ENZYMOLOGY - IV

Original slides: Dr. Sameer Mahjob

Edited by: Mohannad Al-Ja'Awin

Enzyme Inhibition

- Inhibitors are chemicals that reduce the rate of enzymatic reactions. So it doesn't mean we stop the the activity of the enzyme!!

-They are usually specific and they work at low concentrations.

-They block the enzyme but they DON'T usually destroy it.

- Many drugs and poisons are inhibitors of enzymes in the nervous system.

- Inhibitors of the catalytic activities of enzymes provide both pharmacologic agents and research tools for study of the mechanism of enzyme action.

EFFECT OF ENZYME INHIBITION

IRREVRSIBLE

- If the inhibitor is binding to a functional group in the active site of the enzyme by covalent bond or non-covalent bond. And therefore reducing the activity/Stopping the activity of the enzyme

REVERSIBLE

- Binding with loosley bonds
- Then it can be washed away from the solution.

Classification

- based on:

- Their site of action on the enzyme

- Whether they chemically modify the enzyme

- The kinetic parameters they influence. (Vmax/Km)

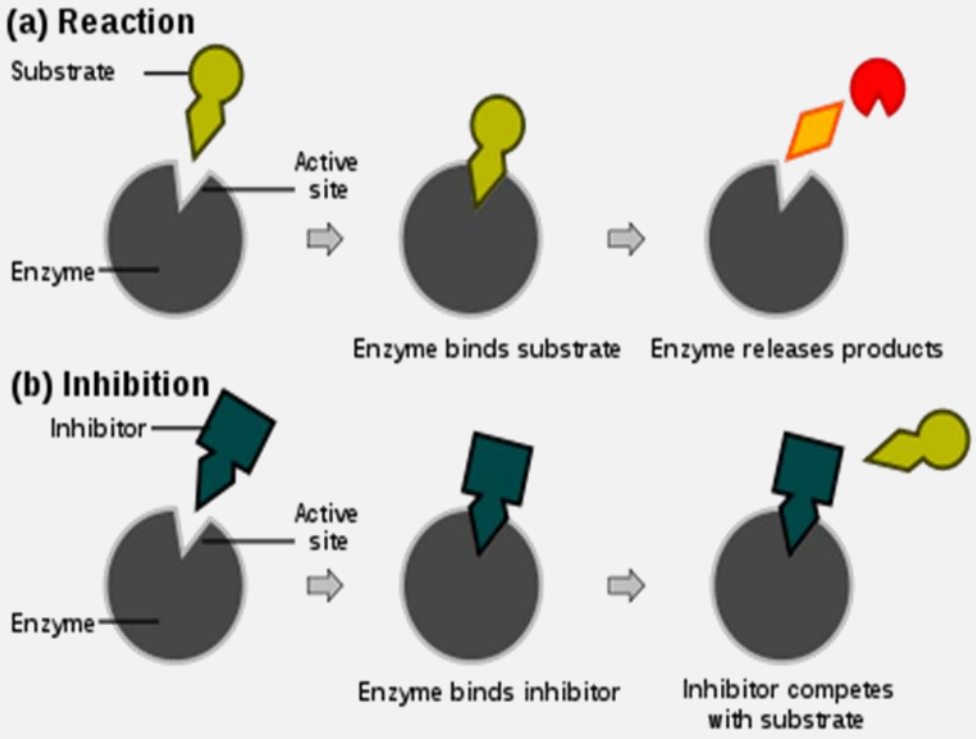
Types of enzyme inhibition:

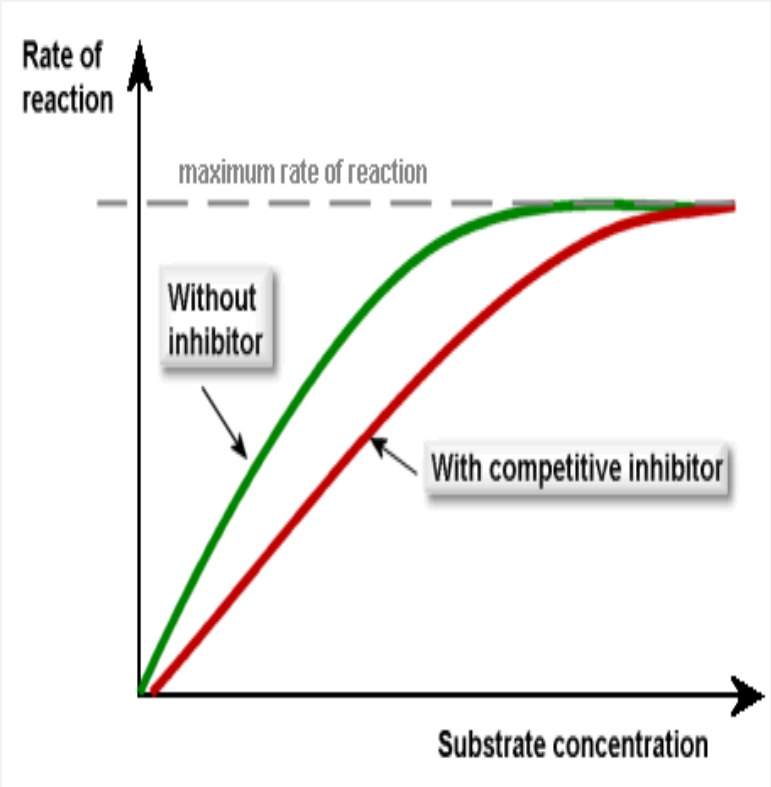
- Competitive inhibition
- Non Competitive inhibition
- Uncompetitive inhibition
- Suicidal inhibition
- Allosteric inhibition
- Feed back inhibition

Competitive enzyme inhibition

A competitive inhibitor

- Has a structure similar to substrate (structural Analog)
- Occupies active site
- Competes with substrate for active site
- Has effect reversed by increasing substrate concentration
- Vmax remains same but Km is increased





IMPORTANT NOTES REGARDING COMPETITIVE INHIBITION

- Best thing we can say about this, is that there is a competition between the substrate and the inhibitor, who has the more concentraion wins!

