ENZYMES

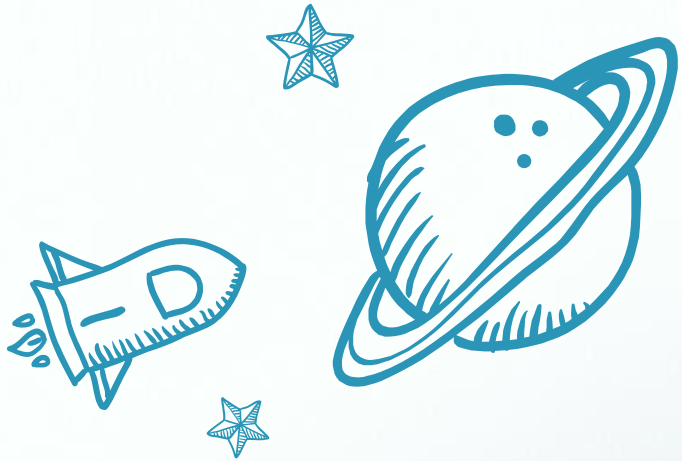
By Dr. Subarna Ghosh
(M.Sc. PhD, Post-doc)

Contents

- ✓ Classification
- ✓ Kinetics
- ✓ Examples of inhibition and inhibitors
- ✓ Modulations

Introduction!

- Enzymes are *biological catalysts* that speed up the rate of the biochemical reaction.
- Most enzymes are three dimensional *globular proteins* (tertiary an quaternary structure).
- Some special RNA species also act as enzymes and are called *Ribozymes* e.g. hammerhead ribozyme.



structure

Structure of Enzyme

The *active site* of an enzyme is the region that binds substrates, co-factors and prosthetic groups and contains residue that helps to hold the substrate.

Active sites generally occupy less than 5% of the total surface area of enzyme.

Active site has a *specific shape* due to tertiary structure of protein.

A change in the shape of protein affects the shape of active site and function of the enzyme.

Active sites

It is further divided into two sites

Binding Sites

- It chooses the substrate and binds it to active site.

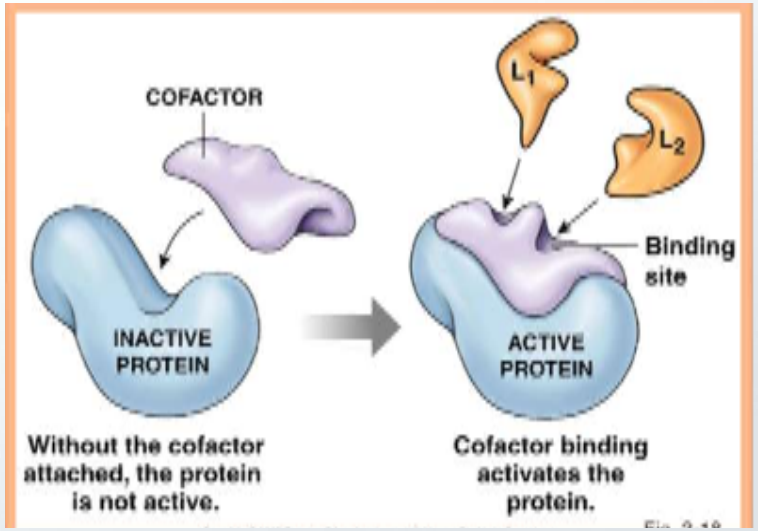
Catalytic Sites

- It performs the catalytic action of enzyme.

CO-FACTORS

Co-factor is the non protein molecule which carries out chemical reactions that can not be performed by standard 20 amino acids.

Co-factors are of two types:
Organic co-factors
Inorganic cofactors



CO-FACTORS

It is further divided into two types

Inorganic Co-Factors

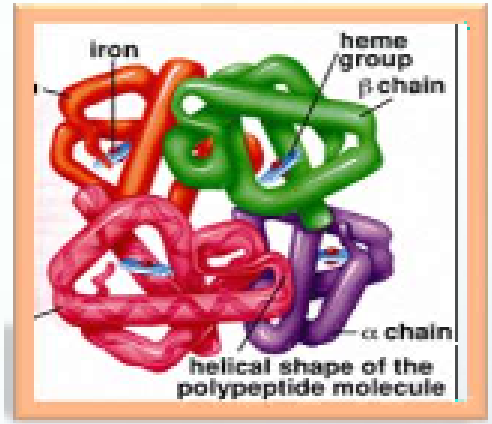
- These are the inorganic molecules required for the proper activity of enzymes.
- Examples
  - ✓Enzyme carbonic anhydrase requires Zn++ for it"s activity.
  - ✓Hexokinase has co-factor Mg++

Organic Co-Factors

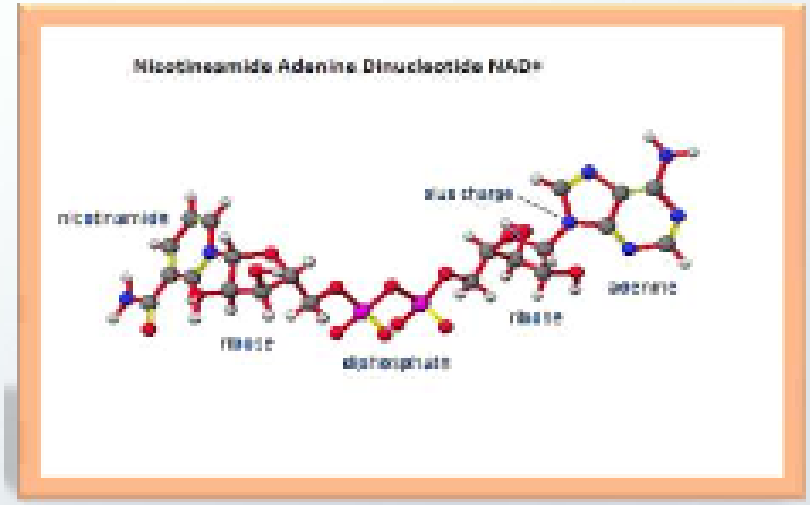
- These are the organic molecules required for the proper activity of enzymes.
- Example
  - ✓Glycogen phosphorylase requires the small organic molecule pyridoxal phosphate.

TYPES OF ORGANIC CO-FACTORS

A prosthetic group is a tightly bound organic co- factor. E. g. Flavins, heme groups and biotin.



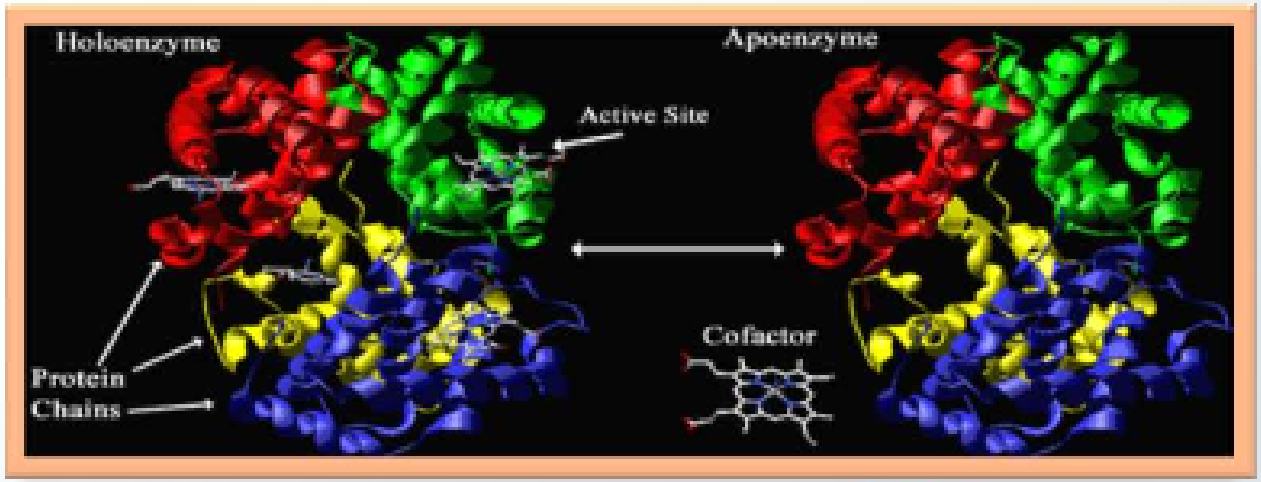
A coenzyme is loosely bound organic co-factor. E.g. NAD+



TYPES OF ORGANIC CO-FACTORS

An enzyme with it"s co-factor removed is designated as *Apoenzyme*.

