بسم الله الرحمن الرحيم

BioChemistry | FINAL 14

Enzymes Pt.6

Written by : Anonymous member

Reviewed by : Rayan Theeb

2. Inhibition

- Enzyme inhibitors can be broadly classified into two main types: **reversible** and **irreversible**.
- Within reversible inhibition, the two primary forms are competitive and non-competitive inhibitors.
- On the other hand, irreversible inhibitors, also called **mechanism-based inhibitors**, disrupt the enzyme's catalytic mechanism. These irreversible inhibitors can be further divided into three categories: covalent inhibitors, transition state analogs, and heavy metals.

MECHANISM-BASED INHIBITORS

- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction
- The term includes:

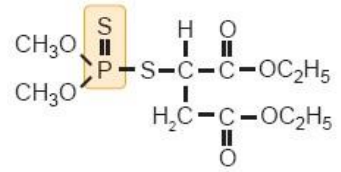
A. Covalent inhibitors

B. Transition state analogs

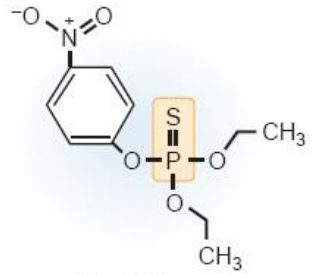
C. Heavy metals

- The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme

Covalent Inhibitors



Malathion



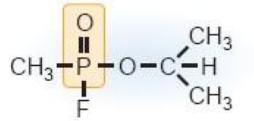
Parathion

- Covalent or extremely tight bonds with active site amino acids
- Amino acids are targeted by drugs & toxins

Covalent inhibitors are substances that bind irreversibly to the enzyme's active site through covalent bonds, thereby blocking its activity. These inhibitors are often classified as poisons, toxins, or drugs.

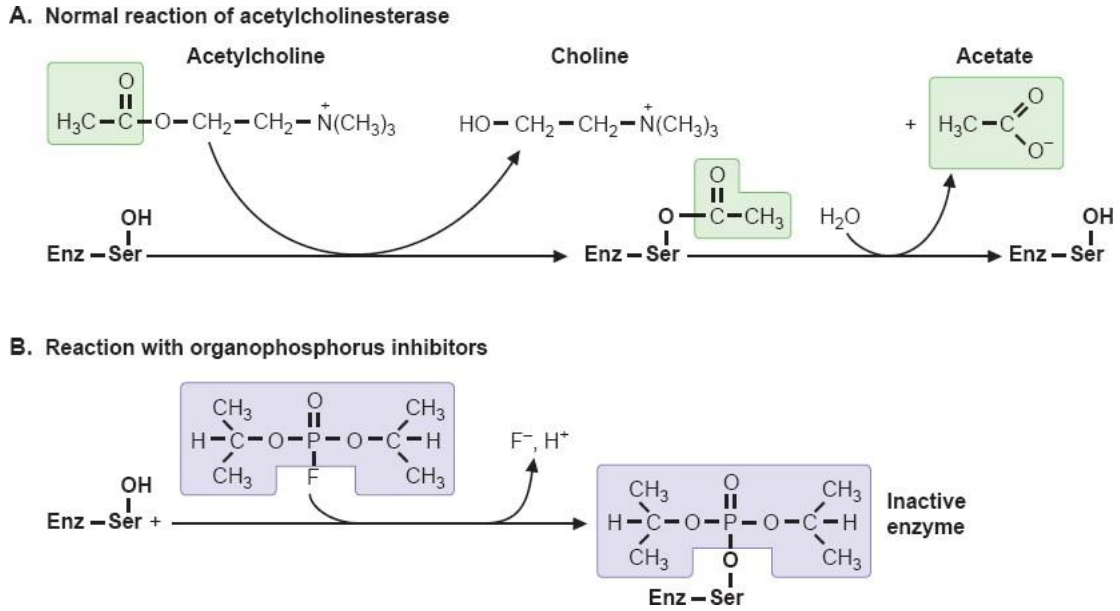
- The lethal compound [DFP] is an organophosphorus compound that served as a prototype for:
  - The nerve gas sarin
  - The insecticides malathion & parathion

Diisopropyl fluorophosphate



Sarin

- DFP also inhibits other enzymes that use serine (ex. serine proteases), but the inhibition is not as lethal

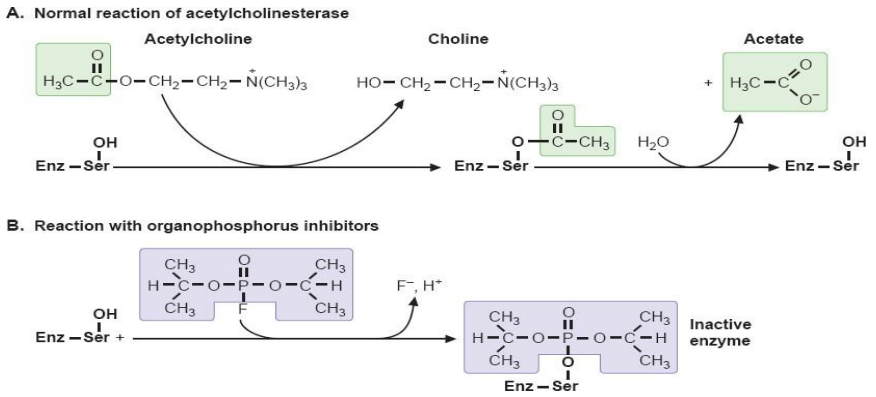


Covalent Inhibitors

Organophosphorus insecticides are highly toxic compounds because they covalently inhibit acetylcholinesterase (AChE), the enzyme responsible for breaking down acetylcholine (ACh). Acetylcholine is a neurotransmitter released from vesicles at the nerve endings of motor neurons at the neuromuscular junction, where it binds to receptors on muscle fibers to trigger contraction.

Acetylcholinesterase normally hydrolyzes acetylcholine into acetate and choline, leading to muscle relaxation. Permanent inhibition of AChE—such as when sarin gas binds to the serine residue at the enzyme's active site—causes the phosphate group to attach covalently to the enzyme. This prevents acetylcholine breakdown, resulting in continuous muscle contractions, a condition called **muscle tetanization.**

Importantly, this effect is most dangerous when it occurs in the **diaphragm**, as persistent contractions deplete ATP and can lead to respiratory failure / arrest, rather than just affecting limb muscles.

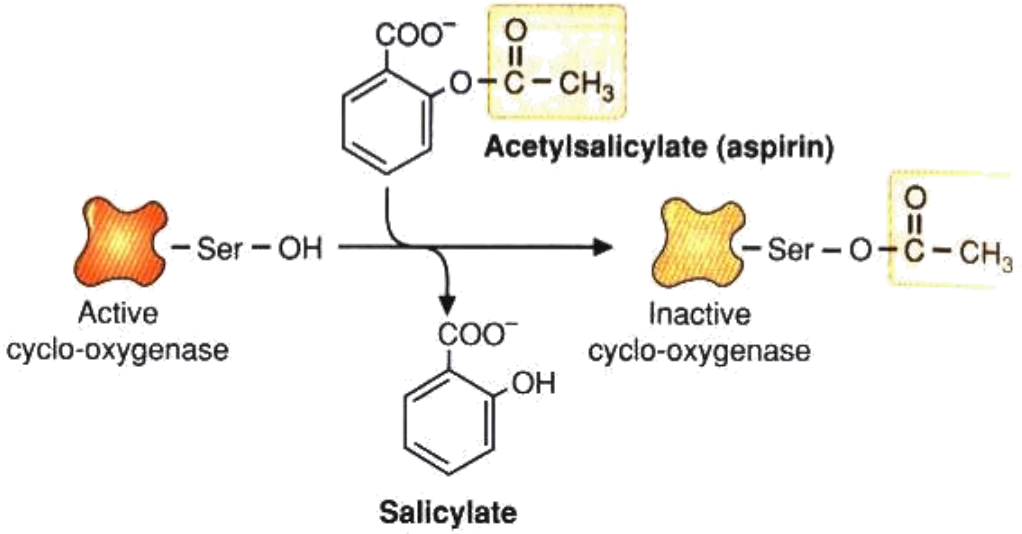


Covalent Inhibitors

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

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

- Another example of covalent inhibitors is aspirin, which irreversibly inhibits the cyclooxygenase (COX) enzyme.
- The active site of COX contains a serine residue, and aspirin (acetylsalicylic acid) has an acetyl group and a salicylic acid moiety (a benzene ring with a carboxyl group).
- During the reaction, the salicylate portion is released, while the acetyl group covalently binds to the oxygen of the serine residue in the active site.
- This covalent attachment results in permanent inhibition of the enzyme.



