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Topic: **Enzyme Inhibition**

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Enzyme Inhibition

•Enzyme inhibition means decreasing or cessation in the enzyme activity.

•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.

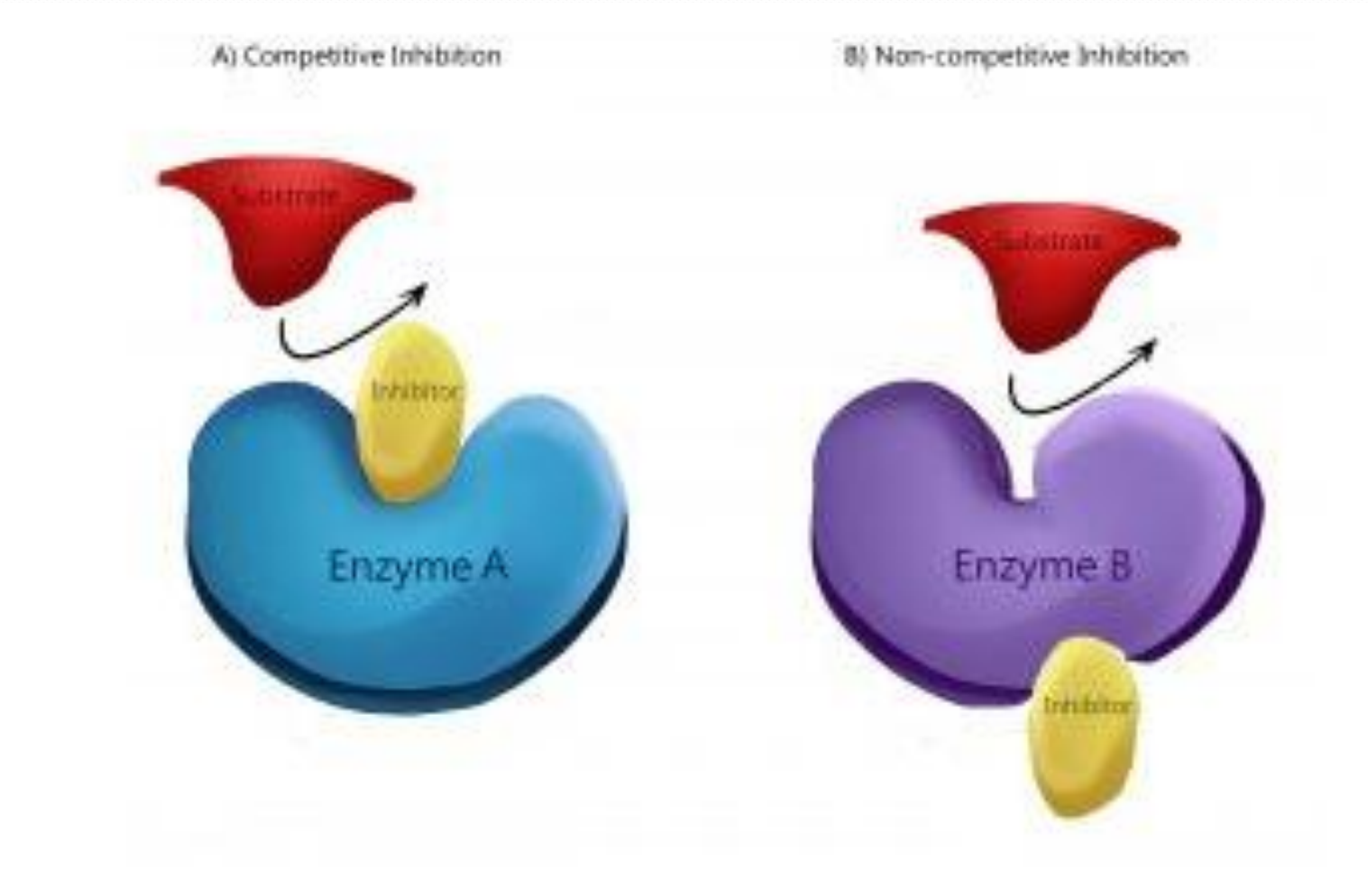
•The inhibitor is the substance that decreases or abolishes the rate of enzyme action.

