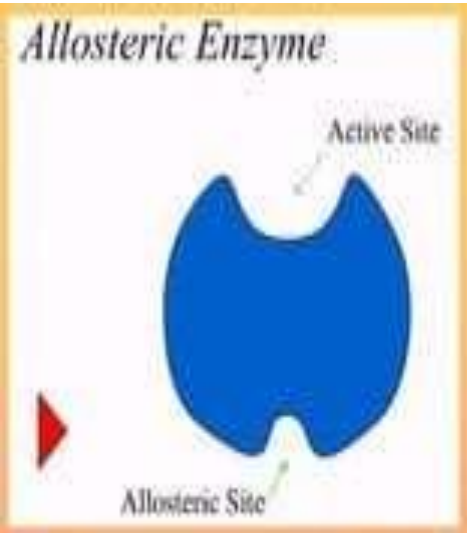
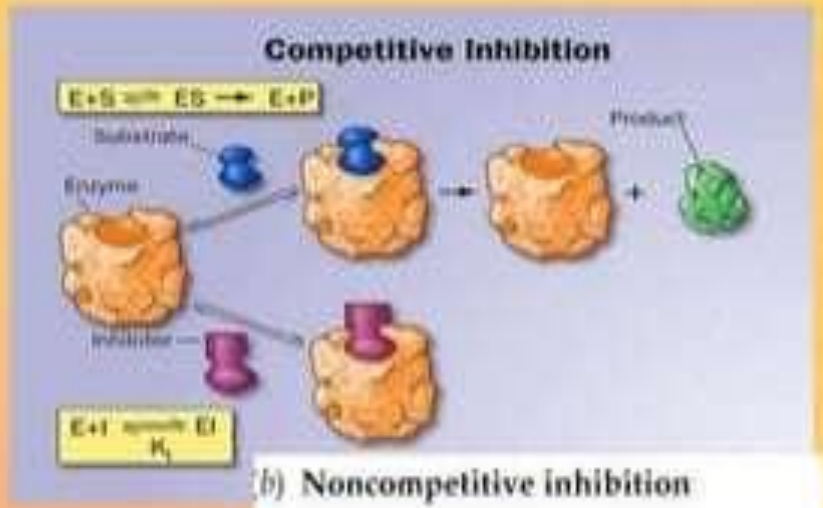
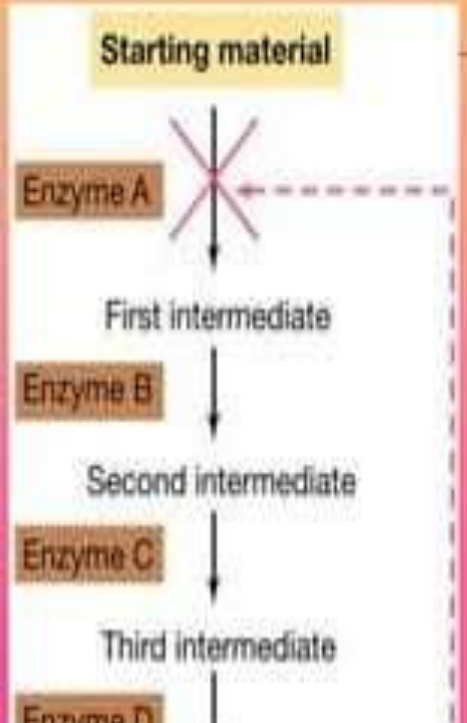