- The inhibitor must mimic the shape of the substrate (Lock and Key) so it can bind to the active site.

- If there was a reaction going on and the substrate is linked to the enzyme at the active site, and then we introduce an inhibitor (with higher concentration than the susbtrate) to the reaction, what will happen?

It will displace the substrate from the active site, so the activity of the enzyme goes down GRADUALLY.

- The precense of this inhibitor decreases the affinity of the enzyme (Because it's been OCCUPIED)

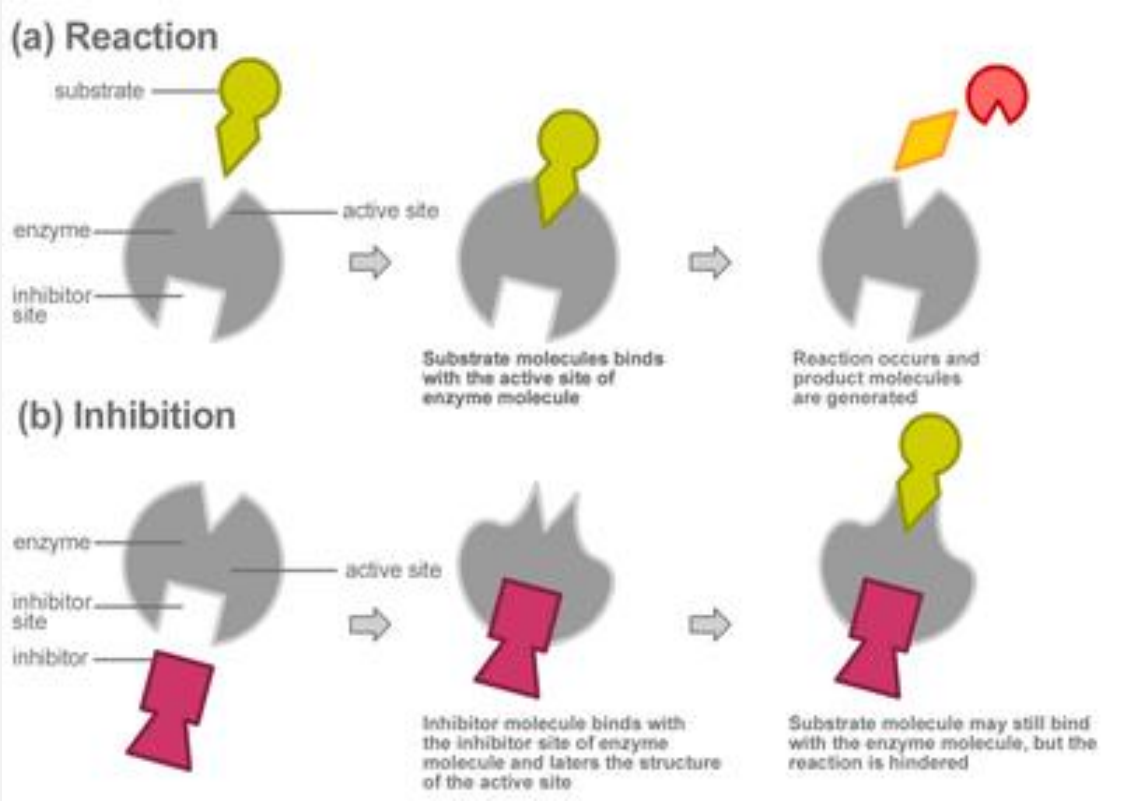
Clinical significance of competitive enzyme inhibitors

Drug	Enzyme Inhibited	Clinical Use
Dicoumarol	Vitamin K Epoxide Reductase	Anticoagulant
Sulphonamide	Pteroid Synthetase	Antibiotic
Trimethoprim	Dihydrofolate reductase	Antibiotic
Pyrimethamine	Dihydrofolate reductase	Antimalarial
Methotrexate	Dihydrofolate reductase	Anticancer
Lovastatin	HMG CoA Reductase	Cholesterol Lowering drug
Alpha Methyl Dopa	Dopa decarboxylase	Antihypertensive
Neostigmine	Acetyl Cholinesterase	Myasthenia Gravis

Non competitive enzyme inhibition

- Noncompetitive inhibitors bind enzymes at sites distinct from the substrate-binding site. (Not the same active site)
- Generally bear little or no structural resemblance to the substrate.
- Binding of the inhibitor does not affect binding of substrate.

- Formation of both EI and EIS complexes is therefore possible.
-The enzyme-inhibitor complex can still bind substrate, its efficiency at transforming substrate to product, reflected by V_{max}, is decreased.
- It would NOT affect the Km.