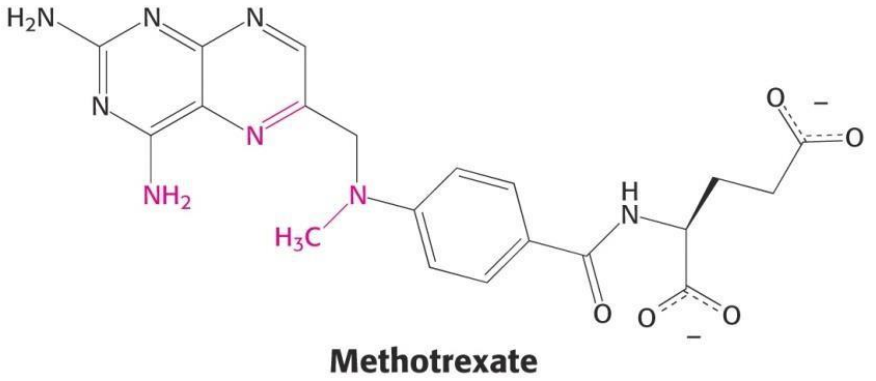
Transition-State Analogs & Compounds that Resemble Intermediate Stages of the Reaction

- Transition-state analogs: extremely potent inhibitors (bind more tightly)
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure)
- Substrate analogs: bind more tightly than substrates
- Known as suicide inhibitors

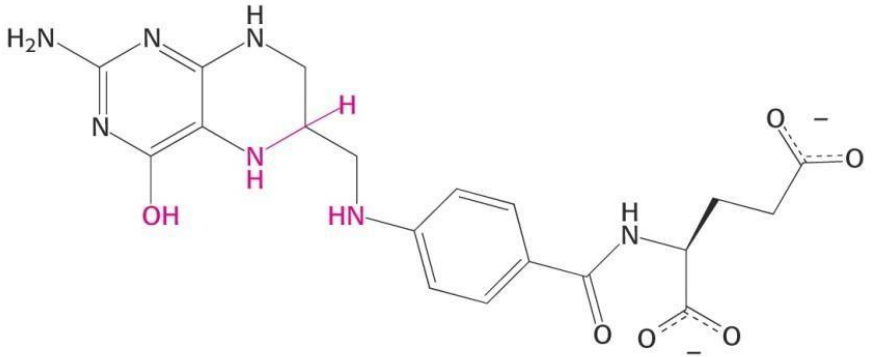
- Transition state analogs are inhibitors that mimic the transition state of a substrate during an enzymatic reaction. These inhibitors often bind very tightly—sometimes covalently—to the enzyme's active site.
- Unlike simple covalent inhibitors, the enzyme initially begins the reaction, but because the analog is not the true substrate, the process cannot proceed. Their affinity for the enzyme is much higher than that of the natural substrate, leading the enzyme to essentially "trap" itself. For this reason, they are also called suicide inhibitors.

Methotrexate

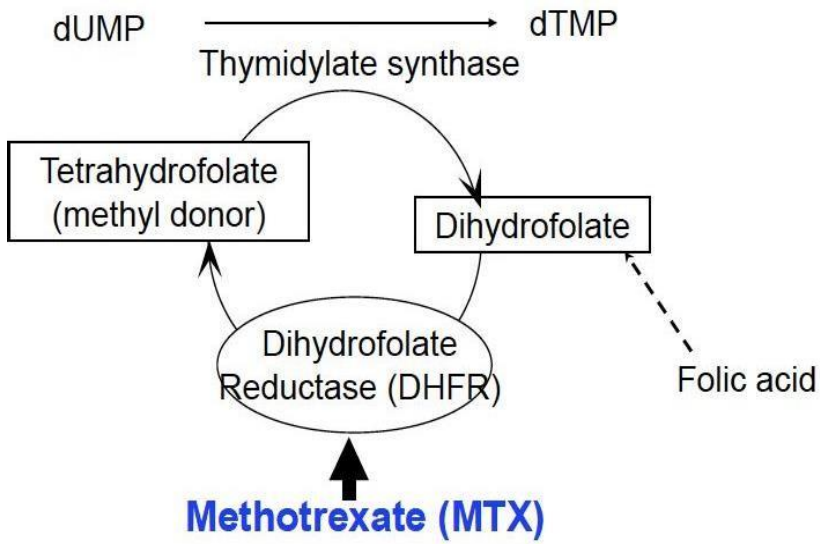
- Synthetic inhibitor
- Anticancerous
- Analog of tetrahydrofolate
- Binds to enzyme a 1000-fold more tightly
- Inhibits nucleotide base synthesis



Methotrexate

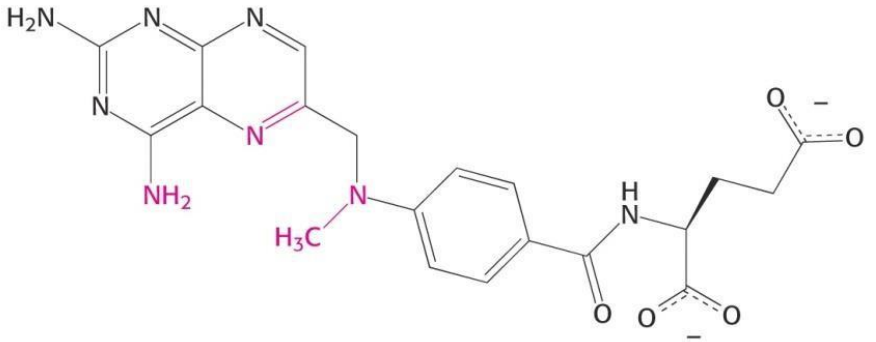


Tetrahydrofolate

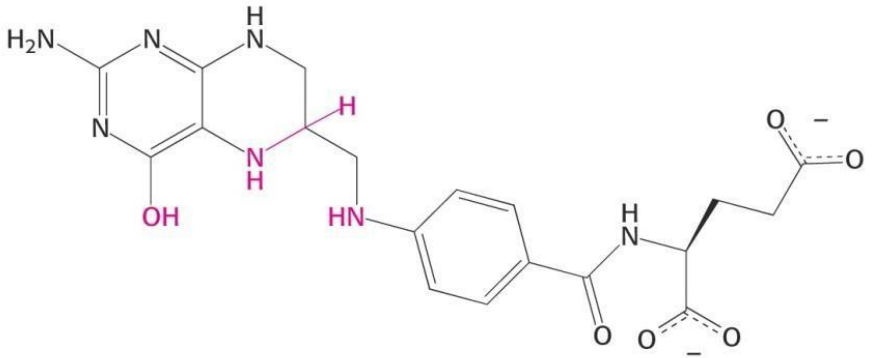


Methotrexate

A key example is methotrexate, one of the most widely used drugs in the treatment of cancers such as breast cancer. Methotrexate acts as a transition state analog of folate derivatives (dihydrofolate and tetrahydrofolate), which are coenzymes derived from vitamin B9 (folic acid).
Normally, tetrahydrofolate plays a critical role in the synthesis of nucleotides. It is required by the enzyme **thymidylate synthase**, which converts uracil into thymidine nucleotides—an essential step in DNA synthesis.

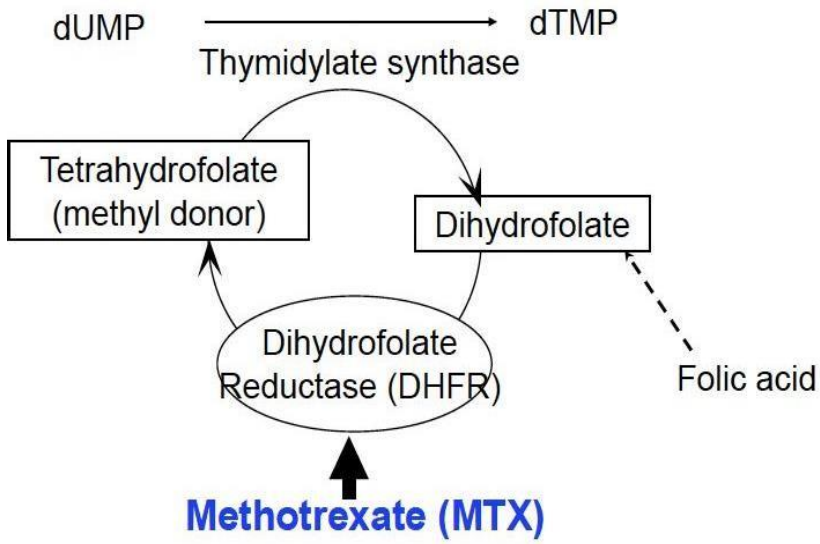


Methotrexate



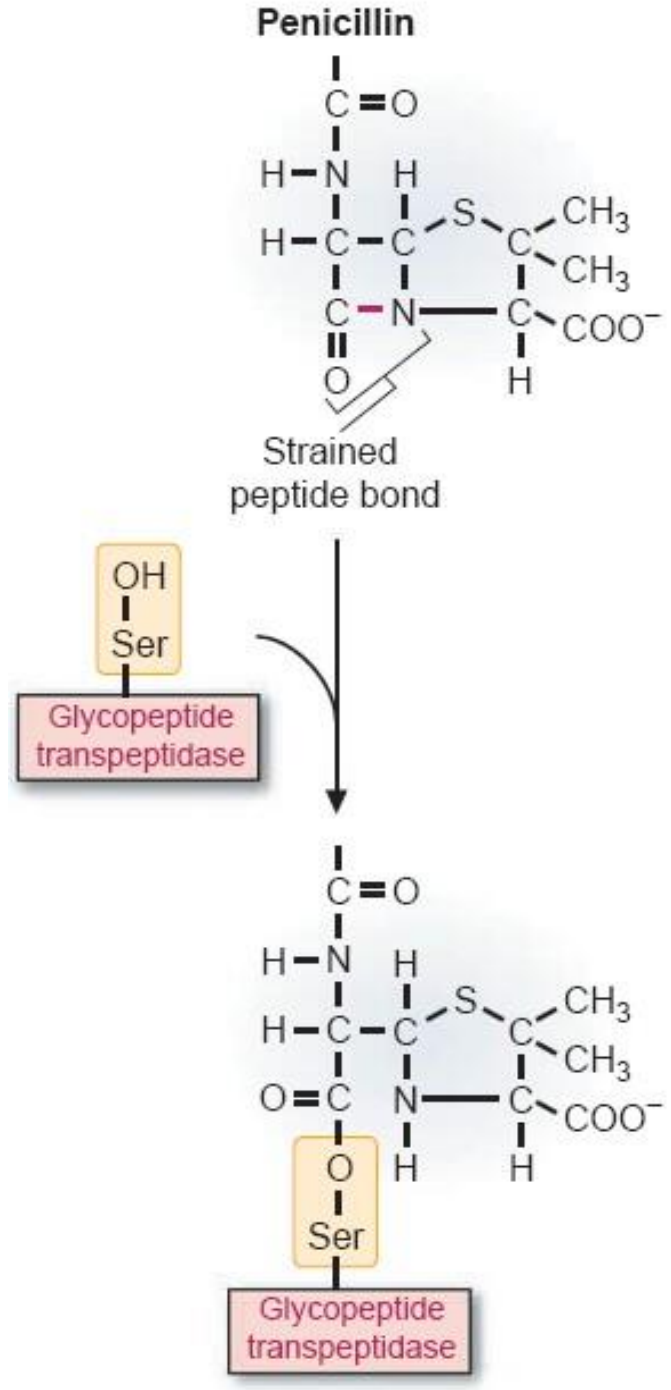
Tetrahydrofolate

In this pathway, tetrahydrofolate is converted to dihydrofolate and then regenerated back to tetrahydrofolate by the enzyme **dihydrofolate reductase** (DHFR), allowing the cycle to continue. Methotrexate closely resembles folate in structure but binds to DHFR with about 1000 times greater affinity than the natural substrate. This blocks the regeneration of tetrahydrofolate, halting nucleotide synthesis and thereby preventing cancer cells from dividing.



