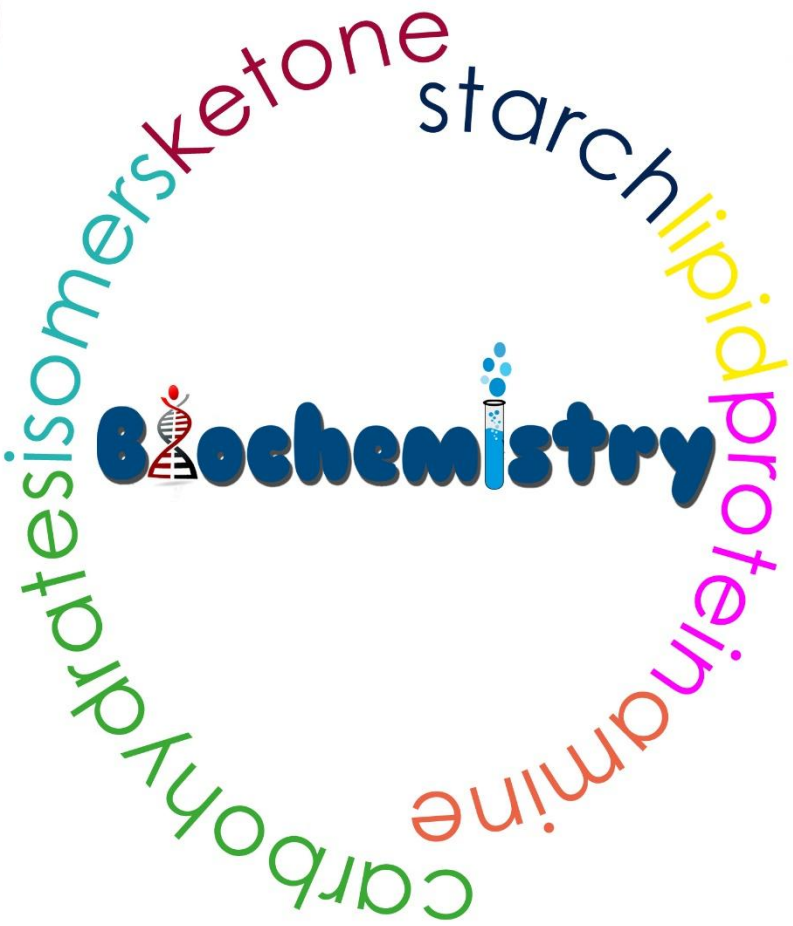


◉Sheet ○Slides

Subject :	Enzymes Regulation
Done by :	Zahra Muneer
Corrected by :	Ziad Fraij
Number :	17

Modes of regulation

1. Isozymes
2. Inhibition
3. Conformational changes
4. Changes in the amount of the enzyme
5. None-specific

2. **Inhibitors**

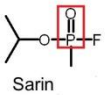
*This is a review for irreversible inhibitors which have been discussed previously, if you choose to skip it, go to page 4.

A. Irreversible Inhibitors "Mechanism Based Inhibitors"
(usually react with the enzyme and change it chemically via covalent bond formation)
As the name implies, "Mechanism Based Inhibitors" interfere with the mechanism of the enzyme.
The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme units.

- *Covalent Inhibitors*
  They bind to the active site covalently.
  Once they bind to the active site they inhibit the reaction.
  Drugs and toxins bind covalently to the active site's amino acids.
  DFP is a lethal organophosphorus compound which serves as a prototype for:
  - The nerve gas Sarin
  - The insecticides (Malathion & Parathion)
    They all combine with the amino acid serine at the

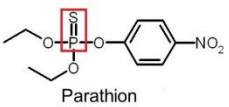
Insecticides: substances used for killing insects.