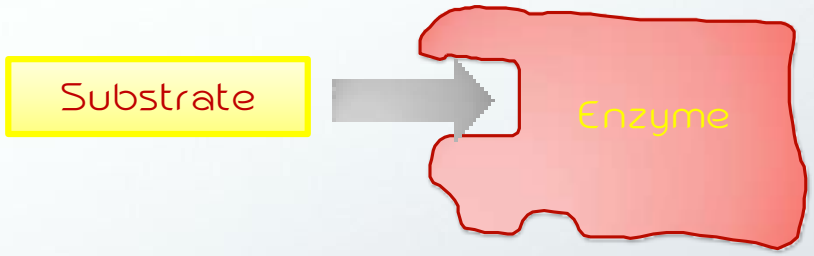
The complete complex of a protein with all necessary small organic molecules, metal ions and other components is termed as *Holoenzyme* or *Holoprotein*.



Substrates

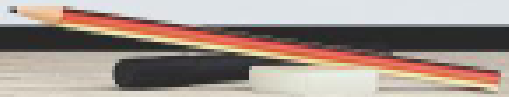
The reactant in biochemical reaction is termed as substrate.

When a substrate binds to an enzyme it forms an enzyme-substrate complex.



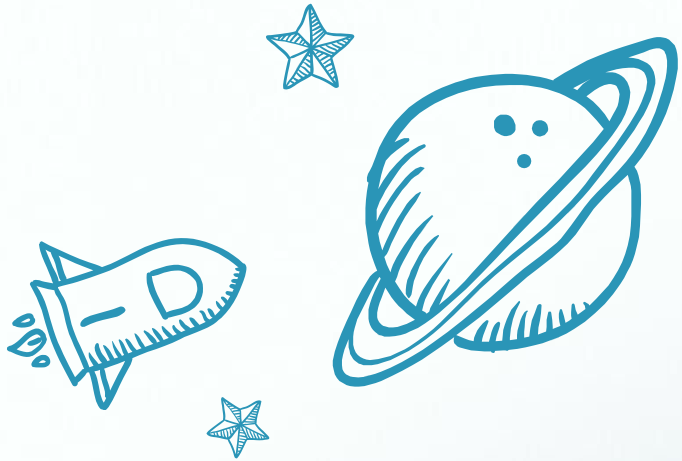
Characteristics of Enzyme

- Enzymes speed up the reaction by lowering the activation energy of the reaction.
- Their presence *does not effect* the nature and properties of *end product*.
- They are *highly specific* in their action that is each enzyme can catalyze one kind of substrate.
- Small amount of enzymes can accelerate chemical reactions.
- Enzymes are sensitive to change in pH, temperature and substrate concentration.
- *Turnover number* (6x106/min) is defined as the number of substrate molecules transformed per minute by one enzyme molecule.


CATALYTIC PROPERTIES OF ENZYMES

- They accelerate the rate of catalysis by lowering the activation energy.
- They are highly specific in nature, because they only catalyse a specific biochemical reaction.
- Enzymes accelerate the forward and reverse reactions to attain the equilibrium.
- **Temperature:** Optimum temperature of enzymes is 20-35°C. They become inactivated at very low temperature and denatured at very high temperature (greater than 45°C).
- **pH:** Most enzymes exhibit optimal activity at pH value between 5 and 9. High or low pH value than optimum value will cause ionization of enzyme which result in denaturation of enzyme.



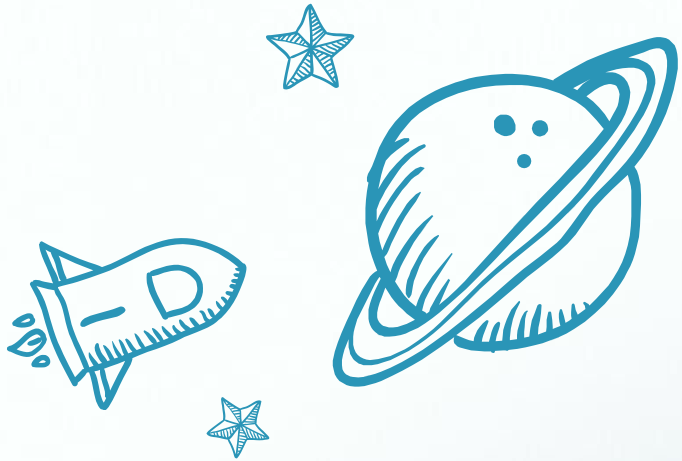
Classification

Classification

- A systematic classification of enzymes has been developed by International Enzyme Commission.
- This classification is based on the type of reactions catalyzed by enzymes.
- There are *six* major classes.
- Each class is further divided into sub classes, sub sub-classes and so on, to describe the huge number of different enzyme- catalyzed reactions.

table to show the classification

ENZYME CLASS	REACTION TYPE	EXAMPLES
Oxidoreductases	Reduction-oxidation (redox)	Lactate dehydrogenase
Transferases	Move chemical group	Hexokinase
Hydrolases	Hydrolysis; bond cleavage with transfer of functional group of water	Lysozyme
Lysases	Non-hydrolytic bond cleavage	Fumarase
Isomerases	Intramolecular group transfer (isomerization)	Triose phosphate isomerase
Ligases	Synthesis of new covalent bond between substrates, using ATP hydrolysis	RNA polymerase



Enzyme kinetics

Introduction

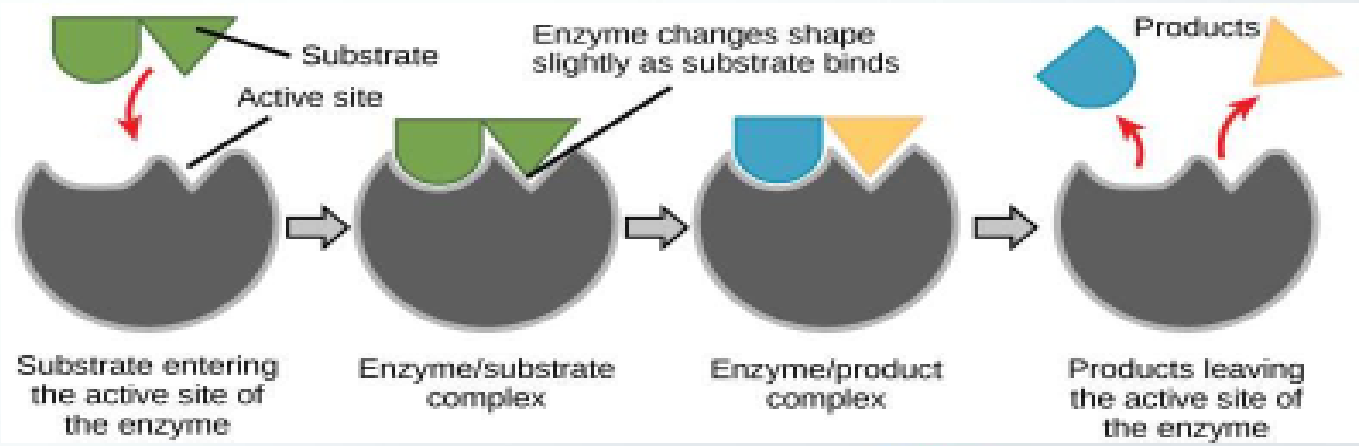
- "It is a branch of biochemistry in which we study *the rate of enzyme catalyzed reactions*."
- Kinetic analysis reveals the number and order of the individual steps by which enzymes transform substrate into products
- Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of that enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme

RATES OF REACTION AND THEIR DEPENDENCE ON ACTIVATION ENERGY

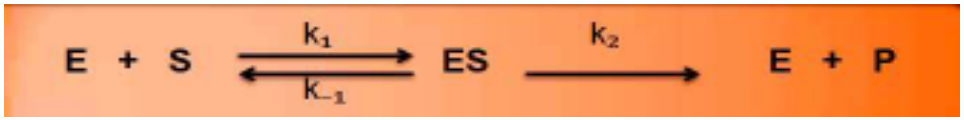
- Activation Energy (Ea): "The least amount of energy needed for a chemical reaction to take place."
- Enzyme (as a catalyst) acts on substrate in such a way that they lower the activation energy by changing the route of the reaction.
- The reduction of activation energy (Ea) increases the amount of reactant molecules that achieve a sufficient level of energy, so that they reach the activation energy and form the product.
- Example: Carbonic anhydrase catalyses the hydration of 10^6 CO_2 molecules per second which is 10^7x faster than spontaneous hydration.