PENICILLIN

- A transition-state analog to *glycopeptidyl transferase or transpeptidase*
- Required by bacteria for synthesis of the cell wall
- The reaction is favored by the strong resemblance between the peptide bond in the β-lactam ring of penicillin & the transition-state complex of the natural transpeptidation reaction
- Inhibitors that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed *suicide inhibitors*

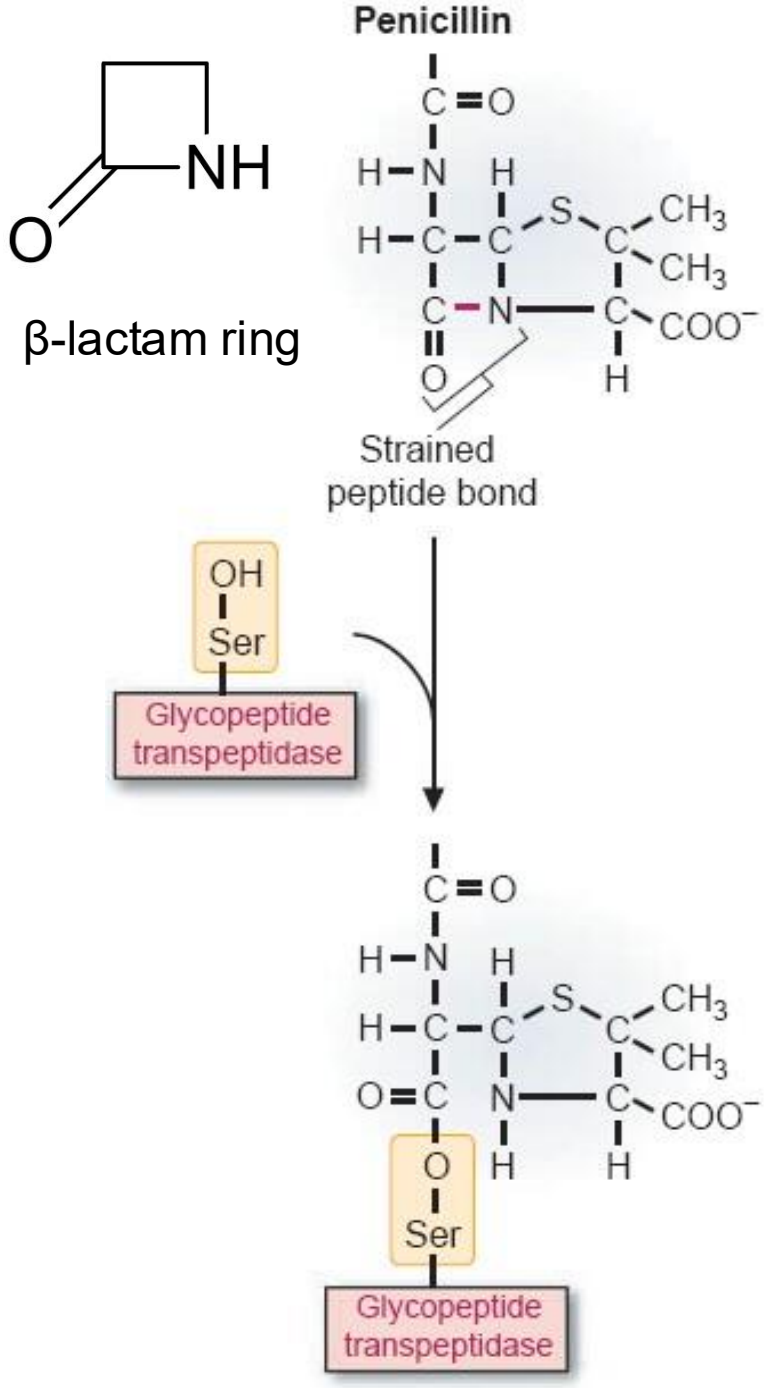


PENICILLIN

- For bacteria to grow, they need enzymes to synthesize their cell wall. One key enzyme in this process is **glycopeptidyl transferase** (also called transpeptidase), which normally forms cross-links in the bacterial cell wall by acting on peptide bonds (specifically amide bonds between a nitrogen and a carbonyl group).

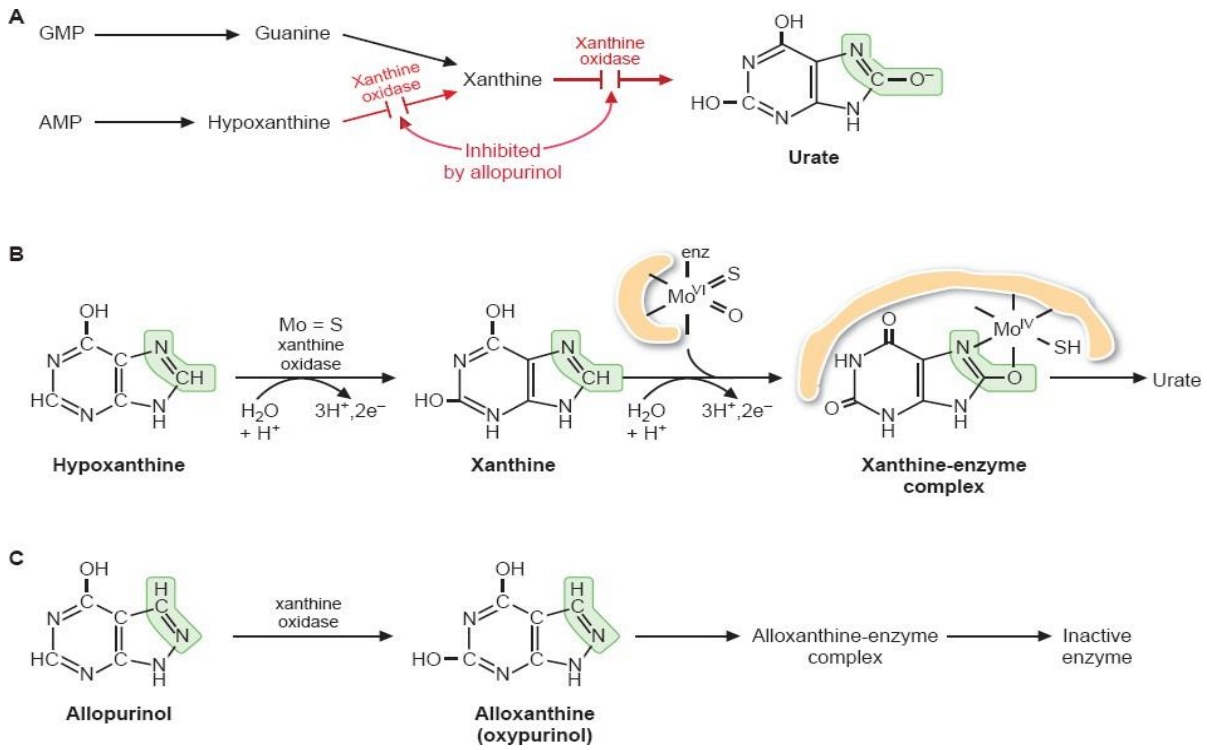
- Penicillin mimics the natural substrate of this enzyme. Its structure contains a β-lactam ring (a nitrogen within a four-membered ring), which resembles the peptide bond. Because of this, transpeptidase mistakenly recognizes penicillin as its substrate and binds to it with higher affinity than to the natural peptide. The enzyme then attempts to catalyze the reaction, but instead of releasing the product, the β-lactam ring of penicillin is irreversibly opened and forms a covalent bond with the serine residue in the enzyme's active site.

- As a result, the enzyme is permanently inactivated, the bacterial cell wall cannot be properly cross-linked, and the bacterium eventually dies.



ALLOPURINOL

- A drug used to treat gout
- Decreases urate production by inhibiting xanthine oxidase
- The enzyme commits suicide by converting the drug to a transition-state analog
- The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions
- Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, a compound that binds very tightly to a molybdenum–sulfide complex in the active site



ALLOPURINOL

Allopurinol is a transition state analog and the most widely used drug for the treatment of gout. Gout is not primarily a protein pathway disorder but is related to the metabolism of nucleotides (nitrogenous bases). When a person consumes a high-protein diet, excess nitrogen enters the body. This nitrogen is used not only to build proteins but also to form nitrogenous compounds, including purine bases such as hypoxanthine and xanthine.

