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Enzymes

Introduction :

The chemical reactions which occur within organisms are collectively known as metabolism. It is estimated that over 1000 different reactions occur in an individual cell. There are two types :anabolism and catabolism

Anabolism	*Catabolism*
Building up processes in cell	**Breaking down process in cell**
Energy required, i.e. endogenic	**Energy is released, i.e. exogenic**
e.g. building of protein molecules	**e.g. deamination**

Both processes need enzymes to speed up the reaction

Enzymes are proteins that increase the rate of reaction by lowering the energy of activation .They catalyze nearly all the chemical reactions taking place in the cells of the body.

Not altered or consumed during reaction.

Reusable

Importance of enzymes

1 - Enzymes play an important role in Metabolism, Diagnosis, and Therapeutics .

2 - All biochemical reactions are enzyme catalyzed in the living organism. •

3 - Level of enzyme in blood are of diagnostic importance e.g. it is a good • indicator in disease such as myocardial infarction.

4 - Enzyme can be used therapeutically such as digestive enzymes.

Properties of enzyme

1. It speeds up chemical reactions but remain undestroyed at the end of the reaction.

2. It works in either direction. i.e. it catalyses the forward and backward reaction to the same extent.

3.An enzyme speeds up the rate of reaction by lowering the activation energy barrier

4.The activity of an enzyme is affected by temperature

5. It works rapidly and therefore is required in small quantity.

6. It is soluble in water and works in aqueous solution in living cells.

7. All enzymes operate only on specific substrates. Only substrates of particular shape will fit the active site of an enzyme.

8. All enzymes are proteins, some may have other associated molecules.

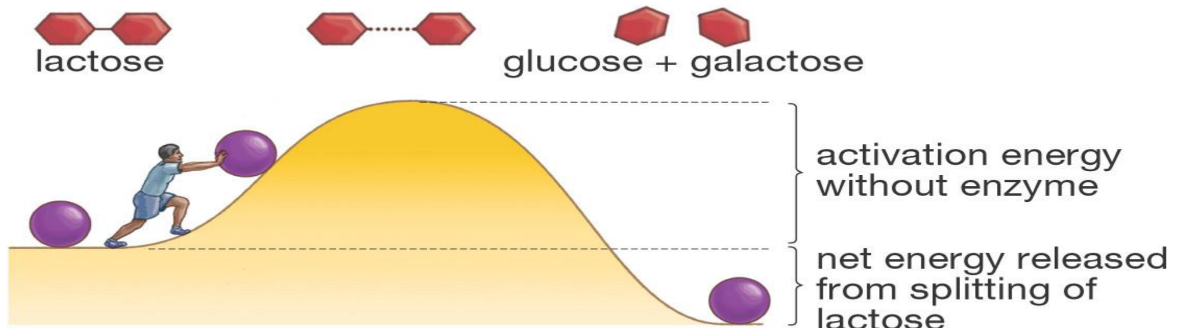
9. Enzyme may be denatured by excessive heat, extreme pH or various chemicals

10. Enzyme activity is affected by pH of the medium. It worked best at an optimum pH.

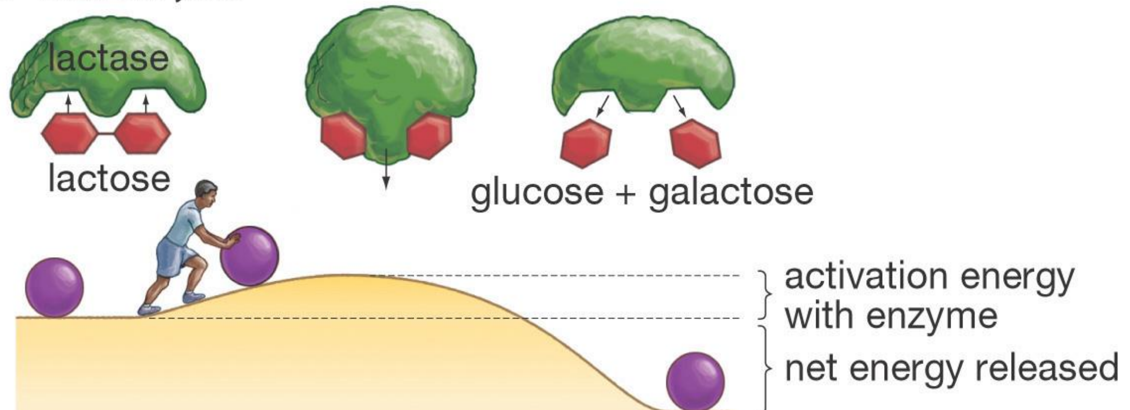
11. Some enzymes work efficiently only in the presence of appropriate co- factors

Activation energy: is the energy required to make the substances react.

