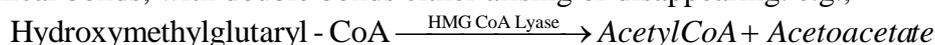
(Class 2)=**Transferases** catalyze the transfer of other groups from one molecule to another. Transferases generally require coenzymes.e.g.,



(Class 3)=**Hydrolases** are also involved in group transfer, but the acceptor is always a *water molecule*.e.g.,



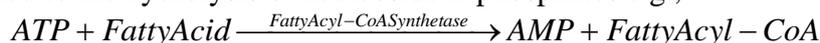
(Class 4)=**Lyases**, often also referred to as “synthases” catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing. e.g.,



(Class 5)=**Isomerases** move groups within a molecule, without changing the gross composition of the substrate.e.g.,



(Class 6)=**Ligases (Synthetases)**: The ligation reactions catalyzed by are energy-dependent and are therefore always coupled to the hydrolysis of nucleoside triphosphates.e.g.,

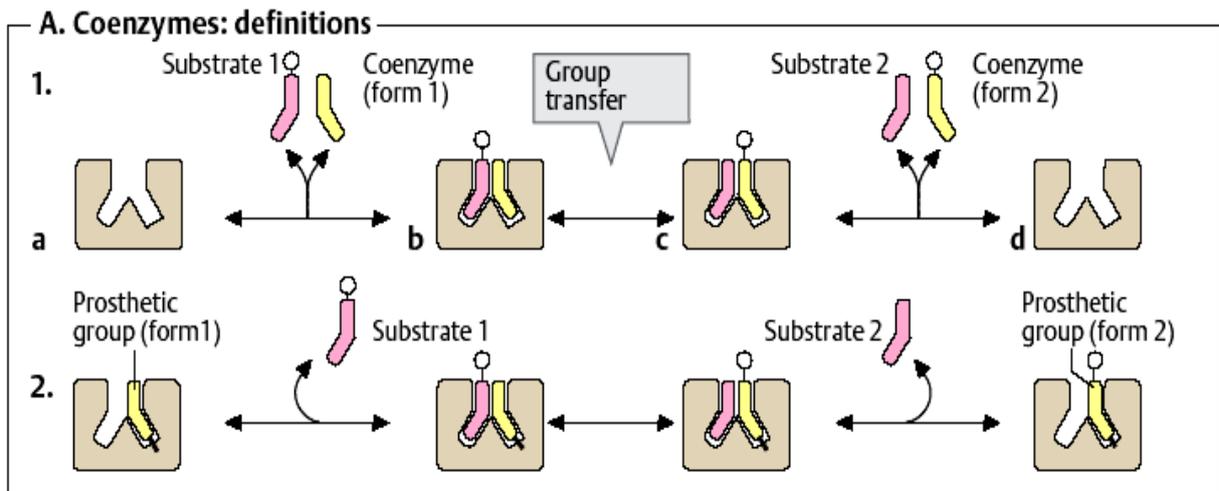


C. The enzyme classes

Class	Reaction type	Important subclasses
1 Oxidoreductases	<p>O = Reduction equivalent</p>	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases		C <sub>1</sub> -Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases		Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")		C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases		Epimerases cis trans Isomerases Intramolecular transferases
6 Ligases ("synthetases")		C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

**PROSTHETIC GROUPS, COFACTORS, & COENZYMES:**

Many enzymes contain small nonprotein molecules and metal ions that participate directly in substrate binding or catalysis. Termed prosthetic groups, cofactors, and coenzymes



Class	Prosthetic group	Example	Coenzyme	Examples of chemical groups transferred
Lipoproteins	Lipids	$\beta_1$ -Lipoprotein of blood	Biotin	$\text{CO}_2$
Glycoproteins	Carbohydrates	Immunoglobulin G	Coenzyme A	Acyl groups
Phosphoproteins	Phosphate groups	Casein of milk	5'-Deoxyadenosylcobalamin (coenzyme B <sub>12</sub> )	H atoms and alkyl groups
Hemoproteins	Heme (iron porphyrin)	Hemoglobin	Flavin adenine dinucleotide	Electrons
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase	Lipoate	Electrons and acyl groups
Metalloproteins	Iron	Ferritin	Nicotinamide adenine dinucleotide	Hydride ion (:H <sup>-</sup> )
			Pyridoxal phosphate	Amino groups
			Tetrahydrofolate	One-carbon groups
			Thiamine pyrophosphate	Aldehydes

**Prosthetic group:** The non-amino acid part (A coenzyme or metal ion) of a conjugated protein that contributes to the protein's function. Prosthetic groups are distinguished by their tight, stable incorporation into a protein's structure by covalent or noncovalent forces. A number of proteins contain more than one prosthetic group.

E.g., pyridoxal phosphate, flavin mononucleotide (FMN), flavin dinucleotide (FAD), thiamin pyrophosphate, biotin, and the metal ions of Co, Cu, Mg, Mn, Se, and Zn.

**Cofactors:** serve as recyclable shuttles—or group transfer reagents—that transport many substrates from their point of generation to their point of utilization. It binds in a transient (cofactors associate reversibly with enzymes or substrates), dissociable manner either to the enzyme or to a substrate such as ATP. Unlike the stably associated prosthetic groups, cofactors therefore must be present in the medium surrounding the enzyme for catalysis to occur.

Chemical moieties transported by coenzymes include methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides.

**Q/**what is the difference between coenzymes and prosthetic group?

**A/** the coenzymes need to move from enzyme to enzyme to carry out their function. While prosthetic group bind to enzymes. To be active for example: ATP is converted to ADP during hexokinase action but another phosphate transfer (catalyzed by another enzymes.) is required to convert ADP back to ATP.

