Biochemistry

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Enzymes

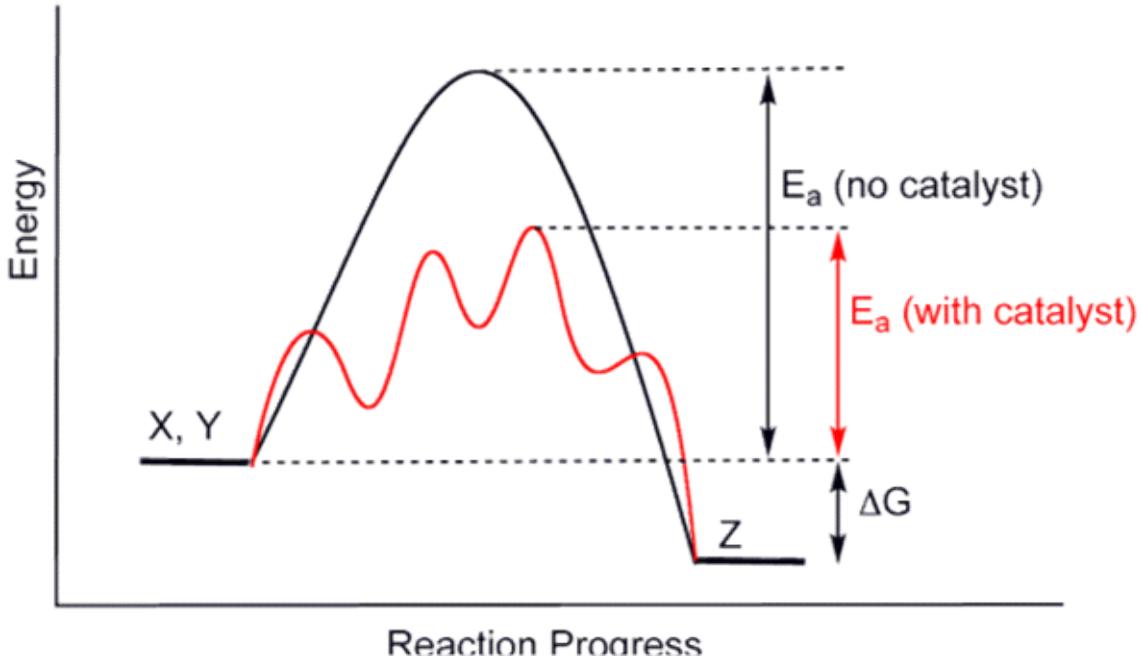
Biocatalysts

Enzymes - catalysts of biological systems (hence are called as biocatalysts. They are remarkable molecular devices that determine the pattern of chemical transformations. They also mediate the transformation of different forms of energy.

The striking characteristics of enzymes are their catalytic power and specificity. Actions of most enzymes are under strict regulation in a variety of ways.

Enzyme converts Substrate to Product **S ->P (X+Y -> Z)**

Catalyst - reduces the energy of activation – speeds up reaction >10000 times



1. Activation energy – energy required to convert molecules of substrate from ground state to transition state
2. In high energy transitional state molecule then can “fall” – undergo reaction spontaneously
3. Enzyme reduces the magnitude of activation energy that has to be supplied to reaction

Enzymes

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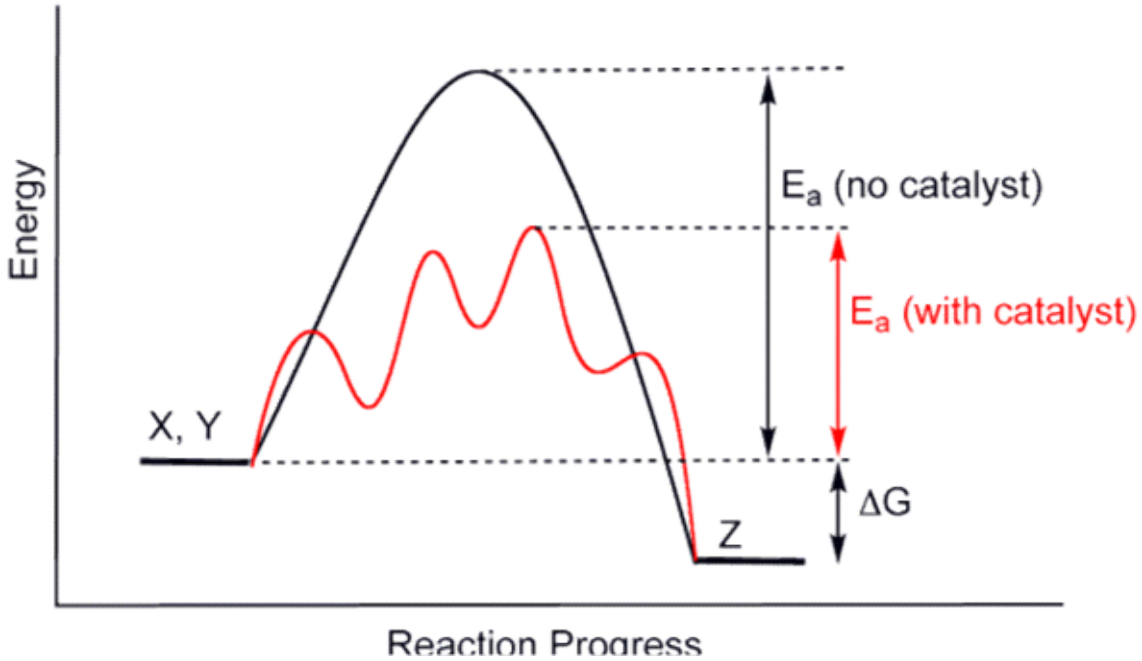


Enzymes by nature:

1. Proteins (dogma)
2. Rybozymes (Nobel 1989)

i. Enzymes follow the physical and chemical reactions of proteins.

ii. They are heat labile.

iii. They are water-soluble.

