Chapter five

Enzymes

Enzymes:

An enzyme is a biomolecule that catalyzes chemical reaction under mild physiological conditions, which increase the rate of reaction by lowering the activation energy without any change in the overall process. Enzymes are produced by the living organism and are present in very small amounts in various cells. Almost all the functions of the body such as digestion, breathing, synthesis, breakdown of carbohydrates, fats, and proteins are catalyzed and controlled by specific enzymes.

The molecule upon which an enzyme acts is called the Substrate [S] and Enzyme will convert the Substrate into Enzyme-Substrate complex (E-S) then to product [P].

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

Proteins nature of enzymes:

All enzymes are proteins with the exception of a small group of RNA molecules, called ribozymes. Enzymes may be:

1- Simple proteins

The enzyme contains only amino acids in their structure.

2- Conjugated proteins

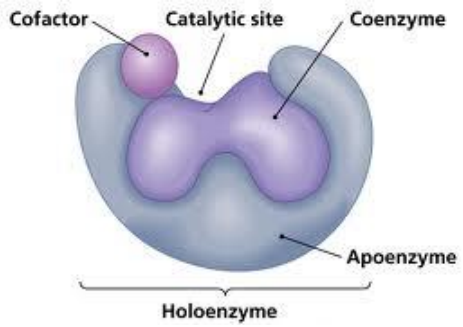
These are enzymes contain proteins linked with a non protein part, the protein part is called apoenzyme and the non protein part is called the prosthetic group (cofactor). The complete structure of apoenzyme and prosthetic group is called holoenzyme.

Holoenzyme = Protein part + Non protein part

Apoenzyme Prosthetic group (Cofactor)

Enzymes require the presence of cofactors to their function. Cofactors may be inorganic (metal) ions such as Zn+2, Fe+2,… ions or organic molecules called coenzymes (almost

coenzymes are derivatives of vitamins. Like NADH, NADPH, and FADH). In the absence of the cofactor, enzymes do not show biologic activity



Properties of enzymes:

1- All enzymes are proteins with the exception of a small group of RNA molecules, ribozymes.

2- They accelerate the reaction, but required only in very small amounts.

3- They have enormous power for catalysis.

4- Enzymes are highly specific for their substrate.

5- Enzymes possess active sites at which interaction with substrate take place.

6- Enzymes lower activation energy.

7- They form substrate complex as intermediates during their action.

8- Some enzymes are regulatory in function.

Localization of enzymes:

Enzymes are localized either:

1- Intracellularly.

2- Extracellularly.

Intracellularly:

Many enzymes usually act within the cells in which they are produced and hence are called intracellular enzymes or endoenzymes, e.g., glycolysis enzymes, Krebs cycle enzymes…..

Extracellularly:

Certain enzymes are secreted out from the cell and catalyze reactions outside the cell origin: and hence are known as extracellular enzymes or exoenzymes. E.g., digestive enzymes: α-amylase, pepsin, trypsin, lipase.

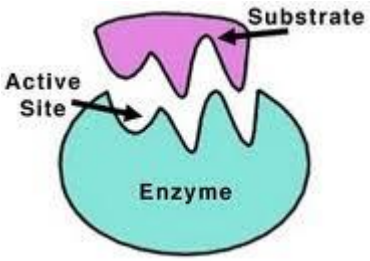
Mechanism of enzyme action:

The substrate [S] binds to enzyme [E] on a specific sites called as the active sites to form enzyme-substrate complex [E-S], which then E-S complex converted to enzyme-product complex [E-P] which further dissociated to form product [P] and free [E]. Free enzyme combine with another molecule of substrate and form product in a similarly way.

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

Active site:

Active site is a region or a place within the enzyme that combined with the substrate and made up of amino acids.



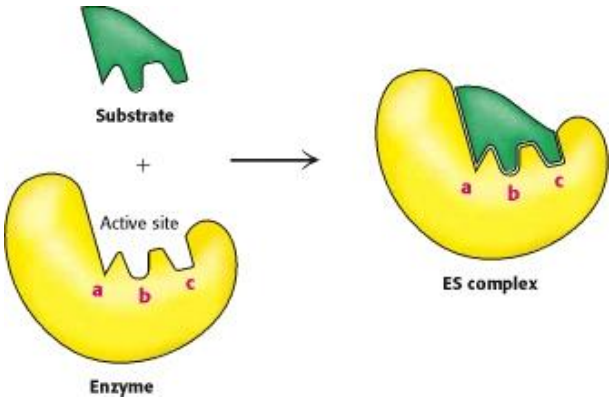
Models of enzyme-substrate complex formation:

Two models for substrate binding to the active site of the enzyme have been proposed to explain the specificity that an enzyme has for its substrate:

1- Lock and key models (Rigid template model or Emil Fisher):