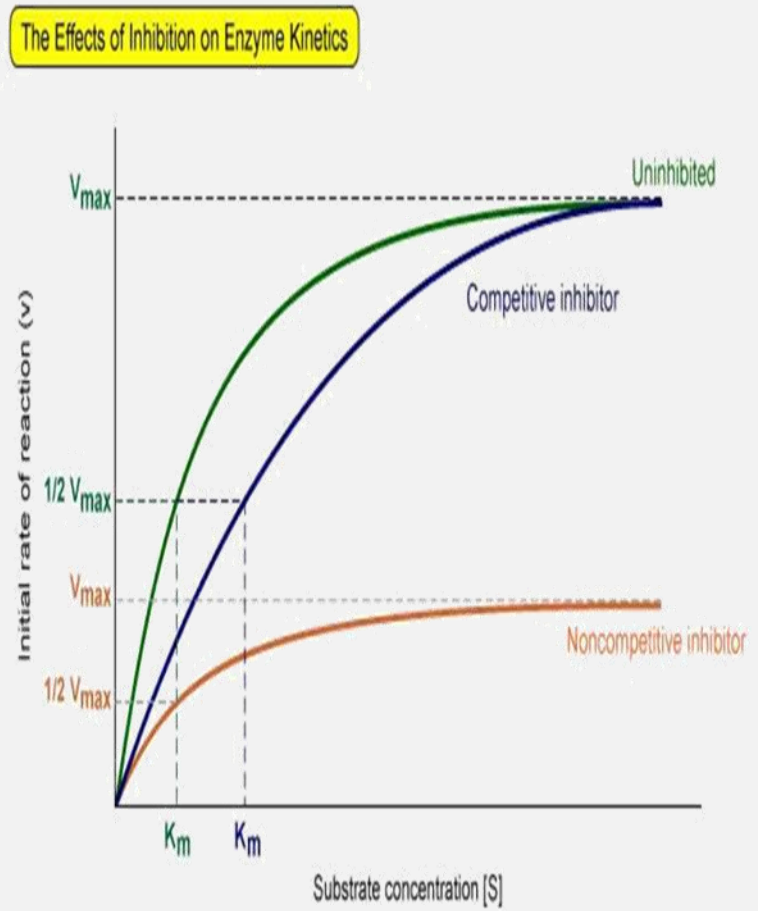
Examples of non competitive enzyme inhibitors

- Cyanide inhibits cytochrome oxidase.

- Fluoride inhibits enolase and hence glycolysis.

- Iodoacetate inhibits enzymes having SH groups in their active sites.
- BAL (British Anti Lewisite, dimercaprol) is used as an antidote for heavy metal poisoning

- Heavy metals act as enzyme poisons by reacting with the SH groups, BAL has several SH groups with which the heavy metal ions bind and thereby their poisonous effects are reduced.



Uncompetitive enzyme inhibition

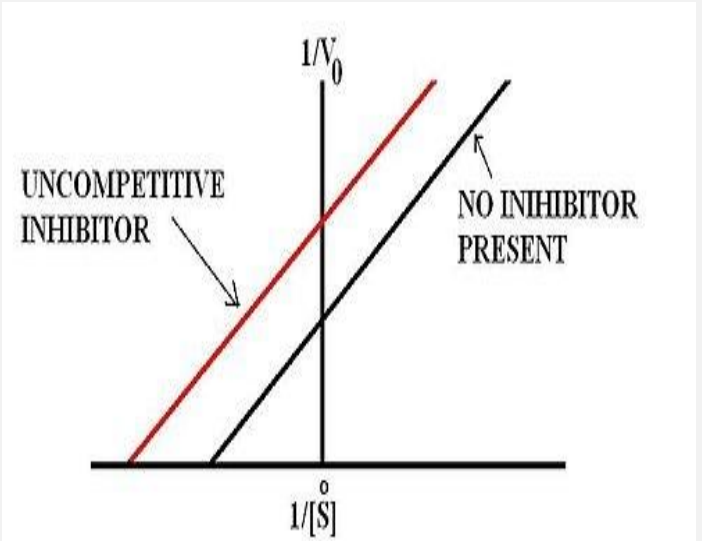
-No similarity in the strucutre between the Inhibitor and the substrate. Binds on another site.