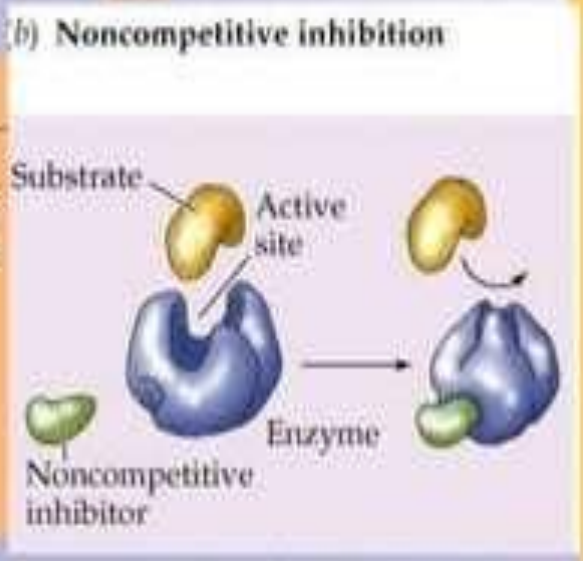


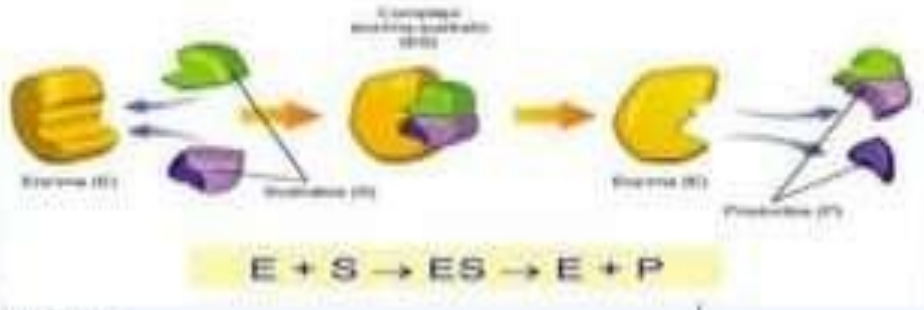


Enzyme Inhibition

Dr.N. Sivaranjani, MD

Asst Prof



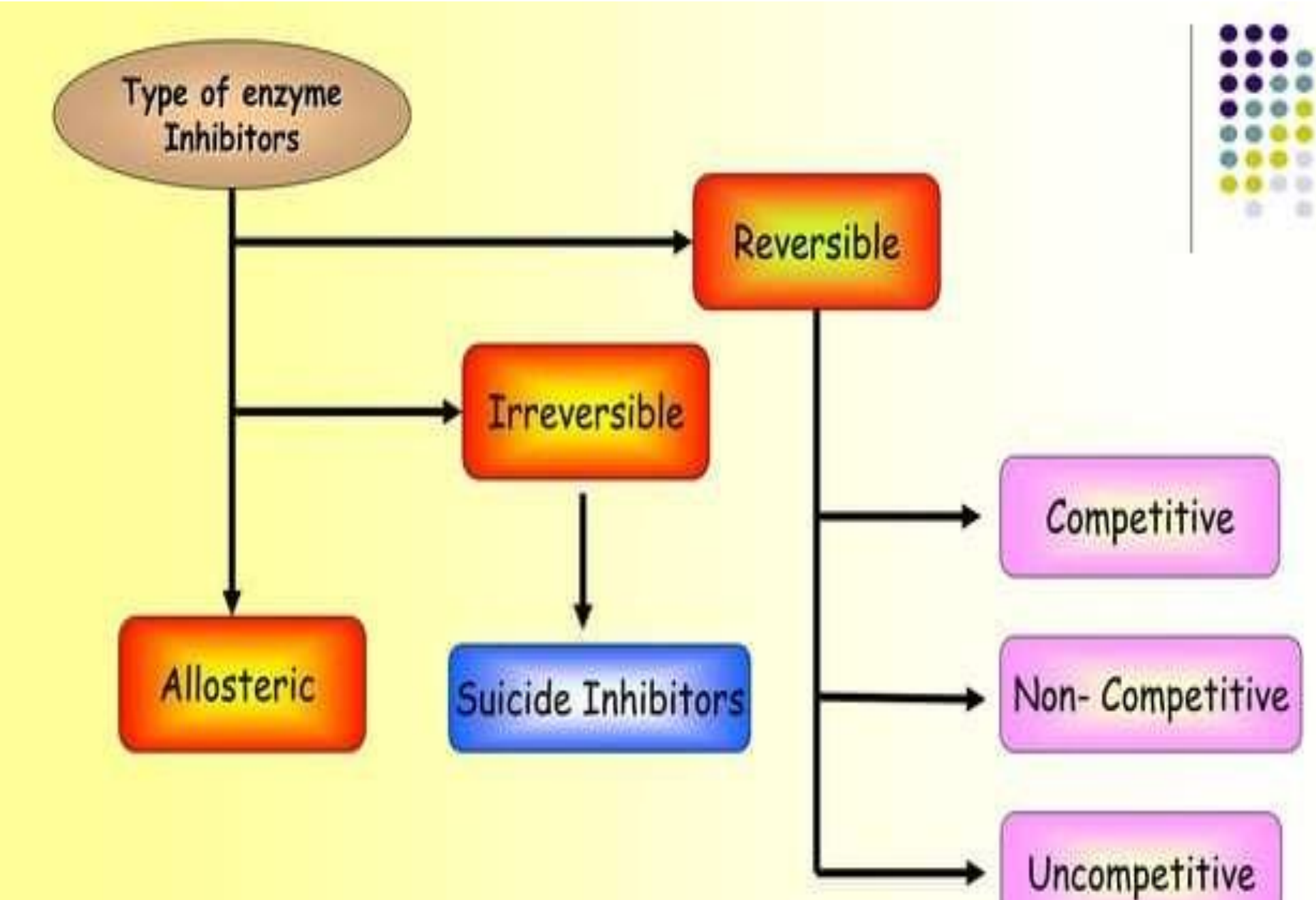


Enzyme Inhibitor

- An Enzyme inhibitor is a compound that decreases or diminish the rate or velocity of an enzyme-catalyzed reaction by influencing the binding of S and /or its turnover number.

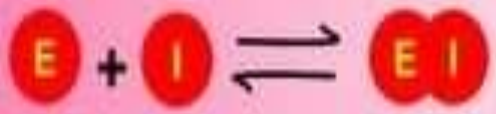
- The inhibitor may be organic or inorganic in nature

- Inhibitors - drugs, antibiotics ,toxins and antimetabolite or natural products of


Type of enzyme Inhibitors
Reversible
Irreversible
Allosteric
Suicide Inhibitors
Competitive
Non- Competitive
Uncompetitive

Reversible Inhibition

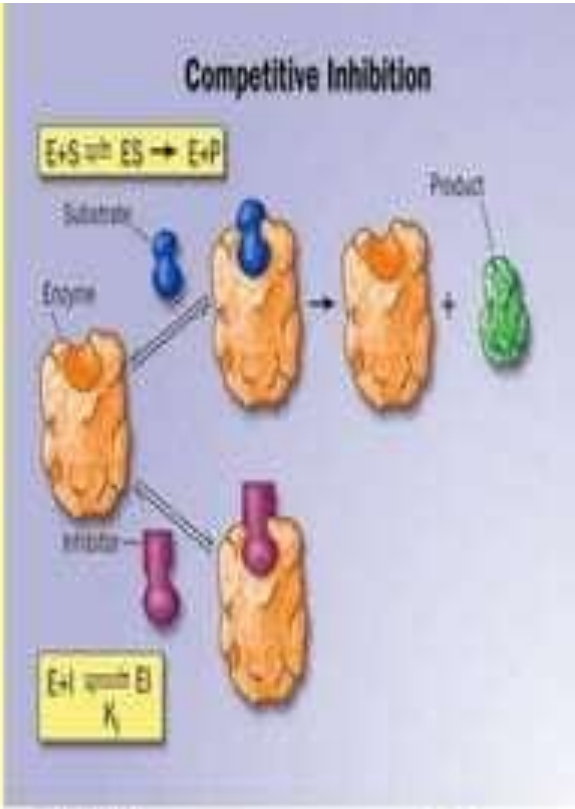
- Inhibitor binds non-covalently (weak interaction) with Enzyme
- If inhibitor is removed - action of E fully restored - Reversible
- An Equilibrium is established between the free inhibitor & EI Complex and is defined by an equilibrium constant (Ki)

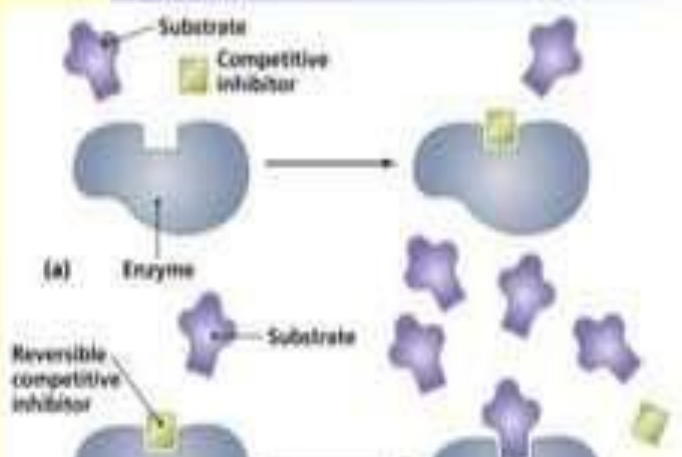


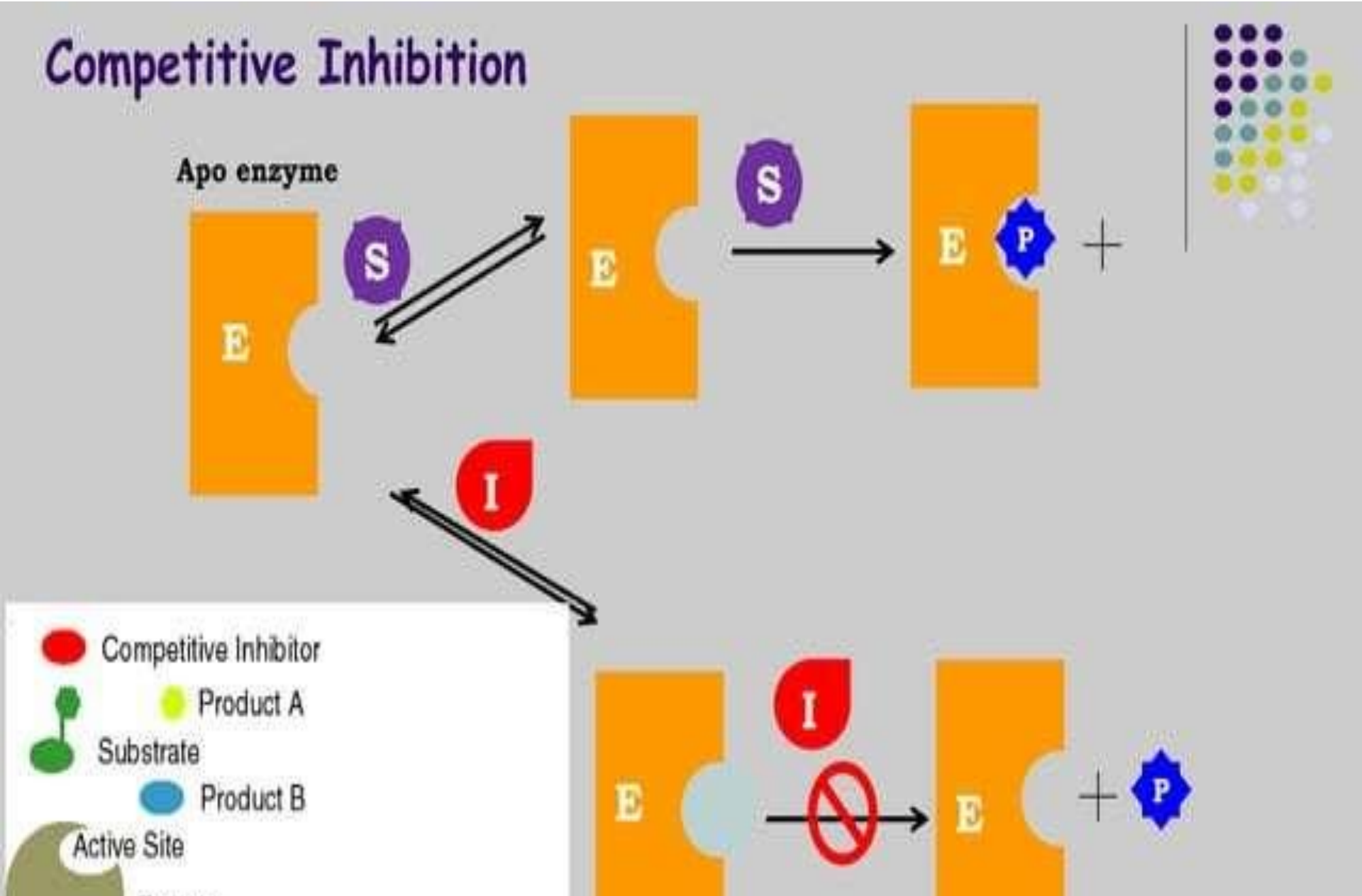
- The activity of Enzyme is fully restored on removing the Inhibitor by dialysis