•The inhibitor may be organic or inorganic in nature

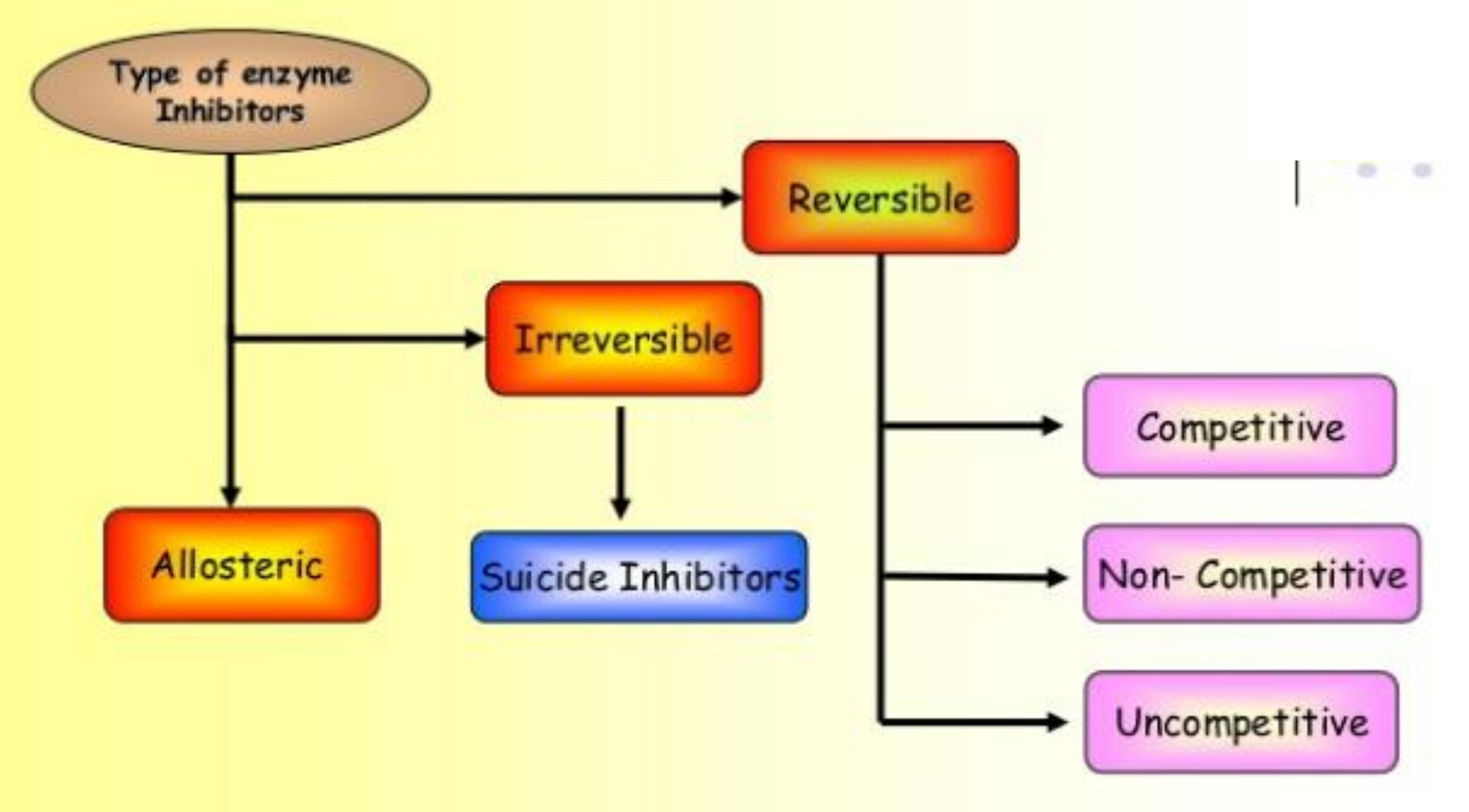
•Inhibitors - drugs, antibiotics ,toxins and antimetabolite or natural products of enzyme reaction.