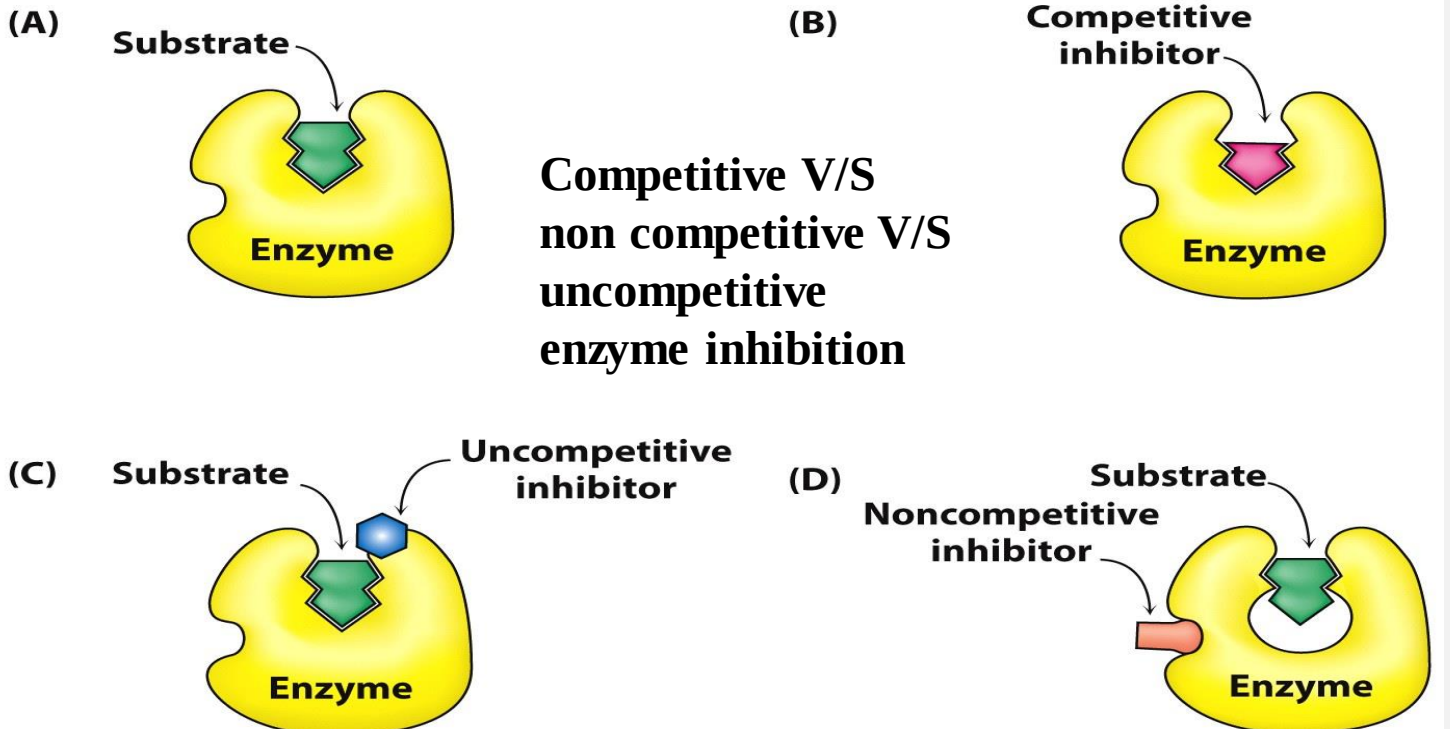
-Where the enzyme would bind? Very close to the active site.

-Inhibitor binds to enzyme- substrate complex

- Both Vmax and Km are decreased

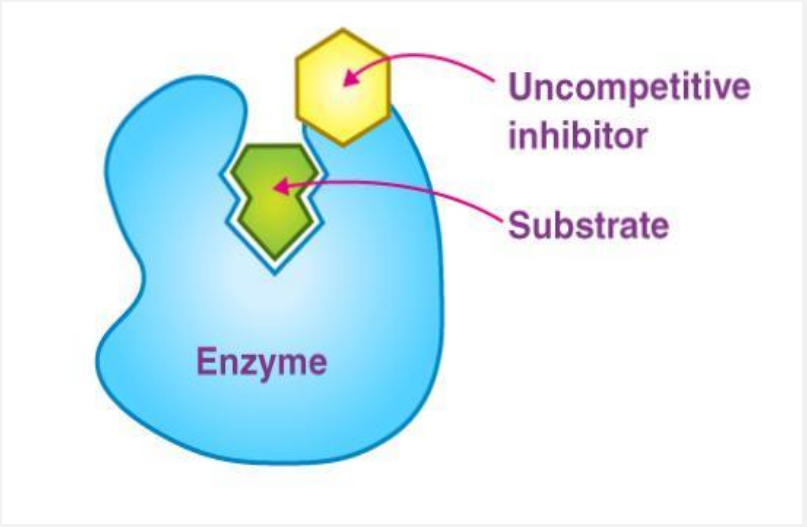
- Such as; Inhibition of placental alkaline phosphatase (Regan isoenzyme) by phenylalanine





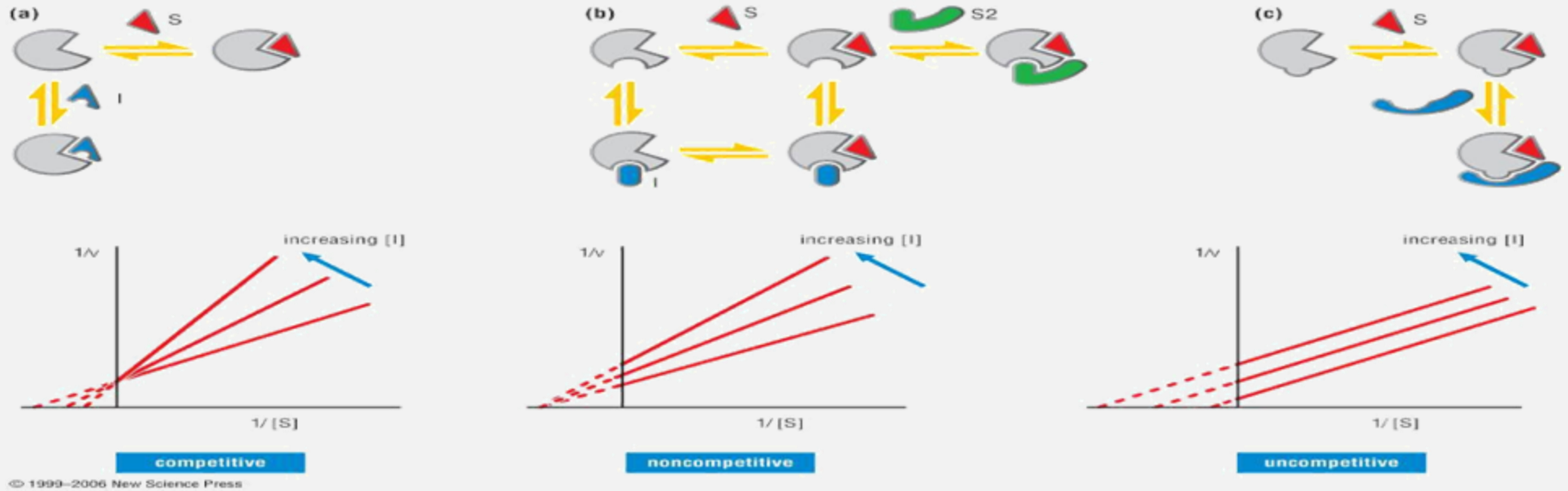
Competitive V/S non competitive V/S uncompetitive enzyme inhibition