Enzymes

CLASSIFICATION

Some Enzymes have unique names – Trypsin, Pepsin etc

Others are named by the TRIVIAL NAMES - by adding the suffix "-ase" to the substrate/process:

Lactase acts on the substrate lactose, and the products are glucose and galactose.
Enzymes that hydrolyse starch (amylose) are termed as amylases;
those that dehydrogenate the substrates are called dehydrogenases.

IUBMB System of Classification (International Union of Biochemistry and Molecular Biology)

EC (enzyme class) followed by 4 digits.

First digit represents the class
Second digit stands for the subclass
Third digit is the sub-subclass or subgroup
Fourth digit gives the number of the particular enzyme in the list.

Enzymes

CLASSIFICATION

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

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. Acetyl choline 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

Enzymes

CLASSIFICATION

Class 1 - oxidation of one substrate with simultaneous reduction of another substrate or co-enzyme

$$AH_2 + B \dashrightarrow A + BH_2$$

$$\text{Alcohol} + NAD^+ \dashrightarrow \text{Aldehyde} + NADH + H^+$$

Alcohol dehydrogenase; IUB name is Alcohol-NAD-oxidoreductase; Code number is EC.1.1.1.1

Class 2 - transfers one group (other than hydrogen) from the substrate to another substrate.

$$A\text{-}R + B \rightarrow A + B\text{-}R$$

$$\text{Hexose} + ATP \rightarrow \text{Hexose-6-phosphate} + ADP$$

Hexokinase and systematic name is ATP-Hexose--6-phosphate-transferase

Class 3 - hydrolyse ester, ether, peptide or glycosidic bonds by adding water and then breaking the bond

$$A + H2O \rightarrow A1 + A2$$

$$\text{Acetyl choline} + H_2O \dashrightarrow \text{Choline} + \text{acetate}$$

Acetyl choline esterase or Acetyl choline hydrolase (systematic). All digestive enzymes are hydrolases.

Enzymes

CLASSIFICATION

Class 4 - remove groups from substrates or break bonds by mechanisms other than hydrolysis

A -> A1 + A2

Fructose-1,6-bisphosphate -------→ Glyceraldehyde-3-phosphate +dihydroxy acetone phosphate
Aldolase

Class 5 - can produce optical, geometric or positional isomers of substrates

A -> A'

Glyceraldehyde-3-phosphate -----→ Di-hydroxy-acetone-phosphate
Triose phosphate isomerase

Racemases, epimerases, cis-trans isomerases are examples of the class

Class 6 - link two substrates together, usually with the simultaneous hydrolysis of ATP

A + B (+ ATP) -> C (+ ADP + Pi)

$$\text{Acetyl CoA} + CO_2 + \text{ATP} \rightarrow \text{Malonyl CoA} + \text{ADP} + \text{Pi}$$

Acetyl CoA carboxylase.

Enzymes

CO-ENZYME

Enzymes may be simple proteins, or complex enzymes, containing a **non-protein part**, called the prosthetic group (LMW) . The prosthetic group is called the **co-enzyme**. It is heat stable.

The protein part of the enzyme is then named the **apo-enzyme**. It is heat labile.

These two portions combined together is called the **holo-enzyme**

APO-ENZYME gives the necessary three dimensional infrastructure for chemical reaction; but the group is transferred from or accepted by the co-enzyme (co-enzyme is essential for the biological activity

Generally, the co-enzymes combine loosely with the enzyme molecules. The enzyme and co-enzyme can be separated easily by dialysis

Most of the co-enzymes are derivatives of vitamin B complex group of substances.

Enzymes

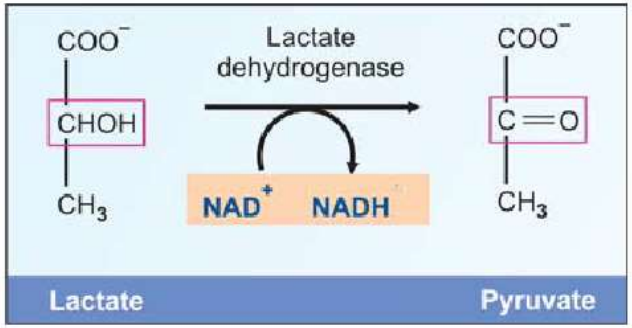
CO-ENZYME type 1

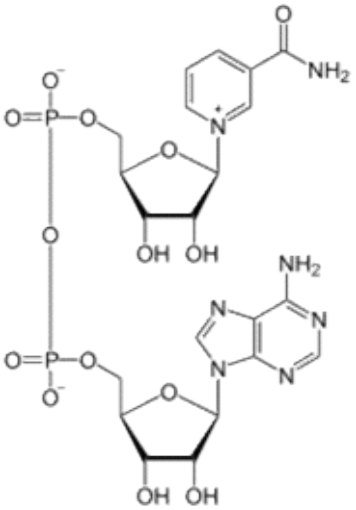
take part in reactions catalyzed by oxidoreductases by donating or accepting hydrogen atoms or electrons

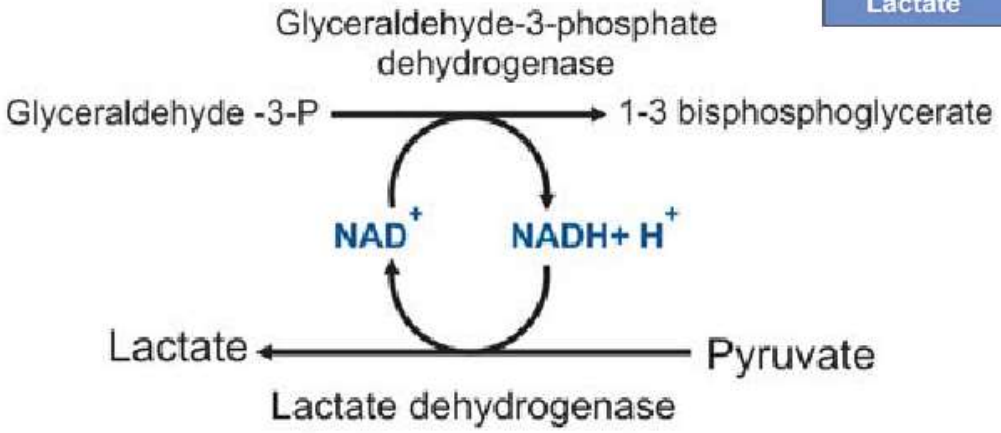
the change occurring in the substrate is counter-balanced by the co-enzymes. Therefore, such co-enzymes may be considered as co-substrates or secondary substrates