Competitive Inhibition

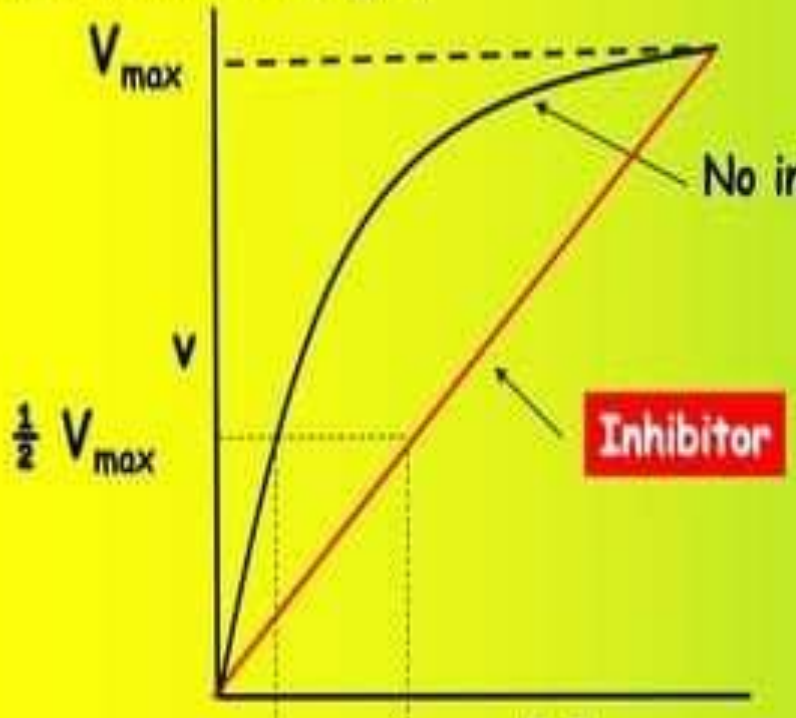
- Inhibitor binds reversibly to the same site that the substrate binds - competes with the S for binding.
- Substrate analogue - I closely resembles the S
- I can be reversed by increasing the conc. of S - reversible
- Degree of inhibition - depend on the conc. of S & I and on the relative affinities of the enzyme for

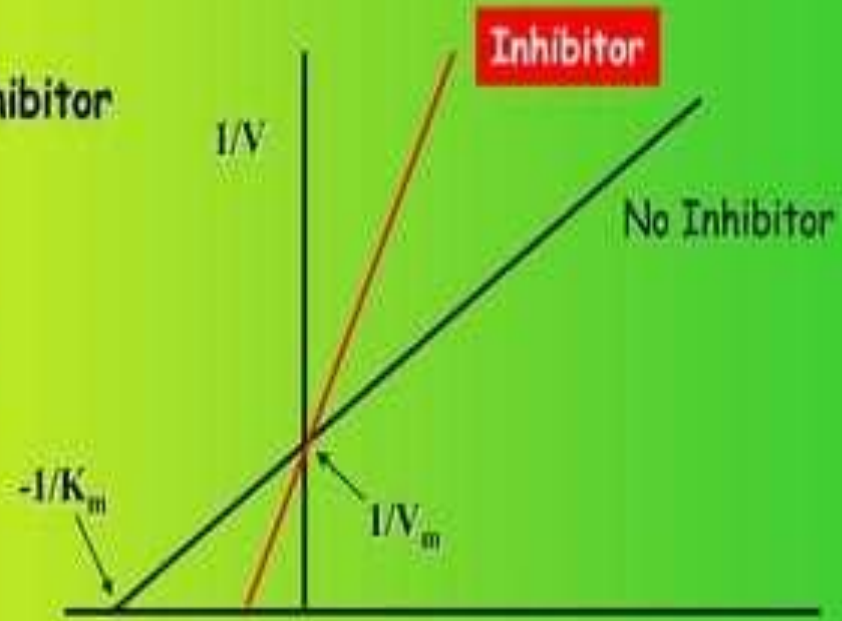




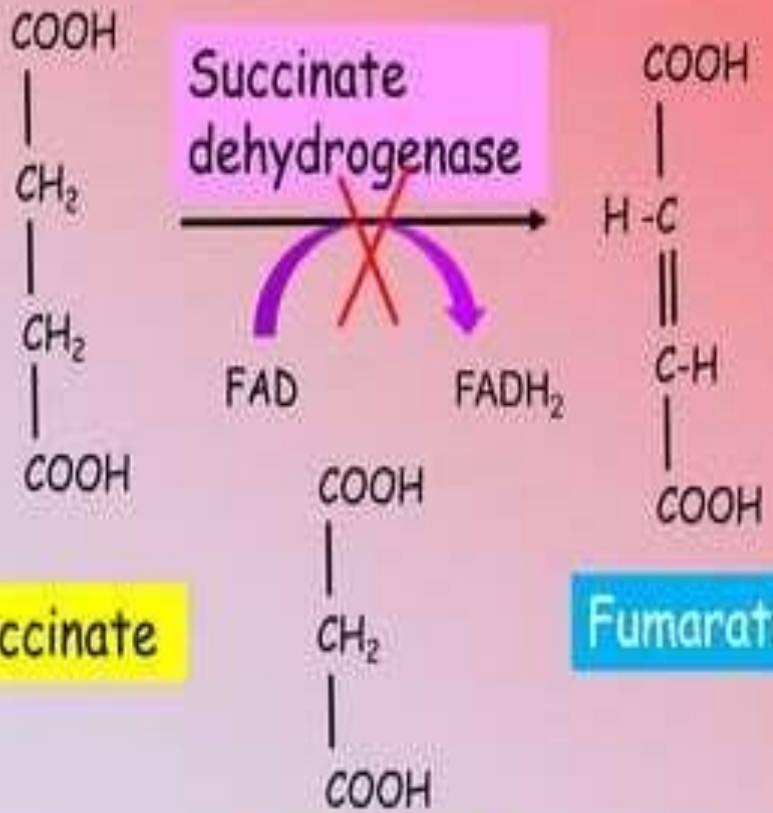

Competitive Inhibition
Apo enzyme
E
S
E
S
E
P
+
I
Competitive Inhibitor
Product A
Substrate
Product B
Active Site
E
I
E
P

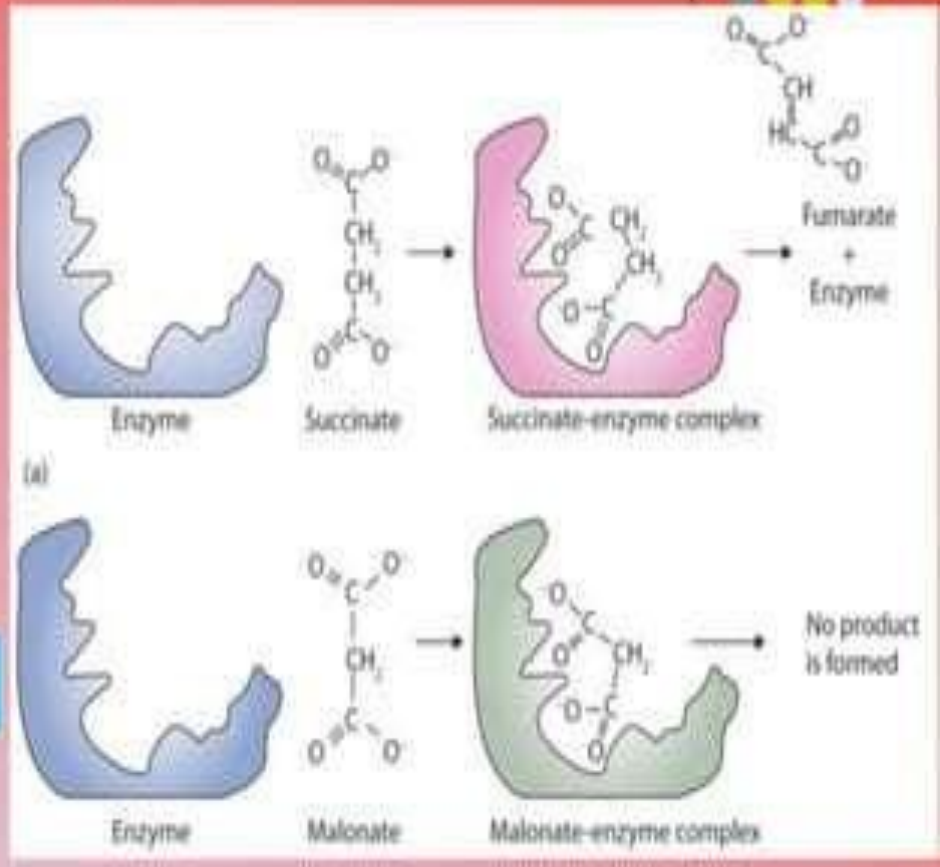
- Velocity is decreased - effective concentration of enzyme is reduced
- Km is increased -affinity of the enzyme towards substrate is apparently decreased in presence of the inhibitor
- Vmax is not changed