IMPORTANT NOTES REGARDING UNCOMPETITIVE INHIBITION

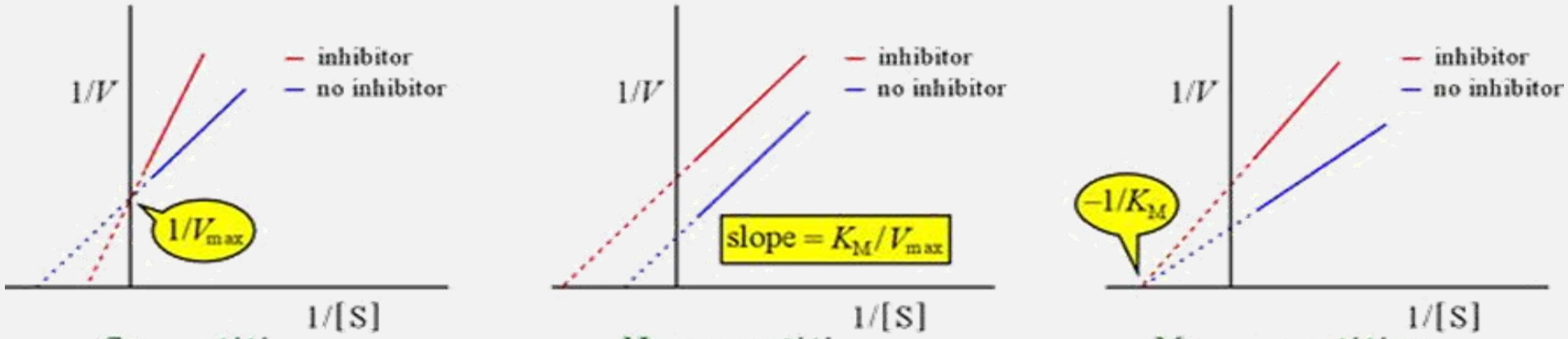


- They only Bind to the OCCUPIED enzyme (ES complex).
- They **distort the active site to prevent the enzyme from being catalytically active without actually blocking the binding of the substrate**.
- Vmax is decreased (Binding on another site to reduce the activity, thus; no products produced)
- Km is decreased (The apparent affinity of the enzyme for the substrate increases, meaning that Km decreases.)

From Protein Structure and Function 2005-2006 Online Update
by Gregory A Petsko and Dagmar Ringe



The Lineweaver-Burk plots for inhibition



Competitive inhibition
K_M increased
V_{max} unaffected

Uncompetitive inhibition
K_M reduced
V_{max} reduced

Noncompetitive inhibition
K_M unaffected
V_{max} reduced

Suicidal inhibition

- Irreversible inhibition

- We activate the enzyme (Naïve enzyme)

- Then Structural analog of the substrate is converted to more effective inhibitor with the help of enzyme to be inhibited.

-The new product irreversibly binds to the enzyme and inhibits further reaction.

- Such as ;

  Ornithine decarboxylase: is irreversibly inhibited by difluormethyl ornithine, as a result multiplication of parasite is arrested .

  Used against trypanosome in sleeping sickness

  Allopurinol: treatement for Gout.

-Allopurinol is oxidized by xanthine oxidase to alloxanthine which is a strong inhibitor of xanthine oxidase

- Aspirin action is based on suicide inhibition

  Acetylates a serine residue in the active center of cyclo-oxygenase .

  Thus, PG synthesis is inhibited so inflammation subsides

- Disulfiram: used in treatment of alcoholism

NB: The active substance in alcohol is Ethanol, it gets converted to acetylaldehyde and it causes:

1. Fatty liver
2. Transformation of the cell
3. Cancer of the Liver.

   Drug irreversibly inhibits the enzyme aldehyde dehydrogenase preventing further oxidation of acetaldehyde which produces sickening effects leading to aversion to alcohol.

Allosteric inhibition

- Some enzymes have **other site** (allosteric site) similar but different from the active site which may or may not physically adjacent to the active site.

-Allosteric: not only applied to enzymes, it's also applied to a group of protiens called allosteric proteins. Ex: Hemoglobin.

-This site binds an effector called the allosteric effector that may be an activator (positive modifier) or inhibitor (negative modifier).