NAD+ is Nicotinamide-Ribose-P-P-Ribose-Adenine

MAJOR CO-ENZYME PAIRS of this type –
NADP – NADPH
NAD - NADH
FAD - FADH2
FMN – FMNH2







Nicotinamide Adenine Dinucleotide (NAD+)

Enzymes

CO-ENZYME type 2

co-enzymes take part in reactions transferring groups other than hydrogen

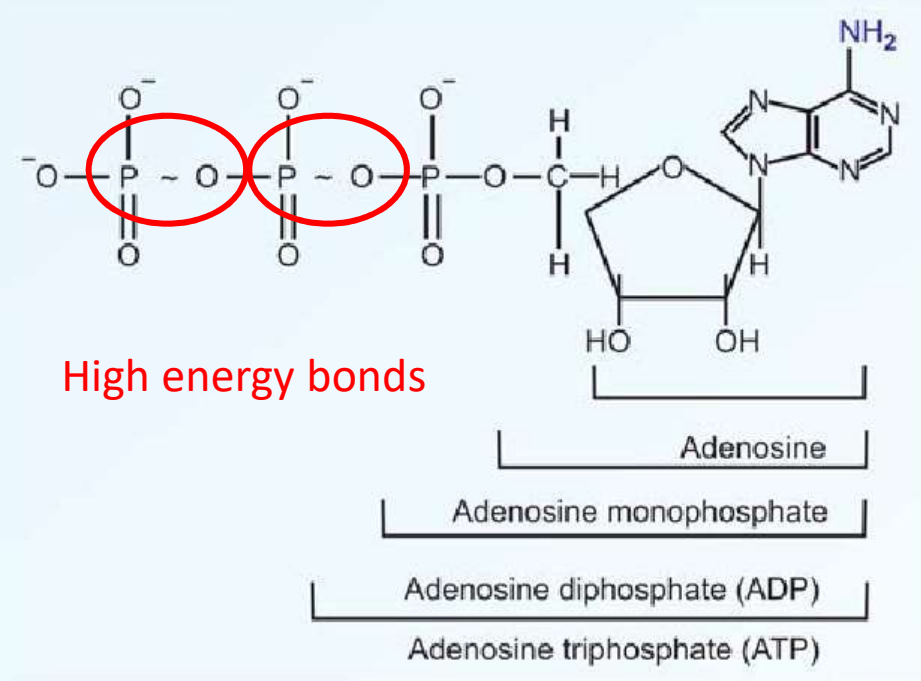
the change occurring in the substrate is counter-balanced by the co-enzymes. Therefore, such co-enzymes may be considered as co-substrates or secondary substrates

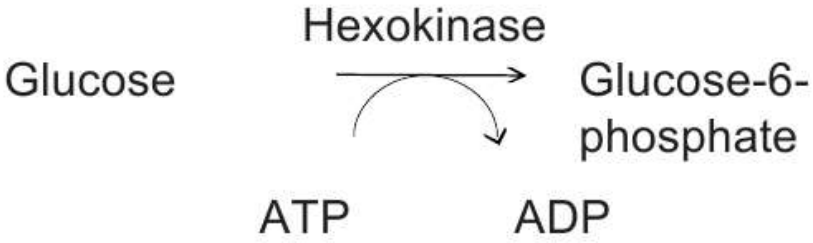
Co-enzyme	Group transferred
Thiamine pyrophosphate (TPP)	Hydroxy ethyl
Pyridoxal phosphate (PLP)	Amino group
Biotin	Carbon dioxide
Coenzyme-A (Co-A)	Acyl groups
Tetra hydrofolate (FH_4)	One carbon groups
Adenosine triphosphate (ATP)	Phosphate

Enzymes

Importance of ATP as cofactor

co-enzymes take part in reactions transferring groups other than hydrogen





Reactions that require energy goes with the help of energy released from hydrolysis of ATP.

Enzymes

Importance of Metals as cofactor

Metal	Enzyme containing the metal
Zinc	Carbonic anhydrase, carboxy peptidase, alcohol dehydrogenase
Magnesium	Hexokinase, phospho fructo kinase, enolase, glucose-6-phosphatase
Manganese	Phospho gluco mutase, hexokinase, enolase, glycosyl transferases
Copper	Tyrosinase, cytochrome oxidase, lysyl oxidase, superoxide dismutase
Iron	Cytochrome oxidase, catalase, peroxidase, xanthine oxidase
Calcium	Lecithinase, lipase
Molybdenum	Xanthine oxidase

Some metals are tightly bound to apo-enzyme (tyrosinkinase)

Some metals just enhances dramatically activity, when present in holo-enzyme

Enzymes

Other Enzyme features – HIGH SPECIFICITY

1. Stereochemical 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 For example - 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. Products formed can be also stereospecific.*

2. Reaction specificity - *one enzyme can catalyse only one of the various reactions. Oxaloacetic acid can undergo several reactions but each reaction is catalysed by its own separate enzyme which catalyses only that reaction and none of the others.*

3. Substrate specificity - *Trypsin hydrolyses proteins after the residues of only lysine and arginine, while chymotrypsin hydrolyses residues of only aromatic amino acids*