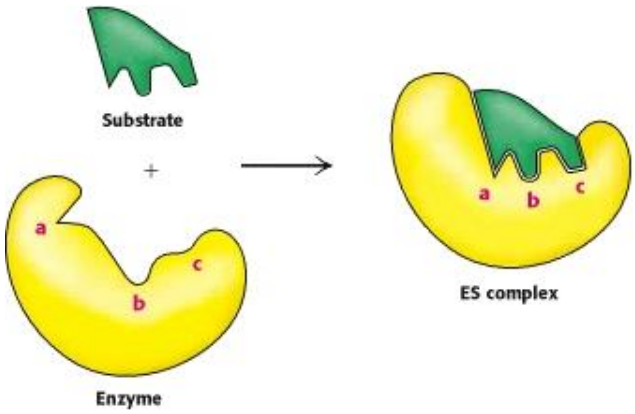
The Lock and key models assumes that the active site of an enzyme is rigid or inflexible (the lock) and the substrate (the key) fits into a specific rigid (the active site) on the enzyme to

form the enzyme-substrate complex just as a key fits into the proper lock. The lock and Key hypothesis does not explain the broad specificity of some enzymes. In addition, the molecular shape of active sites is not always complementary to that of the substrate.



2- Induced fit model (Koshland model):

The induced fit model assumes that the active site of an enzyme is flexible, not rigid. The interaction between enzyme and substrate causes to change in the shape of the active site of enzyme to form the enzyme-substrate complex.



Nomenclature and classification of enzymes:

There are several ways of naming enzymes**:**

A- **Trivial names of some enzymes,** which have historical names that have no relationship to either the substrates or the reactions that they catalyze. A few examples include catalase, trypsin, pepsin, and chymotrypsin. In these cases, the names of the enzymes and the reactions that they catalyze must simply be memorized.

B- **Some enzymes were named by attaching the suffix -ase** to the name of the substrates e.g. urease, which catalyze the hydrolysis of urea and lactase catalyzes the hydrolysis of the disaccharide lactose.

C- **Some enzymes were named according to the type of the reaction** e.g. aminotransferase**.**

D- **To standardize enzyme nomenclature, the International Union of Biochemistry and Molecular Biology (IUBMB) classifies the enzymes by giving each enzyme a number**. This number called: Enzyme commission or Enzyme code (EC) and it contains 4 digits**:**

First digit: indicates the class of the enzyme. There are 6 classes of enzymes**.**

Second digit: indicates the functional group upon which the enzyme acts e.g. -OH, -CHO.

Third digit: indicates the acceptor (coenzyme) e.g. NAD, FAD**.**

Fourth digit: It indicates the serial number of enzyme (the substrate).

For example: Lactate dehydrogenase:

EC =1.	1.	1.	27
Class1 (Oxido reductase)	Enzyme act on CH-OH	The acceptor is NAD	Serial number

Alcohol Dehydrogenase

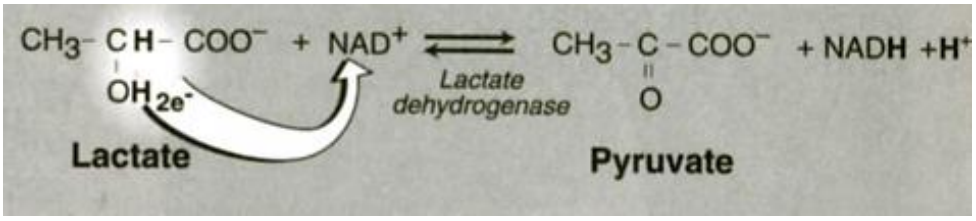
EC =1.	1.	1.	1
Class1 (Oxido reductase)	Enzyme act on CH-OH	The acceptor is NAD	Serial number

Classification of enzymes

According to the IUBMB system, Enzymes are classified into six major classes as follows:

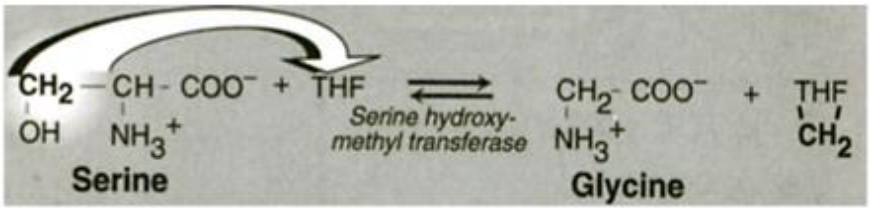
1-Oxido reductase (EC=1):

Oxidoreductases are enzymes that catalyze oxidation–reduction (redox) reactions; adds oxygen or removes 2 hydrogen atoms from substrate; requires a cofactor such as NAD+, NADP+ or FAD;. Like lactate dehydrogenase.



2-Transferase (EC=2):

Transferases are enzymes catalyze the transfer of some group from one molecule to another molecule (except hydrogen). Like aminotransferase or hexokinase.