-And then it causes a conformational change to the enzyme.

-The allosteric effector is usually a metabolite or a product resulting from the process of metabolism.

- Enzymes having these sites are called allosteric enzymes.

ALLOSTERIC INHIBITION

- In case of positive modefier: The conformational change in the active site is made to improve the suitability of a substrate to activate the reaction.
- In case of a negative modefier: The conformational change in the active site is made to block the substrate from binding to the active site; and therefore cancelling the reaction.
- In the case of negative modefier: simply it's changing the active site suitability to the substrate (Lock and Key).

- Inhibitor is not a substrate analogue.

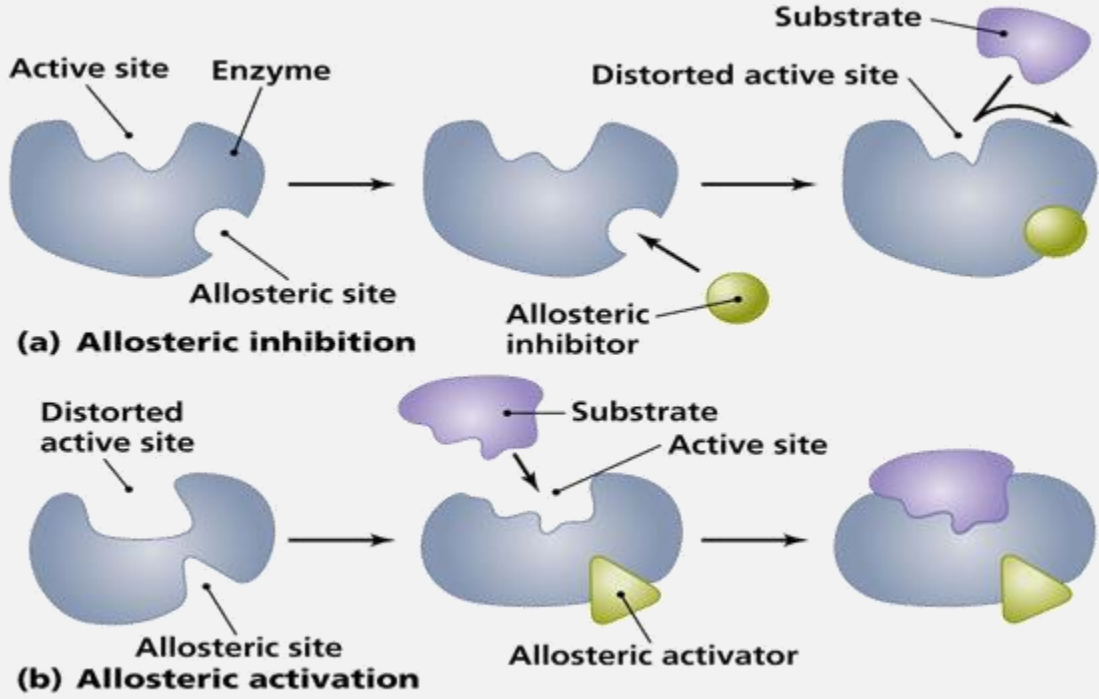
- Partially reversible, when excess substrate is added.

- Km is usually increased (K series enzymes).

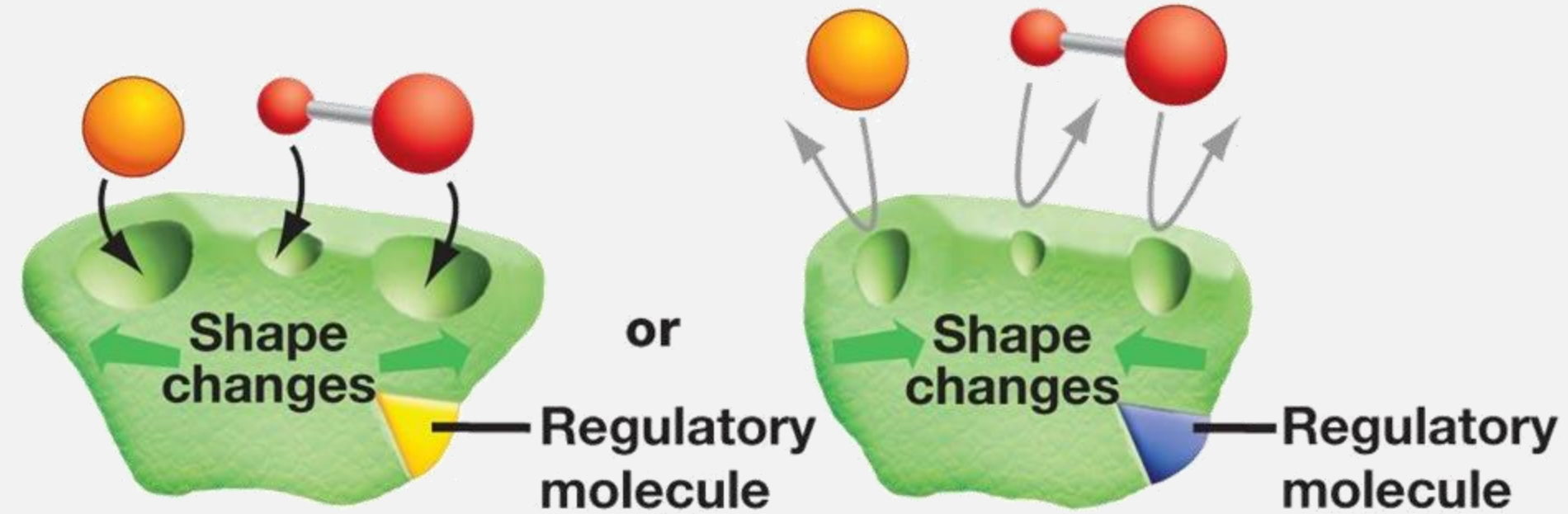
- Vmax is reduced (V series enzymes). So the affinity would decrease.