4. 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.*

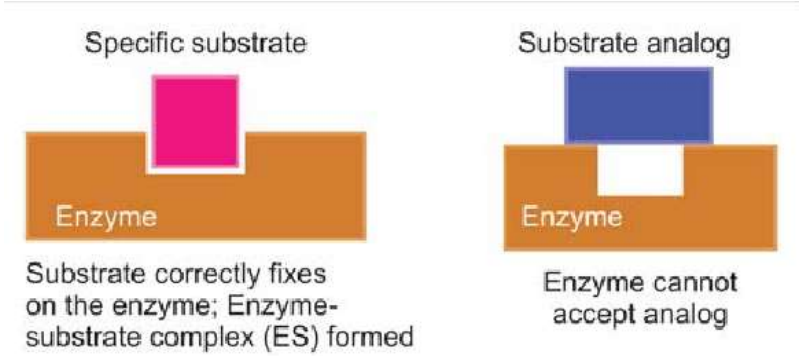
Enzymes

Mechanism of action – Michaelis Menten

$$E + S \leftrightarrows E\text{–}S\ \text{Complex} \rightarrow E + P$$

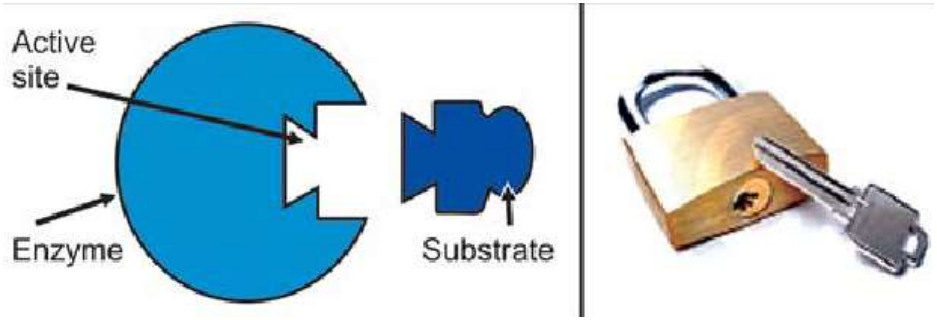
the enzyme molecule (E) first combines with a substrate molecule (S) to form an enzyme-substrate (ES) complex which further dissociates to form product (P) and enzyme (E) back

Complex formation



Fisher – TEMPLATE

KEY-LOCK



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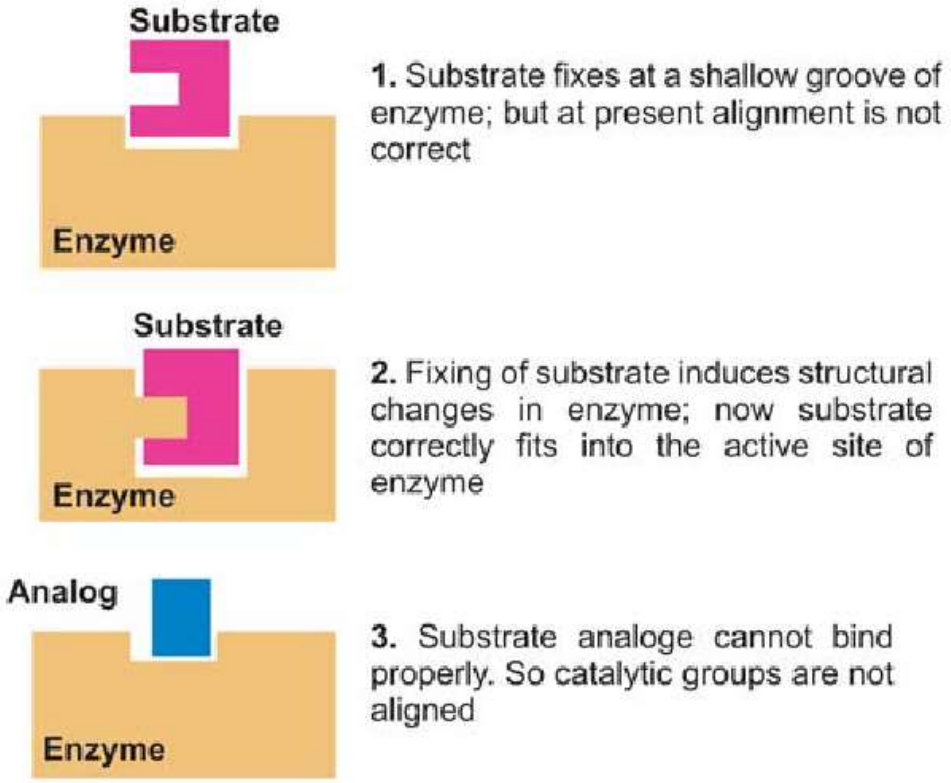
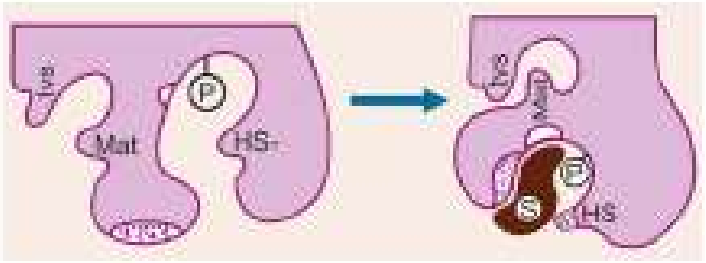