MICHAELIS-MENTEN MODEL FOR ENZYME KINETICS

- Michaelis–Menten kinetics is one of the simplest and best-known models of enzyme kinetics.
- This model explain how an enzyme can cause kinetic rate enhancement of a reaction and why the rate of a reaction depends on the concentration of enzyme present.



- According to this model the enzyme reversibly bind with substrate to form an ES complex that subsequently yields product.

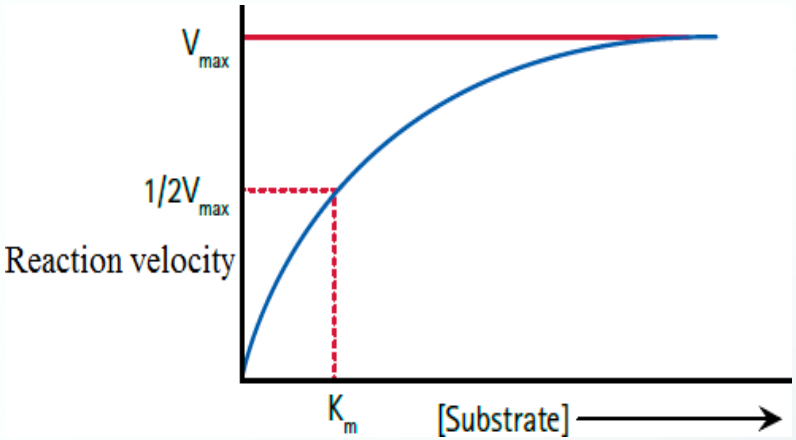


where:

S- Substrate; E- Enzyme; ES-Enzyme substrate complex ; P- product;
K1, K-1 K2- Rate constants

- The mechanism of enzyme catalyzed reactions is studied by making
- kinetic measurements on enzyme-substrate reaction systems.
- The simple kinetic model is obtained, when plotting the rate of catalysis, V (reaction velocity), v/s the substrate concentration, [S] (fixed enzyme concentration).

PLOT OF REACTION VELOCITY WITH RESPECT TO SUBSTRATE CONCENTRATION



- From this plot,

reaction velocity,V can be expressed as:

- V = V max [S] /Km +[S]
- K m =K -1 + K 2/K 1

Km and vmax

Km

- It is the substrate concentration, [S] at which half maximum
- velocity of reaction is observed.
- K m implies that half of the active sites on the enzymes are filled.
- It is the measure of the binding strength between substrate and the enzyme.
- where lower K m value indicates a strong affinity between the substrate and enzymes.

Vmax

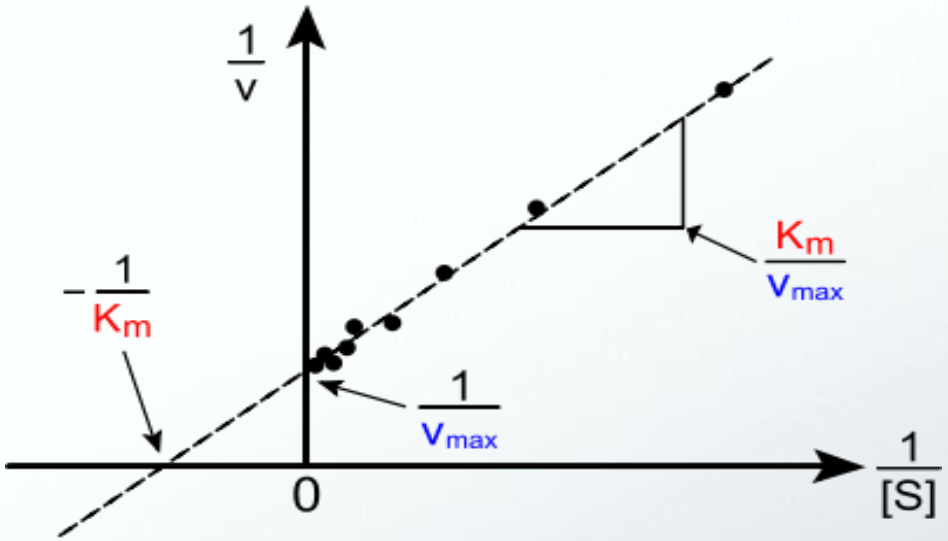
- It is the maximum velocity of reaction or rate at which the enzyme catalyzed a reaction under given conditions.
- Vmax is reached when all enzyme sites are saturated with the substrate.
- This will happen when substrate concentration [S] is greater than K m.

KINETIC DATA

LINEWEAVER–BURK PLOT

- The Lineweaver–Burk plot is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934.
- V = V max [S] /Km +[S]
- Taking the reciprocal of this equation gives
- 1/V= Km +[S]/V max [S]Km= [Km /V max].1/[S]+1/Vmax

LINEWEAVER–BURK PLOT



- This plot was widely used to determine Km and Vmax, before the wide availability of powerful computers and non-linear regression software.

Kinetics: its Advantage and Disadvantage

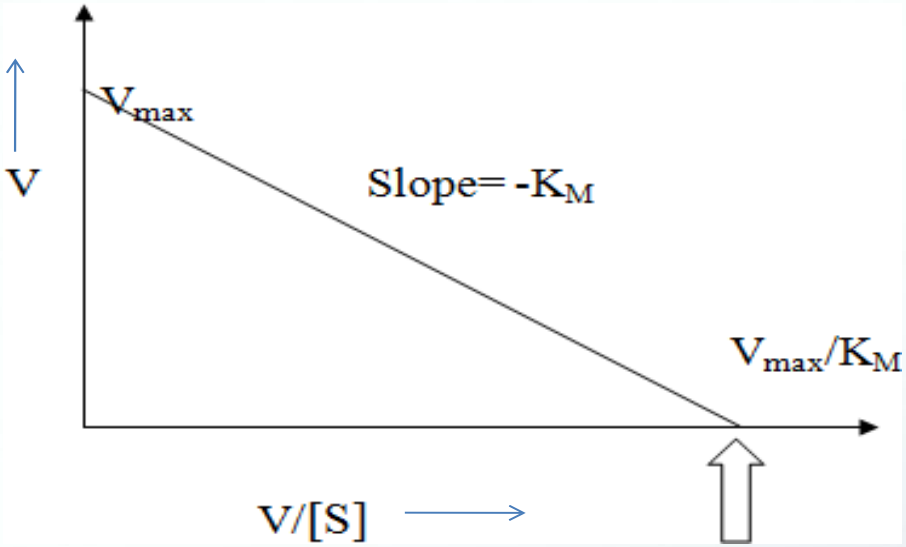
Advantage

+ This plot estimate the values of Km and Vma more precisely and in best precision.