•Enzymes need to be tightly regulated to ensure the levels of the products do not rise the desired levels. This is accomplished by enzyme inhibition.

Competitive and Non- competitive inhibitors



Types of Inhibition



Types of Inhibition

1. Reversible
   - Competitive
   - Non- Competitive
   - Uncompetitive
2. Irreversible
   - Suicide Inhibitors
3. Allosteric

•Reversible and irreversible inhibitors are chemicals which bind to an enzyme and supress its activity.

•One method it binds permanently to the enzyme called irreversible wherever others bind transiently called reversible

Reversible Inhibition

✓Inhibitor binds non-covalently (weak interaction) with Enzyme

✓If inhibitor is removed – action of E fully restored

✓Reversible inhibitors bind to an active site called competitive or another site called non- competitive inhibitors.

✓They bind to enzyme with noncovalent interactions, such as hydrogen bonds, ionic bonds, and hydrophobic interactions

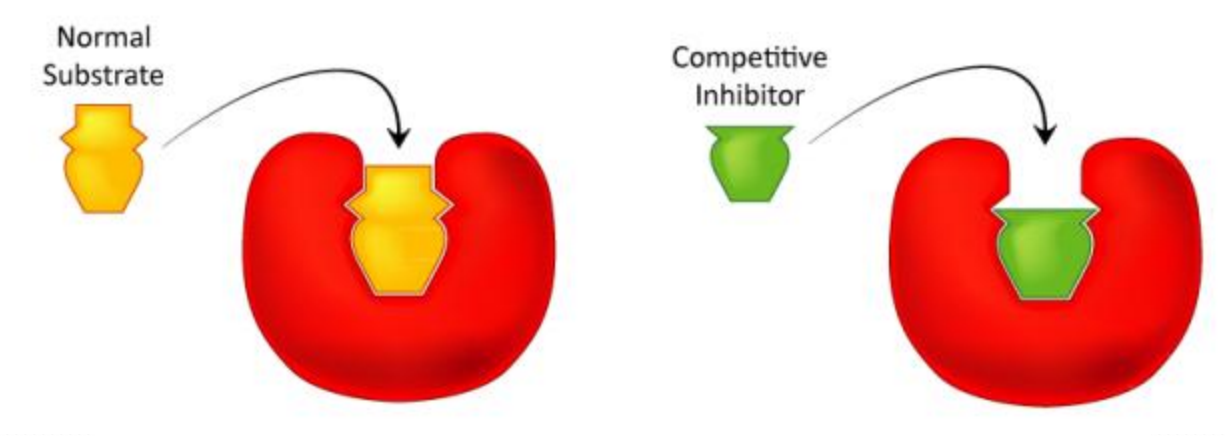
✓An Equilibrium is established between the free inhibitor & EI Complex and is defined by an equilibrium constant (Ki) E + I E I