- When the inhibitor binds the allosteric site, the configuration of the active site is changed so that the substrate can not bind properly.

- Most allosteric enzymes possess quaternary structure.



(b) Allosteric regulation



Allosteric activation

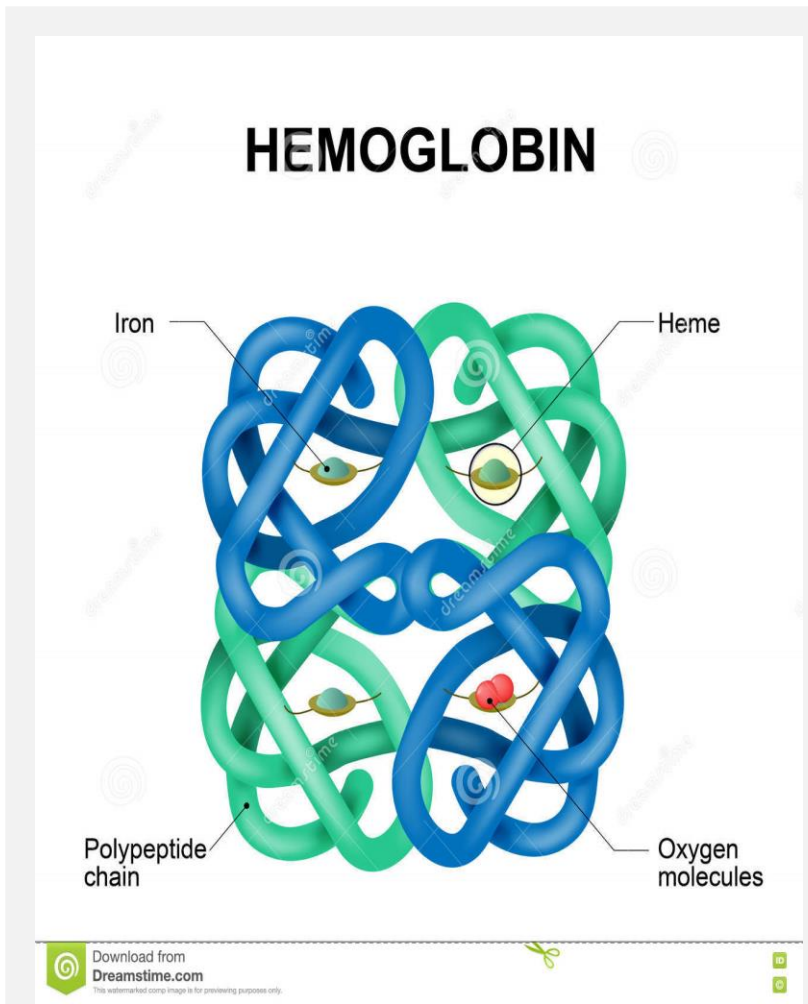
The active site becomes available to the substrates when a regulatory molecule binds to a different site on the enzyme.

Allosteric deactivation

The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.

ALLOSTERIC PROTEINS: HEMOGLOBIN

- How many molecules enters the center of hemoglobin? 2alpha and 2 beta and in the center of the chains there is heme group, and in the center if it, we find ferrous atom.
- How many oxygens can be carried out by the heme group? 4 Oxygens.
- Are the 4 oxygens bind to the heme group at one time or gradually?
- Cooperatively. Alpha and beta chains are linked together by a group bonds to form a diamer.
- If No oxygen binded to the heme group, The hemoglobin is in the tense state. And when it bind gradually, it to transform into relaxed state.
- Why not at a one time bond? Because of the oxidation of the ferrous into the ferric. so we avoid the oxidation of the ferrous into ferric at once so it can bind to other oxygens.
- If the hemoglobin was oxidized prematurely (at one time) we call it Methemoglobin.
- So we have an enzyme inside the erythrocytes called met hemoglobin reductase enzyme that can reduce the oxidized Methemoglobin into normal hemoglobin.



Switching off

- When the inhibitor is present it fits into its site and there is a conformational change in the enzyme molecule:

1) The enzyme's molecular shape changes.
2) The active site of the substrate changes.

- The substrate cannot bind with the enzyme and the reaction slows down. (**Slows Down**)

- When the inhibitor concentration diminishes the enzyme's conformation changes back to its active form. (**Switching On**)

- This is not competitive inhibition but it is reversible.

Example: Phosphofructokinase -1(PFK-1)

- It catalyses: phosphorylation of fructose-6-phosphate into fructose 1, 6 biphosphate.

- It has an allosteric site for an ATP molecule (**the inhibitor**).