Disadvantage

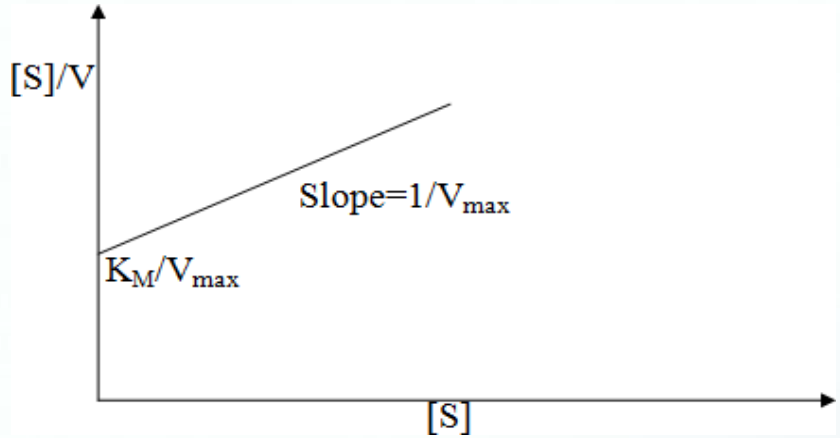
+ It emphasizes points at lower concentration and compresses data
+ points obtained at high concentration.

EADIE–HOFSTEE PLOT



- Eadie–Hofstee diagram is a graphical representation of enzyme kinetics in which reaction velocity is plotted as a function of the ratio of velocity and substrate concentration.
- V = V max.(-K m V/[S])

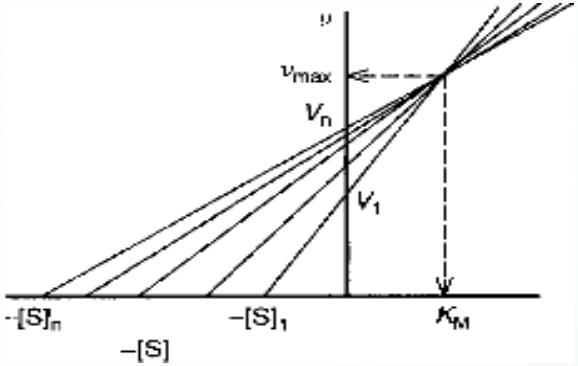
HANES – WOOLF PLOT



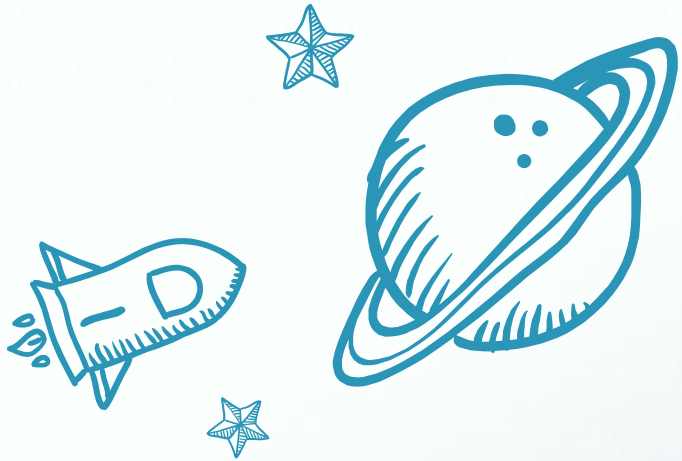
- Another linear representation of the Michaelis-Menten equation is he Hanes- Woolf plot.
- Thus a plot of [S]/V v/s [S] is linear, with a slope of I/V max, The y- intercept gives K m/V max, and the x-intercept gives –K m.

$$\frac{[S]}{v} = \frac{1}{V_{max}}[S] + \frac{K_M}{V_{max}}$$

EISENTHAL AND CORNISH-BOWDEN PLOT



- This plot provide the best estimates of Km and Vmax.
- 'V' is plotted on the y-axis and a corresponding negative value of [S] is plotted on the x-axis.
- A straight line is then drawn, passing through the points on the two axis and extending beyond the " point of intersection."
- This is repeated for each set of V and [S] values.
- A horizontal line drawn from the point of intersection to the y-axis provides the V max, value, whereas a vertical line from the point of intersection to the x-axis provides the K m value.



Inhibition

HANES – WOOLF PLOT

+ Enzyme inhibitors are low molecular weight compounds that combine with the enzyme to form an enzyme-inhibitor complex, either reducing or completely inhibiting the catalytic activity of the enzyme.

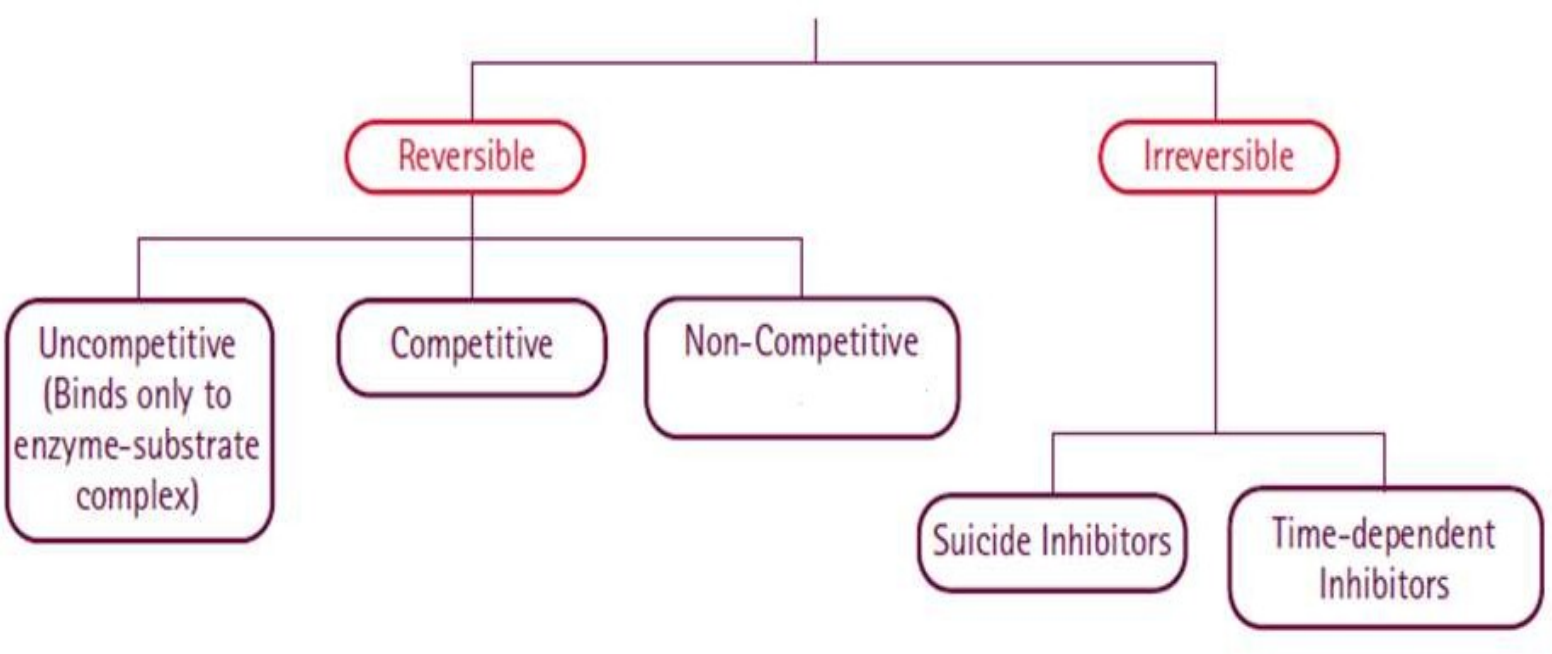
CLASSIFICATION OF ENZYME INHIBITOR

+ Enzyme inhibitors are low molecular weight compounds that combine with the enzyme to form an enzyme-inhibitor complex, either reducing or completely inhibiting the catalytic activity of the enzyme.