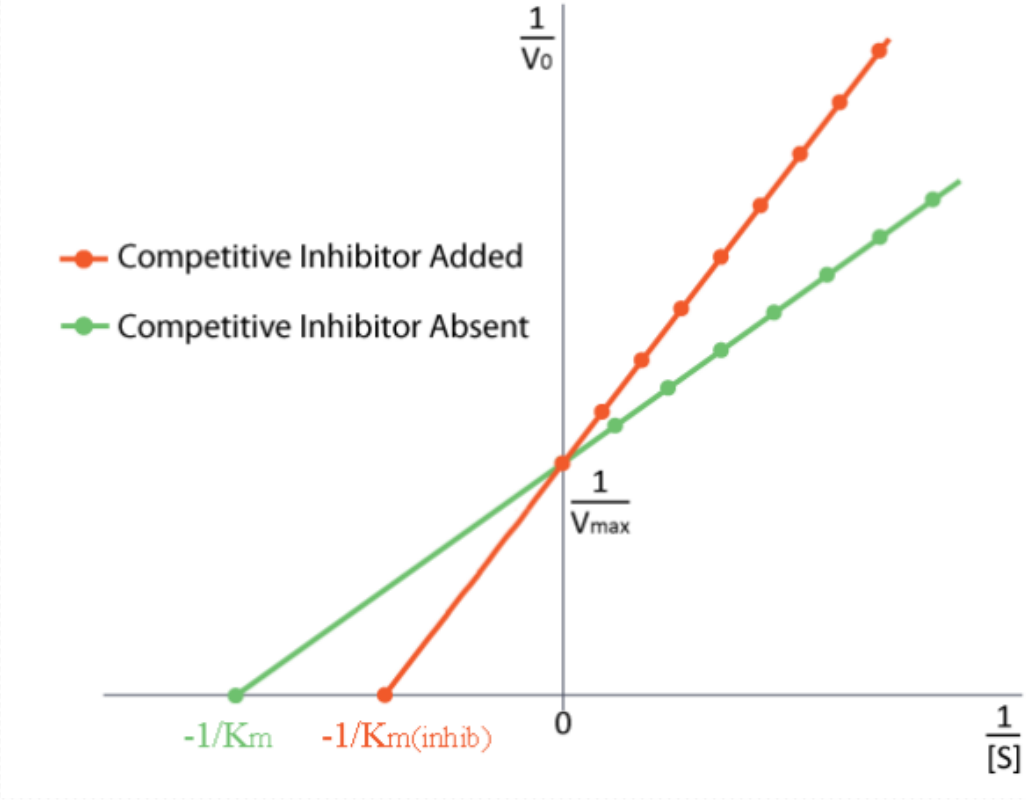
✓The activity of Enzyme is fully restored on removing the Inhibitor by dialysis

✓**Reversible inhibitors** can be classified as competitive, non-competitive or uncompetitive.

1. Competitive Inhibition

- Inhibitor binds reversibly to the same site that the substrate binds - competes with the S for binding.
- I can be reversed by increasing the conc. of S – reversible Substrate analogue – I closely resembles the S
- Degree of inhibition - depend on the conc. of S & I and on the relative affinities of the enzyme for S & I
- Km is increased -affinity of the enzyme towards substrate is apparently decreased in presence of the inhibitor
- Vmax is not changed No inhibitor Inhibitor Vmax
- ½ Vmax Km New Km [s] v 1/Vm -1/Km 1/S 1/V No Inhibitor -1/Km Inhibitor
- Malonate is a competitive inhibitor of SDH COOH CH_2 CH_2 COOH Succinate dehydrogenase FAD $FADH_2$ Succinate COOH H -C C-H COOH COOH CH_2 COOH Malonate Fumarate Similarity in three dimensional structure b/w S and I


Normal
Substrate
Competitive
Inhibitor


1
V0
Competitive Inhibitor Added
Competitive Inhibitor Absent
1
Vmax
-1/Km
-1/Km(inhib)
0
1
[S]

2. Non-Competitive Inhibition

✓A second type of inhibition employs inhibitors that do not resemble the substrate and bind not to the active site, but rather to a separate site on the enzyme

✓The effect of binding a non-competitive inhibitor is significantly different from binding a competitive inhibitor because there is no competition.

✓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.

✓ In the case of competitive inhibition, the effect of the inhibitor could be reduced and eventually overwhelmed with increasing amounts of substrate.