Nerve Gases

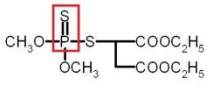


Sarin

Insecticides



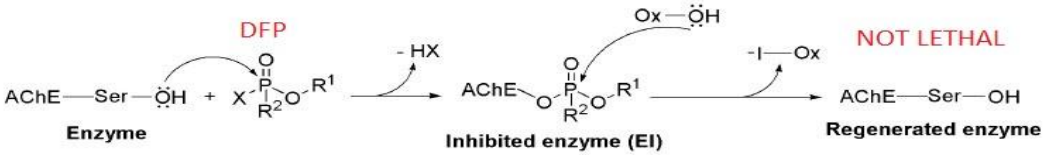
Parathion



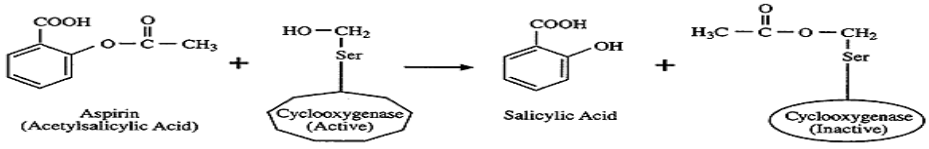
Malathion

active site of the enzyme acetylcholinesterase, once the enzyme is inhibited it accumulates and nerve impulses cannot be stopped, causing prolonged muscle contraction. Paralysis occurs and death may result since the respiratory muscles are affected.

DFP also inhibits other enzymes that has serine Like serine proteases, but the inhibition is not as lethal.



- Aspirin (acetylsalicylic acid): covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase)



Reminder: Non steroidal anti inflammatory drugs (NSAIDS), like Aspirin, inhibit the synthesis of prostaglandins and thromboxanes, accounting for their anti-inflammatory, analgesic, and antipyretic effects.

Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme

- *Transition state analogs* "Suicide Inhibitors"
  - Potent inhibitors, they bind more tightly to the active site than substrates do.
  - Mechanism: they bind to the active site so the enzymes define them as the substrate so they

start reacting but they can't go further than the first step.

- They're used as drugs
- Drugs cannot be designed that precisely mimic the transition state because they're highly unstable structures.
- Inhibitors that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed suicide inhibitors

Examples:

- Methotrexate: it is a synthetic inhibitor, anticancerous, and an analog of tetrahydrofolate, it binds to the enzyme (Dihydrofolate reductase DHFR) 1000-fold more tightly than the substrate, and inhibits nucleotide base synthesis.
- Penicillin: A transition-state analog to glycopeptidyl transferase or transpeptidase, it weakness the cell wall of the bacterium.
- Allopurinol: A drug used to treat gout, it decreases urate production by inhibiting xanthine oxidase, The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions so the allopurinol binds to xanthine oxidase, which oxidizes allopurinol to oxypurinol, a compound that binds very tightly and irreversibly to a molybdenum–sulfide complex in the active site.

- *Heavy Metals*
  - They bind tightly to a functional group in an enzyme
  - They affect the CNS
  - They're nonspecific for the enzymes they inhibit

  Examples:

  Mercury (Hg), lead (Pb), aluminum (Al), and iron (Fe)
  - Mercury (Hg): it is nonspecific and inhibits enzymes with sulfhydryl group in the active site.

    It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity.
  - Lead (Pb): it inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc.

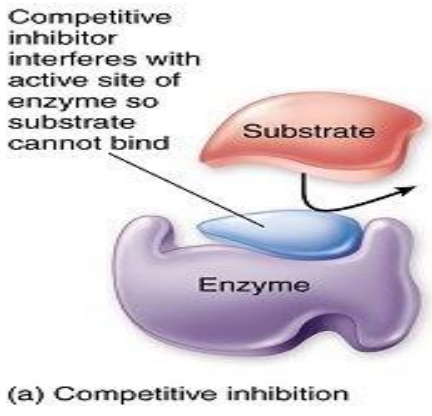
    Its developmental & neurological toxicity may be caused by its ability to replace Ca+2 in several regulatory proteins that are important in the central nervous system and other tissues.

    *End of review.