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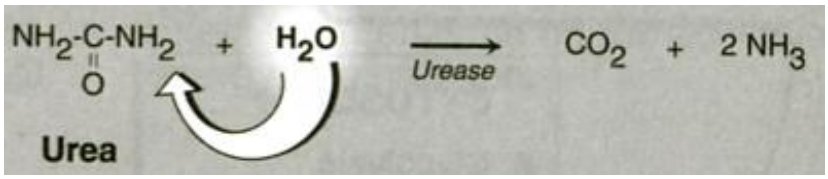
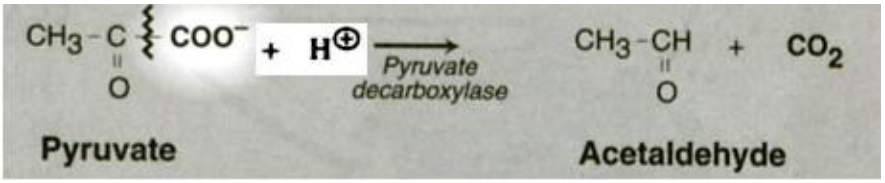
3- Hydrolase (EC=3):

Hydrolase are Enzymes that catalyze the cleavage of C-O, C-N, C-C, and some other bonds with the addition of water. Like: all digestive enzymes like α- amylase, pepsin, trypsin,…



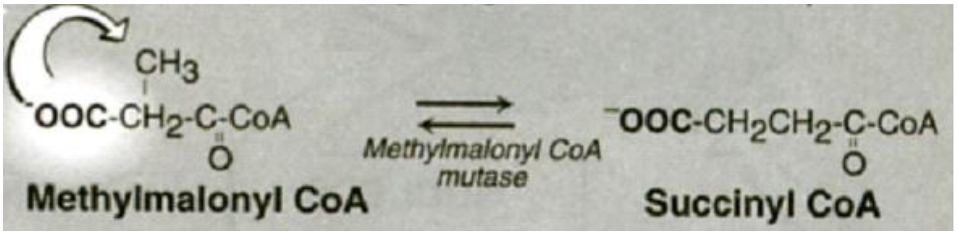
4- Lyases (EC=4):

Lyases catalyze the cleavage of C-O, C-N, and C-C bonds without adding water. Like: pyruvate decarboxylase.



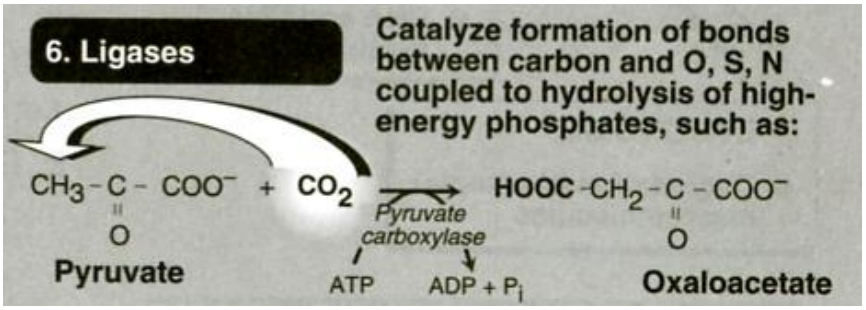
5-Isomerase (EC=5):

Isomerase are enzymes catalyses rearranges the functional groups within a molecule and catalyze the conversion of one isomer into another. Like phosphoglycerate mutase, glucose-6-phosphate mutase, isomerase, and epimerase.



6- Ligases (Synthetase EC=6):

Ligases are enzymes link two substrates together with the hydrolysis of ATP to ADP and Pi. They may form or broken of C-O, C-N, C-C, and C-S. Like glutamine synthetase or peptide synthetase, and pyruvate carboxylase,….

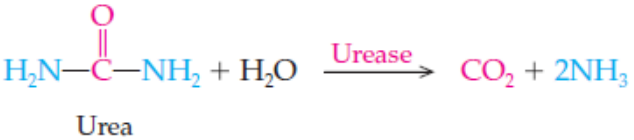


Specificity of enzyme action:

Enzyme specificity is the ability of an enzyme to bind on one or few substrates and catalyze a reaction. There are three types of specificity:

1- Absolute specificity:

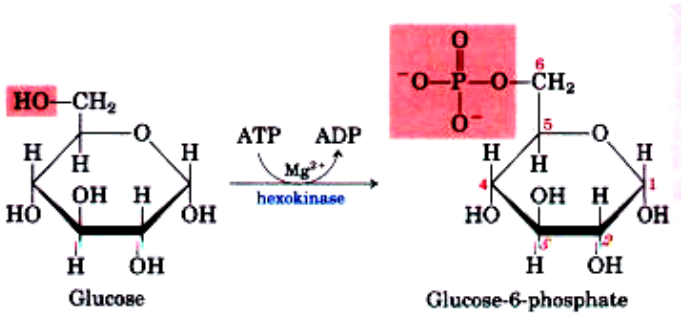
Some enzymes will act only on one substrate and catalyze one reaction. For example, the enzyme urease catalyzes the hydrolysis of urea:



The catalysis does not take place when the structure of urea is changed, e.g., N-methyl urea and thiourea are not hydrolyzed by ureaes.

2- Group specificity (non absolute specificity):

These enzymes can catalyze a group of structurally similar compounds, e.g., peptidase, hexkinase, and lipase.