Normally, hypoxanthine is converted to xanthine, and xanthine is further converted into uric acid by the enzyme xanthine oxidase. Uric acid has very low solubility in body fluids; when present in excess, it forms crystal deposits in the joints. These urate crystals restrict joint movement and cause the severe pain typical of gout.

Allopurinol is a structural analog of hypoxanthine, differing by a rearrangement of nitrogen and carbon atoms. When allopurinol is administered, xanthine oxidase mistakenly converts it into oxypurinol (alloxanthine). However, oxypurinol is not a suitable substrate for the enzyme, and it binds tightly to xanthine oxidase, thereby blocking its activity. This inhibition prevents further production of uric acid, reducing crystal formation and alleviating gout symptoms.

Heavy Metals

- Tight binding of a metal to a functional group in an enzyme
- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)
- Relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity
- Mercury: binds to so many enzymes, often at reactive sulfhydryl groups in the active site
  - It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity
- Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc
  - Its developmental & neurologic 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

Heavy Metals

Mercury is considered an irreversible inhibitor of many enzymes in the central nervous system (CNS). It is not selective for a single enzyme; instead, it can inactivate any enzyme that contains a thiol (-SH) group in its active site by binding to it and forming a stable, irreversible complex. This permanently inhibits the enzyme's function.

Mercury has historically been used in some preservatives (for example, thimerosal in certain vaccines as an antibacterial agent). Concerns arose in the past about a possible link between mercury-containing preservatives and autism in children. However, extensive scientific research has shown no causal relationship between vaccines (including those with thimerosal) and autism. Because of public concern, many countries reduced or eliminated thimerosal from routine childhood vaccines, even though evidence did not support the link.

Lead is another important example of a heavy metal inhibitor. It has historically been used in paints to make them shinier, and young children may ingest lead-containing paint chips or dust without realizing it. Lead in paint is inhibited to use in some countries like US. Lead is toxic because it can replace calcium in enzymes and biological systems, as it has a higher affinity for certain enzyme active sites than calcium does. This leads to permanent inhibition of those enzymes.

Like mercury, lead is non-selective; it does not target a single enzyme but instead affects many enzymes, especially those that require calcium for their activity.

In general, heavy metals have much higher affinity for enzyme active sites compared to normal physiological cofactors (like calcium, zinc, or magnesium). The degree of inhibition depends on both the concentration of the heavy metal and its affinity for the binding site.

Reversible Inhibitors

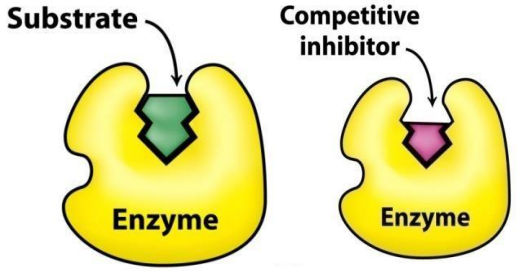
- Characterized by a rapid dissociation of the enzyme-inhibitor complex
- Usually these inhibitors bind through non-covalent interactions & inhibitor maintains a reversible equilibrium with the enzyme
- Reversible inhibitors can be divided into two classes: competitive & noncompetitive
- The double-reciprocal plots are highly useful for distinguishing among these inhibitors

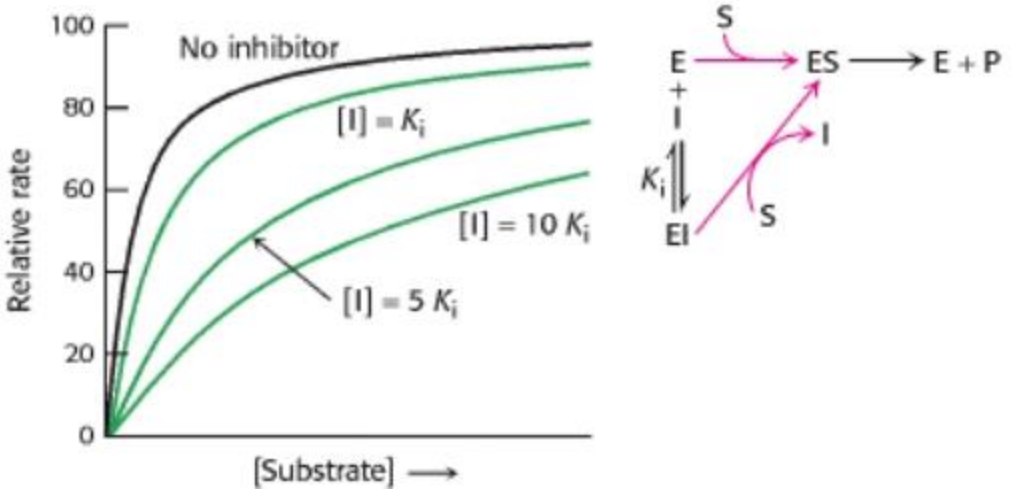
Reversible Inhibitors

Reversible inhibitors are subdivided into three categories:
Competitive inhibitors
Non-competitive inhibitors
Uncompetitive inhibitors (a mixed type with features of both)

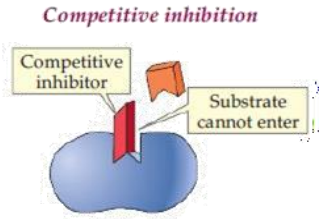
Competitive inhibition
As the name suggests, competitive inhibitors compete with the substrate for binding to the enzyme's active site.
The outcome depends on two factors:
Affinity (how strongly the inhibitor or substrate binds to the active site).
Concentration (the amount of substrate or inhibitor present).
A substrate with higher concentration can overcome an inhibitor with lower affinity, and vice versa.

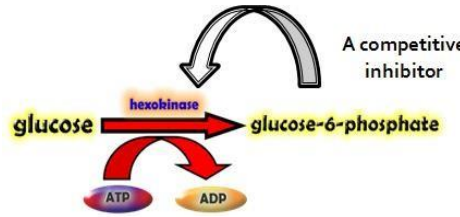
Competitive inhibition

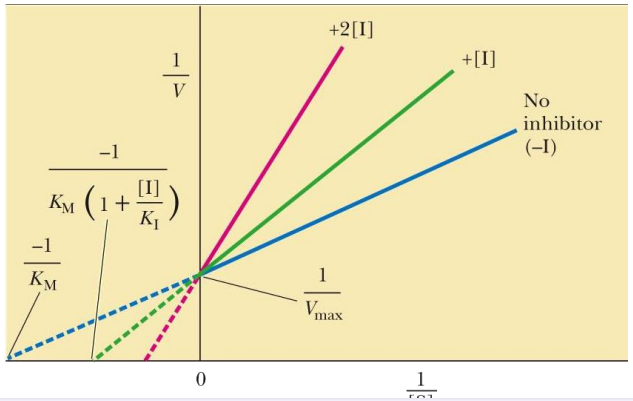


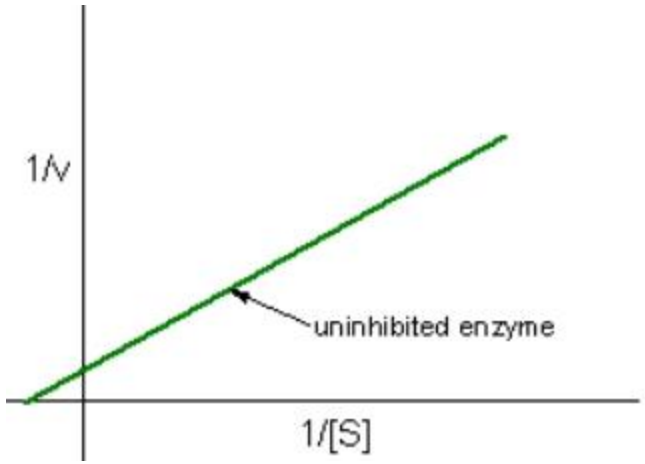


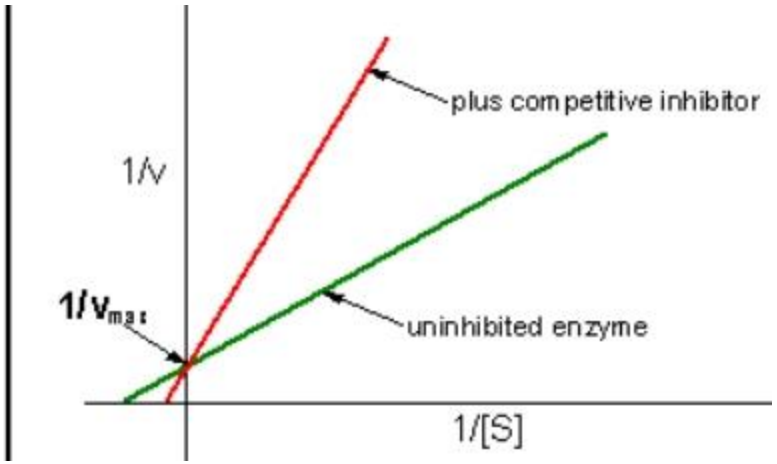
- The inhibitor competes with substrate
- Increasing [S] can overcome the inhibition (V_{max})
- Does K_M change?
- Significance (ex. Hexokinase)

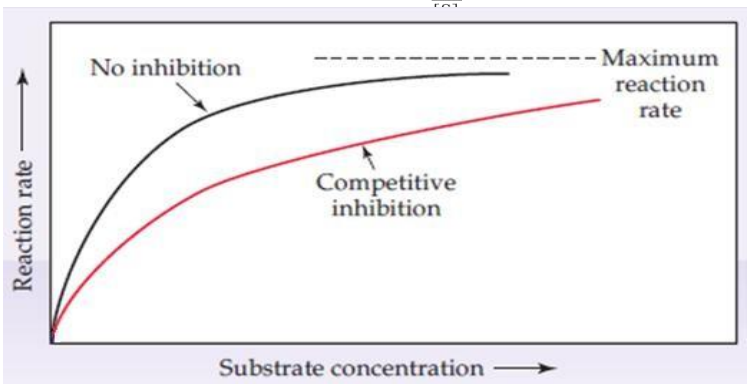












Kinetic effects

In enzyme kinetics, we measure reaction velocity (y-axis) versus substrate concentration (x-axis). The two key parameters are:

Vmax = maximum velocity of the reaction.