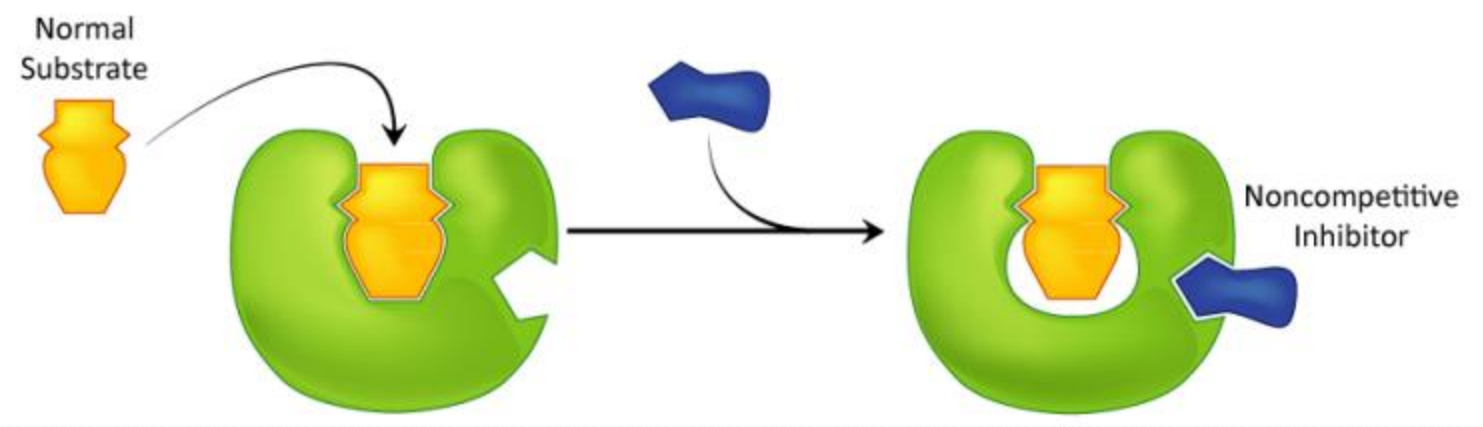
✓With non-competitive inhibition, increasing the amount of substrate has no effect on the percentage of enzyme that is active.

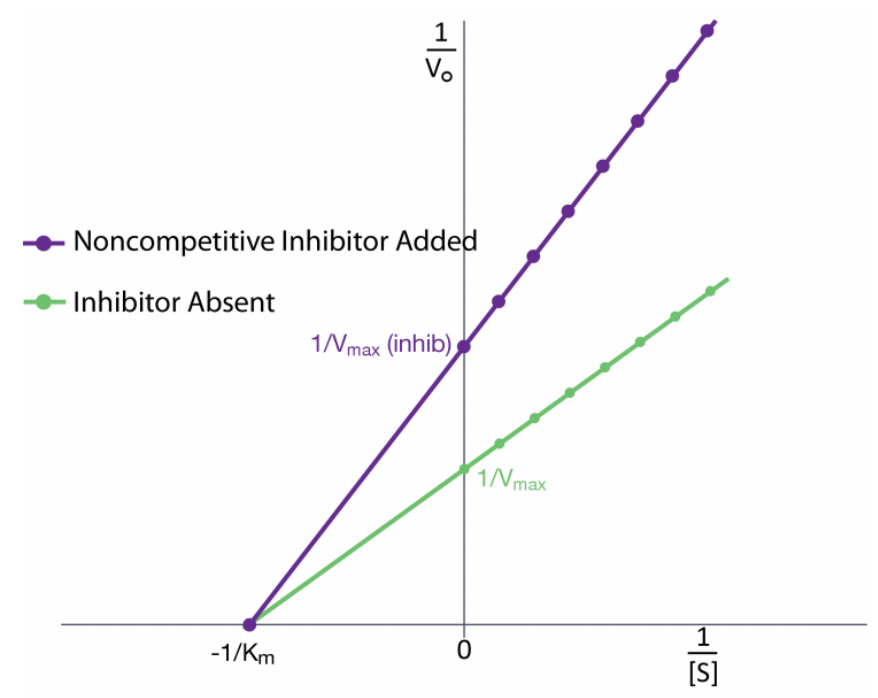
✓Indeed, in non-competitive inhibition, the percentage of enzyme inhibited remains the same through all ranges of [S].