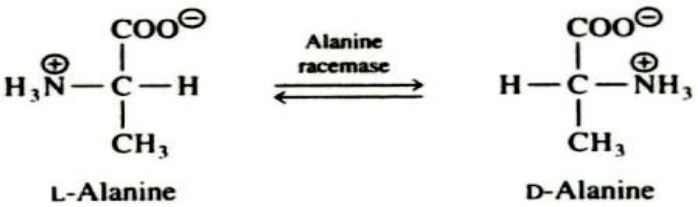


3- Stereo specificity:

a- Optical specificity:

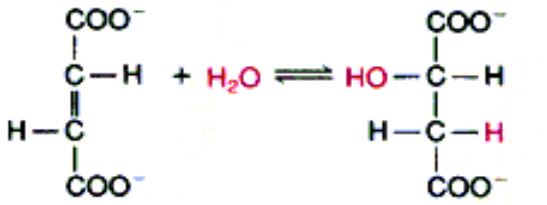
These enzymes are capable of acting only with one form of two optical isomers L or D. Human enzymes are specific for L-amino acid and D- carbohydrates and the enzymes involved in digestion and metabolism recognize only those particular stereo isomers.

And some enzymes, interconvert the two optical isomers of a compound. For example: alanine racemase.



b- Geometrical specificity:

Some enzyme reacts with one form, the cis or trans. For example: Fumarase catalyze the interconversion of trans fumaric acid and malic acid.



trans fumaric acid **malic acid**

Isoenzymes or isozymes:

Isoenzymes are multiform (isomers) of the same enzyme that catalyze the same reaction but differ in structure and in the kinetic constant Km and Vmax. For example:

Lactate dehydrogenase (LDH) which is catalyzes the reversible oxidation of lactate to pyruvate.

LDH is a tetramer, made up of two polypeptide M (Muscle) type and H (Heart) type.

These two polypepetide chains (subunit) combined in 5 different ways so that there are 5 isoenzymes of LDH

Type	Composition	Location
LDH1	H4	Heart
LDH2	H3M	Heart
LDH3	H2M2	Brain and kidney
LDH4	HM3	Liver and Muscle
LDH5	M4	Muscles

Creatine kinase is dimmer, that are made up of two types of polypeptide chains, which may be either M (Muscle) type or B (Brain) generating three isoenzymes:

Type	Composition	Location
CK1	BB	Brain
CK2	BM	Heart
CK3	MM	Muscle

Zymogene (proenzymes):

Some enzymes are synthesized in an inactive forms called zymogene or proenzymes, which must be cleaved (a part of the polypeptide or a few amino acid residue) to be activated. For example:

Proenzymes of the Digestive Tract		
Proenzyme	**Activator**	**Enzyme**
Proelastase	Trypsin	Elastase
Trypsinogen	Trypsin	Trypsin
Chymotrypsinogen A	Trypsin + chymotrypsin	Chymotrypsin
Pepsinogen	Acid pH + pepsin	Pepsin
Procarboxypeptidases	Trypsin	Carboxypeptidase A, carboxypeptidase B

The secretion of the enzyme in proenzyme (inactive form) to:

1- Prevents auto digestion of tissues

2- Prevents intravascular coagulation of blood

Units of enzyme activity

Enzymes are never expressed in terms of their concentration (as mg or pg etc.), but are expressed only as activities. Various methods have been introduced for the estimation of enzyme activities (particularly for the plasma enzymes). The International Commission of Enzymes is measured enzyme activity in **international units (I.U.).** One **I.U.** is defined as the amount of enzyme that produces 1μmol of product per minute. A new unit for measuring enzyme activity called the ***katal,*** has recently been introduced. One **katal (kat)** denotes the conversion of one mole substrate per second (mol/sec).

One katal = 6 x 10^7 international units.

Or one IU =16.67×10^{-9} kat

Factors affecting the rate (velocity) of enzyme reaction:

Various factors that affect enzyme activity are:

1- Temperature.

2- PH.

3- Enzyme concentration -

4- Substrate concentration -

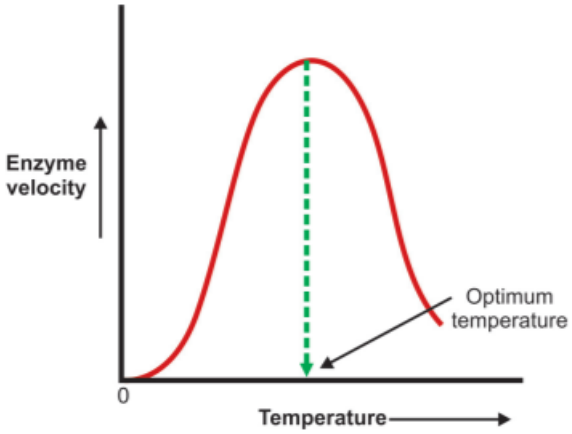
5- Inhibitor.

1-Temperature:

The velocity of enzyme reaction increases with increase in temperature of the medium, reaches a maximum and then falls. The temperature at which maximum amount of substrate is converted to the product per unit called the optimum temperature (O.T).

Above the optimum temperature, the velocity of reaction decrease and the enzyme are denaturated by heat and become inactive. Most of the body enzymes have the O.T close to 35-45^{O}C.

O.T is that temperature at which the activity of the enzyme is the maximum.