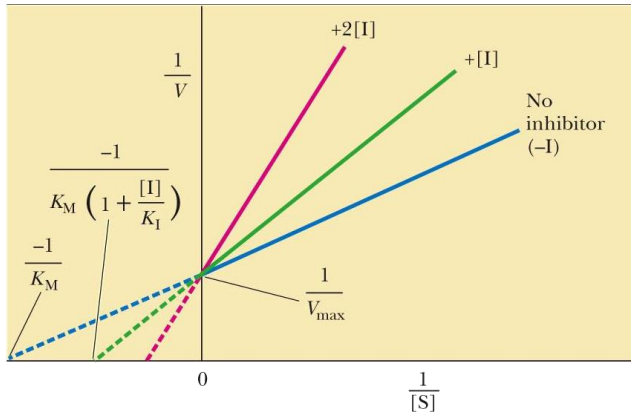
Km = substrate concentration at half Vmax (an indicator of affinity).

With competitive inhibition:

Vmax remains unchanged (because adding enough substrate can still reach maximum velocity).

Km increases (because more substrate is needed to outcompete the inhibitor and reach half-maximal velocity).

On a Michaelis-Menten plot, the curve shifts to the right.



The Lineweaver-Burk plot is a double-reciprocal plot used in enzyme kinetics, where:

Slope = Km / Vmax

Y-intercept = 1 / Vmax

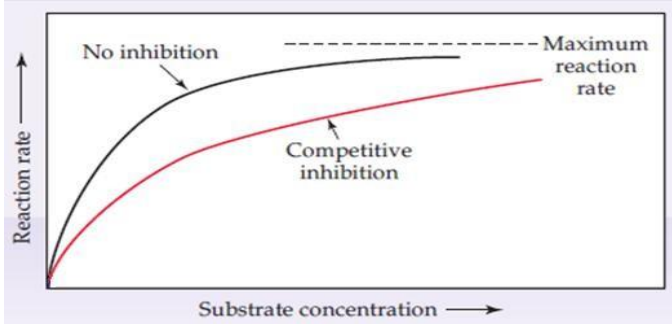
X-intercept = -1 / Km

When a competitive inhibitor is added:

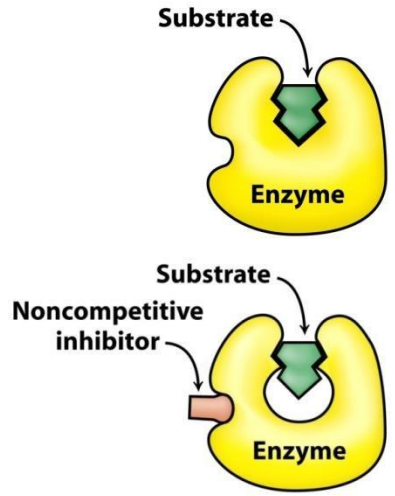
The slope increases (since Km increases while Vmax remains constant).

Vmax stays the same, so the y-intercept (1/Vmax) does not change.

Because Km is larger, the x-intercept (-1/Km) shifts closer to the origin.

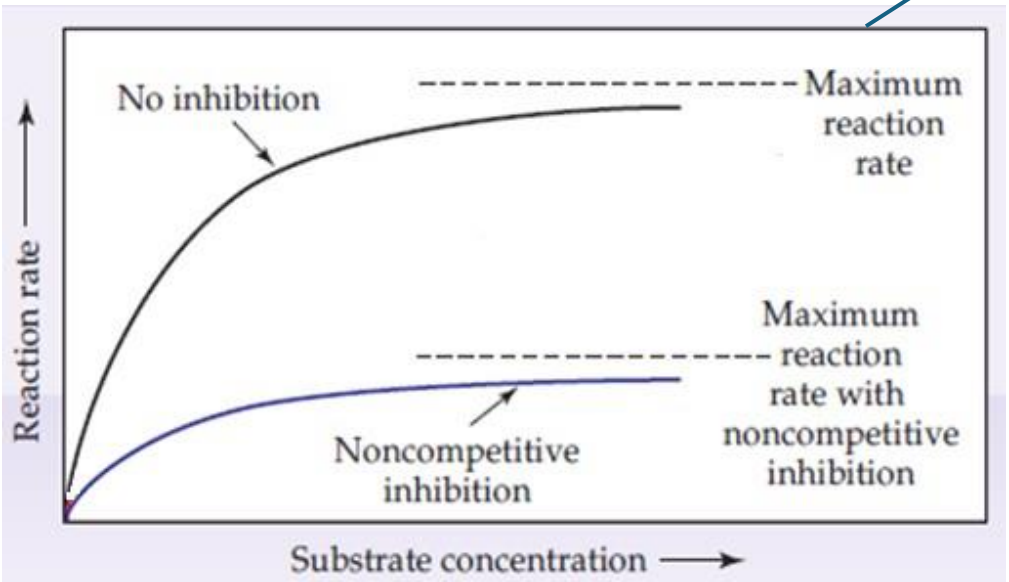


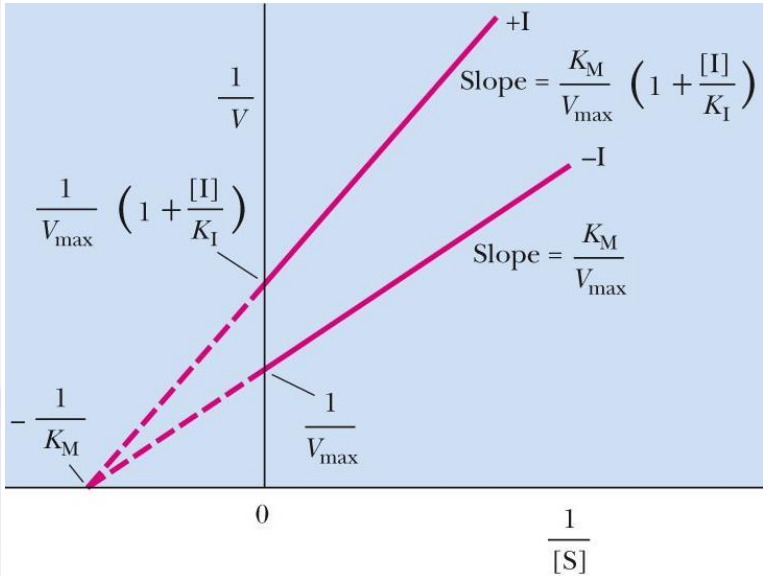
Noncompetitive inhibition

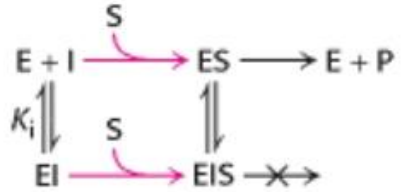


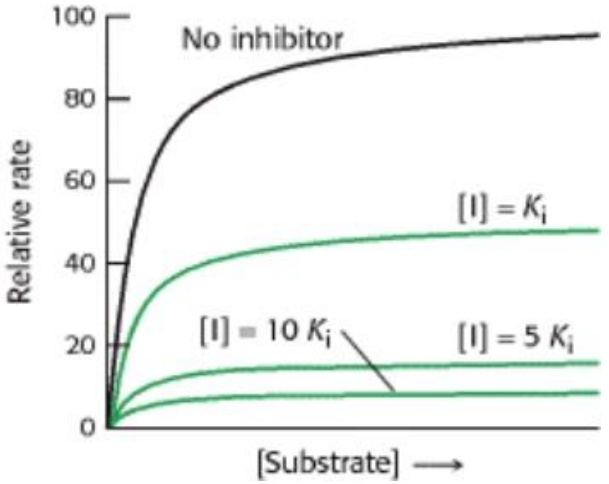
- The inhibitor binds at a site other than the active site
- The complex does not proceed to form product or has a lower efficiency
- V_{max} vs. K_M
- Can we reach V_{max}?

This plot is seen in two situations:
1- Enzyme concentration effect
2- Noncompetitive inhibition effect









Noncompetitive inhibition

Non-competitive inhibition
(a type of irreversible inhibition)
A non-competitive inhibitor binds to a site other than the active site.
It does not compete with the substrate for binding. Instead, it changes the geometry (conformation) of the enzyme, making the active site less efficient at converting substrate into product.
As a result, increasing substrate concentration cannot overcome the inhibition.

Kinetic effects
Vmax decreases → because the overall catalytic efficiency of the enzyme is reduced, and maximum velocity cannot be reached even at high substrate concentrations.
Km remains unchanged → because the substrate can still bind to the enzyme with the same affinity; the problem is with catalysis, not binding.

Lineweaver-Burk plot
Slope (Km/Vmax) increases → since Vmax is lower while Km is unchanged.
Y-intercept (1/Vmax) increases → because Vmax is reduced.
X-intercept (-1/Km) remains the same → since Km is unchanged.

