Enzymes

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☒ Classification of Enzymes

Enzymes are sometimes considered under two broad categories:

(a) **Intracellular enzymes** - They are functional within cells where they are synthesized.

(b) **Extracellular enzymes** – These enzymes are active outside the cell; all the digestive enzymes belong to this group.

The **I**nternational **U**nion of **B**iochemistry (**lUB**) appointed an Enzyme Commission in 1961.This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes.

Enzymes are divided into **six** major classes in that order. Each class on its own represents the general type of reaction brought about by the enzymes of that class. The six classes of the enzymes are as in the table 1 below:

	Classification	Distinguishing Feature
1.	**Oxidoreductases**	**$A_{red} + B_{ox} \rightarrow A_{ox} + B_{red}$ Hydrolysis reactions**
	Oxidases	**Use oxygen as an electron acceptor but do not incorporate it into the substrate**
	Dehydrogenases	**Use molecules other than oxygen (e.g., NAD^+) as an electron acceptor**
	Oxygenases	**Directly incorporate oxygen into the substrate**
	Peroxidases	**Use H_2O_2 as an electron acceptor**
2.	**Transferases**	**A-B+C → A+B-C Transfer of functional groups**
	Methyltransferases	**Transfer one-carbon units between substrates**
	Aminotransferases	**Transfer NH_2 from amino acids to keto acids**
	Kinases	**Transfer PO_3~ from ATP to a substrate**
	Phosphorylases	**Transfer PO_3~ from inorganic phosphate (P,) to a substrate**
3.	**Hydrolases**	**$A\text{-}B + H_2O \rightarrow A\text{-}H + B\text{-}OH$**
	Phosphatases	**Remove PO_3~ from a substrate**
	Phosphodiesterases	**Cleave phosphodiester bonds such as those in nucleic acids**

	Proteases	**Cleave amide bonds such as those in proteins**
4.	**Lyases**	**A(XH)-B → A-X+B-H Elimination reaction**
	Decarboxylases	**Produce CO_2 via elimination reactions**
	Aldolases	**Produce aldehydes via elimination reactions**
	Synthases	**Link two molecules without involvement of ATP**
5.	**Isomerases**	**A-B-C → A-C-B**
	Racemases	**Interconvert L and D stereoisomers**
	Mutases	**Transfer groups between atoms within a molecle**
6.	**Ligases**	**A+B+ATP →A-B +ADP +Pi Bond formation coupled with ATP hydrolysis**
	Carboxylases	**Use CO_2 as a substrate**
	Synthetases	**Link two molecules via an ATP-dependent reaction**

1- **Oxidoreductases** : Oxidation-reduction reactions are very common in biochemical pathways and are catalyzed by a broad class of enzymes called oxidoreductases. Whenever an oxidation-reduction reaction occurs, at least one substrate gains electrons and becomes **reduced**, and another substrate loses electrons and becomes **oxidized**.

2- **Transferases** : Transferases catalyze group transfer reactions—the transfer of a functional group from one molecule to another. If the transferred group is a high-energy phosphate, the enzyme is a kinase; if the transferred group is a carbohydrate residue, the enzyme is a *Glycosyltransferase; if it is a fatty acyl group, the enzyme is an *acyltransferase. Another subset of group transfer reactions consists of transamination. In this type of reaction, the nitrogen group from an amino acid is donated to an alpha - keto acid, forming a new amino acid and the alpha - keto acid corresponding to the donor amino acid. Enzymes catalyzing this last type of reaction are called *transaminases or aminotransferases. The coenzyme ***pyridoxal*** phosphate is required for all transaminases. When the physiologically important aspect of the reaction is the compound synthesized, the transferase may be called a **synthase**. For example, the enzyme commonly called

glycogen synthase transfers a glucosyl residue from UDP-glucose to the end of a glycogen molecule. Its systematic name is **UDP-glucose-glycogen glycosyltransferase**.

3- Hydrolases: In hydrolysis reactions, C-O, C-N, or C-S bonds cleaved by the addition of H2O in the form of OH^- and H^+ to the atoms forming the bond . The enzyme class names specify the group being cleaved (e.g., the enzyme commonly named chymotrypsin is a **protease**, a hydrolase that cleaves peptide bonds in proteins).

4- Lyases. The lyase class of enzymes consists of a diverse group of enzymes cleaving C-C, C-O, and C-N bonds by means other than hydrolysis or oxidation. Some of the enzymes catalyzing C-C bond cleavage are called ***aldolases**, ***decarboxylases** (when carbon dioxide is released from a substrate), and ***thiolases** (when the sulfur-containing nucleophile of cysteine or CoASH is used to break a carbon-carbon bond).