As heat is often the source of activation energy, enzymes often dispense with the need for this heat and so allow reactions to take place at lower temperature

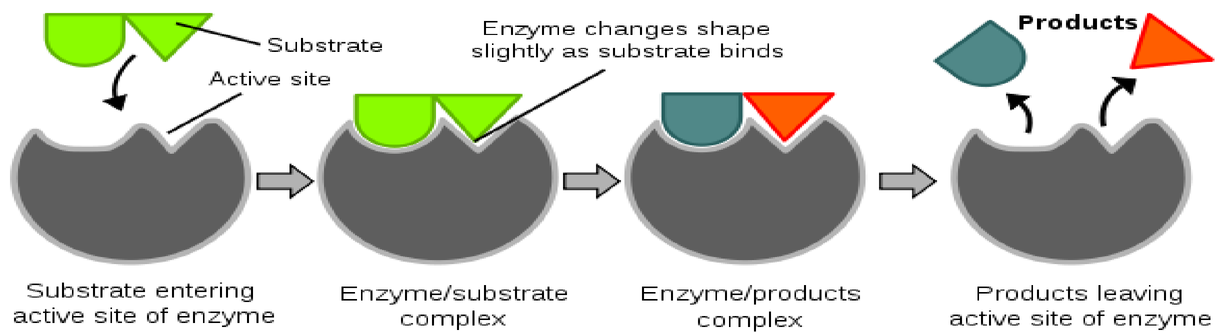


Enzymes Lower a Reaction's Activation Energy



<u>Active site:</u>

The area on the enzyme where the substrate or substrates attach to is called the <u>active site</u>. Enzymes are usually very large proteins and the active site is just a small region of the enzyme molecule.



In enzymatic reactions, the substance at the beginning of the process, on which an enzyme begins it's action is called <u>substrate</u>

SUBSTRATE

- The reactant in biochemical reaction is termed as substrate.
- When a substrate binds to an enzyme it forms an enzyme-substrate complex.

Substrate
Joins
Enzyme

Enzyme-Substrate Interactions:

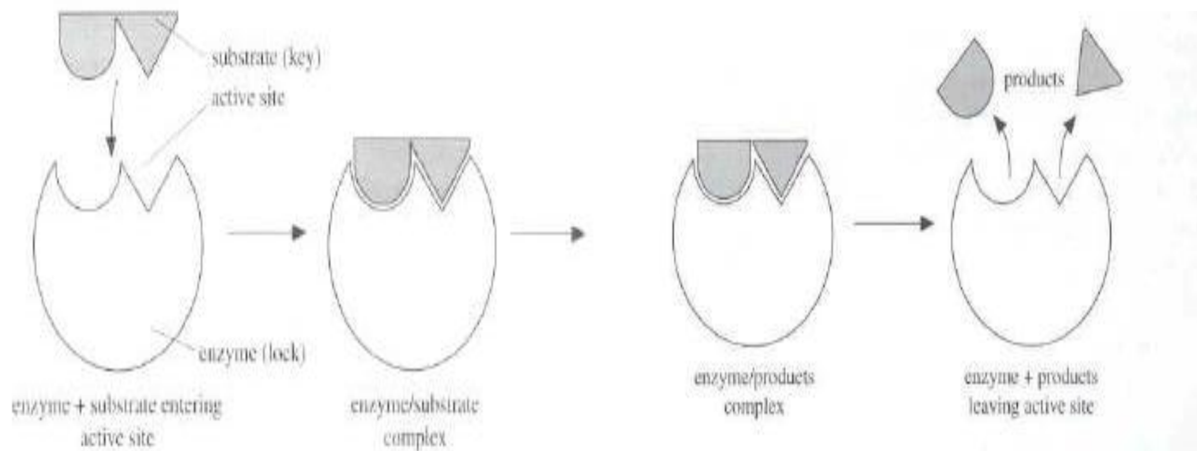
Formation of Enzyme substrate complex by:

Lock-and-Key Model

Induced Fit Model

Mechanism of Enzymatic Actions :

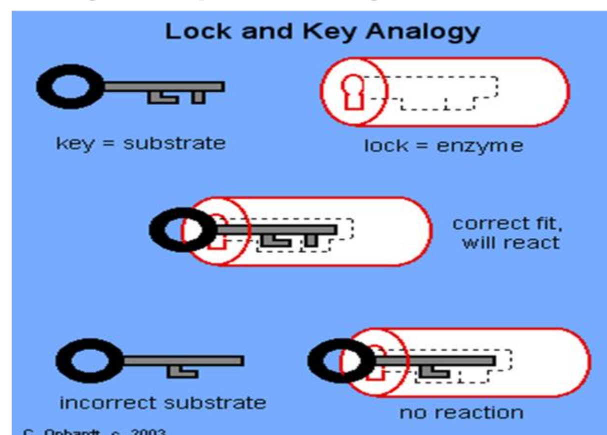
Lock and Key Hypothesis



Lock-and-Key Model

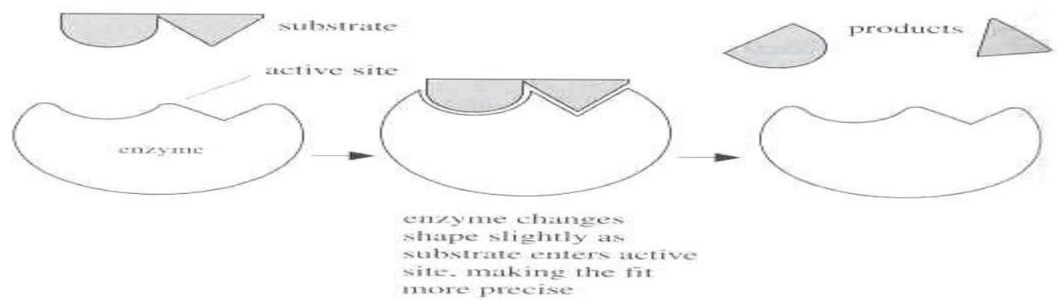
Only the specific substrate molecules with the right shape can fit into the active site to form enzyme-substrate complex, thus the substrate and enzyme molecules act as the lock and key respectively

This explains enzyme specificity
This explains the loss of activity when enzymes denature