✓KM for non-competitively inhibited reactions does not change from that of uninhibited reactions. This is because, as noted previously, one can only measure the KM of active enzymes and KM is a constant for a given enzyme.

✓Km value is unchanged - I do not interfere with the binding of S to E Vmax decreases - I cannot be overcome by increasing the conc. of S No inhibitor Inhibitor Vmax Vmax

✓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


Normal
Substrate
Noncompetitive
Inhibitor


1
Vo
Noncompetitive Inhibitor Added
Inhibitor Absent
1/Vmax (inhib)
1/Vmax
-1/Km
0
1
[S]

3. Uncompetitive Inhibition

✓A third type of enzymatic inhibition is that of uncompetitive inhibition, which has the odd property of a reduced Vmax as well as a reduced KM.

✓The explanation for these seemingly odd results is rooted in the fact that the uncompetitive inhibitor binds only to the enzyme-substrate (ES) complex.

✓The inhibitor-bound complex forms mostly under concentrations of high substrate and the ES-I complex cannot release product while the inhibitor is bound, thus explaining the reduced Vmax.

✓The answer lies in the fact that the inhibitor-bound complex effectively reduces the concentration of the ES complex. By Le Chatelier's Principle, a shift occurs to form additional ES complex, resulting in less free enzyme and more enzyme in the forms ES and ESI (ES with inhibitor).

✓Decreases in free enzyme correspond to an enzyme with greater affinity for its substrate.

✓Uncompetitive inhibition both decreases Vmax and increases an enzyme's affinity for its substrate.

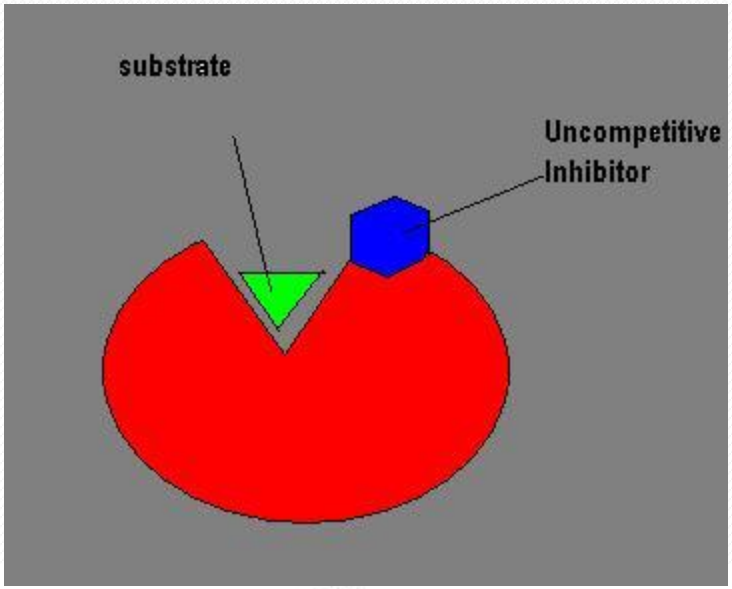
✓I binds only to the ES complex , not to free E