+ **Based on the type of binding of enzyme and inhibitor**

1. Reversible inhibitor
2. Irreversible inhibitor

Based on the binding between enzyme and inhibitor



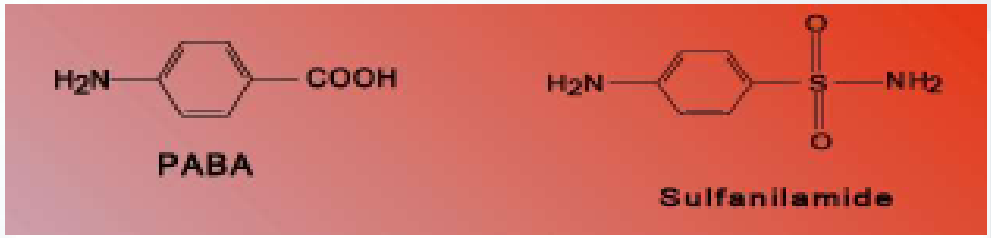
REVERSIBLE INHIBITOR

- Reversible inhibitors are bind to enzyme with non-covalent interactions, such as hydrogen bonds, ionic bonds, and hydrophobic interactions.
- Three types. Described in detail in the following slides.

a. COMPETITIVE INHIBITOR

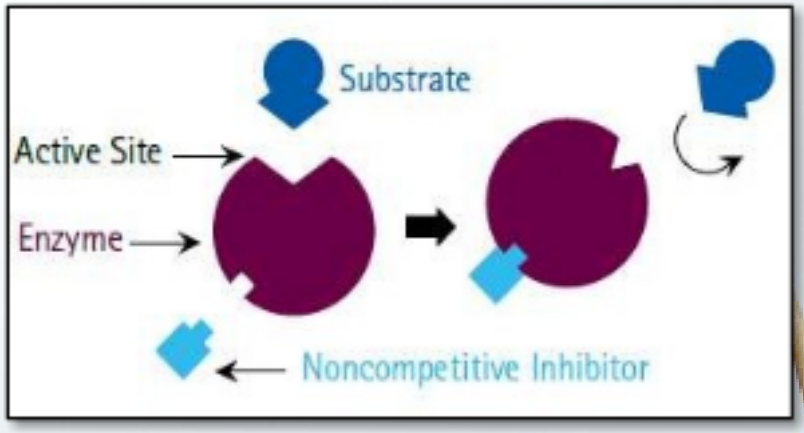
- This inhibitor has structural similarities with the substrate so, the inhibitor competes with the substrate for the active site.
- If the inhibitor binds more tightly than the substrate, then it is an effective competitive inhibitor.
- In competitive inhibition, the inhibitor can bind only to the free enzyme and not with the enzyme-substrate complex.
- Hence inhibition can be overcome by increasing the concentration of substrate in the reaction mixture.

Example: The antibacterial action of sulfanilamide which is a structural analog of PABA. Sulfanilamide inhibits the bacterial enzyme dihydropteroate synthetase which catalyzes the incorporation of PABA into 7,8-dihydropteroic acid.



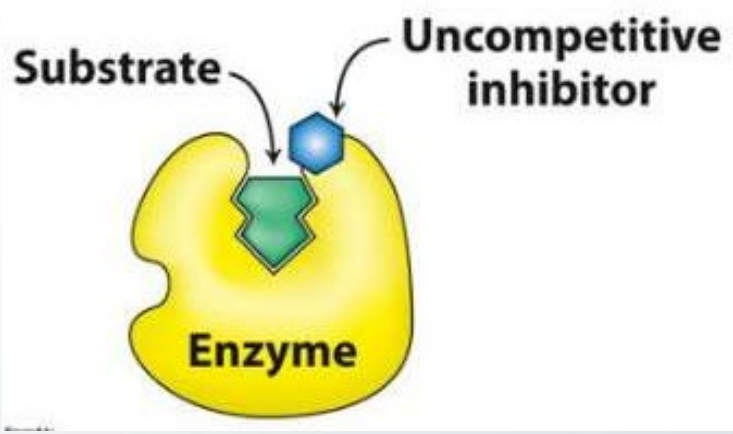
b. NON COMPETITIVE INHIBITOR

- In noncompetitive inhibition, the binding of the inhibitor reduces enzyme activity, but does not affect the binding of substrate.
- These inhibitors bind non covalently to sites other than the substrate binding site.
- Inhibitor binding does not influence the availability of the binding site for substrate.
- Binding of the substrate and the inhibitor are independent of each other and inhibition cannot be overcome by increasing substrate concentration.



c. UNCOMPETITIVE INHIBITOR

- Uncompetitive inhibitors bind only with the enzyme-substrate complex.
- The inhibitor does not bind to the active site of the enzyme and it does not have to resemble the substrate.



IRREVERSIBLE INHIBITOR

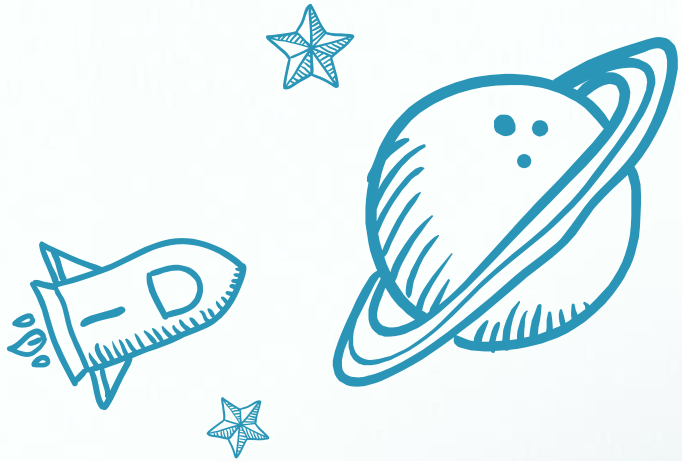
- This type of inhibition involves the covalent attachment of the
- Inhibitor to the enzyme.
- The catalytic activity of enzyme is completely lost.
- It can only be restored only by synthesizing molecules
- It is of two types.

a. SUICIDAL INHIBITOR

- They are relatively un-reactive until they bind to the active site of the enzyme.
- In the first few steps of the reaction it functions like a normal substrate, but then it is converted into a very reactive compound that combines with the enzyme to block its activity.

b. TIME-DEPENDENT INHIBITORS

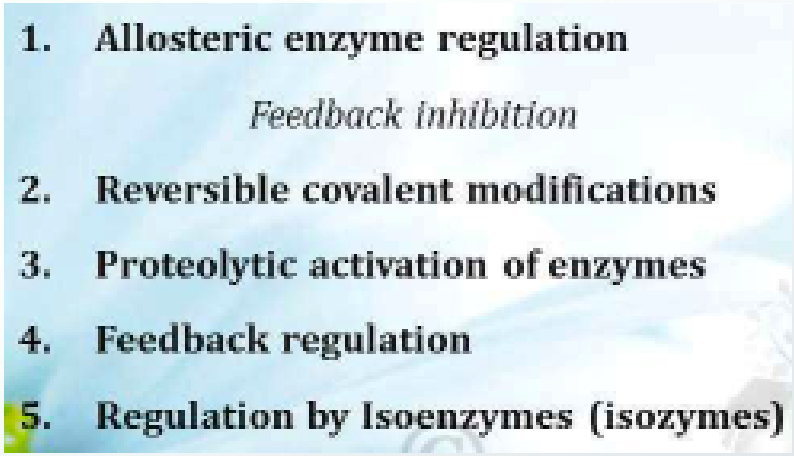
- Time-dependent inhibitors are those that exhibit slow binding to the enzyme.
- The observed onset of inhibition is slower.



Enzyme Modulation

What is enzyme modulation

- An enzyme's catalytic activity can be directly controlled through structural alterations that influence the enzyme's substrate-binding affinity.
- The Mechanism of modulation is as follows:



Enzyme modulation

Definition: *"Process by which cells can turn on, turn off, or modulate the activities of various metabolic pathways by regulating the activity of enzyme"*

- Enzymes have extraordinary catalytic power
- Couse thousand fold increase in chemical reactions
- Can convert millions of substrate molecules into products in fraction of time
- It is very essential to control the activity of enzyme in order to regulate the metabolic activities in the cell

Enzyme modulation mechanism

- Enzyme regulation will permit changing needs of the cell to meet its energy and resource demands
- If a product is available in excess, enzyme regulation could then divert the resources to other needy reactions
- If a product is in demand, it could activate pathways to produce more of the biomolecule that is needed

What are enzyme regulator or modulator?