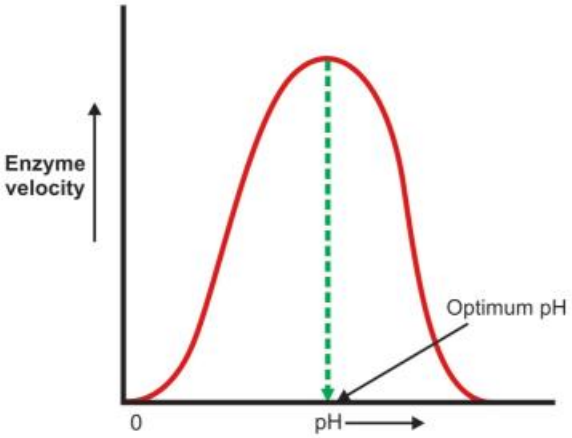


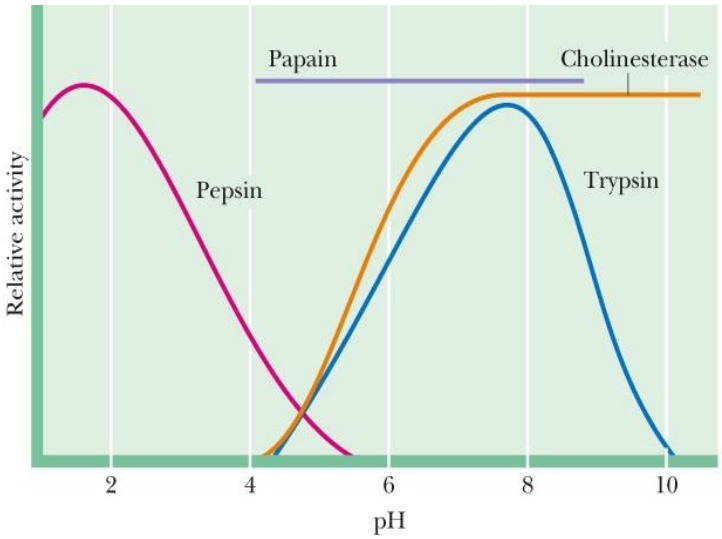
Enzymes from humans, who maintain a body temperature of 37 ºC, generally exhibit stability at temperature up to 45-55 ºC. Enzymes from microorganisms that inhabit natural hot springs or hyper thermal vents on the ocean floor may be stable at or above 100 ºC**.**

2- Effect of PH:

Velocity of an enzyme catalyzed reaction is dependent upon PH. The velocity is increase when PH is increased, reaches a maximum, the enzyme activity at this maximum PH is called maximum PH or optimum PH (O.PH). Above the O.PH the velocity of reaction decrease and the enzyme are denaturated. The O.PH of most enzymes are in the range of 4-9.Whilesome enzymes like pepsin PH=2, arginase 8.9-9.9, alkaline phosphate =10-11.

O.PH is that PH at which the enzyme activity is the maximum.

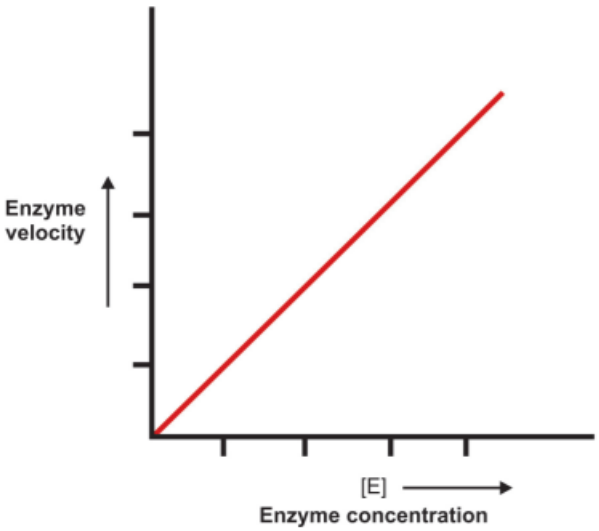




Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

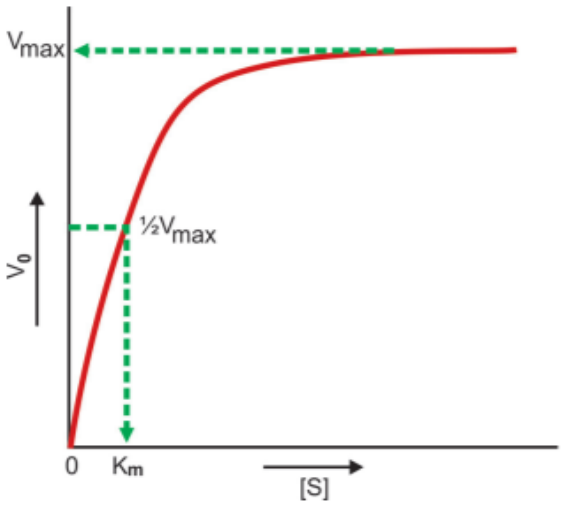
3- Enzyme concentration:

The velocity of enzyme catalyzed reaction is directly proportional to the concentration of enzyme. When the concentration of enzyme is increase, the rate of reaction is increased too, when the substrate concentration is in large excess in the medium.