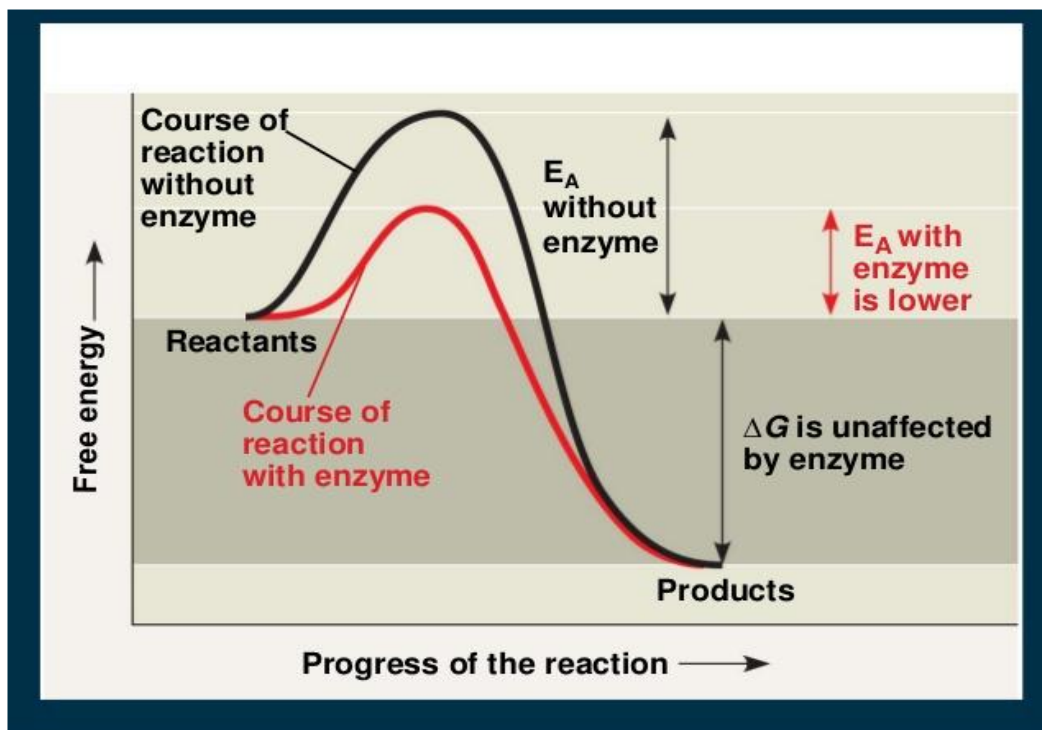


Induced Fit model

In the presence of substrate the active site of the enzyme may change shape to fit the substrate i.e. the enzyme is flexible and moulds to fit the substrate molecule. This theory is stated based on the nature of enzyme --- protein molecule is flexible enough to allow conformational changes

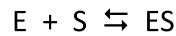


The induced fit theory of enzyme action

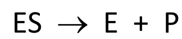


Enzyme Catalyzed Reactions

When a substrate (S) fits properly in an active site, an **enzyme-substrate (ES) complex** is formed:



Within the active site of the ES complex, the reaction occurs to convert substrate to product (P):

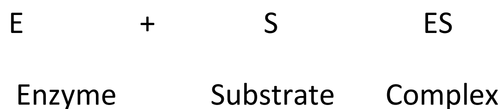


The products are then released, allowing another substrate molecule to bind the enzyme

this cycle can be repeated millions (or more times per minute).

Enzyme and substrate combine to form complex

Step 1:

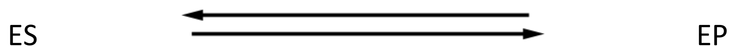


E + S ES

Enzyme Substrate Complex

Step 2:

An enzyme-product complex is formed.

ES  EP

Step 3:

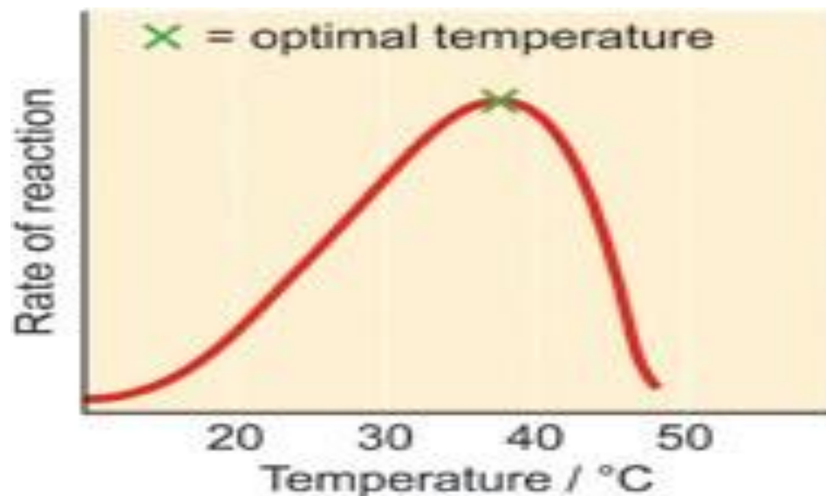
Product is released

What Affects Enzyme Activity?