Malonate is a competitive inhibitor of SDH





Similarity in three dimensional

Antibacterial action of sulpha drugs (sulfonamide) - structural analog of PABA

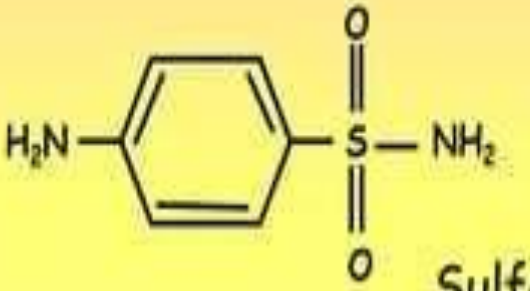
PABA + 7,8-dihydropteroic acid

Bacteria — Pteroid synthetase

Folic acid

PABA- para amino benzoic acid

Sulfonamide inhibits the bacterial enzyme



Sulfanilamide

Non toxic to human -human cannot synthesize Folic acid

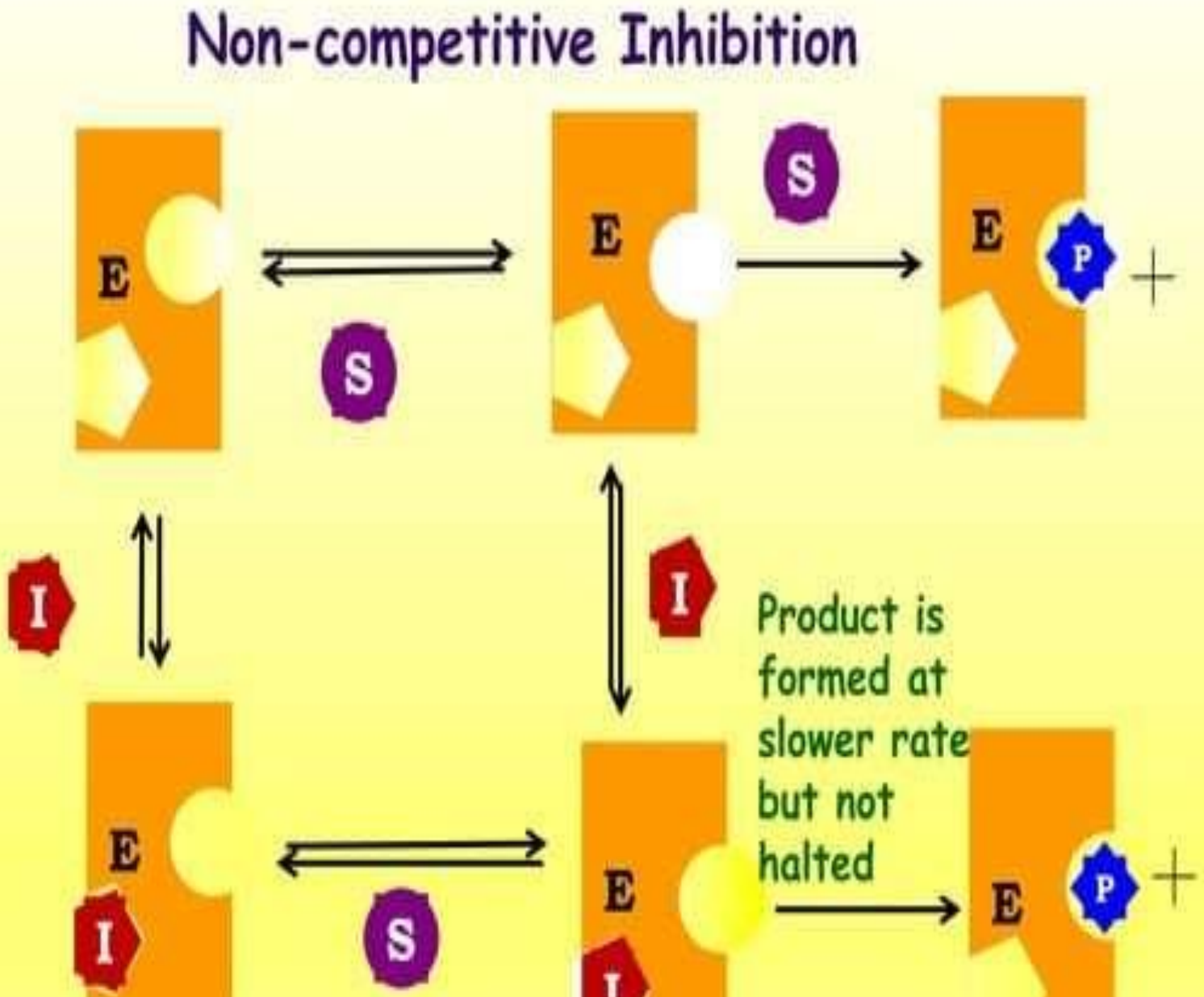
Clinically useful Competitive Inhibition

Drugs	Target Enzyme	Therapeutic Use
STATINS - Atorvastatin , simvastatin	HMG CoA reductase	Decrease plasma Cholesterol level - Antihyperlipidemic agents
Allopurinol	Xanthine oxidase	Gout
Methotrexate	Dihydrofolate reductase	Cancer
Captopril & Enalapril	Angiotensin converting enzyme	High blood pressure
Dicoumarol	Vit.K-epoxide-reductase	Anti-coagulant

Non-competitive Inhibition

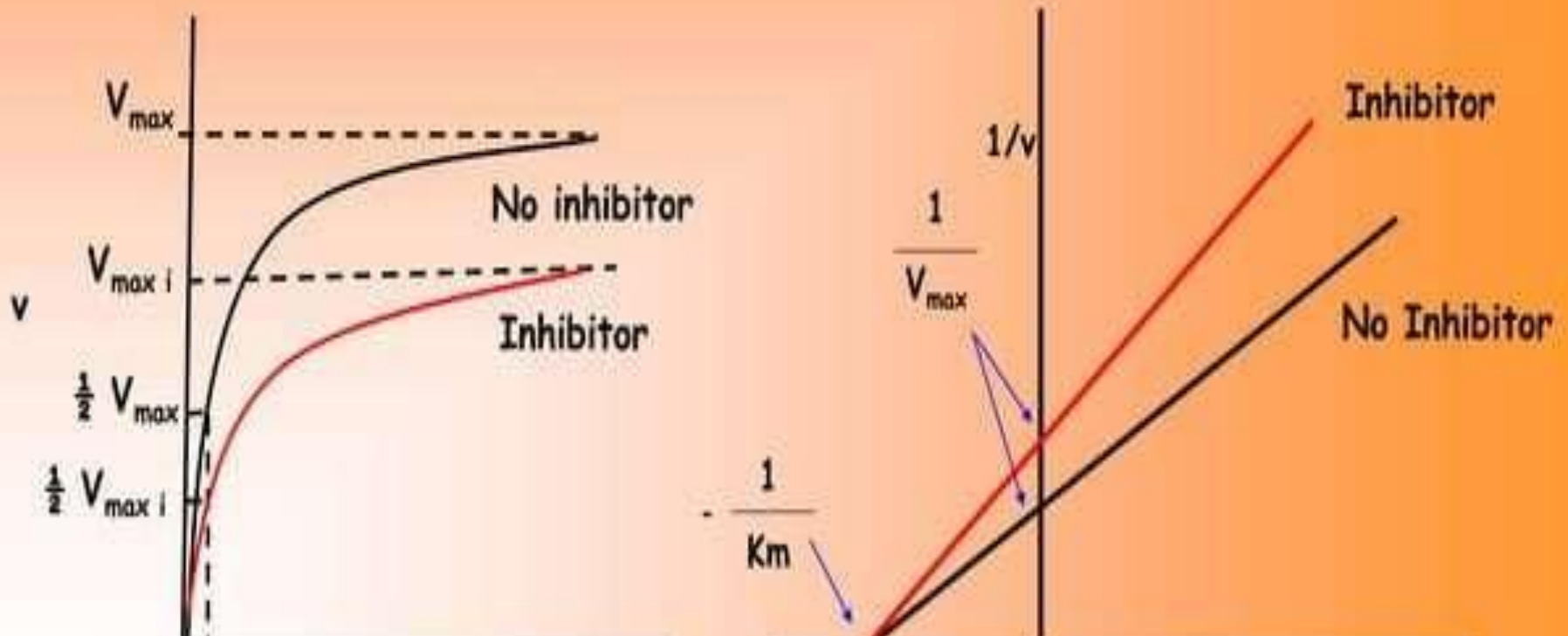
- Inhibitor binds at a site other than the active site of the enzyme
- I has no structural resemblance to the S - No competition for binding
- Increase in the S conc. does not relieve this I
- I & S binds at different site - formation of both EI and EIS complexes is possible.
- EIS - forms product at a slower rate than ES
- Reaction is slowed down but not halted.