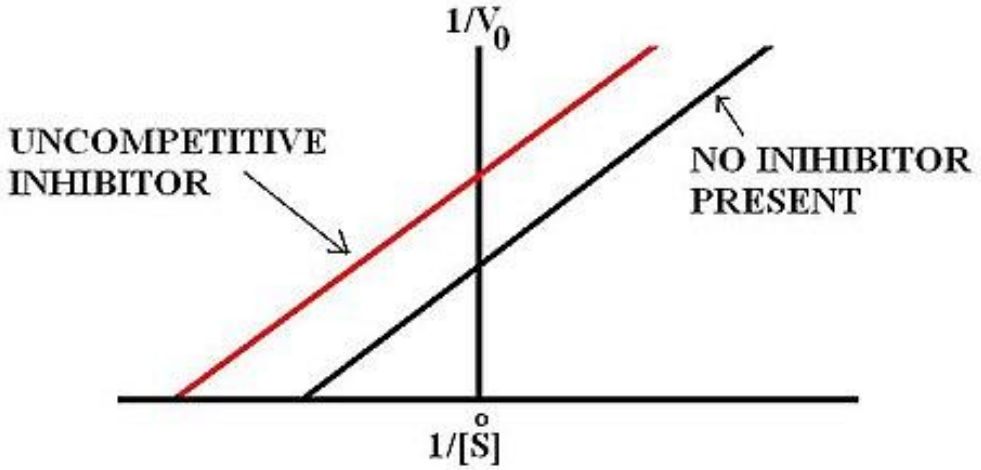
✓I - cause structural distortion of the active site - E catalytically inactive

✓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 E S E + P + I ESI Inhibitor Vmax Vmax i ½ Vmax Km


substrate
Uncompetitive
Inhibitor


1/V0
UNCOMPETITIVE
INHIBITOR
NO INIHIBITOR
PRESENT
1/[S]

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

Suicide Inhibition

✓In contrast to the first three types of inhibition, which involve reversible binding of the inhibitor to the enzyme, suicide inhibition is irreversible because the inhibitor becomes covalently bound to the enzyme during the inhibition and thus cannot be removed.

✓Specialized form of Irreversible inhibition

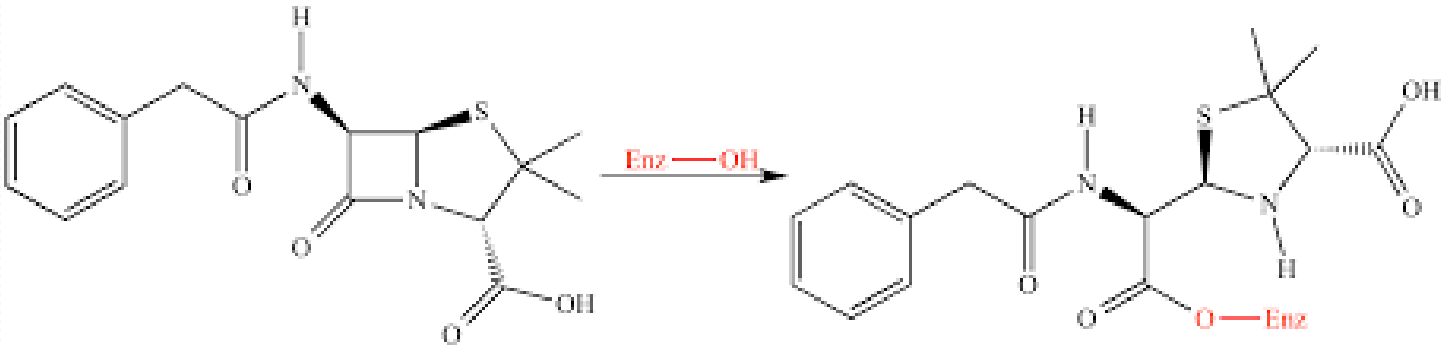
✓Suicide inhibition rather closely resembles competitive inhibition because the inhibitor generally resembles the substrate and binds to the active site of the enzyme.

✓The primary difference is that the suicide inhibitor is chemically reactive in the active site and makes a bond with it that precludes its removal.

✓Such a mechanism is that employed by penicillin (Figure 4.10.5), which covalently links to the bacterial enzyme, D-D transpeptidase and stops it from functioning.