B. Reversible inhibitors: they usually bind to the active site through non-covalent interactions and then dissociate.
The double-reciprocal plots are highly useful for distinguishing among these inhibitors

- *Competitive Inhibitors*

  Inhibitors that compete with the substrate for the active site. What determines if the active site will bind to the inhibitor or the substrate?



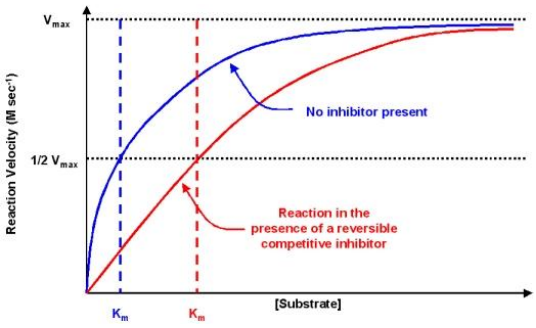
(a) Competitive inhibition

- Concentration, since it is a reversible (non covalent interaction) pathway we can overcome inhibition by increasing the substrate's concentration.
- Affinity

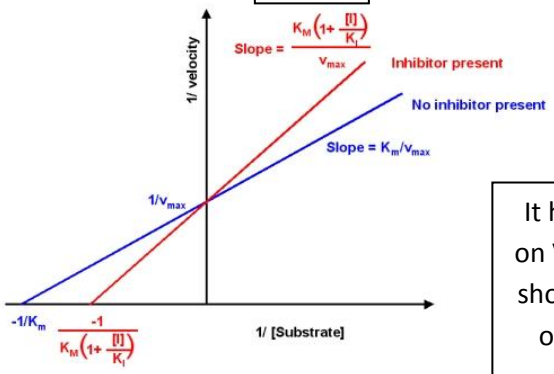
  Example: glucose-6-phosphate (a competitive inhibitor with the substrate glucose) inhibits hexokinase.*

  *It does not inhibit glucokinase.

Plot 1



Plot 2



It has no effect on Vmax so they should intersect on the y axis (1/V)

The effect of competitive inhibitor on:

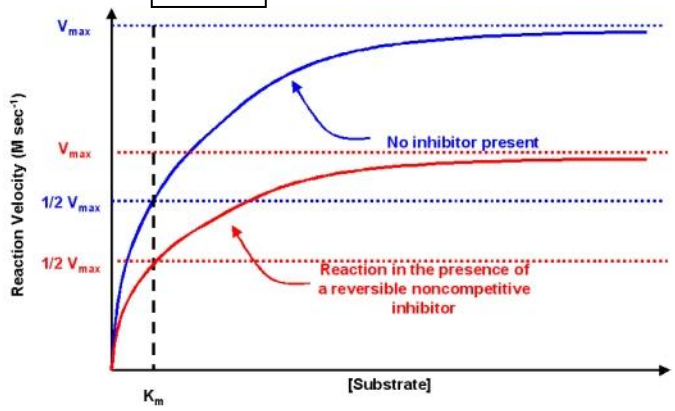
Vmax	Km
Since it's a property for the enzyme, competitive inhibitors have no effect on Vmax because in this situation it is constant for a certain enzyme whether it's saturated or not	Since we're increasing the substrate's concentration to overcome inhibition, competitive inhibitors increase km; because it equals the substrate's concentration needed to reach half of the Vmax

Plot 1	Plot 2
The reaction will shift to the right as we need more substrate (Km) to reach the same Vmax	The slope will increase and the curve will shift to the left since the slope is equal to Km/Vmax and Km is increased while Vmax is constant

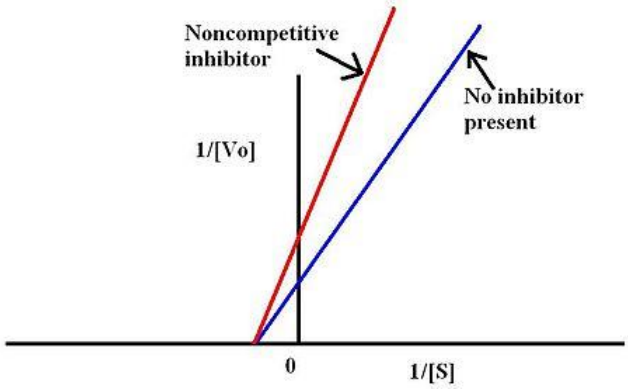
- Non-Competitive inhibitors
  They are inhibitors that bind at a site other than the active site, so they don't compete with the substrate. They cause some conformational changes in the active site so that the substrate can bind but the enzyme isn't as efficient as it was before the conformational change (before the inhibitor was bound to it).