Non-competitive Inhibition



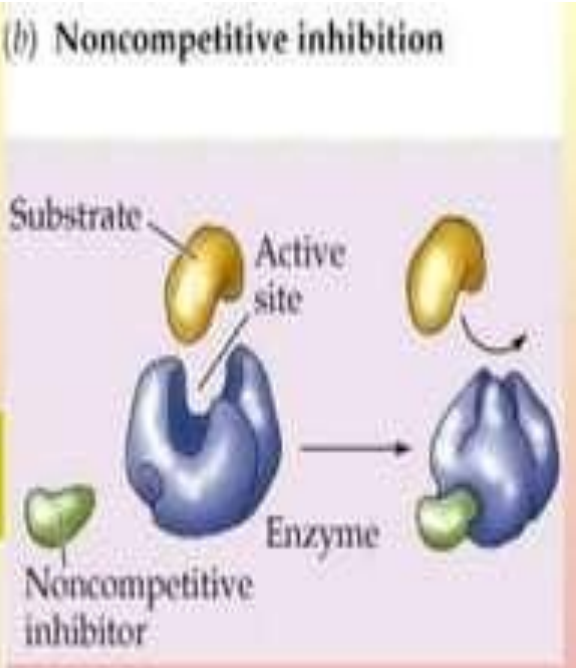
K_m value is unchanged - I do not interfere with the binding of S to E

V_{max} decreases - I cannot be overcome by increasing the conc. of S



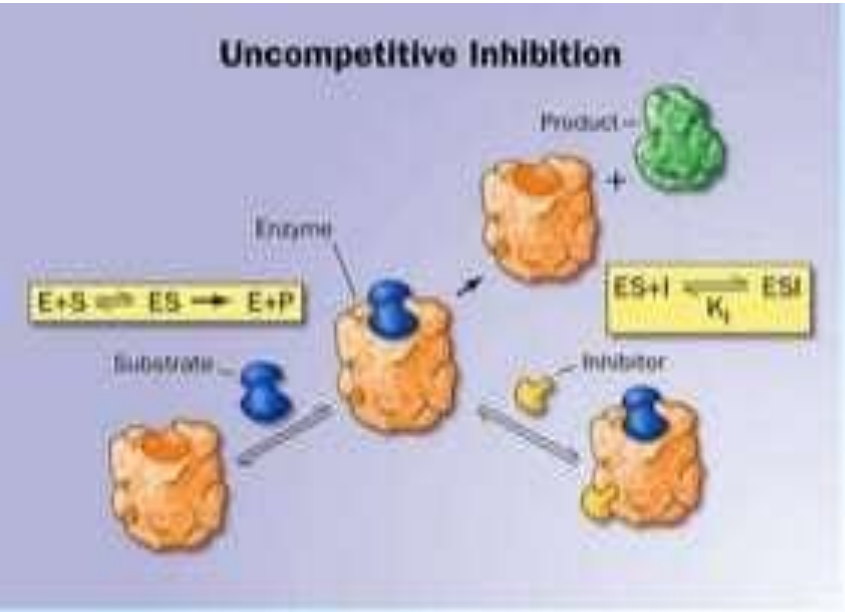
Non competitive inhibitor

Inhibitor	Enzyme inhibited
Heavy metals - Ag^{2+} ,Hg^{2+} , Pb^{2+}	Binding with cysteinyl SH gr of E
Pepstatin	Pepsin
Soyabean trypsin inhibitor	Trypsin
Ethanol or narcotic drugs	Acid phosphatase



Uncompetitive Inhibition

- I binds only to the ES complex , not to free E
- I - cause structural distortion of the active site - E catalytically inactive
- I can't be reversed by increasing the [S] since I doesn't compete with S for the same binding site
- Inhibition of placental alkaline phosphatase (Regan iso-enzyme) by phenylalanine .



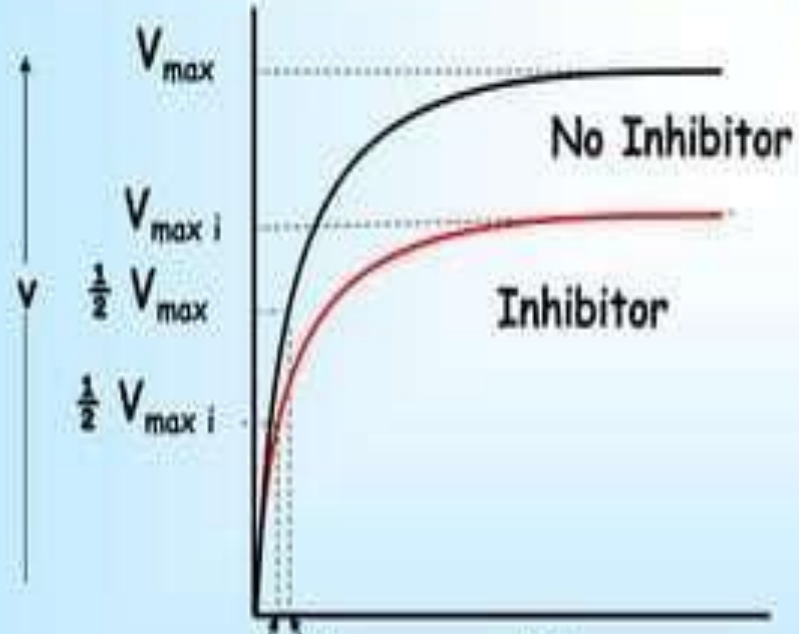
$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

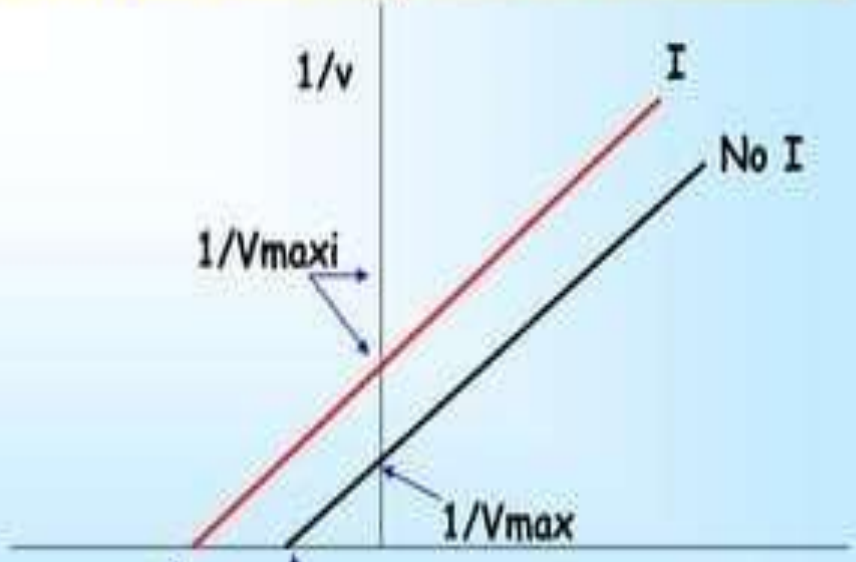
$$ES + I \rightleftharpoons ESI$$

Vmax = Decreases

Km = Decreases

I has no affinity for free E





Irreversible Inhibition

- Inhibitor binds covalently (strong) with the enzyme irreversibly
- so it can't dissociate from the enzyme