This broad class of enzymes also includes ***dehydratases** and many ***synthases**. Dehydratases remove the elements of water from two adjacent carbon–carbon bonds to form a double bond. Certain enzymes in this group, such as certain group transferases, are commonly called synthases when the physiologically important direction of the reaction favors the formation of a carbon–carbon bond (e.g., citrate synthase).

5- Isomerases : Many biochemical reactions simply rearrange the existing atoms of a molecule, that is, create isomers of the starting material . Enzymes rearranging the bond structure of a compound are called isomerases, whereas enzymes catalyzing movement of a phosphate from one atom to another are called **mutases**.

6- Ligases : Ligases synthesize C-C, C-S, C-O, and C-N bonds in reactions coupled to the cleavage of a high-energy phosphate bond in ATP or another nucleotide. ***Carboxylases***, for example, add CO2 to another compound in a

reaction requiring ATP cleavage to provide energy . Most carboxylases require the coenzyme *biotin*. Other ligases are named ***synthetases*** (e.g., fatty acyl CoA synthetase). Synthetases differ from the synthases mentioned under "lyases" and group "transferases" in that synthetases derive the energy for new bond formation from cleavage of high-energy phosphate bonds, and synthases use a different source of energy.

Cofactor & Coenzyme

Some enzymes depend for activity only their proteins structure [simple proteins], while others require one or more non-protein component [conjugated proteins]. Cofactor may be metal ion often act as additional cofactors (Zn2+, Mg2+, Mn2+ & Fe2+) . A metal ion cofactor can be bound directly to the enzyme or to a coenzyme . Coenzyme A small organic molecule, acting as a cofactor in a conjugated enzyme

- Organic molecules, heat stable
- Derived from water soluble vitamins or vitamin derivatives
- Usually function as intermediate carriers of functional groups of specific molecules

Examples of Metal as cofactor:

- ✓ Alcohol dehydrogenase **Zn^{++}**
- ✓ Kinases(phosphotransformer) **Mg^{++}**
- ✓ Cytochromes **Fe^{++}** or **Fe^{+++}**
- ✓ Cytochromeoxidase **Cu^{++}**

Examples of coenzyme and function:

- ✓ Pyridoxalphosphate - Amino transfer
- ✓ NAD+, NADP - in H+ transfer
- ✓ FAD, FMN - in H+ transfer

- ✓ COQ - in H+ transfer
- ✓ Coenzyme A - Acyl group transfer
- ✓ Biotin - Addition of CO2
- ✓ Thiamine pyrophosphate -Removal of CO2

•If the coenzyme is tightly bound to the enzyme molecule, it is called a prosthetic group.

Isoenzymes:

Isoenzymes are different forms of an enzyme that catalyze the same reaction in different tissues in the body

- They have slight variations in the amino acid sequences of the subunits of their quaternary structure

For example, lactate dehydrogenase **(LDH)**, which converts lactate to pyruvate, consists of five isoenzymes

• **LDH (LDH1, LDH2, LDH3, LDH4, LDH5)** .

All of these can be measured in blood, because LDH can be found in many tissues in the body total LDH is not specific for heart disease. Normally, the level of LDH-2 is greater than LDH-1.However, after a heart attack LDH-1 higher than LDH-2 , the LDH1 level rises within 24-72 hours after heart attack, peak in 3-4 days and returns to normal level through 14 days.

Creatine kinase **CK, CK-1 BB, CK-2 MB, CK-3 MM.**

- CK2 is undetectable (2%) in serum for healthy individuals, and elevated to 20% in the first 6-18 hrs after myocardial infarction.

Used as a earliest reliable indicator of myocardial infarction.

Medical Uses of Enzymes

- **Tissue plasminogen activator (TPA)** activates the enzyme *plasminogen* that dissolves blood clots

•Used in the treatment of MI (myocardial infarction) .

There is no direct test to measure urea in the blood

–**Urease** converts urea into ammonia, which is easily measured & is used as urea indicator

- **Blood Urea Nitrogen (BUN)** is used to measure kidney function

High urea levels in the blood indicate kidney malfunction

Enzyme inhibition

Enzyme are protein and they can inactivated by agents that denaturant them. The chemical substance which inactive the enzyme called as inhibitors and the process is called as enzyme inhibition .Inhibitors are sometimes referred to as negative modifier, they may be small inorganic ions, or organic substance. Enzyme inhibitions are classified under two major groups:-

1- Competitive inhibition (reversible).

2-Non-competitive inhibition (irreversible or reversible).