4- Substrate concentration:

At a low concentrations of substrate, the initial velocity of reaction increases proportionally with the increase in substrate concentration. Further, increase in Substrate concentration, the velocity of the reaction increase less but not proportionally. At high Substrate concentration, maximal velocity is constant and the velocity of reaction remains unchanged however, much the Substrate concentration is increased. This is called as maximum velocity (Vmax). The Vmax is the velocity of the reaction at high Substrate concentration when all active site of the enzyme are filled with the substrate.



Effects of substrate concentration [S] on enzyme activity keeping enzyme concentration constant

where, V_0 : initial velocity

Vmax : maximum velocity

Km : 1/2 Vmax = Michaelis Menten constant

[S] : substrate concentration

The reason of these, that at low Substrate concentrations, active site of the enzyme will be in the uncombined with S. At maximum velocity all the enzyme is saturated with substrate and present as the E-S complex, so that any further increase in [S], has no effect on the velocity and Vmax is attained.

The Substrate concentration [S] where the velocity of the reaction V_0 is one-half of the **maximum velocity** (1/2 Vmax) is called **Michael constant** (Km). Km indicates the affecting of the enzyme for the substrate, low Km indicates higher affinity of the enzyme for the substrate and vice versa.

The relationship between reaction rate and Substrate concentration is described mathematically by the Michaels –Menton equation as follows:

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

Some times at higher Substrate concentration at maximum velocity, 1/2Vmax and Km may be difficult to determine.

A more accurate method of determining values for Vmax and Km uses lineweaver- Burk equation by taking the reciprocal of the Michaels –Menton equation:

$$\frac{1}{v} = \left(\frac{K_m}{V_{\max}[S]} + \frac{1}{V_{\max}} \right)$$

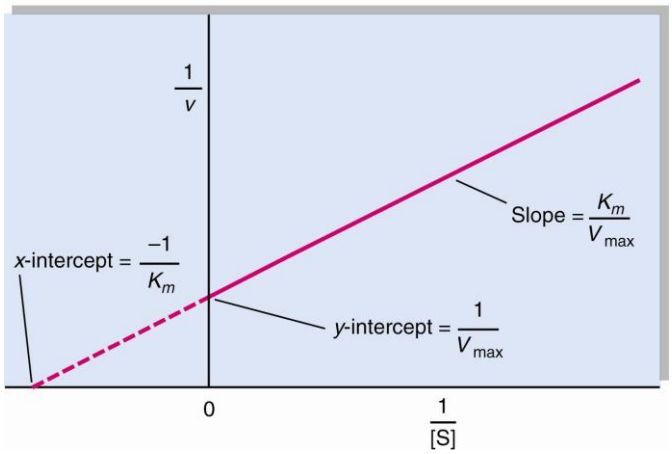
Where 1/V is plotted against 1/[S], a straight line is obtained. Where the intercept on the X-axis is equal to -1/Km and the intercept on the Y-axis is 1/Vmax.

Linear Plots Can Be Derived from the Michaelis-Menten Equation

The Lineweaver-Burk equation.

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

The Lineweaver-Burk double-reciprocal plot.



5- Inhibitors:

Inhibitors are chemical compounds that bind to the enzyme which decrease or stop the activity of an enzyme. The inhibitor may be organic or inorganic in nature like: drugs, antibiotics, toxins, and natural products of the enzyme reaction.

Inhibitors are classified based on whether they bind to active site or any other side (rather than the active site) on the enzymes:

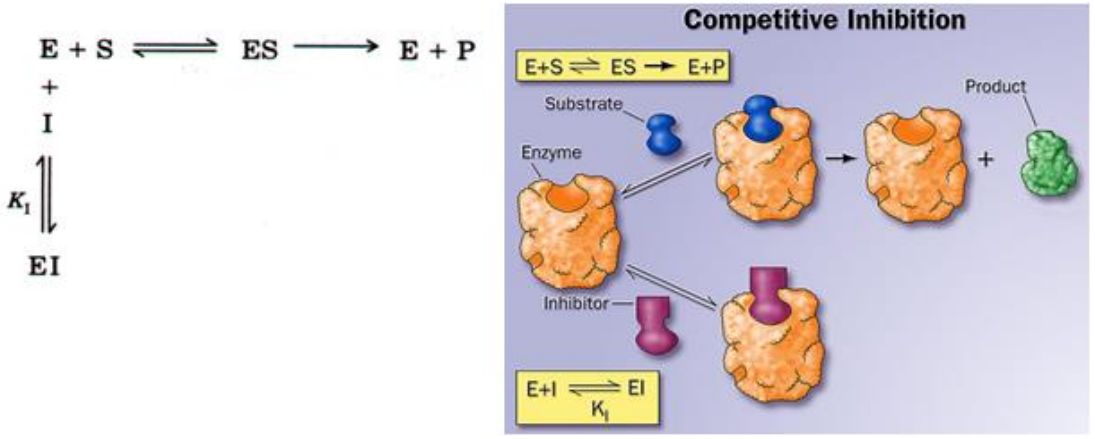
1. Competitive inhibitions

2. Non competitive inhibition

3. Uncompetitive inhibition

1. Competitive inhibitions:

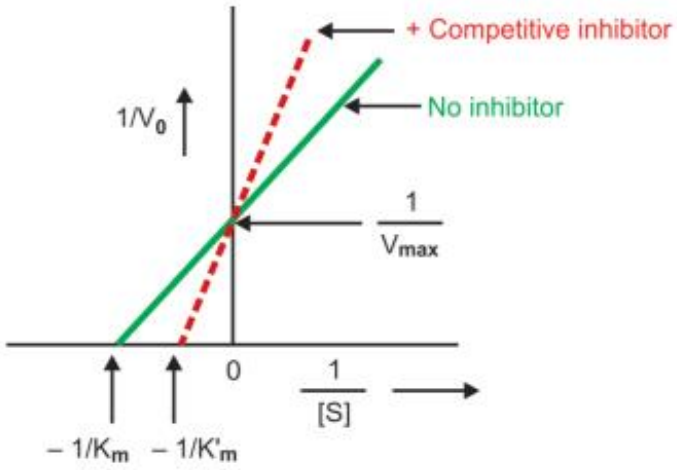
In competitive inhibition, the inhibitor is a structural similar to the substrate, so inhibitor [I] competes with the substrate for the active site of enzyme. Therefore, E-S complex is not formed, instead E-I complex formation take place. E-I can not be converting to product. Hence the rates of the reaction will decrease. Thus by increasing the substrate concentration [S], it pushes the inhibitor from the active site and combines with the enzyme to form E-S complex, which can be converted to product.



Like Inhibition of the enzyme succinate dehydrogenase by malonate . Malonate, can be combined with the succinate dehydrogenase instead of succinate. The inhibition can be removed by increasing the concentration of succinate.

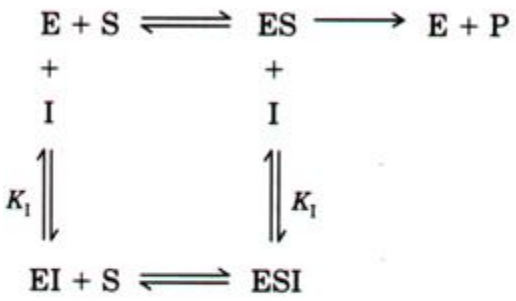
Substrate	Product	Competitive inhibitor
COO^-	COO^-	
CH_2	CH	COO^-
SDH →	‖	CH_2
CH_2	HC	COO^-
COO^- (2H)	COO^-	
Succinate	Fumarate	Malonate

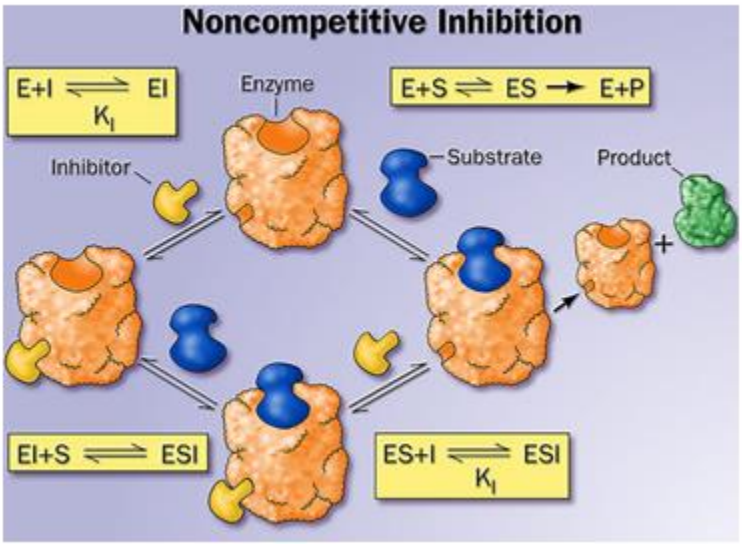
Km is increased whereas Vmax remains unchanged. Enzyme affinity of the substrate is decrease. The effect of inhibitor can be removed by increasing the concentration of the substrate.



2. Non-competitive inhibition:

In this type of inhibition no competition occurs between substrate and inhibitor. Inhibitor is usually structurally different from the substrate and the inhibitor binds with the enzyme at a site (place) other than the active site. The inhibitor may combine with E and E-S complex, forming E-I and ESI complexes.