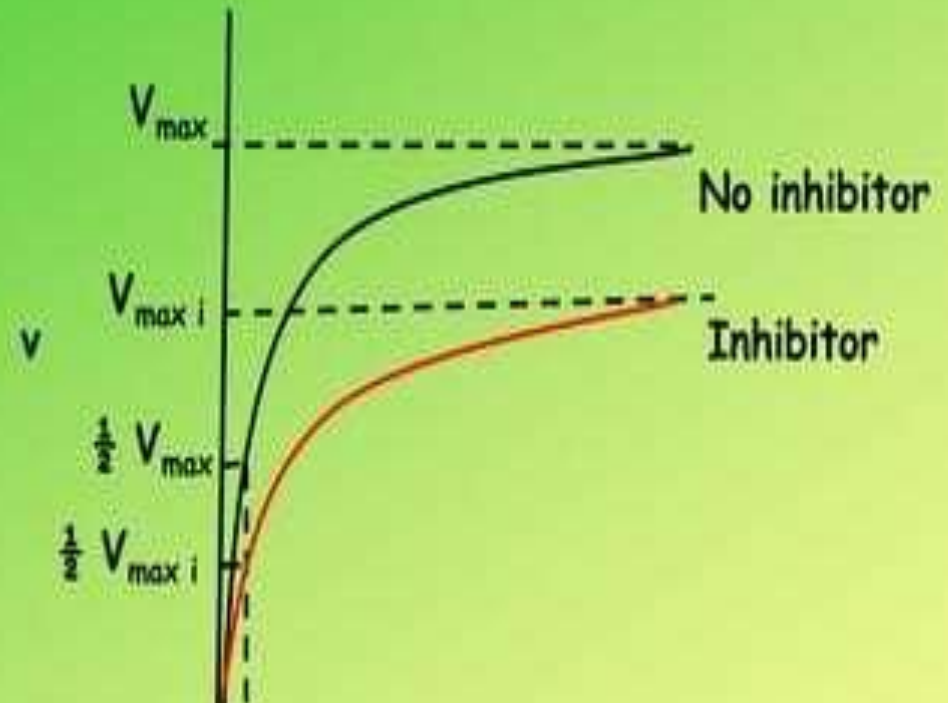
- Inhibitor cause conformation change at active site of the E- destroying their capacity to function as catalysts.

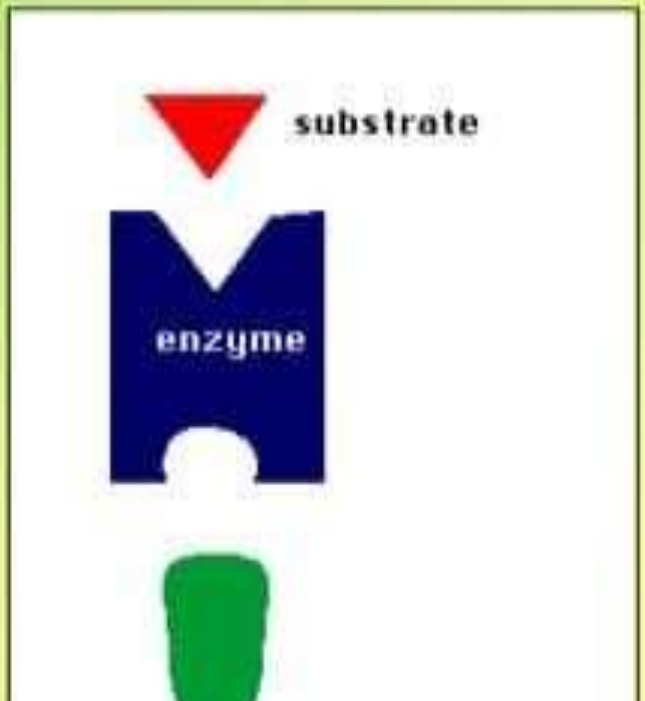
- Enzyme activity is not regained on dialysis / by increasing the conc. of S

- A variety of poisons, such as iodoacetate, OP poisoning and oxidizing agents act as irreversible inhibition.

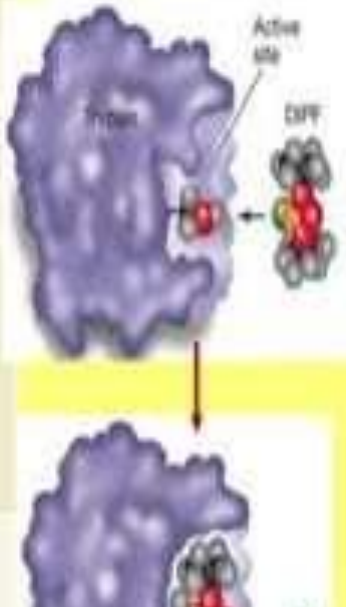
Irreversible Inhibition

In terms of kinetics – irreversible is similar to non competitive inhibition
Vmax – Decreased
Km – No change





Inhibitors	Enzyme inhibited	Therapeutic uses
Disulfiram	Aldehyde dehydrogenase	Treatment of Alcoholism
Cyanide	Cytochrome oxidase	Inhibits respiratory chain
Fluoride	Enolase	Inhibits Glycolysis
Melathion	Acetylcholine esterase	Organophosphorus insecticide
Di-isopropyl fluorophosphate	Serine proteases, Acetylcholine esterase	Nerve gas
BAL - British Anti	reacts with the SH group	Antidote for heavy metal

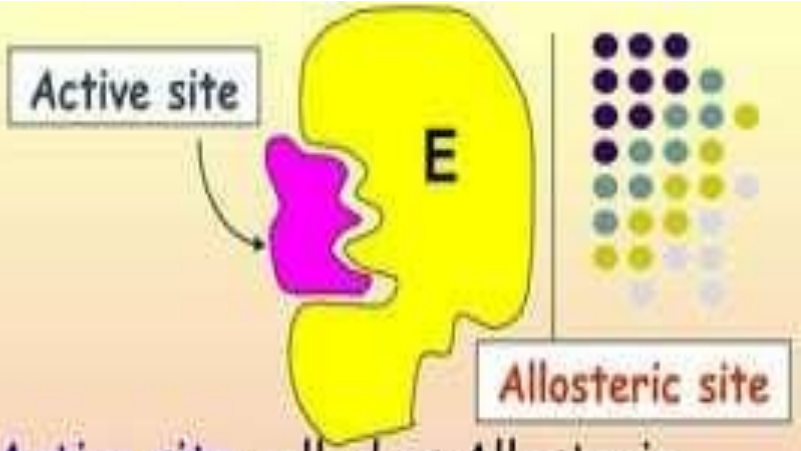


Suicide Inhibition

- Specialized form of Irreversible inhibition
- Also known as Mechanism based inactivation
- I makes use of the enzyme's own reaction mechanism to inactivate it
- Inhibitor (structural analog) is converted to a more effective inhibitor with the help of the E to be inhibited
- E literally commits suicide - they utilize normal E reaction mechanism to