**Apoenzyme + Prosthetic group or Cofactor** (Inactive) → **HoloEnzyme** (Active)

## THE ACTIVE SITE:

The **active site** is a cleft or pocket of three dimensional small portion of enzyme molecule at which the substrate contact with. The properties of the active sites:

1. Take small size of the enzyme molecules.
2. It's a group of sequences of a.a. folded by three dimensional structure.
3. It represents the catalytic site of the enzyme.
4. substrate binds with the enzyme by multiple weak attractions at the active site.
5. Active sites are cleft or cervices at which the substrate binds with.

## Factors influencing Enzyme catalyzed reactions:

\*Enzymes, being proteins, are usually denaturated by high temperature or by pH values more than a few units away from neutrality. This denaturation is in most cases irreversible

### 1/ Effect of temperature:

There is an optimal temperature at which the reaction is most rapid. Above or below this temperature, the reaction rate decreases sharply, generally due to heat denaturation of the enzymes.

- The optimal temperature approximates those of the environment of the cell. the enzymes of human has the optimal temperature of body =37.5°C
- When temperature increases, there is consequently loss of secondary, tertiary structure & parallel loss of biological activity.

**Note:** IF a cell extract having catalytic activity loses this activity when boiled (denaturated), the catalyst probably was an enzymes.

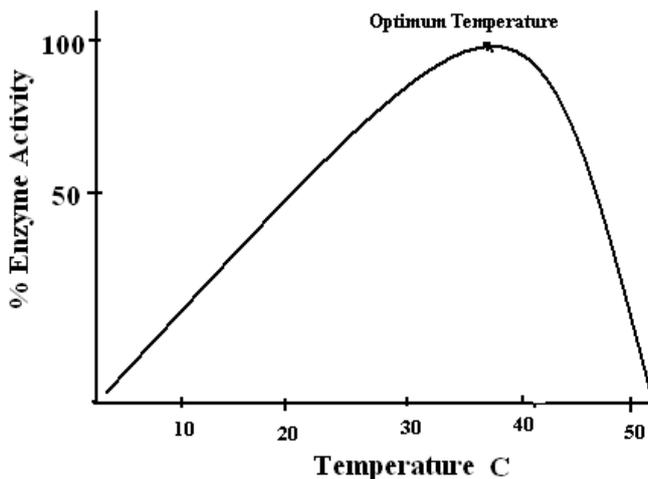
### 2/PH: Every enzyme has an optimum pH (or pH range) at which it has maximal activity.

pH changes affects the ionic state of the enzymes & substrate. Optimal activity of enzymes is generally observed between pH values of (5-9). However, a few enzymes e.g. pepsin are active at pH values outside this range. The shape of pH activity shape (Figure) determined by these factors

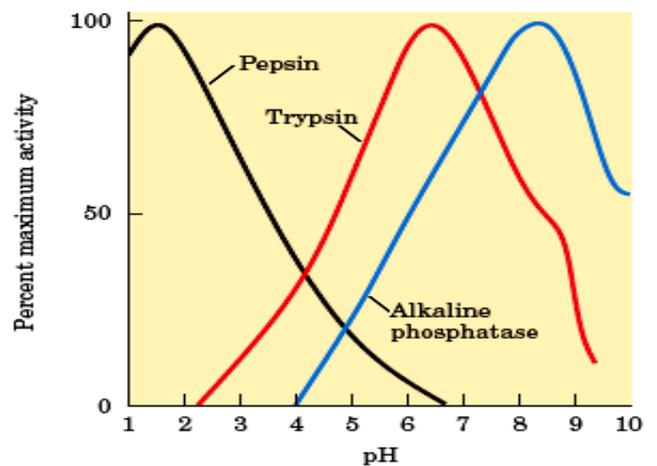
1/ Enzymes denaturation at high or low pH values

2/ Effects on the charged state of the substrate or enzymes.

The charge changes may affect by changing the structure or by changing the functional groups in substrate binding or catalysis.



**Figure:** Effect of temperature on the enzymes activity



**Figure:** The pH optima of some enzymes.

### 3/ Enzymes concentration: the initial velocity of an enzymes catalyzed reaction is directly proportional to the enzyme concentration.

Since enzymes concentration is not changed and only affect rates of the forward and back reaction, not rate constants, they cannot affect equilibrium constants ( $K_{eq}$ ), as follow:



$$Rate_1 = k_1[E][S] \quad , \quad Rate_{-1} = k_{-1}[E][P]$$

At equilibrium:  $K_{eq} = \frac{K_1}{K_{-1}} = \frac{[E][P]}{[E][S]} = \frac{[P]}{[S]}$

**4/ Substrate conc:** the velocity increases as the substrate conc. is increased up to a point where the enzymes. Is said to be saturated. For many enzymes, the rate of catalysis (v) varies with the substrate conc. [s] (v) is defined as the number of moles of product formed per second. (v) varies with the substrate conc. in a manner shown in the following figure:

The relationship between velocities,  $V_{max}$  substrate conc. was discussed previously.

**5/ Oxidation:** sulfhydryl (-SH) groups of many enzymes are essential for enzymes activity. Oxidation of these (SH) groups forming disulfide linkages (S-S) leads to conformational changes.

e.g: Dehydrogenates enzymes are active when (-SH) present in reduced form and inactive in (S-S) from (oxidized form).

e.g. Ribonuclease are active when (-S-S-) present & inactive when (-SH) present in the enzymes.

**6/Radiation:** Enzymes are highly sensitive to short wavelength (high energy) such as (UV, X,  $\beta$ , or  $\gamma$  – rays). This due to oxidation of the enzymes by peroxides formed by high energy radiation.