  When increasing the substrate's concentration can we reach the Vmax?
  No. first of all the inhibitor is not competing with the substrate so increasing the substrate's concentration has no effect but since the enzyme's efficiency is now different we'll have a lower Vmax.

Plot 1



Plot 2



Vmax	Km
Since it's a non competitive inhibitor, it'll result in an enzyme with less efficiency which results in a lower Vmax So the Vmax changes.	There's no competition on the active site so we need as much substrate as we need without inhibition to reach Vmax. Relationship between Km and [substrate]: [substrate] = Km when half of the enzymes are saturated (1/2 Vmax) So Km doesn't change.

<u>Plot 1</u>	<u>Plot 2</u>
It has a lower Vmax but the same Km	Because the Vmax value got smaller, 1/Vmax got bigger, and the slope which is Km/Vmax got bigger (Km stayed the same)

3. Conformational changes.

They are changing between deactivated (inhibited) and activated.

Types of conformational regulation can rapidly change an enzyme from an inactive form to a fully active conformation.

A. Allosteric activation and inhibition

Reminder: Allosteric Enzymes are enzymes that change their conformational ensemble upon binding of an effector, which results in an apparent change in binding affinity at a different ligand binding site or they are multi-subunit enzymes with catalytic subunit(s) and regulatory subunit(s).

The Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes because they are not simple enzymes.

Behaviour of allosteric enzymes: Cooperative›› Sigmoidal plot

Remember: Hemoglobin's behaviour is cooperative; when O2 binds to one subunit this causes a higher affinity to O2 in the other subunits so they change their mode from T to R in sequence (sequential. It could be concerted; we'll talk about it later). The same idea applies to Allosteric Enzymes.

When the modifier is a molecule other than the substrate, then it is known as a heterotropic modifier.

If the modifier is the same as the substrate, then it is called a homotropic modifier.

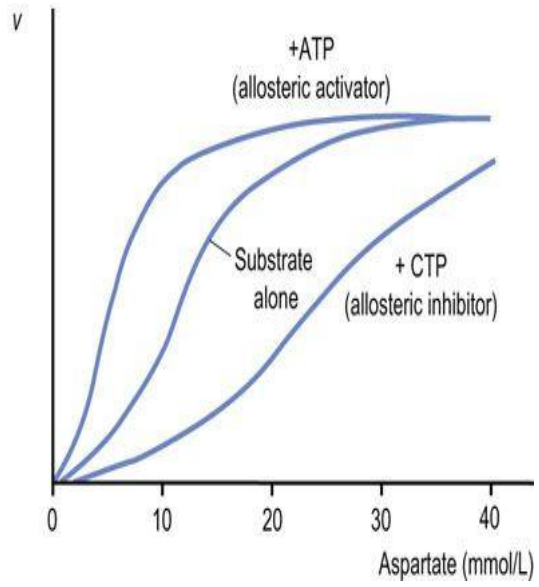
- **Not all enzymes follow Michaelis-Menten equation**

  For example: Chymotrypsin (Specificity for aromatic residues mainly. Also, hydrolysis of ester bonds "hyperbolic") vs. ATCase (synthesis of CTP & UTP for RNA and DNA synthesis "sigmoidal")

Hemoglobin (conjugated>> sigmoidal plot) vs. Myoglobin (simple>> hyperbolic plot).