- **Three factors:**
  1. **Environmental Conditions**
  2. **Cofactors and Coenzymes**
  3. **Enzyme Inhibitors**

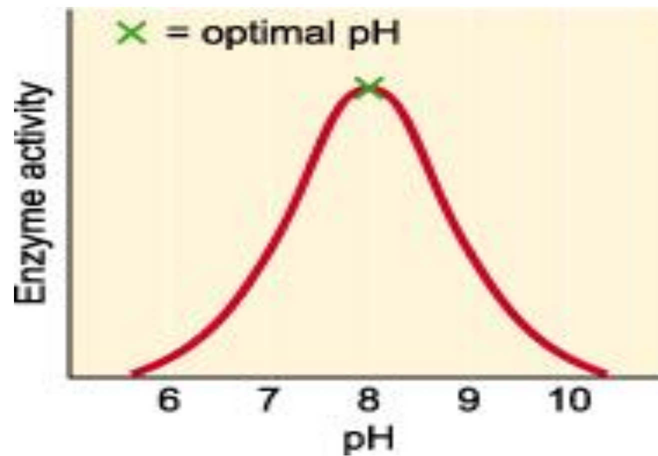
Temperature:

As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (37 °C) for the enzymes in human cells.
Above this temperature the enzyme structure begins to break down (**denature**) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.



pH

Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness



Effect of Product

Accumulation of products of the reaction causes the inhibition of enzyme activity for some enzymatic reactions, this form of control will limit the rate of formation of the product when the product is under used. In biological systems, however, the product is usually removed as it becomes a substrate for a succeeding enzyme in a metabolic pathwany.

Effect of Activators and Co-enzymes

The activity of many enzymes is dependent on the activators (metallic ions) like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, etc. and coenzymes for their activity .
In absence of these activators and coenzymes enzymes become functionally inactive.

Co-factors :It is a non- protein substance which is essential for some enzymes to function efficiently. There are three types of cofactor :

a) Activators : are substances which are necessary for the functioning of certain enzymes. They may assist in forming the E-S complex by moulding either the enzyme or substrate molecule into a more suitable shape. e.g. Enzyme thrombo kinase, which converts prothrombin into thrombin during blood clotting, is activated by Calcium ions ; Salivary amylase requires the presence of chloride ions before it converts starch into maltose

b) Coenzymes : are non-protein organic substances which are essential to the efficient functioning of some enzymes, but are not themselves bound to the enzyme.

Many coenzymes are derived from vitamins.

e.g. Nicotinamide adenine dinucleotide (NAD) act as a coenzyme to dehydrogenases by acting as a hydrogen acceptor in the Krebs Cycle

c) Prosthetic groups : are organic , non-protein molecules and bound to the enzyme themselves. e.g. Haem is an iron-containing prosthetic group. it may function as electron carrier and oxygen carrier in haemoglobin. It is also found in catalases and peroxidases, which catalyse the decomposition of hydrogen peroxide to water and oxygen

ENZYME KINETICS

The study of enzyme reaction rates and how they change in response to changes in experimental parameters is known as ***Enzyme kinetics.***

One of the key factors affecting the rate of a reaction catalyzed by an enzyme *in vitro (in laboratory) is the* substrate Concentration[S]

Why study enzyme kinetics (reaction rates)?

1- measurement of ***velocity = reaction rate***

2 - compare enzymes under *different conditions, or from different tissues or* organism**s**

3 - compare activity of same enzyme with different substrates (**understand *specificity)***

4 - measure ***amount or concentration of one enzyme in a mixture by its activity***

5 - measure ***enzyme purity (specific activity = amount of activity/amount of*** **protein**)

6 - study/distinguish different types of ***inhibitors mechanism***

7 - development of specific ***drugs (enzyme*** inhibitors)

BINDING = the essence of enzyme action

binding of substrate to form an ES complex

• Binding occurs at Active site of enzyme.

  • Subsequent chemical events can then occur.

• **Binding uses *multiple weak interactions:***

1. hydrogen bonds

2. salt links

3. van der Waals interactions

4. hydrophobic effect

Michaelis-Menten Equation

Enzyme catalyzed reactions occur in two stages as shown in the following equations

K1, K2 and K3 are rate constant. The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration.

The relationship between reaction rate and substrate concentration is described mathematically by the '**Michaelis- Menten** equation' as follows:

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

where, V0 = initial reaction velocity (is the rate of reaction as soon as enzyme and substrates are mixed). Vmax = maximum velocity (is observed when all active sites on the enzyme are filled with substrate).

Km = Michaelis-Menten constant, [is the substrate concentration, at which the reaction rate is half of its maximum velocity (Vmax)].