Koshland –

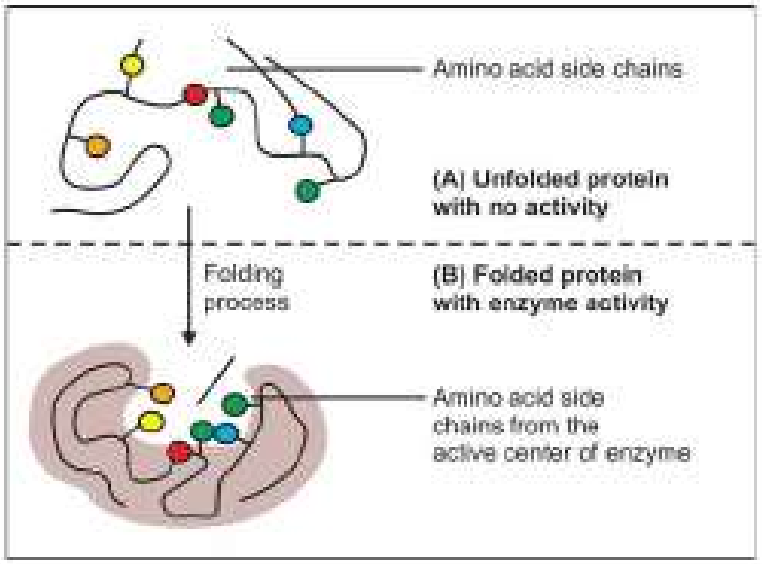
INDUCED FIT



Enzymes

Active center

Catalysis occurs at the **active center or active site**



Name of enzyme	Important amino acid at the catalytic site
Chymotrypsin	His (57), Asp (102), Ser (195)
Trypsin	Serine, Histidine
Thrombin	Serine, Histidine
Phosphoglucomutase	Serine
Alkaline phosphatase	Serine
Acetyl cholinesterase	Serine
Carbonic anhydrase	Cysteine
Hexokinase	Histidine
Carboxypeptidase	Histidine, Arginine, Tyrosine
Aldolase	Lysine

Enzymes

Kinetics

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} E\text{-}S \underset{K_4}{\overset{K_3}{\rightleftharpoons}} E + P$$

Rate of formation of ES

Rate of formation = K_1 [Et] –[ES] [S]

Rate of dissociation of ES

Rate of dissociation = K_2 [ES] + K_3 [ES]

Steady state is attained when rate of formation of ES is equal to rate of dissociation,

$$K_1 [Et] - [ES] [S] = K_2 [ES] + K_3 [ES]$$

$$[ES] = \frac{K_1 [Et] [S]}{K_1 [S] + K_2 + K_3}$$

Now let us simplify further by defining K_m (the Michaelis-Menten Constant) as $\frac{K_3 + K_2}{K_1}$

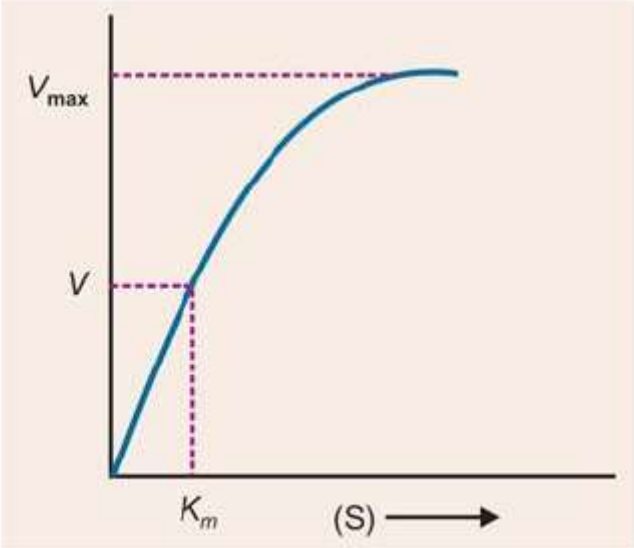
$$V_o = \frac{V_{max} [S]}{[S] + K_m.}$$

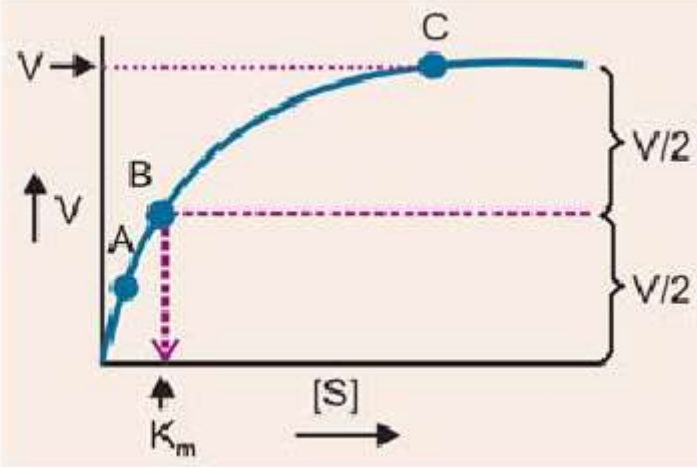
Enzymes

Kinetics

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} E\text{-}S \underset{K_4}{\overset{K_3}{\rightleftharpoons}} E + P$$

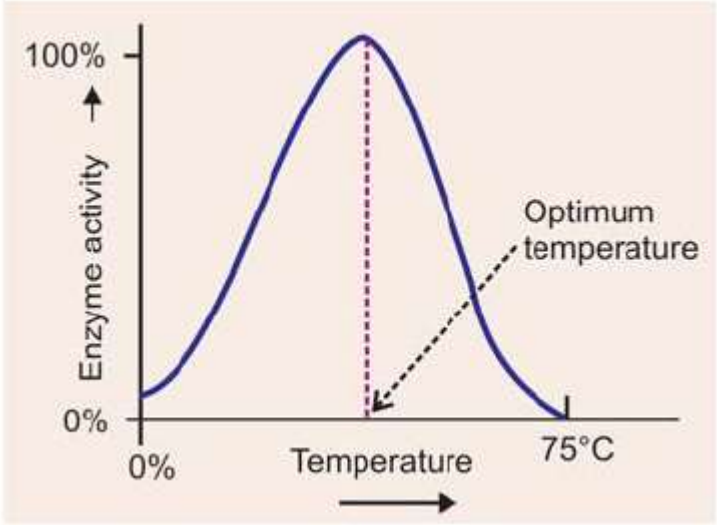
Km is equal to substrate concentration at which the velocity is half the maximum