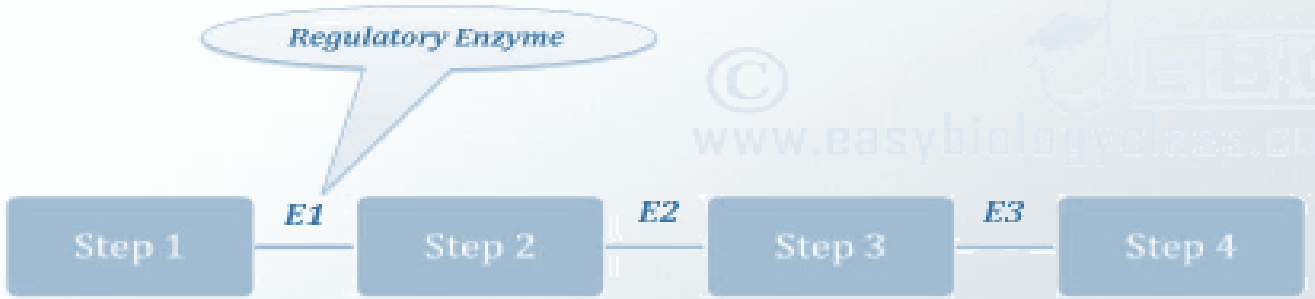
- In cellular metabolic activities, many enzymes work together in a sequence to carry out the given metabolic process
- Example, glycolysis pathway includes ten sequential steps each catalyzed by specific enzymes
- In such an enzymatic process, were more than one steps are involved, the reaction product of one enzymatic reaction acts as the substrate for the next enzyme

What are enzyme regulator or modulator?

- Regulatory enzyme definition: "In a multi-step enzymatic process, there will be one enzyme which will be responsible for the overall rate of that process"
- This critical rate limiting enzyme is called the **regulatory enzyme**
- Regulatory enzyme shows enhanced or decreased catalytic activities in response to other molecules (signals) in the cells

Which is the regulatory enzyme in the multistep metabolic pathway?

- First enzyme of multi-enzyme sequence acts as the regulatory enzyme
- First reaction is the excellent place to regulate a metabolic pathway
- This is because catalysis of even the first few reactions consumes much metabolic energy which can be diverted to other more important processes



1. Allosteric regulation

- Allosteric enzymes are a class of regulatory enzymes
- **Definition**: *A type of enzyme regulation by the reversible non covalent binding of regulatory molecules to the enzyme*
- Regulatory molecules are called allosteric **modulators** or allosteric **effectors**
- Allosteric enzymes have additional conformations induced by the binding of modulators
- Conformational changes induced by the allosteric modulators can produce more active or less active forms of enzyme

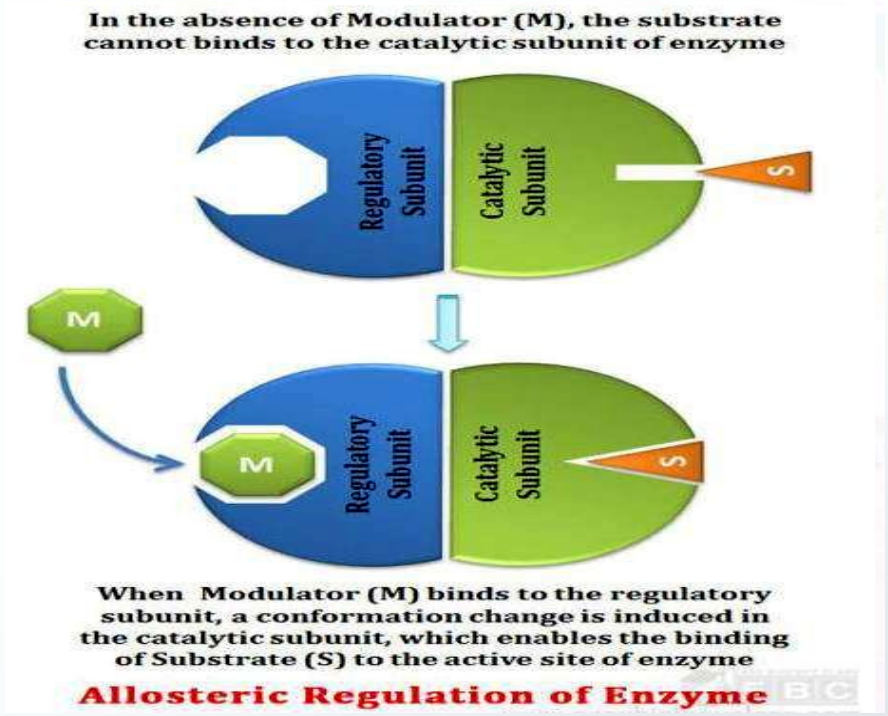
1. Allosteric regulation

- Allosteric modulators may be **inhibitory (+)** or **stimulatory (-)**
- Two types of Allosteric enzymes based on the nature of modulator:
  - ***Homotropic allosteric enzymes***
  - ***Heterotropic allosteric enzymes***
- In most cases, the substrate itself acts as the modulator
- Allosteric enzymes having the substrate and modulators are same are called **homotropic allosteric enzymes**
- Binding of modulator causes conformational changes in the enzyme

1. Allosteric regulation

- Conformational changes affect the subsequent enzymatic activity
- If modulator is any molecule other than substrate, the enzyme is called **heterotropic allosteric enzyme**
- Allosteric modulators are not to be considered as competitive or non competitive inhibitors
- Allosteric enzymes possess one or more **regulatory or allosteric sites**
- Allosteric sites acts as the binding site of modulator
- Modulator and modulator binding site on enzyme are very specific similar to the substrate specific for its active site

1. Allosteric regulation



1. Allosteric regulation

- Allosteric enzymes are larger & more complex than non-allosteric enzymes
- Allosteric enzymes possess many sub-units
- *Aspartate transcarbamoylase (an allosteric enzyme), which catalyze an early reaction in the biosynthesis of pyrimidine nucleotides, has 12 polypeptide chains organized into catalytic and regulatory subunits. Enzymes with several modulators have different and specific binding sites for each*
- In most of the allosteric enzymes, substrate binding side and modulator binding sites are on different subunits

1. Allosteric regulation

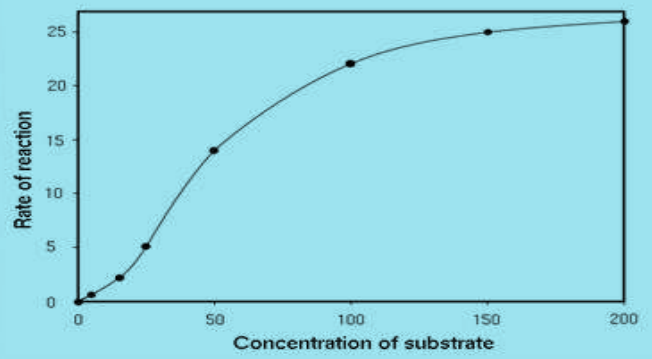
- Substrate binding site is called **catalytic subunit** or C subunit
- Modulator binding subunit is called the **regulatory subunit** or R subunit
- Binding of a positive or stimulatory **modulator (M)** to its specific site on the regulatory subunit is communicated to the catalytic subunit through conformational changes
- This change renders the activation of catalytic subunit
- Activation enables the binding of substrate (S) with higher affinity

1. Allosteric regulation

- Once the modulator is dissociate form the regulatory subunit, the enzyme reverts to its inactive or less active form
- Allosteric enzymes shows variations in enzyme kinetic parameters
- Allosteric enzymes **do not** follow Michaelis-Menten Kinetics
- Allosteric enzymes **do not** show the usual hyper-parabolic curve when the initial velocity V_o is plotted against substrate concentration [S]
- They shows a **sigmoid curve** when the velocity is plotted against substrate concentration