1- Competitive inhibition

When the active site of an enzyme is occupied by a substance other than the substrate of that enzyme, its activity is inhibited. The type of inhibition of this kind is known as a competitive inhibition. This is a type of reversible inhibition. In such

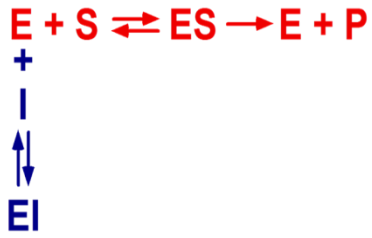
inhibition, both the ES and EI (enzyme inhibitor) complex are formed during reaction. However, the actual amounts of ES and EI will depend on:-

- ✓ Affinity between substrate and inhibitor / enzyme.
- ✓ Actual concentration (amounts) of substrate and inhibitor present.

So, the affinity of the substrate for the enzyme is progressively decreased with the increase in concentration of inhibitor, which lowering the rate of enzymatic reaction . However, when the concentration of substrate is increased, the effect of inhibitors can be reversed forcing it out from EI complex. Few example of competitive inhibitor are given in table.

Enzyme	Substrate	Competitive inhibitor
Lactate dehydrogenase	Lactate	Oxalate
Succinate dehydrogenase	Succinate	Malonate
HMG-CoA reductase	HMG-CoA	HMG

HMG (3-hydroxy-3-methyl-glutaryl-CoA reductase)



Example of Competitive inhibition in biological system – Used clinically

1- Allopurinol

- Uric acid is formed by oxidation of hypoxanthine by the enzyme “xanthine oxidase”.

- Allopurinol structurally resembles hypoxanthine, and thus by competitive inhibition inhibits the enzyme xanthine oxidase thus reducing uric acid formation.
- Allopurinol is used for treatment of gout.

2- MAO inhibitors

- The enzyme “monoamine oxidase” (MAO) oxidizes pressor amines , catecholamine, epinephrine and norepinephrine. They are best known as effective antidepressants, especially for treatment-resistant depression and atypical depression. They are also used to treat panic disorder, social anxiety disorder, Parkinson's disease, and several other disorders.
- Ephedrine and amphetamine have similar structure to catecholamine , thus they can competitively inhibit the enzyme “MAO” and prolong the action of pressor amines .

3- Methotrexate

A drug used for cancer therapy. Chemically it is 4- amino-N10 methyl folic acid. The drug resembles folic acid structurally. Hence it competitively inhibits the enzyme “folate redutase “and prevents formation of F-H4 (tetrahyrofolate).

4- Anti-histamines

inhibit histidine decarboxylase, which converts histidine to histamine

2-Noncompetitive inhibition

This is of different two types namely:-

1- Reversible

2- Irreversible

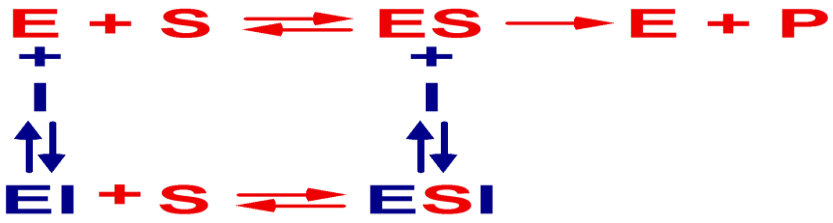
This occurs when the inhibitors not resembling the geometry of the substrate, do not exhibit mutual competition. Most probably, the sites of attachment of the substrate and inhibitor are different .the inhibitor binds reversibly with a site on enzyme other than the active site. So, the inhibitors may combine with both free

enzyme and ES complex. This probably brings about the changes in three dimensional structure of the enzyme inactivating it catalytically.

-If the inhibitor can be removed from its site of binding without affecting the activity of the enzyme, it is called as Reversible Noncompetitive inhibition.

However, if the inhibitor can be removed only at the loss of enzymatic activity, it is known as irreversible non-competitive inhibition.

Table gives the differences that are observed between competitive and noncompetitive inhibition.



☒ Differentiation of competitive and Noncompetitive inhibitions

Competitive inhibitions	Noncompetitive **inhibitions**
Reversible	Reversible or irreversible
Inhibitor and substrate resemble each other in structure	Dose not resemble
Inhibitor binds to the active site	Inhibitor dose not binds the active site
Inhibitor cannot bind with ES complex	Inhibitor can bind with ES complex
Lowers the substrate affinity to enzyme	Dose not change substrate affinity for the enzyme
Complex is E-I	Complex is E-I or E-S