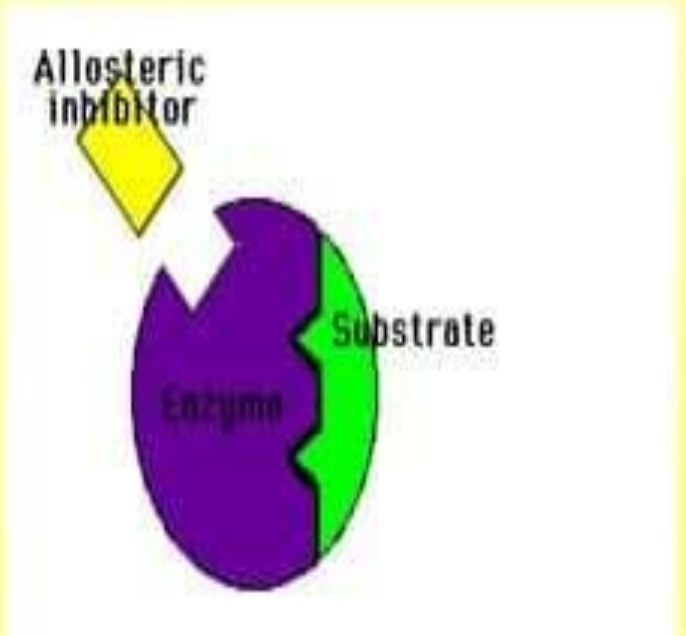
Drugs	Product	Target Enzyme	Therapeutic Use
Allopurinol	Alloxanthin	Xanthine Oxidase	Gout
5-fluorouracil	Fluorodeoxy uridylate	Thymidylate synthase	Cancer
Aspirin	acetylates serine residue in the active center of cyclo-oxygenase	Cyclo-Oxygenase	Non Steroidal Anti-inflammatory Drug (NSAID)
Difluro methyl ornithine (DFMO)	Irreversible covalent complex with the co-enzyme	Ornithine Decarboxylase	Trypanosomiasis (sleeping sickness)

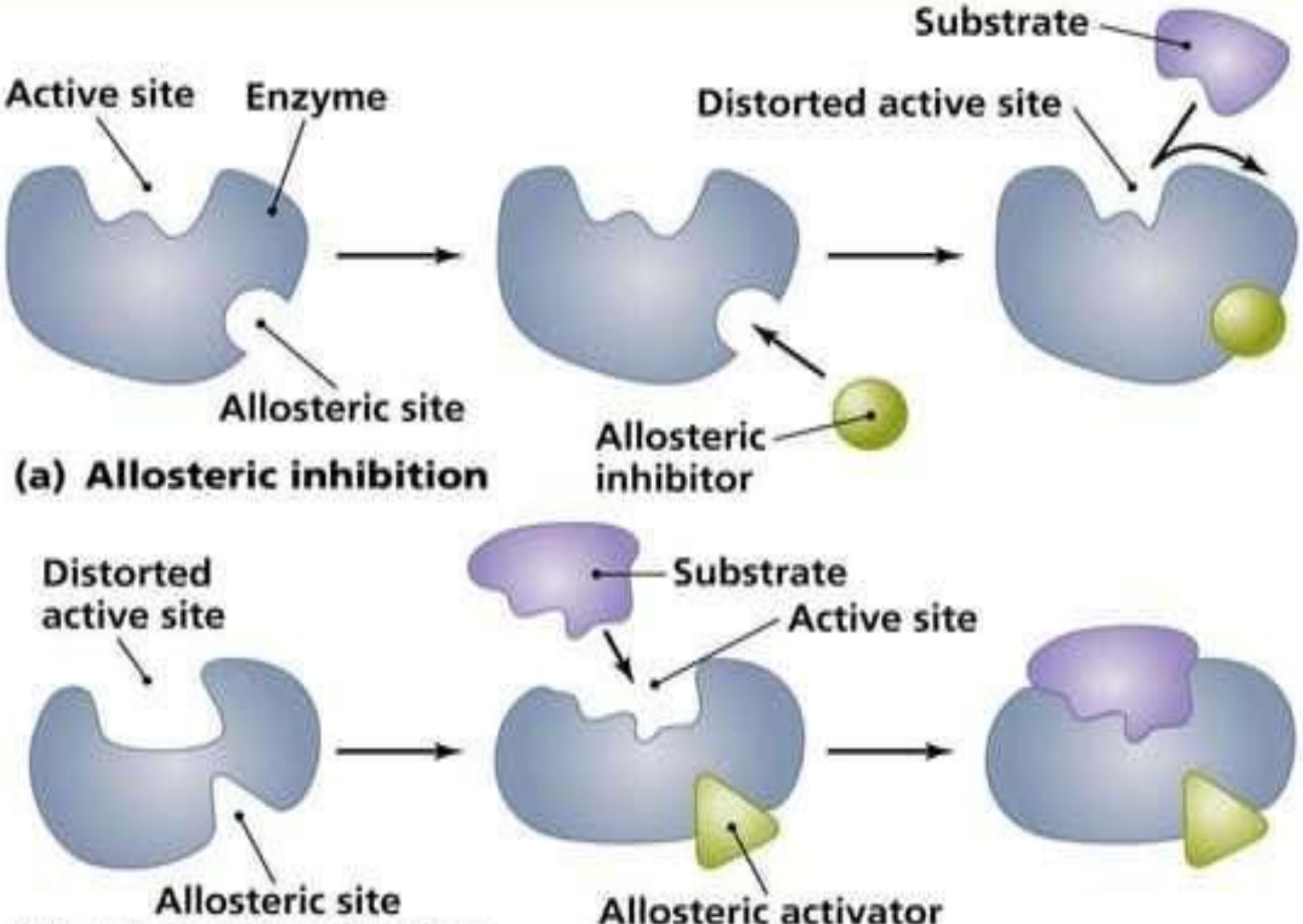
Allosteric Inhibition



- Some E possess additional site other than the Active site called as Allosteric sites, E - Allosteric E.
- They are unique site on protein molecule
- Allosteric Effectors- substances bind at Allosteric site & regulate E activity
- **Positive Allosteric effectors** - E activity is increased
- **Negative Allosteric effectors** - E activity is decreased
- Allosteric enzyme - sigmoidal curve

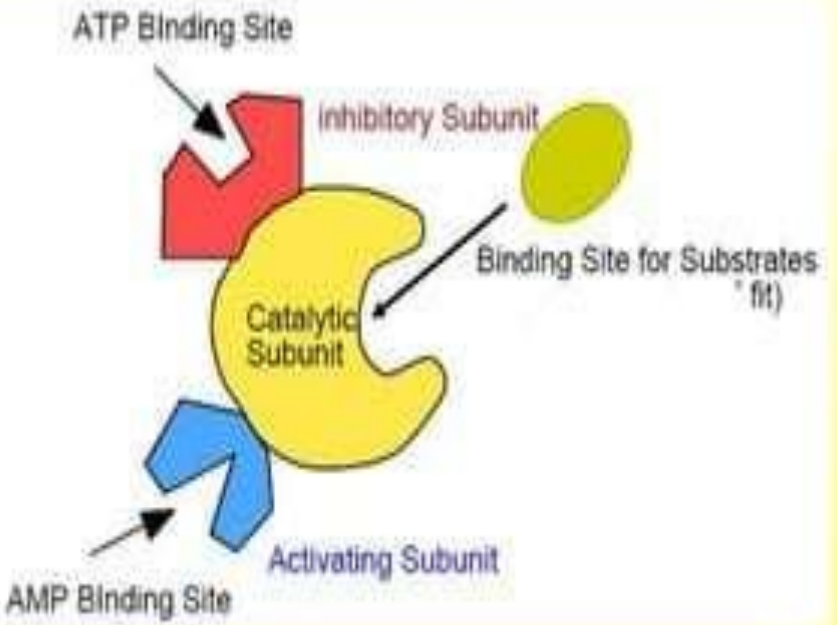
- Inhibitor is not a substrate analog.
- It is partially reversible, when excess substrate is added.
- Km is usually increased.
- Vmax is reduced.
- When an inhibitor binds to the allosteric site, the configuration of catalytic site is modified such that substrate cannot bind properly