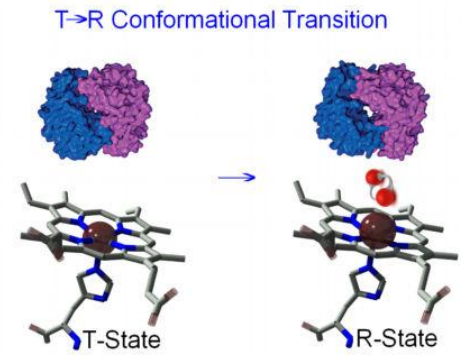
- The substrate concentration at half of the Vmax is called (K0.5). Why can't we call it Km? Because allosteric enzymes don't follow the Michaelis-Menten equation and Km is Michaelis-Menten constant but it follows the same concept.
- Do allosteric enzymes have a Vmax?
- Of course! Because they are enzymes.

- Adding an inhibitor will result in increasing (K0.5). Vmax may be affected or not depending on the enzyme inhibitor.
- Adding an activator will result in decreasing (K0.5). Vmax cannot be affected because it is the highest velocity per unit of time.



- How can we differentiate between simple and allosteric enzymes from the plot?
- Simple> Hyperbolic
- Allosteric> Sigmoidal

- When we add an inhibitor it will shift to the right. It has less affinity to bind to a substrate and it will become more sigmoidal.
- When we add an activator it will shift to the left because we'll be needing less substrate to achieve the same velocity it will become less sigmoidal (more nearly hyperbolic) but never hyperbolic because it's not a simple enzyme.



Allosteric enzymes have two conformations: more active (R) & less active or inactive (T)

The equilibrium ratio (T/R) is called L and assumed to be high

As L (T/R) increases, the shape becomes more sigmoidal

Usually enzymes exist in the T conformation most of the time because naturally enzymes are inhibited and activated once they're needed to function.

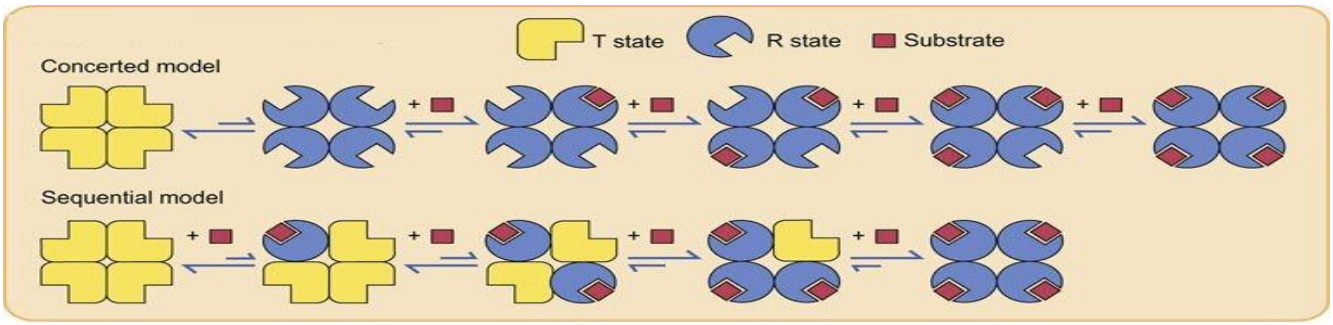
The default of the enzyme is T>R so the L ratio is usually high.

Adding inhibitor	Adding activator
Encourages T conformation Since T increases so the ratio T/R (L) increases More sigmoidal	Encourages R conformation Since R increases so the ratio T/R (L) decreases Less sigmoidal

There are two models explaining how allosteric proteins or enzymes work:

Sequential model: is when subunits change from T to R in a sequence pattern.

Concerted model: is when all subunits change from T to R once a substrate is attached.



Since allosteric enzymes are made from catalytic and regulatory subunits, experiments have shown that once you cleave the regulatory subunit from the catalytic subunit and add a substrate it will bind to the catalytic subunit and it will act as a simple enzyme which will result in a hyperbolic plot.