[S] = Substrate concentration

Every enzyme has the characteristics Vmax and Km

Significance of Km (Michaelis Constant)

. The Km value of an enzyme depends on the substrate and environmental conditions such as pH, temperature and ionic strength.

The Michaelis constant, **Km has two significances**:

1. Km provides a measure of the substrate concentration required for significant catalysis to occur.

2. It is a measure of the affinity of the enzyme for its substrate, a high Km indicates weak binding and a low Km indicates strong binding with its substrate.

Significance of Vmax (Maximal Velocity)

• The Vmax of a reaction is an index of the catalytic efficiency of an enzyme. The Vmax is useful in comparing the activity of one enzyme with that of another

Lineweaver-Burk Plot or Double-Reciprocal Plot

Lineweaver-Burk plots are used to obtain values for Vmax and Km. A more accurate method of determining values for Vmax and Km uses Lineweaver-Burk equation

. This equation is obtained by taking the reciprocal of the Michaelis-Menten equation.

ENZYME INHIBITION

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called **inhibitor**. Two general classes of inhibitors are recognized according to whether the inhibitor action is **reversible or irreversible**

1. Reversible inhibitor

2. Irreversible inhibitor.

Reversible Inhibitor

Reversible inhibitors bind to enzymes through **noncovalent** bonds and the activity of the enzyme is restored fully when the inhibitor is removed from the system.

• Different types of reversible inhibitors are:

i. Competitive or substrate analogue inhibitor

ii. Noncompetitive inhibitor

iii. Uncompetitive inhibitor.

Competitive or Substrate Analogue Inhibitor

• A competitive inhibitor is usually a structural analogue of the substrate. The chemical structure of the inhibitor (I) closely resembles that of the substrate (S) and binds to the enzyme at the active site, forming an EI complex rather than ES-complex

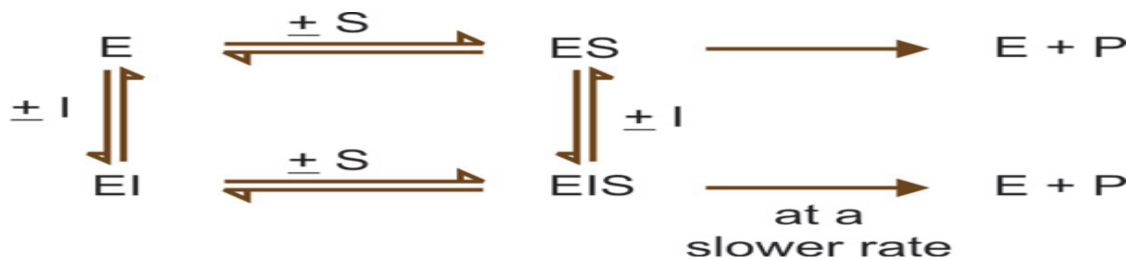
The classical example is the competitive inhibition of **succinate dehydrogenase by the malonate.** Succinate dehydrogenase is one of the enzymes of the citric acid cycle. Succinate dehydrogenase is inhibited by malonate which resembles succinate.

• Many drugs act as competitive inhibitors

Noncompetitive Inhibitors

As the name implies, in this type of inhibition no competition occurs between substrate and inhibitor. Inhibitor is usually structurally <u>different</u> from the substrate.

• It binds at a site on the enzyme molecule <u>other than the substrate-binding site</u> and thus there is no competition between inhibitor and substrate.



Ascaris parasites (worm) contain **pepsin and trypsin inhibitors, which inhibit noncompetitively**

action of pepsin and trypsin, that is why ascar is worm is not digested in human intestine.

For noncompetitive inhibition, the **Km value is unchanged while Vmax is lowered**

Uncompetitive Inhibitor

Uncompetitive inhibitor can bind only to the <u>enzyme-substrate (ES) complex</u>. It does not have affinity for free enzyme. Uncompetitive inhibitor **decreases both Vmax and Km.**This form of inhibitor is rare with single substrate but more common in multiple substrate reaction

Irreversible Inhibitor •

An irreversible inhibitor binds with an enzyme tightly **covalently and forms a stable complex.** •

• An irreversible inhibitor cannot be released by dilution or dialysis or simply by •
increasing the concentration of substrate.