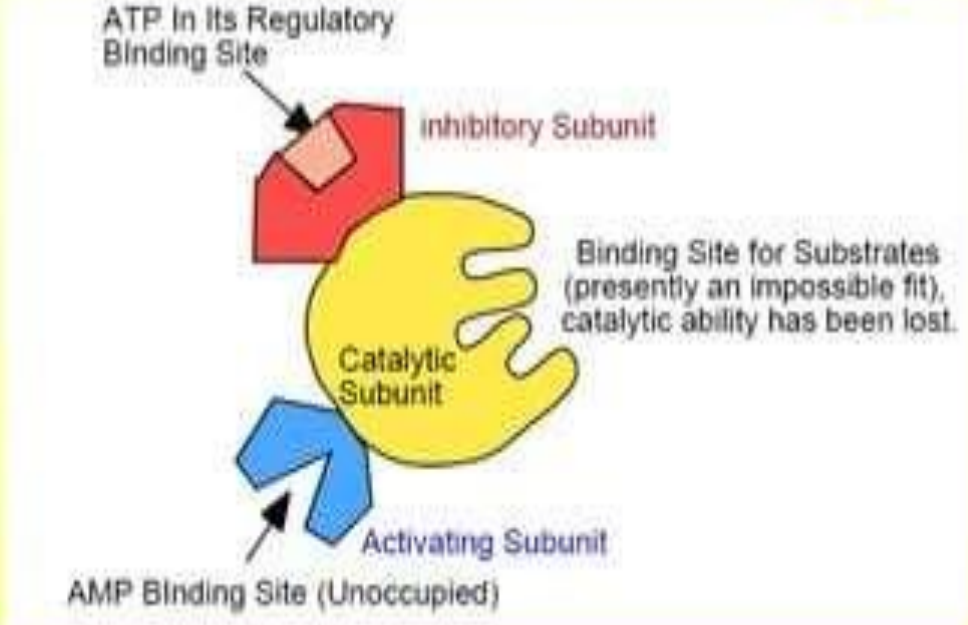



Substrate
Active site
Enzyme
Distorted active site
Allosteric site
Allosteric inhibitor
(a) Allosteric inhibition
Distorted active site
Substrate
Active site
Allosteric site
Allosteric activator

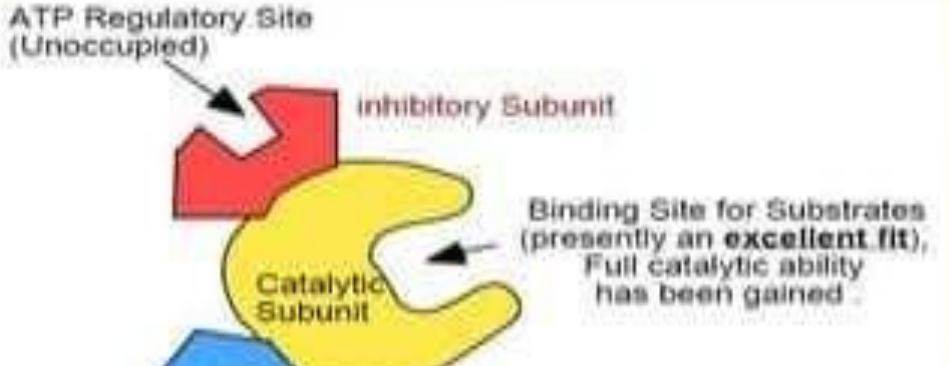
Pathway	Enzyme	Inhibitor	Activator
Glycolysis	Phosphofructokinase-1	ATP & citrate	AMP
TCA cycle	Isocitrate dehydrogenase	ATP	ADP
Glycogenolysis	Glycogen phosphorylase	ATP	AMP
Gluconeogenesis	Fructose 1,6 bisphosphatase	AMP	ATP & citrate
	Pyruvate carboxylase	-	Acetyl coA
Fatty acid synthesis	Acetyl coA carboxylase	-	Citrate

Fructose-6-phosphate → (PFK) → Fructose-1,6-bisphosphate

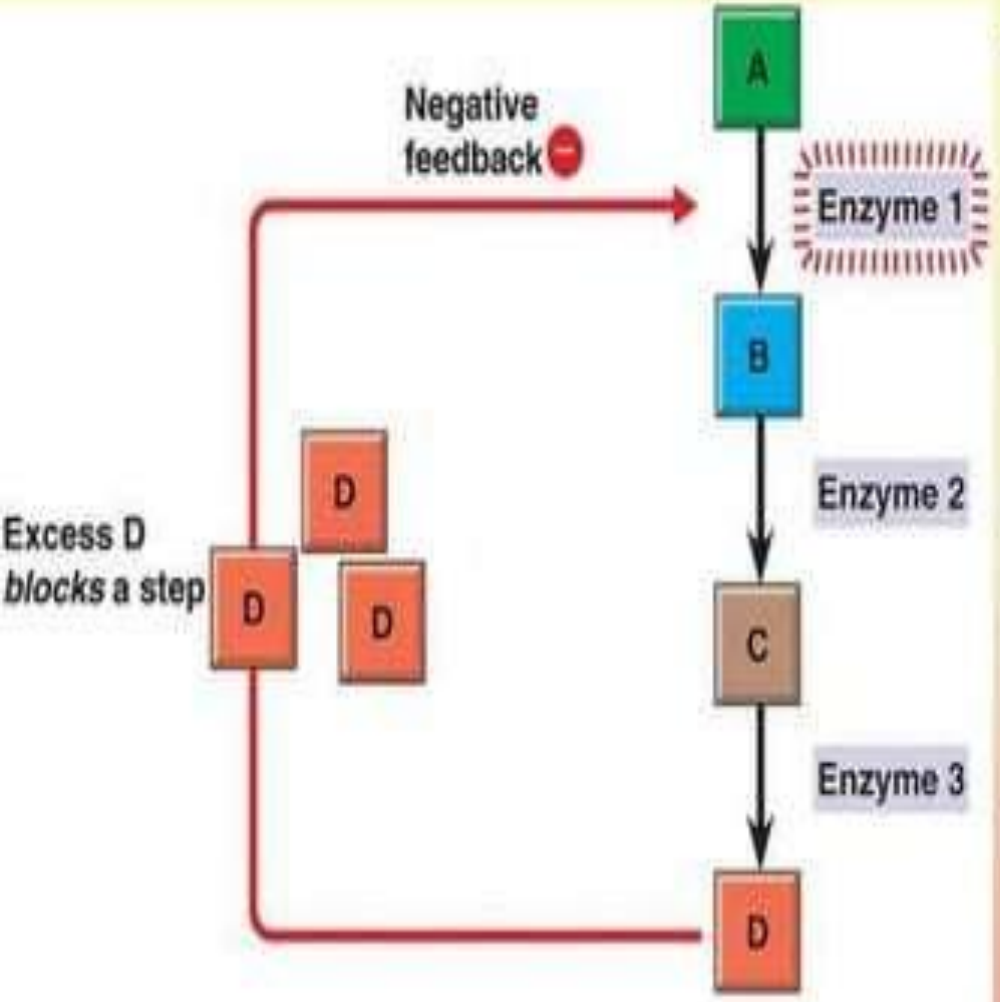




PFK is a quaternary protein and has two allosteric regulatory sites and a catalytic



Negative Feedback inhibition / End product inhibition



- Specialized type of Allosteric inhibition
- Required to control the metabolic pathways for efficient cellular function.
- End product of metabolic reaction produced in excess inhibits the first or regulatory enzyme in the sequence.
- The end products are controlling their own rate of production

Product	E inhibited	Pathway
Heme	ALA synthase	Heme synthesis
Cholesterol	HMG CoA reductse	Cholesterol synthesis
Glucose -6-phosphate	Hexokinase	Glycolysis
Acyl coA	Acetyl CoA carboxylase	Fatty acid synthesis
CTP	Aspartate Transcarbamoylase	Pyrimidine synthesis

Competitive inhibition	Non competitive	Irreversible inhibition	Allosteric inhibition	Suicide inhibition	Feedback inhibition
Structural similarity to S	No structural similarity	Bind tightly to E by covalent bonds	I binds to Allosteric site of the E	I makes use of the E's own Rn mechanism to inactivate it	End products inhibits earlier E of the metabolic pathway
Compete with S for active site Inc. S removes E	Do not compete Binds to site different from active site Rn slowed down	Cause conformational change in active site of E			
Km - Inc. Vmax - no change	Km - no change Vmax - dec.	Km - no change Vmax - dec.	Km - inc. Vmax -dec.	-	-
Drugs Sulfonamides, Statins , Methotrexate	Heavy metal poisons	Cyanide , OP poisoning, Iodoacetate	PFK -1 - ATP and Citrate	Allopurinol , Fluorouracil	Heme - ALA synthase Cholesterol -

Importance of Enzyme Inhibition

- For understanding the regulation of enzyme activity within the living cells
- Useful in elucidating the cellular metabolic pathways by causing accumulation of intermediates
- Identification of the catalytic / functional groups at the active site of E
- Provide information about substrate specificity of the enzyme
- Useful to study the mechanism of catalytic activity
- Enzyme inhibitors have therapeutic applications - some drugs useful in medicine appear to function by inhibiting certain E.

Most drugs are Competitive or Suicide inhibitors.


Thank You