- **Switching on:** When the level of ATP in the cell falls (↑ ratio of ADP to ATP)➔ no ATP binds to the allosteric site of PFK-1➔ so, the enzyme's conformation changes and the active site accepts substrate molecules➔ causing activation of glycolysis➔ The respiration pathway accelerates ➔

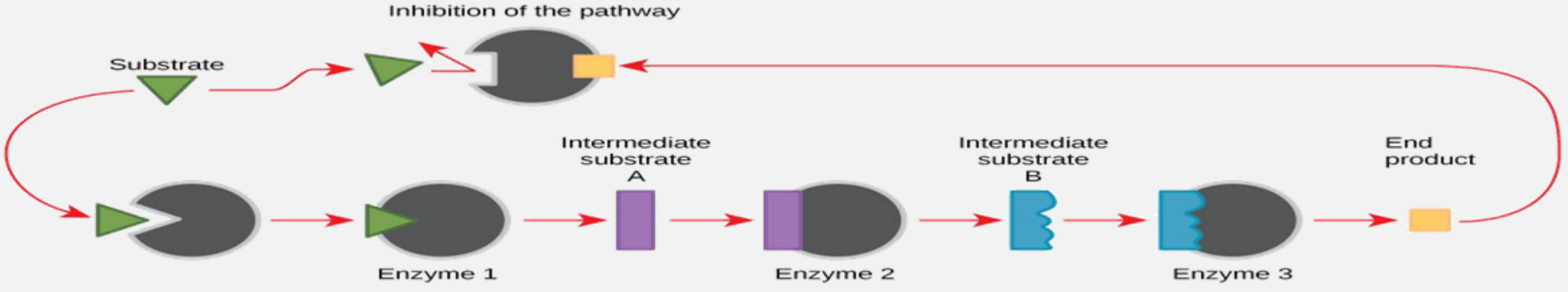
- **Switching off:** the level of ATP in the cell increases (↑ ratio of ATP to ADP in the cell)➔ ATP molecules can fit into the allosteric site of PFK-1 molecules➔ The enzyme's conformation changes again➔ stops accepting substrate molecules in the active site➔ respiration slows down.

Feed back(end point) inhibition

Cell processes consist of series of pathways controlled by enzymes.

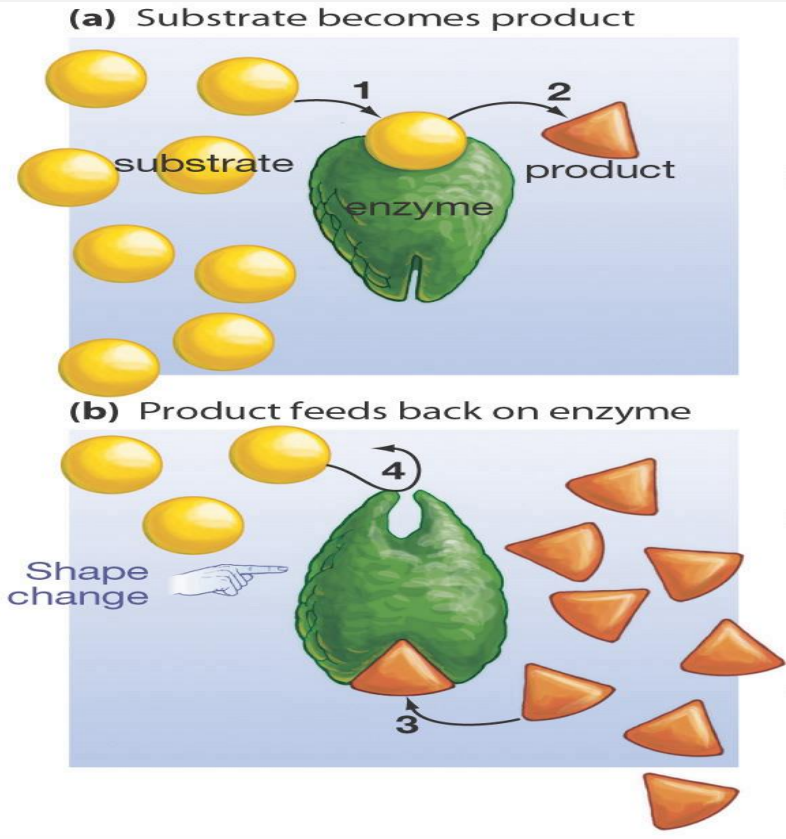
$$A \xrightarrow{e_A} B \xrightarrow{e_B} C \xrightarrow{e_C} D \xrightarrow{e_D} E \xrightarrow{e_E} F$$

- Each step is catalyzed by a different enzyme ($\mathbf{e_A}$, $\mathbf{e_B}$, $\mathbf{e_C}$ etc).
- **Negative Feedback:** End product inhibits the key regulatory enzyme, thus the cell can control (Active/Inhibit) this metabolic pathway.
- The first step (controlled by $\mathbf{e_A}$) is often controlled by the end product (F), therefore negative feedback is possible.



The cell itself undergo this type of inhibition according to its requirements

- The end products are controlling their own rate of production.
- There is no build-up of intermediates (B, C, D and E).
- ➢ the inhibition should be accompanied by inability to synthesis intermediates ➔ Because usually these intermediates cannot be utilized in another pathways ➔ their accumulation should be associated with more excretion and if they are not excreted the will cause diseases.
- Usually such end product inhibition can affect allosterically.



- Accumulated product binds at a site other than the active site to bring about conformational changes, so as to inhibit the binding of the substrate ➔ the rate of reaction declines.

- Note: Good control points are committing steps (very negative activation energy (not easily reversed)

THANK YOU