• Irreversible inhibitors can be divided into three categories: •

i. Group specific inhibitors •

ii. Substrate analogue inhibitor or affinity labels •

iii. Suicide inhibitor or mechanism based inactivation. •

ENZYME CLASSIFICATION

According to the IUB system, enzymes are classified into six major classes as follows:

1. EC-1 : Oxidoreductases

2. EC-2 : Transferases

3. EC-3 : Hydrolases

4. EC-4 : Lyases

5. EC-5 : Isomerases

6. EC-6 : Ligases.

EC-1 Oxidoreductases Those enzymes that catalyze oxidation-reduction reactions, are included in this class .Enzymes in this category include :

• Dehydrogenases

• Reductases

• Oxidases

• Peroxidases

EC-2 Transferases

Those enzymes that catalyze the transfer of a group such as, ***amino, carboxyl, methyl or phosphoryl, etc.*** *from one* molecule to another are called transferases.

Some common enzymes in this category include :

• Amino transferase or transaminase

• Kinase

• Transcarboxylase

EC-3 Hydrolases

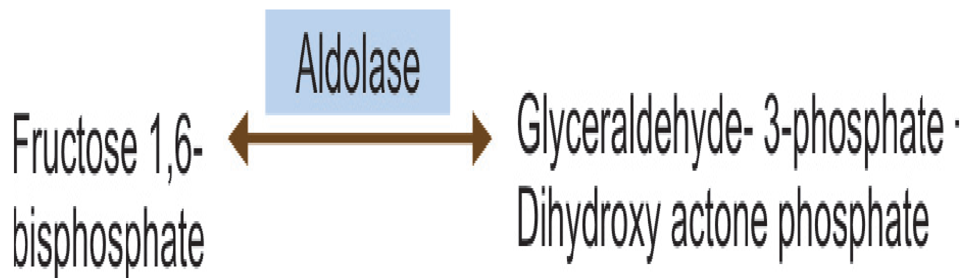
Enzymes of this class catalyze the cleavage of **C-O, C-N, C-C and some other bonds with the addition of** water.Some common enzymes in this category are:

• Acid phosphatase

• All digestive enzymes like α-amylas

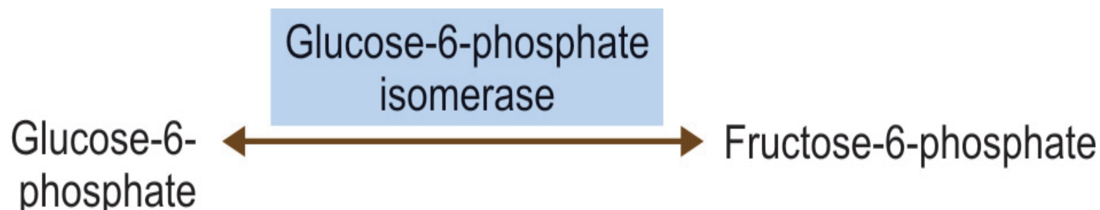
EC-4 Lyases

Lyases catalyze the cleavage of **C-O, C-C and C-N bonds** by means other than hydrolysis or oxidation, giving rise to compound with double bonds or catalyze the reverse reaction, by the addition of group to a double bond. synthase,(not synthetase of group EC-6) is used in the name.



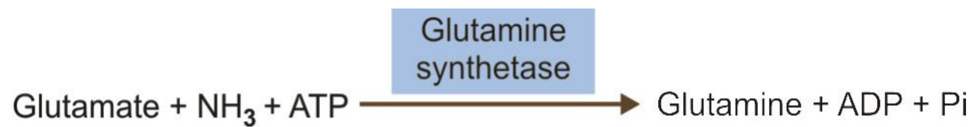
EC-5 Isomerases

Isomerases catalyze intramolecular structural rearrangement in a molecule. They are called **epimerases, isomerases or mutases,**



EC-6 Ligases (Synthetases)

Ligases catalyze the joining of two molecules coupled with the hydrolysis of ATP.



Reference ; Essential of Biochemistry by Naik_P. 2012