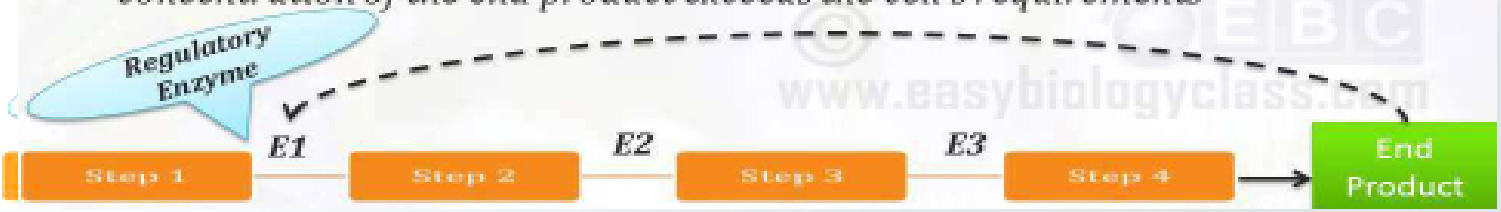
1. Allosteric regulation

- The Lineweaver-Burk plot also shows difference from usual enzymes
- Lineweaver-Burk plot of an allosteric enzyme will be upward concave shaped
- Feedback inhibition is a type of allosteric regulation



1. Allosteric regulation by feedback inhibition

- *Feedback inhibition and feedback regulation are different terms*
- Feedback inhibition is a specific type of allosteric enzymatic activity regulation mechanism in cells
- **Definition**: *in some multi-enzyme pathways, the regulatory enzyme is specifically inhibited by the end product of the pathway whenever the concentration of the end product exceeds the cell's requirements*



1. Allosteric regulation by feedback inhibition

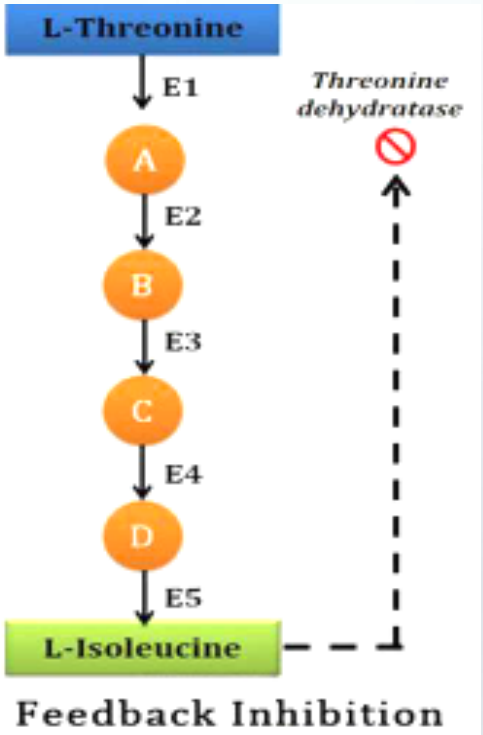
- When the regulatory enzyme reaction is slowed, all subsequent enzymes operate at reduced rates as their substrates are also depleted
- Rate of production of the pathway's end product is thereby brought into balance with the cell's needs
- This type of regulation by the end product inhibiting to the first enzyme of the multi-enzymatic pathway is called feedback inhibition

1. Example of Allosteric regulation by feedback inhibition

- Most cited example of allosteric feedback inhibition is the biosynthesis of L-isoleucine from L-threonine in bacteria
- In bacteria the conversion of L-threonine to L-isoleucine occurs in **five** steps
- Each step is catalyzed by a specific enzyme
- First enzyme in this system is **Threonie dehydratase**
- Threonine dehydratase is **inhibited by isoleucine**, the product of the last reaction (**end product**)
- This is an example of heterotropic allosteric inhibition
- Isoleucine is quite specific as an inhibitor

1. Example of Allosteric regulation by feedback inhibi

- Any other intermediate in this sequence cannot inhibit threonine dehydratase
- Also, any other enzyme in the sequence is not inhibited by isoleucine
- Isoleucine binds not to the active site of the enzyme
- It binds to another specific site on the enzyme molecule, the regulatory site
- The binding is non-covalent and readily reversible



Feedback Inhibition

1. Example of Allosteric regulation by feedback inhibition

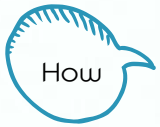
- When the isoleucine concentration decreases, the isoleucine binds to the regulatory site of threonine dehydratease detaches and making the enzyme active again
- Thus threonine dehydratase activity responds rapidly and reversibly to fluctuations in the cellular concentration of isoleucine

2. Regulation by reverse covalent modification

How

- Catalytic activity is modulated by reversible covalent modification of enzyme
- More than 500 molecules are known to modify enzymes by this method
- There will be separate enzyme for adding and removing of modifying groups
- Most common modifying groups include:
  - Phosphoryl group
  - Acetyl group
  - Adenylyl group
  - Uridylyl group
  - Methyl group
  - Amide group
  - Carboxyl group
  - Hydroxyl group

2. Regulation by reverse covalent modification

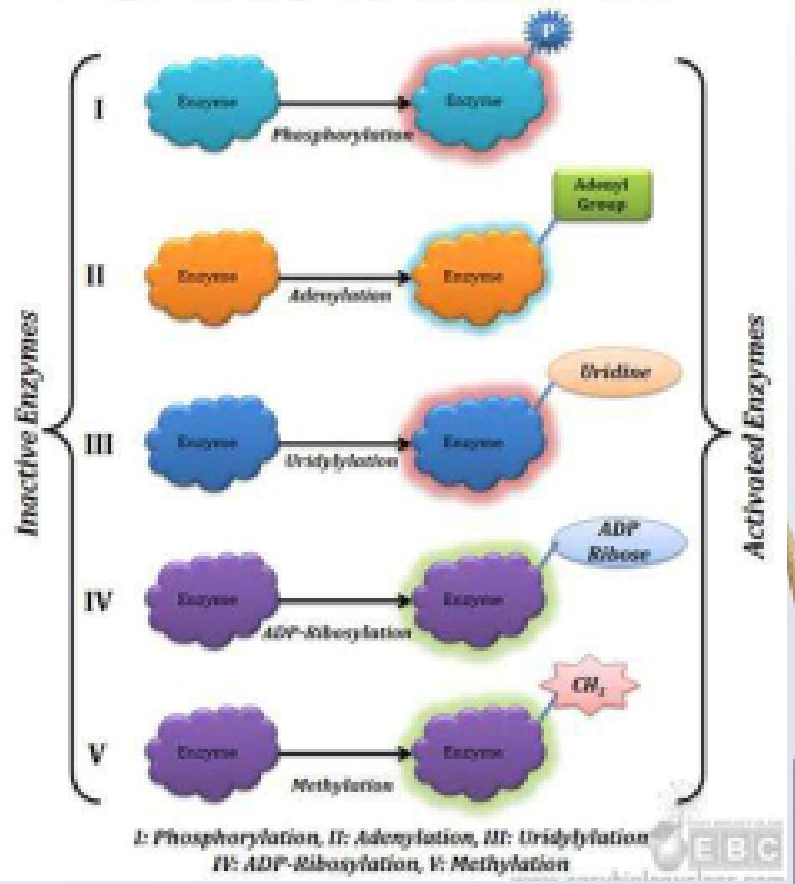


- Common reversible covalent modifications of enzyme:

1. **Phosphorylation:** most common type, addition of phosphate group to Tyr, Ser, Thr and His residue of protein
2. **Adenylation:** addition of adenine to Tyr residue of protein
3. **ADP-ribosylation:** addition of ADP ribose to Arg, Gln, Cys and Diphthamide of protein (dipthamide is a modified Histidine residue)
4. **Methylation:** addition of methyl group to Glu residue of protein