Enzymes

1. Effect of Temperature



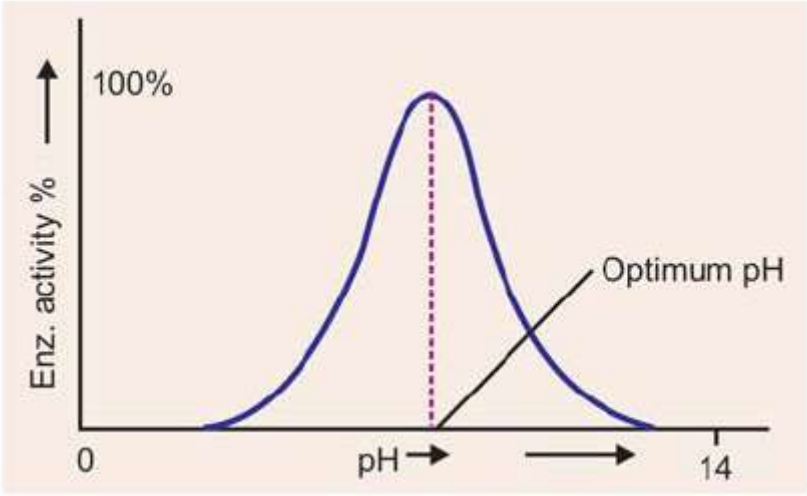
Temperature increases total energy of the system so that activation barrier is passed with less effort. Usually reaction velocity doubles every 10 grad Celsius rise. However. there is impact of destructualization of active center of catalyst.

2. Effect of pH

The optimum pH of most enzymes lies in the range of 4–9.

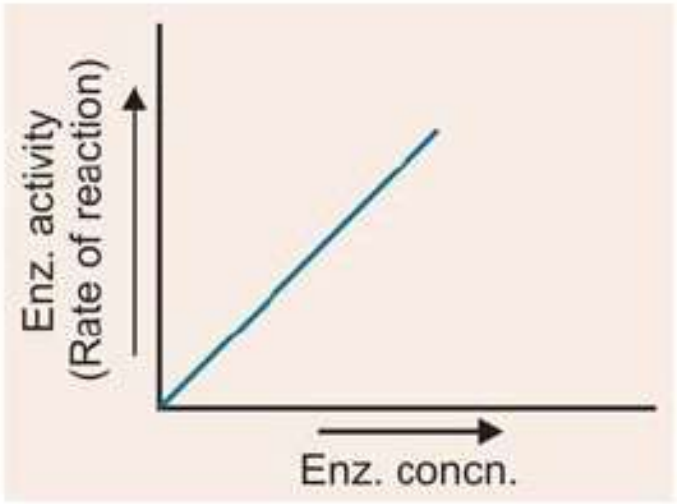
• Hydrogen ions in the medium may alter the ionization of active site or substrates. Ionisation is a requirement for ES complex formation.

• pH may influence the separation of coenzyme from holoenzyme complex. At a very low or high pH the H-bonds may be inactivated in the protein structure, destroying its 3D structure.



Enzymes

3. Effect of Enzyme Concentration



In the beginning velocity of the enzymatic reaction is directly proportional to the enzyme concentration. When the substrate conc. is in large excess exceeding that of Vmax, because enzyme is the limiting factor in the enzyme-substrate reaction and providing more enzyme molecules enables the conversion of progressively larger numbers of substrate molecules

4. Effect of Product Concentration

Products formed as a result of enzymatic reaction may accumulate and this excess of product may lower the enzymatic reaction by occupying the active site of the enzyme. If the actual chemical step of reaction is reversible - under certain conditions of high concentration of products a reverse reaction may be favoured forming back the substrate

5. Effect of Activators and Coenzymes

The activity of certain enzymes is greatly dependent of metal ion activators and coenzymes.

6. Effect of Modulators and Inhibitors

Whenever the active site is not available for the binding of the substrate the enzyme activity may be reduced. The substances which stop or modify the enzymatic reaction are called inhibitors or modulators.

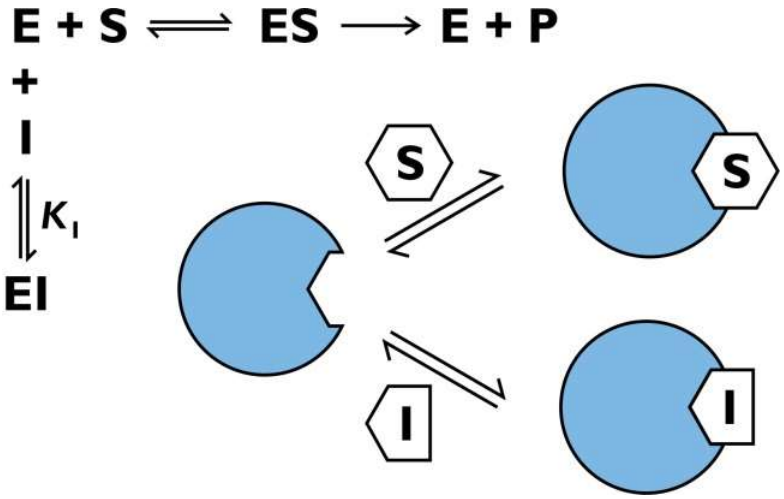
Enzymes

ENZYME INHIBITION

The chemical substances which inactivate the enzymes are called as inhibitors and the process is called as enzyme inhibition. Inhibitors are sometimes referred to as negative modifier.

Competitive inhibition (Reversible)

When the active site or catalytic site of an enzyme is occupied by a substance other than the substrate of that enzyme, its activity is inhibited.