REGULATION THROUGH CONFORMATIONAL CHANGES

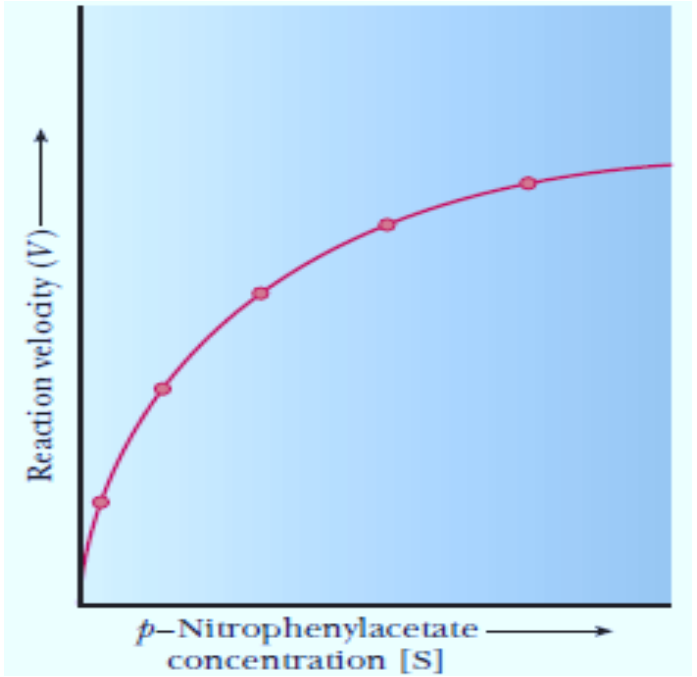
- **These regulatory mechanisms include**
  - **A. Allosteric activation and inhibition;**
  - **B. Phosphorylation or other covalent modification;**
  - **C. Protein-protein interactions between regulatory & catalytic subunits or between two proteins;**
  - **D. Proteolytic cleavage**

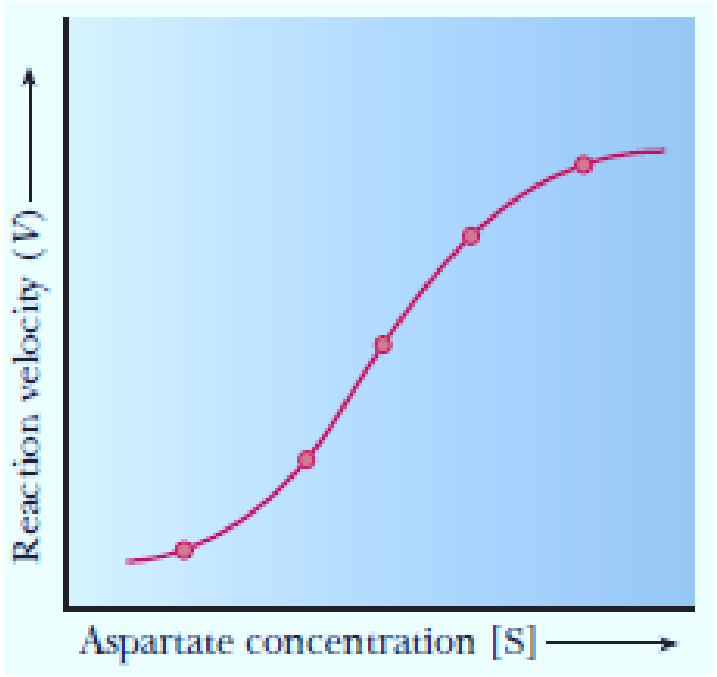
- **These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation**

Not all enzymes follow Michaelis-Menten equation; Chymotrypsin vs. ATCase

- **Chymotrypsin: Specificity for aromatic residues mainly. Also, hydrolysis of ester bonds**
- **Aspartate transcarbamoylase (ATCase): synthesis of CTP & UTP for RNA and DNA synthesis**

$$\text{Carbamoyl phosphate} + \text{Aspartate} \rightarrow \text{Carbamoyl aspartate} + HPO_4^{2-}$$





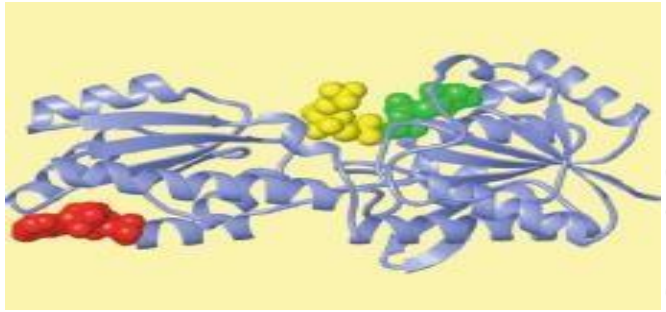
Not all enzymes follow Michaelis-Menten equation; Chymotrypsin vs. ATCase

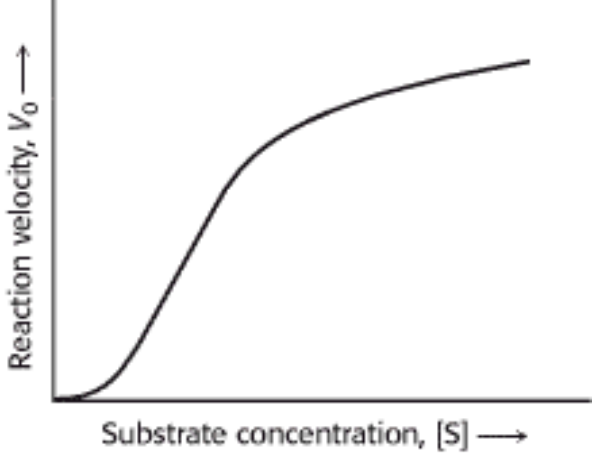
Allosteric regulation

The term allosteric means “regulation from a site far away.”
Enzymes that are allosteric are usually multi-subunit proteins.
The catalytic subunit carries out the enzymatic reaction.
The regulatory subunit controls the enzyme’s activity by binding effectors.
Effectors can be:
Activators → increase enzyme activity.
Inhibitors → decrease enzyme activity.
Effectors can also be classified as:
Homotropic effectors → the same molecule that binds at the catalytic site also regulates activity (e.g., substrate acting as its own effector).
Heterotropic effectors → a different molecule from the substrate regulates enzyme activity.

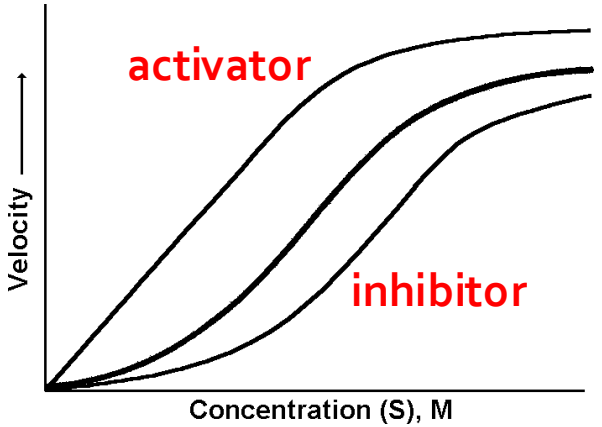
Kinetics of allosteric enzymes
Allosteric enzymes do not follow classical Michaelis-Menten kinetics.
Instead of Km, they use the term K0.5, which refers to the substrate concentration at half-maximal velocity.
Vmax is not altered by allosteric effectors.

Allosteric regulation





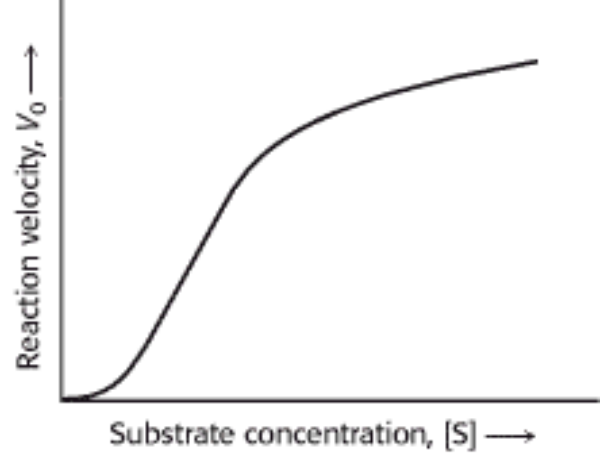
- What are allosteric enzymes? A multi- subunit enzyme with catalytic subunit(s) and regulatory subunit(s)
- Binding triggers a conformational change in the active site
- The Michaelis-Menten model can't explain the kinetic properties
- The effect of the modulators (allosteric modifiers)
- Homotropic vs. heterotropic
- The substrate concentration at half of the V_{max} is called ($K_{0.5}$)
- Allosteric inhibitors have a much stronger effect on enzyme velocity