Examples of Allosteric regulation:

- **ATCase (**Aspartate transcarbamoylase)
- As the name applies the substrate is Aspartate
- CTP and UTP are the products. They are used for neucleotide synthesis (DNA and RNA synthesis).
- ATCase and Hb are allosteric proteins (cooperative behaviour)
- It has 12 subunits, 6 catalytic and 6 regulatory
- CTP is an inhibitor of ATCase (feedback inhibition)
- ATP is an activator
- Since the substrate is Aspartate and the inhibitor is CTP, it is a heterotropic effector.

When we have high concentration of ATP it means we need more nucleotides for DNA and RNA so we need UTP and CTP, so an increase in the concentration of ATP means activation to make UTP and CTP.

B. *Phosphorylation or other covalent modification*

When you hear phosphorylation, kinase should be the first thing to come to your mind

Kinase adds phosphate group

Phosphotase removes phosphate group

Why phosphate? Why is it effective?

- It adds two negative charges: new electrostatic interactions and accordingly affects conformation.
- Can form three or more hydrogen bonds: specific interactions with hydrogen-bond donors.
- Can take place in less than a second or over a span of hours.
- Rapid and transient regulation of enzyme activity - REVERSIBLE
- Often causes highly amplified effects. How? When a kinase adds phosphate on another kinase it activates it and then the kinase adds phosphate on other enzymes which activates lots of other reactions and so on.

- Phosphate can be added only on a hydroxyl group so you can find it on Ser, Thr, and Tyr.

- Mostly, ATP is the donor

- You should keep in mind that Phosphorylation does not lead always to activation of enzymes like in glycogen synthase where it leads to inhibition instead.

- Glycogen phosphorylase which adds a phosphate group on a terminal glucose in glycogen so it breaks off. It can exist in two states ("A" state (active) and "B" state (inactive)). It has two Serines that are away from the active site. If these two serines are phosphorylated by a kinase the enzyme becomes active. And if a phosphatase removes the phosphate groups it returns to the inactive state (the "B" state).

  The two forms of the enzyme both phosphorylase "B" and phosphorylase "A" exist as equilibria between an active R state and a less-active T state.

  • Phosphorylase "B" is usually inactive because the equilibrium favours the T state.

  • Phosphorylase "A" is usually active because the equilibrium favours the R state.

  The transition of phosphorylase "B" between the T and the R state is controlled by the energy charge of the muscle cell.

  <u>When inactive, equilibrium favours T more than R</u>

  Protein kinase A (PKA)

  What activates Kinase ?

  Protein kinase A (PKA): refers to a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP)

cAMP: is referred to as a hormonal 2nd messenger. Increase in the concentration of cAMP will lead to binding to protein like protein Kinase A (A is due to cyclic AMP)
Protein kinase A is allosteric; it has 4 subunits; 2 catalytic and 2 regulatory. The regulatory subunits bind to 4 cAMP which leads to the separation of the regulatory subunits from the catalytic subunits and the catalytic subunits are now active.

Adrenaline (epinephrine) →↑cAMP→ activates protein kinase A → phosphorylates & activates glycogen phosphorylase kinase → phosphorylates & activates glycogen phosphorylase → phosphorylates glycogen (this is called phosphorylation cascade)
A phosphorylation cascade is a sequence of events where one enzyme phosphorylates another, causing a chain reaction leading to the phosphorylation of thousands of proteins (this is why it's amplified).

- Phosphodiesterase breaks down cAMP converting it to AMP so it will not bind to protein kinase A.

Other covalent modifiers:
You should just know them briefly

- Adenylylation (addition of adenylyl group). AMP (from ATP) is transferred to a Tyr hydroxyl by a phosphodiester linkage. The addition of bulky AMP inhibits certain cytosolic enzymes.
- Uridylylation (addition of uridylyl group).

- ADP-ribosylation (addition of adenosine diphosphate ribosyl group): inactivates key cellular enzymes.
- Methylation: masks a negative charge & adds hydrophobicity on carboxylate side chains.
- Acetylation: masks positive charges when added to lysine residues.

C. Protein-protein interactions between regulatory & catalytic subunits or between two proteins;

D. Proteolytic cleavage