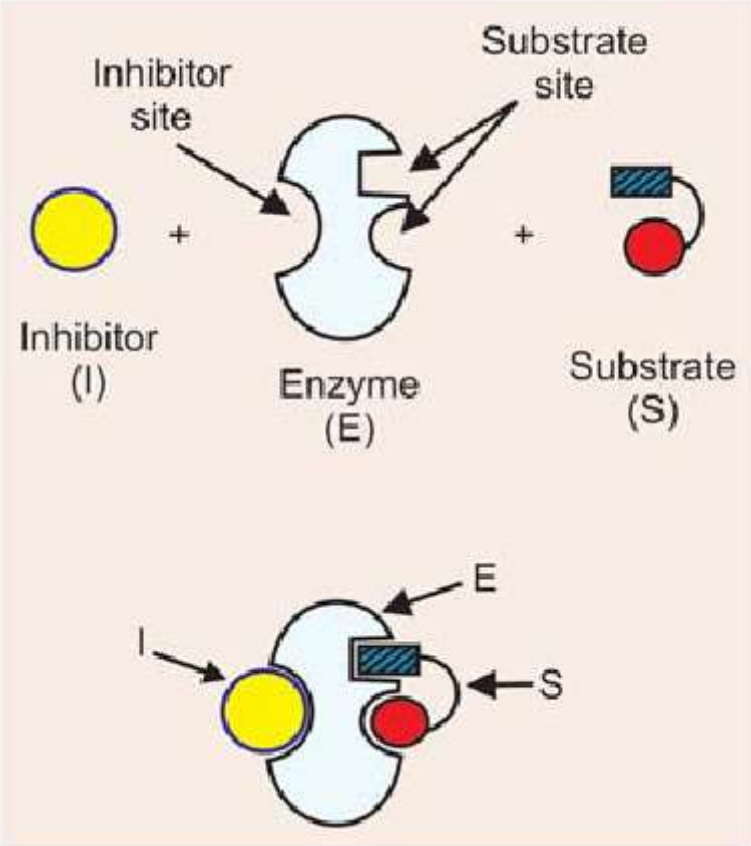
So the affinity of the substrate for the enzyme is progressively decreased with the increase in conc. Of inhibitor lowering the rate of nzymatic reaction. Thus, the Km is high, but Vmax is the same in competitive inhibition. However, when the concentrated substrate is increased, the effect of inhibitor can be reversed forcing it out from EI complex.

Enzymes

ENZYME INHIBITION

Non-competitive inhibition (Irreversible or reversible)

This occurs when the substances 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.



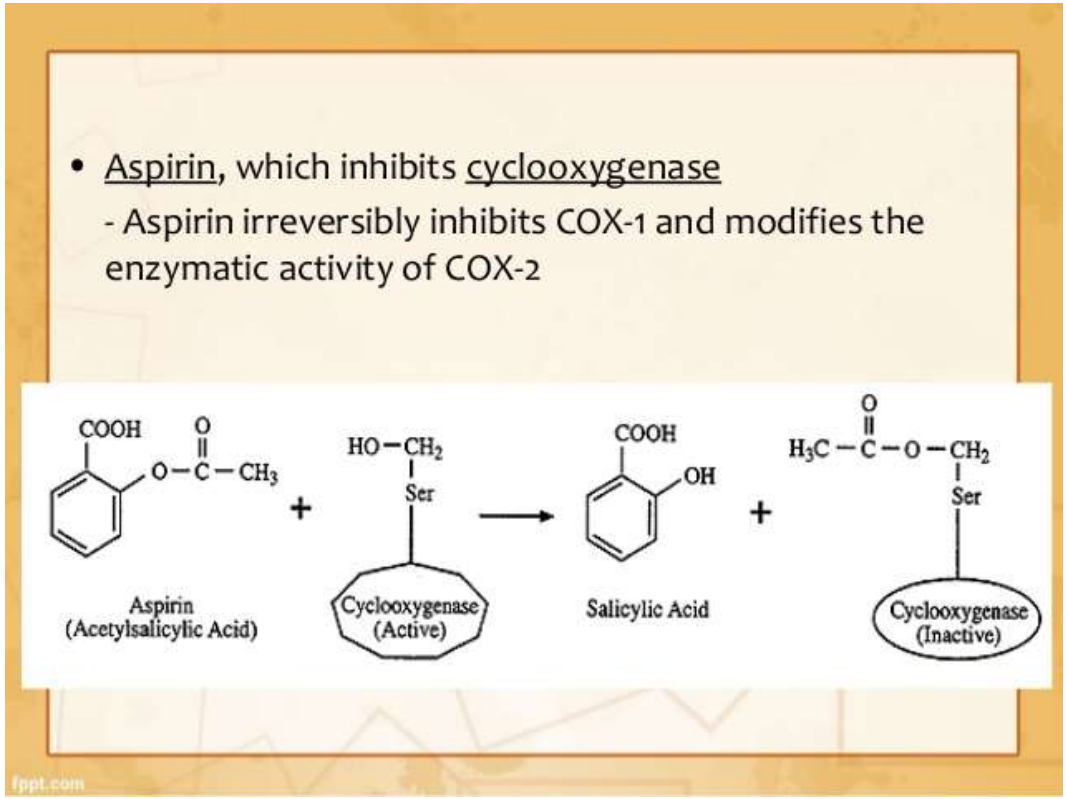
So the inhibitor may combine with both free enzyme and ES complex. This probably brings about the changes in 3D structure of the enzyme inactivating it catalytically. In non-competitive inhibition Vmax is lowered, but Km is kept constant. If the inhibitor can be removed from its site of binding without affecting the activity of the enzyme, it is called as Reversible-Non-competitive Inhibition. However, if the inhibitor can be removed only at the loss of enzymatic activity, it is known as Irreversible Non-competitive Inhibition. However, the kinetic properties in case of both are the same.