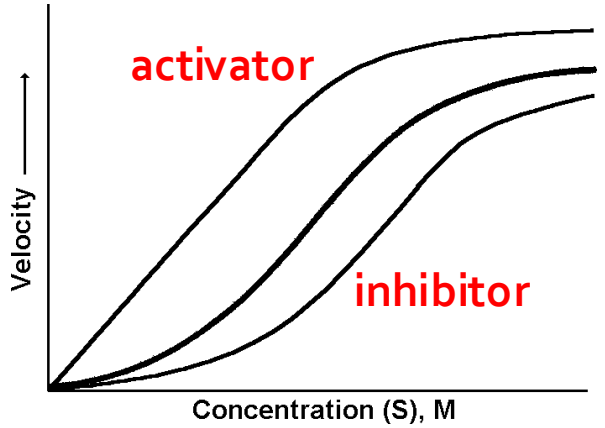


The effect of modifiers on V_{max} & $K_{0.5}$

Allosteric regulation

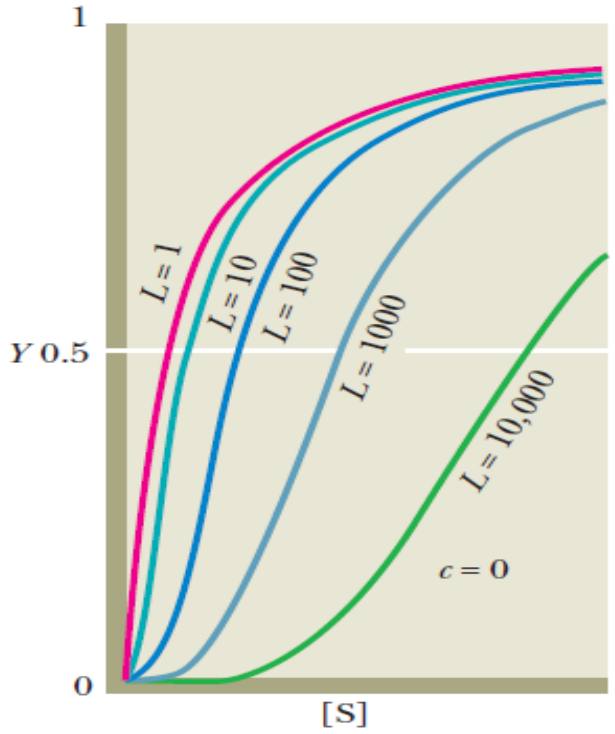
Graphical behavior
By definition, allosteric enzymes show a sigmoidal (S-shaped) curve for velocity vs. substrate concentration.
In the presence of an allosteric **inhibitor**, the curve shifts to the **right** (decreased affinity).
In the presence of an allosteric **activator**, the curve shifts to the **left** (increased affinity).



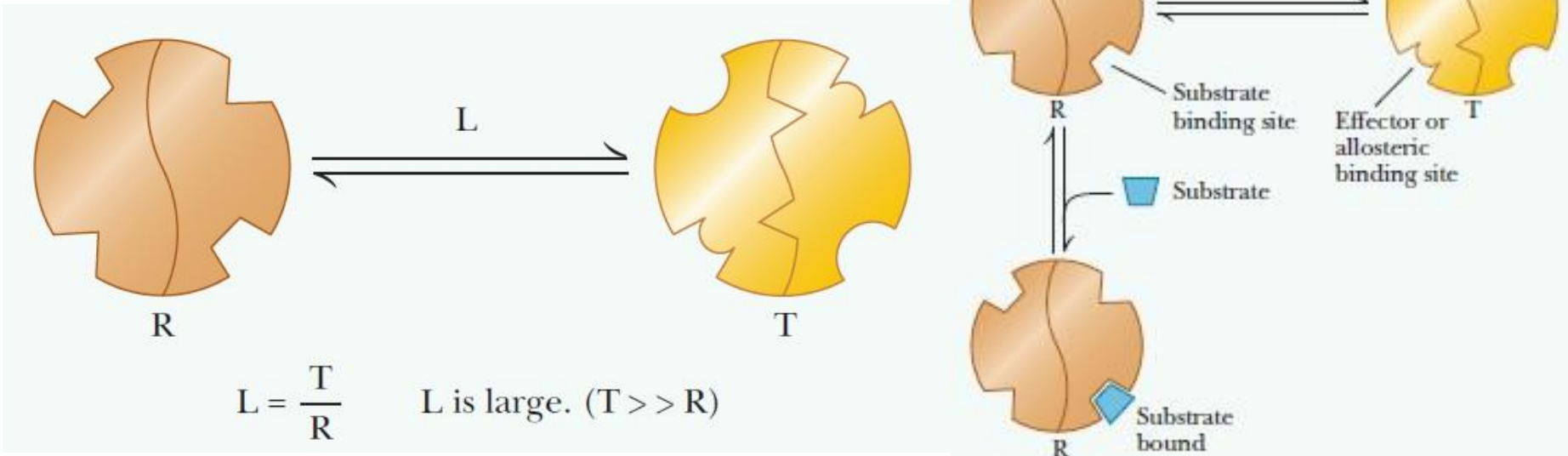


How do allosteric enzymes work?

- 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 (and shifts to the right)



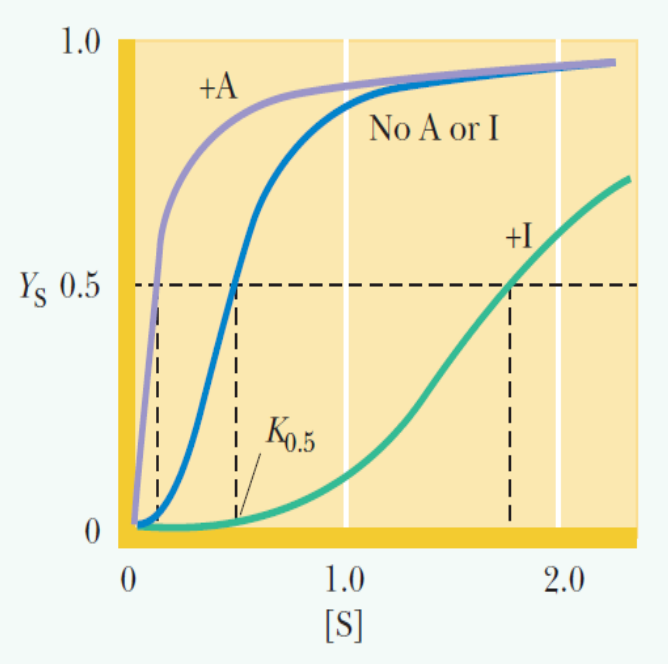
"The T/R ratio is usually high in the body, meaning most allosteric enzymes are kept in the less active T state until regulation occurs. This allows precise control of enzyme activity when needed."

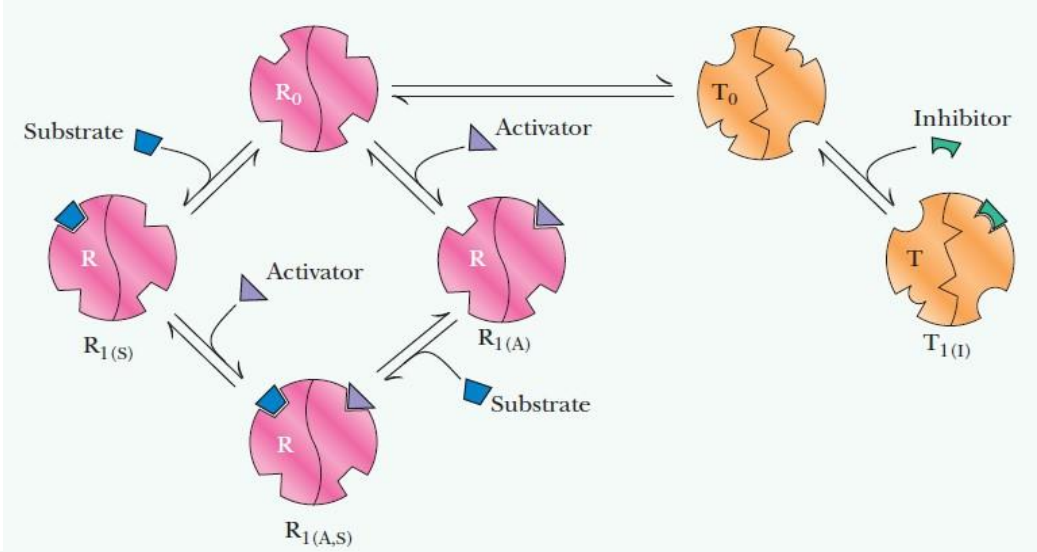


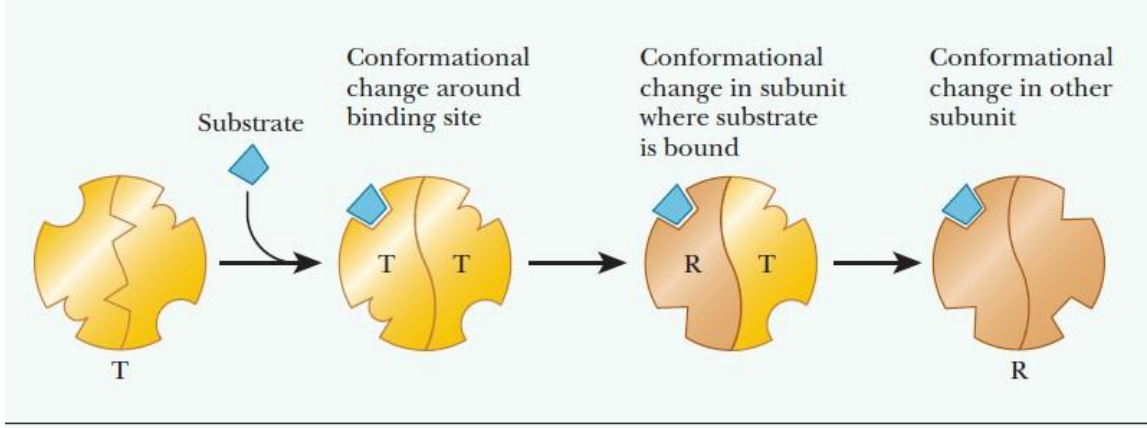
Concerted or sequential?