Example

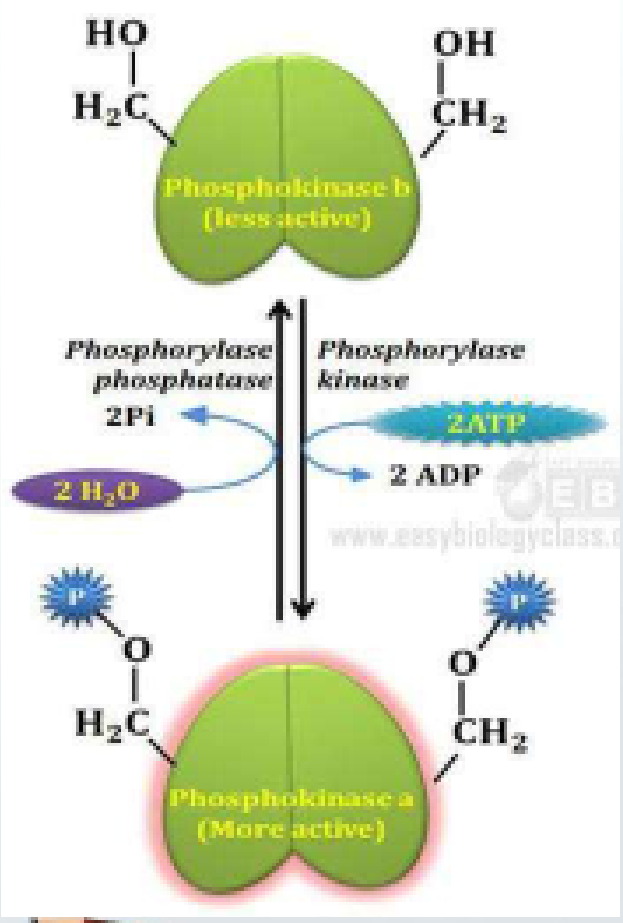
2. Regulation by reverse covalent modification



2. Regulation by reverse covalent modification by phosphorylation

Mechanism

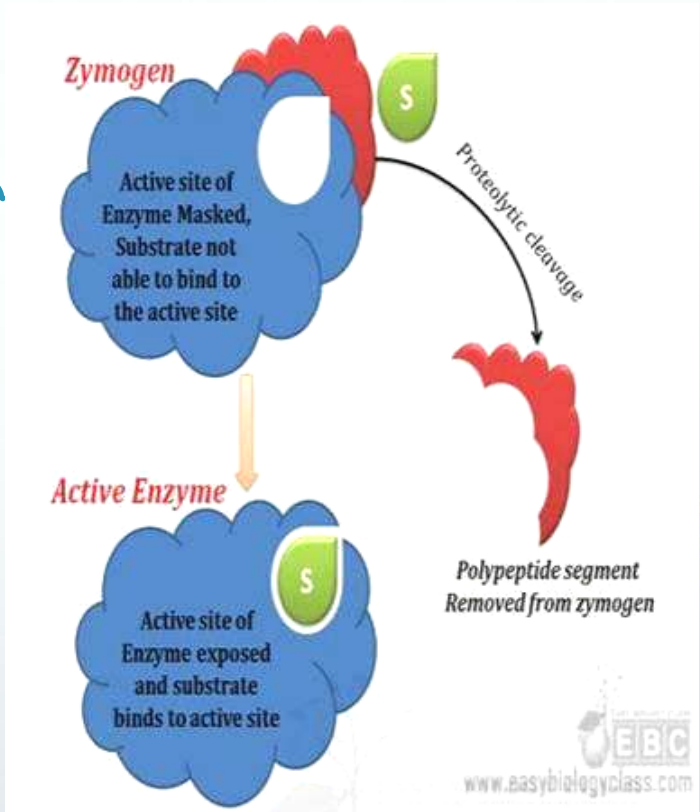
Regulation of Glycogen phosphorylase activity by covalent modification: Phosphorylase exists in two forms. More active Phosphorylase a and less active phosphorylase b. Specific phosphorylation of less active phosphorylase b on Ser 14 on each subunit by two molecule of ATP by phosphorylase kinase enzyme produces the more active phosphorylase a enzyme. Similarly the de-phosphorylation by the enzyme Phosphorylase phosphatase produces the less active Phosphorylase b enzyme



3. Regulation of enzyme by proteolytic enzyme

Mechanism

- Enzymes regulated by proteolytic cleavage method are produced first as inactive forms
- Inactive form of enzyme is called **zymogen or pro-enzyme**
- Inactive nature of zymogens is duet the fact that the active site will be masked or covered by part of polypeptide chain



Zymogen Activation by Proteolytic Cleavage

3. Regulation of enzyme by proteolytic enzyme

- Zymogens are later converted to active enzymes
- Conversion is done by the removal of specific parts of the enzyme by proteolytic cleavage
- Specific cleavage causes conformational changes that expose the active site of enzyme
- This type of activation is irreversible (once enzyme is activated, it cannot be made inactive)

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Mechanism

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examples

Chymotrypsinogen	→	Chymotrypsin
Trypsinogen	→	Trypsin
Pepsinogen	→	Pepsin
Procarboxypeptidase	→	Carboxypeptidase
Proelastase	→	Elastase
Procollagen	→	Collagen
Procaspase	→	Caspase
Prothrombin	→	Thrombin
Fibrinogen	→	Fibrin

3. Regulation of enzyme by proteolytic enzyme

- Significance of enzyme production as zymogen:
  - Helps to prevent the autocatalytic damage of cellular components
  - Assist in the mobilization of enzyme in the cell
  - Can be converted to active forms when it is needed
  - Can be stored for long time as zymogen
  - Hal-life of zymogens are usually more than its active enzymes

4. Regulation of enzyme by feedback regulation

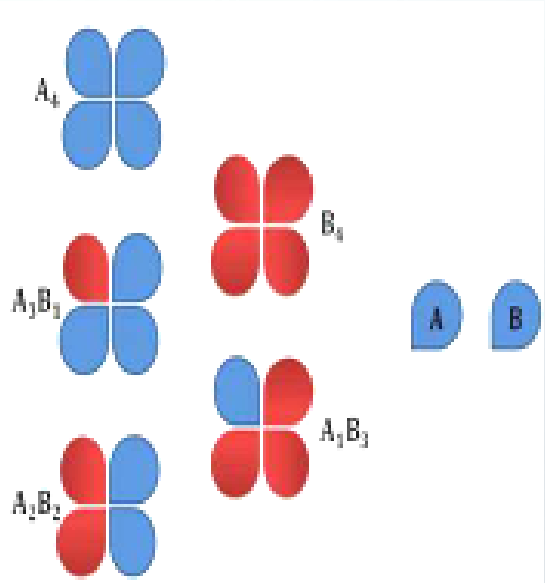
- Feedback regulation is different from feedback inhibition
- A type of enzymatic activity regulation
- Here the end product of an enzymatic pathway directly inhibit the synthesis of concerned enzyme by interfering with the gene of that enzyme
- Enzyme is not directly inhibited by the end product
- End product reduces the concentration of enzyme by inhibiting the synthesis of new enzyme molecules
- Best example is the reduction of HMG CoA reductase enzyme by dietary cholesterol

5. Regulation of enzyme by isozyme

- Not a direct method of enzyme regulation
- Isozymes are enzyme doing similar catalytic function but having different amino acid sequences
- They have different kinetic parameters
- The K_m values, V_{max} and V_o of isozymes varies
- Km denotes the affinity of enzyme towards its substrate
- Isozymes enable the cell to catalyze same reaction in different conditions of the cells

5. Regulation of enzyme by isozyme

- Mammalian Lactate dehydrogenase (LDH) is a classic example for isoenzyme
- Mammalian LDH is a tetramer of two types subunits (A subunit and B subunit)
- LDH exists in five different forms based on the tetrameric association of A and B subunits
- They are A4, A3B1, A2B2, A1B3 and B4



Isoenzymes of Lactate Dehydrogenase (LDH)

5. Regulation of enzyme by isozyme

- Kinetic parameters of all these isoforms varies
- Different tissues express different isoenzymes appropriate to their metabolic needs
- By regulating the relative amounts of A and B subunits the cells of various tissues can regulate the enzymatic activity in specific tissues
- Example: in the liver the A4 type of LDH predominates where as in heart the B4 type predominates. In brain the A1B3 type is the most common one


Thank you !!