Enzymes

ENZYME INHIBITION

Non-competitive inhibition (Irreversible or reversible)

SUICIDE INHIBITION - It is a special type of irreversible noncompetitive inhibition. In this type of inhibition, substrate analogue is converted to a more effective inhibitor with the help of the enzyme to be inhibited. The so formed new inhibitor binds irreversibly with the enzyme

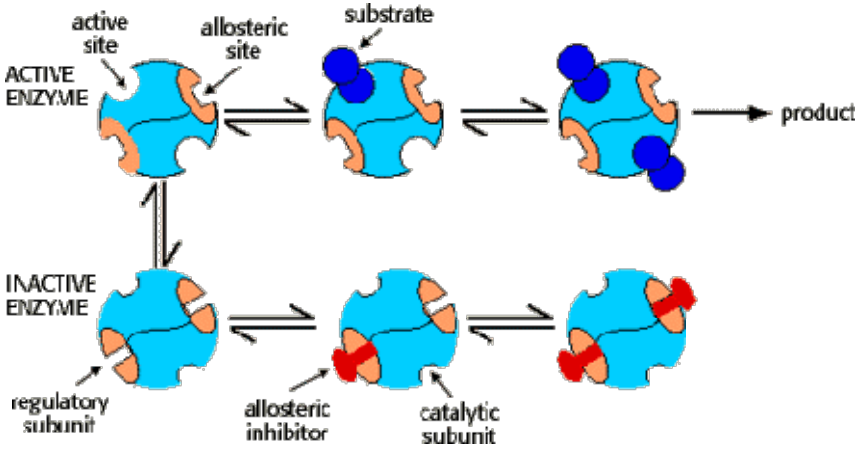


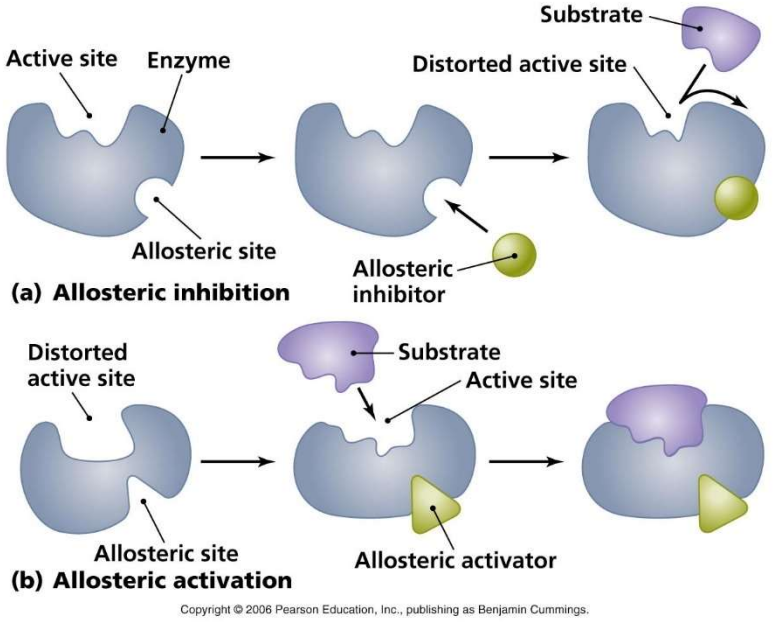
Enzymes

ENZYME INHIBITION

Allosteric regulation

Enzymes with multiple subunits have quaternary structure. One consequence of multiple subunits is that individual catalytic subunits each have their own active site. This means that an enzyme with quaternary structure can bind more than one substrate molecule. Allostery means "different shape." Allosteric enzymes change shape between active and inactive shapes as a result of the binding of substrates at the active site, and of regulatory molecules at other sites.



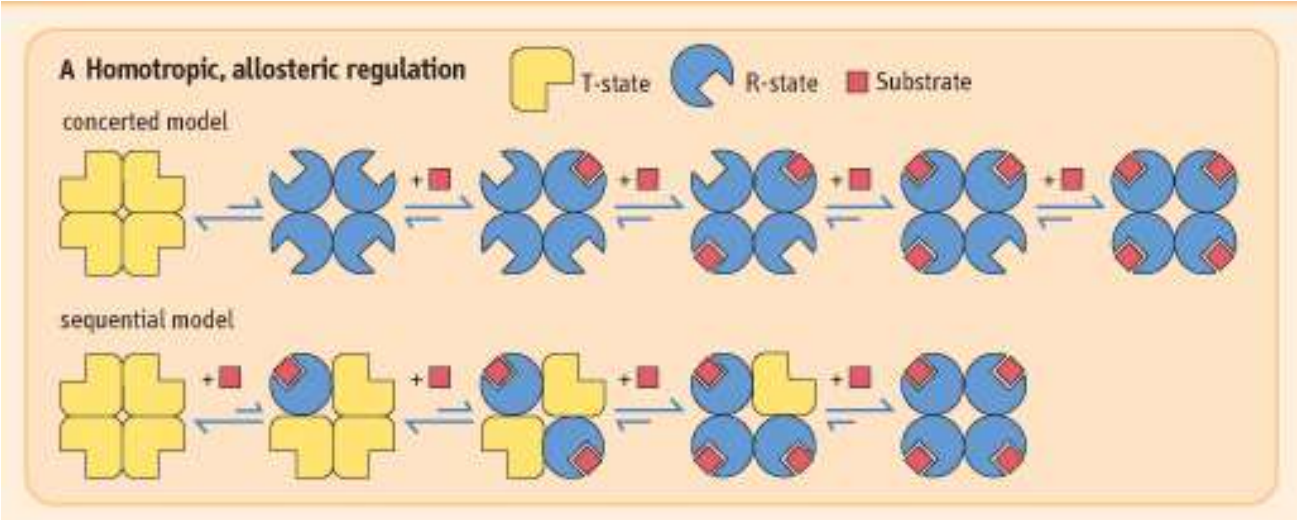


Enzymes

ENZYME INHIBITION

Allosteric regulation

In oligomeric enzymes, the allosteric site and active site are located on different subunits. Changes in the enzyme-substrate interaction due to the allosteric effects of regulatory molecules other than the substrate are called heterotropic allosteric modulations. Allos teric activators and inhibitors exhibit respectively positive and negative cooperativities with the substrates. Binding of substrate to one protomer enhances the binding of the same to another protomer or another substrate binding site on the same enzyme molecule. When the binding of a substrate enhances the interaction between the allosteric enzyme and more molecules of the same substrate it is homotropic allosteric effect.



Literature biochemistry

1. Lehninger Principles of Biochemistry (Nelson D.L., Cox M.M.)
2. Principles and Techiniques of Biochemistry and Molecular Biology (Wilson K., Walker J.)