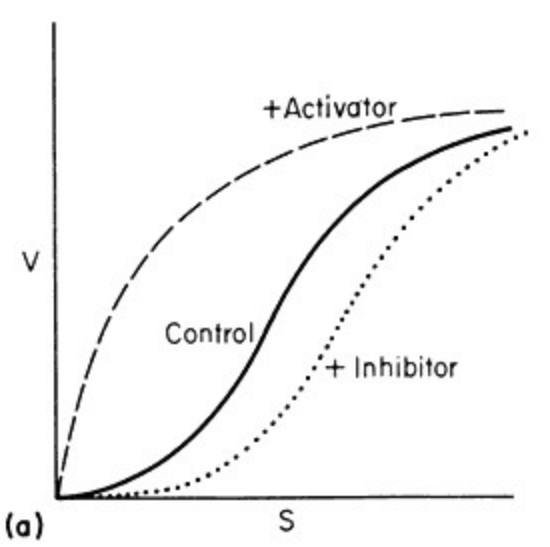
✓Since the normal function of the enzyme is to make a bond necessary for the peptido-glycan complex of the bacterial cell wall, the cell wall cannot properly form and bacteria cannot reproduce.

✓If one were to measure the kinetics of suicide inhibitors under conditions where there was more enzyme than inhibitor, they would resemble non-competitive inhibition's kinetics because both involve reducing the amount of active enzyme by a fixed amount in a set of reactions.



Allosteric Inhibition

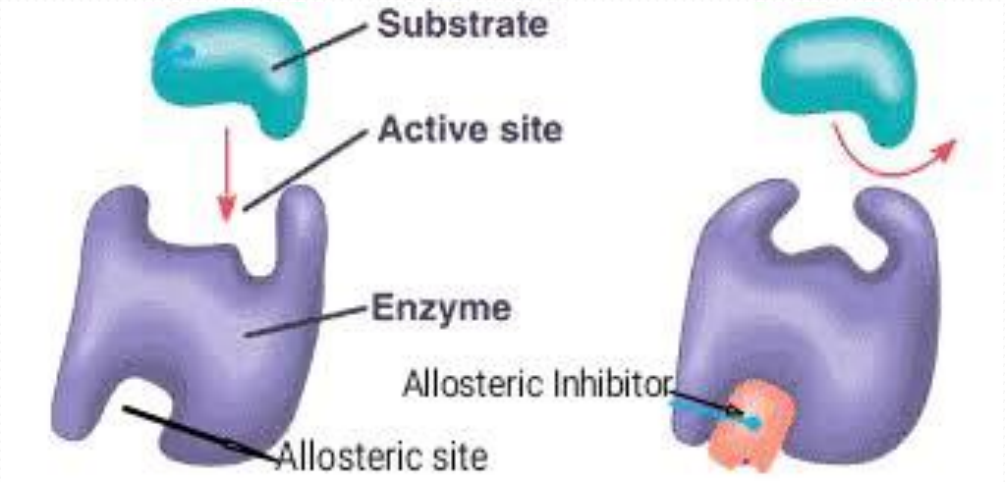
- Some E possess additional site other than the Active site called as Allosteric sites
- They are unique site on protein molecule Allosteric Effectors– substances bind at Allosteric site & regulate E activity
- Positive Allosteric effectors – E activity is increased
- Negative Allosteric effectors – E activity is decreased
- Allosteric enzyme – sigmoidal curve Active site Allosteric site
- 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 Vmax Vmax i ½ Vmax Km [s] v ½ Vmax i No inhibitor Allsoteric Inhibitor Km

➢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 is a quaternary protein and has two allosteric regulatory sites and a catalytic site. PFK Fructose-1,6-bisphosphate



Importance of Enzyme Inhibition

1. For understanding the regulation of enzyme activity within the living cells
2. Useful in elucidating the cellular metabolic pathways by causing accumulation of intermediates
3. Identification of the catalytic / functional groups at the active site of E
4. Provide information about substrate specificity of the enzyme
5. Useful to study the mechanism of catalytic activity
6. Enzyme inhibitors have therapeutic applications – some drugs useful in medicine appear to function by inhibiting certain E.
7. Most drugs are Competitive or Suicide inhibitors.