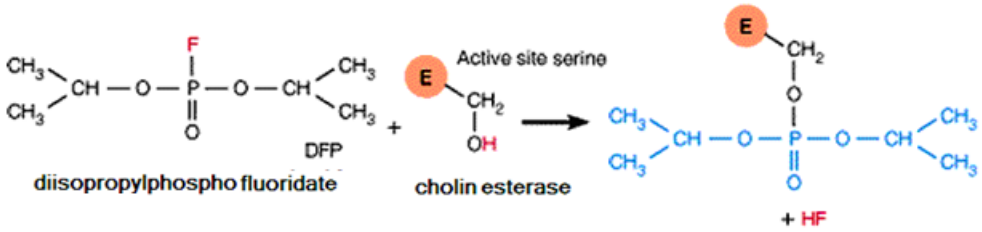


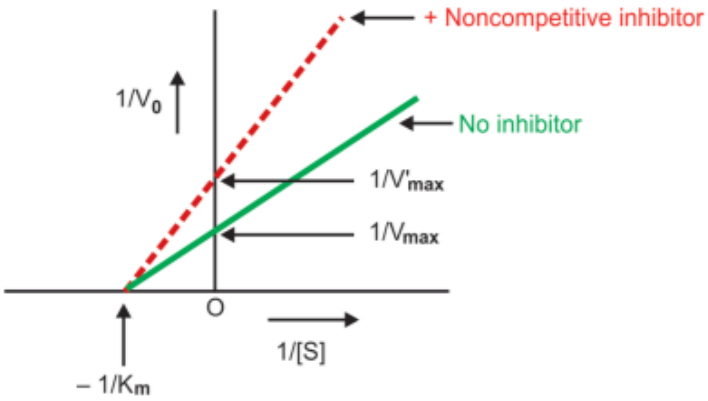


Heavy metal ions (Ag+,Pb2+,Hg2+etc.) can non-competitively inhibit the enzymes by binding with cysteinyl sulfhydryl groups. The general reaction for Hg2+is shown below.

$$E\text{-}SH + Hg^{2+} \rightleftharpoons E\text{-}S \ldots Hg^{2+} + H^{+}$$

Nerve gases and pesticides, containing organophosphorus (like Diisopropylphosphofluoride DIPF), combine with hydroxyl group in serine residues present in the enzyme acetylcholine esterase and inhibited acetylcholine esterase enzyme which involved in the transmission of nerve impulses. Acetylcholine esterase catalyzes the hydrolysis of acetylcholine, a neurotransmitter.

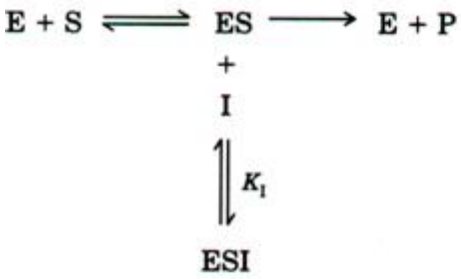


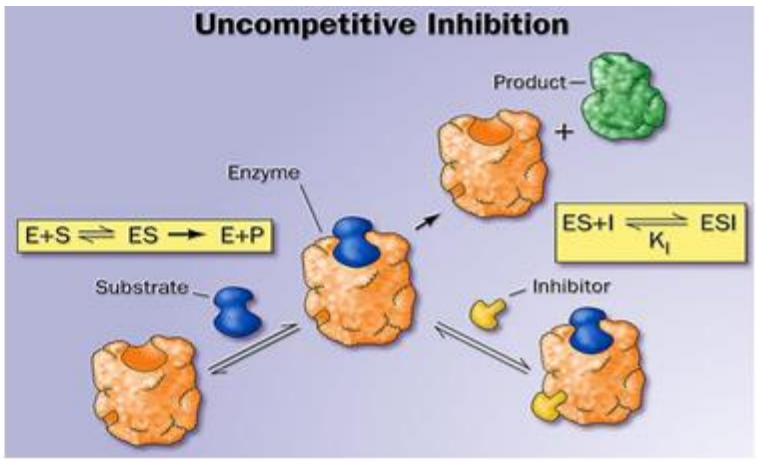


Km is unchanged while Vmax is lowered. The effect of inhibitor can not be removed by increasing the concentration of the substrate.

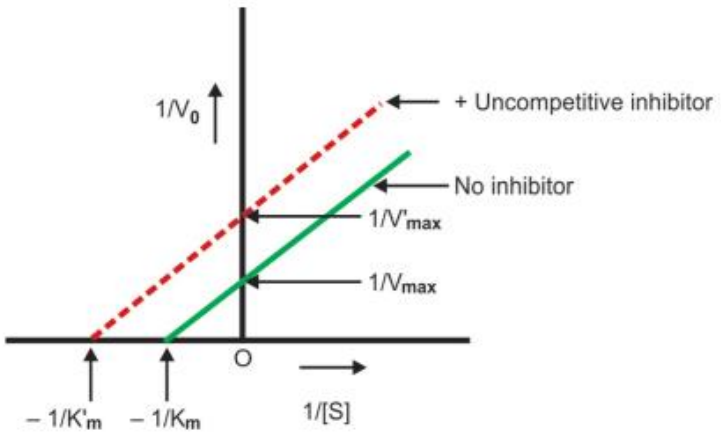
3. Uncompetitive inhibition:

Here inhibitor does not have any affinity for free enzyme. Inhibitor binds to enzyme – substrate complex, but not to the free enzyme.





Like inhibition of placental alkaline phosphatase by phenyl alanine.



Uncompetitive inhibitor **decreases both Vmax** and **Km**. The effect of inhibitor can not be removed by increasing the concentration of the substrate.