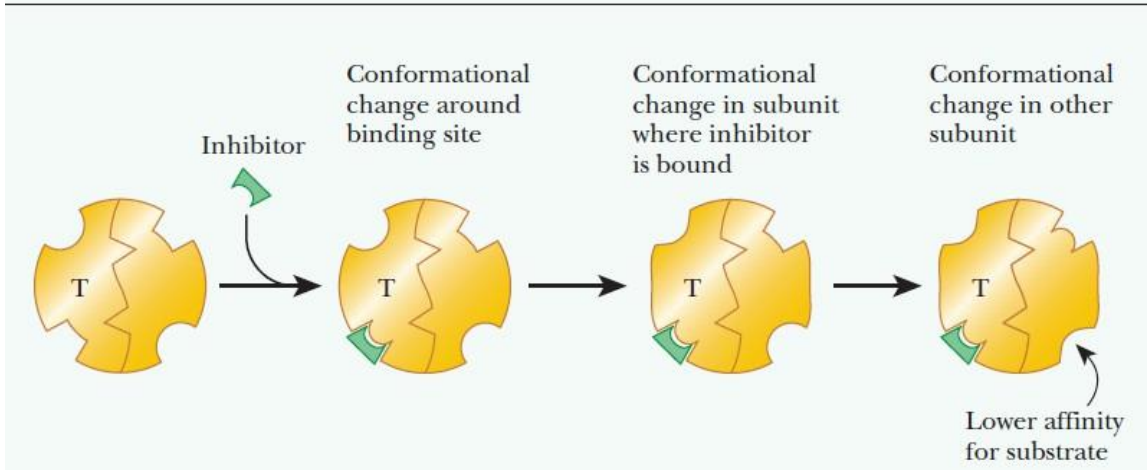
- **Either substrate or activator must be increased to overcome the effects of the allosteric inhibitor**
- **Conformational change**

Some enzymes or processes follow a concerted (all-or-none) mechanism, some follow a sequential (stepwise) mechanism, and some can adopt both mechanisms depending on the conditions or effectors present.

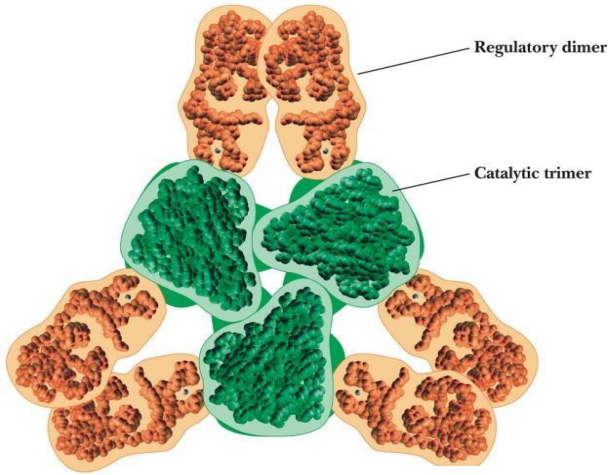




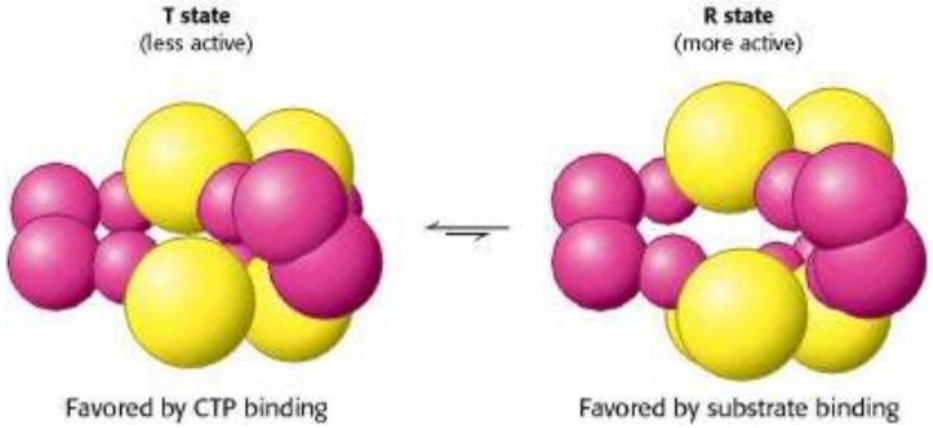


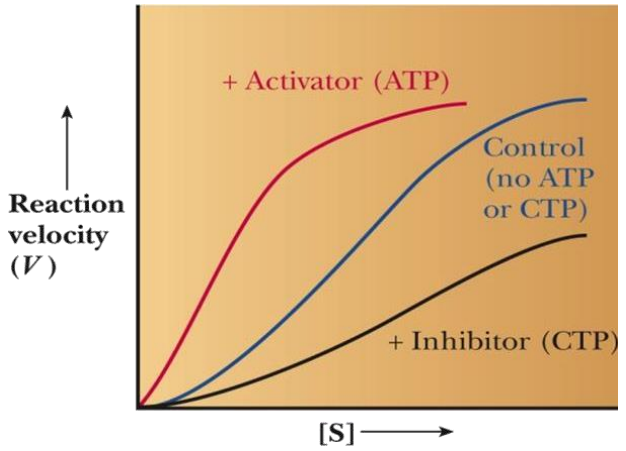


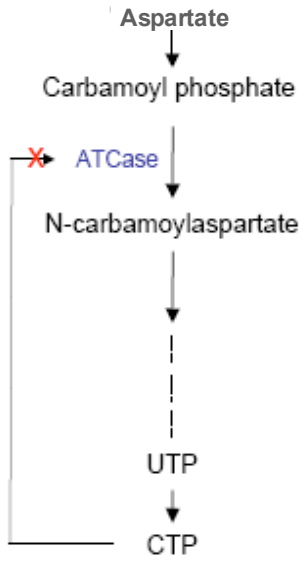
Allosteric regulation – ATCase "synthesis of pyrimidine nucleotides"

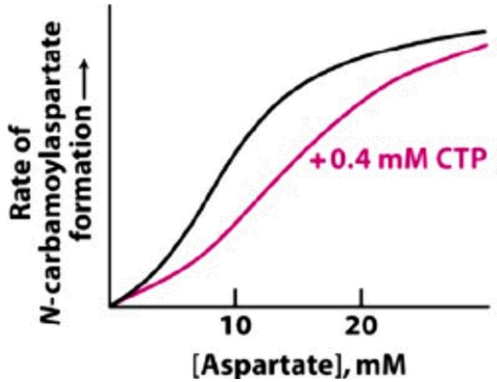


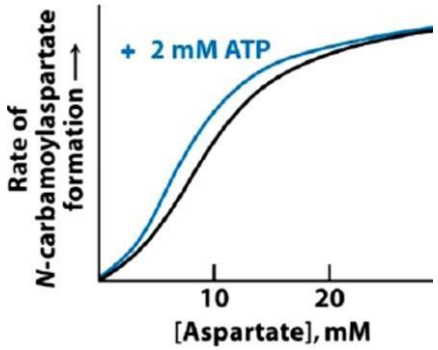
- ATCase and Hb are allosteric proteins (cooperative behavior)
- Catalytic can be separated from regulatory (hyperbolic)
- Cooperativity in relation to substrate
- CTP is an inhibitor of ATCase (feedback inhibition),
  ATP is an activator



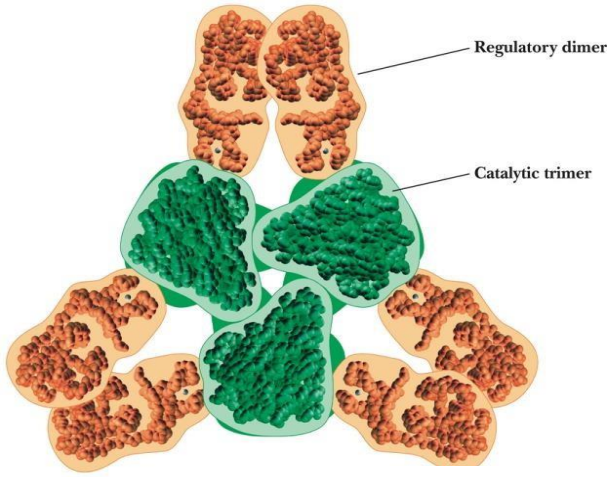






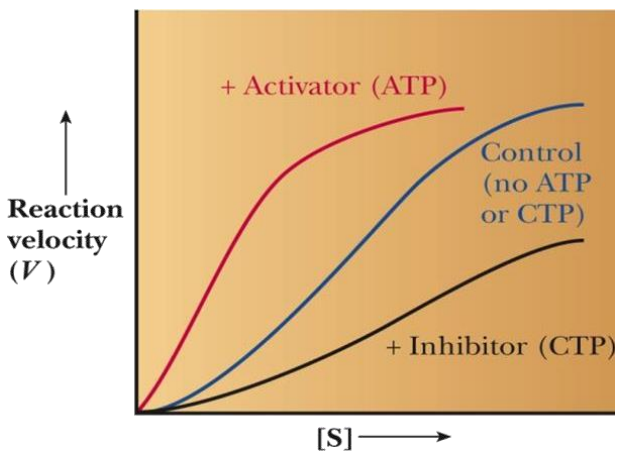


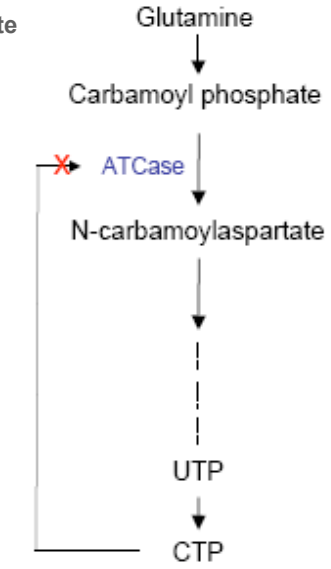
Allosteric regulation – ATCase
"synthesis of pyrimidine nucleotides"



Aspartate Carbamoyltransferase (ATCase)
Function: Catalyzes the first step in pyrimidine biosynthesis, converting aspartate and carbamoyl phosphate eventually into UTP, which is further converted to CTP. These nucleotides are essential for DNA and RNA synthesis.

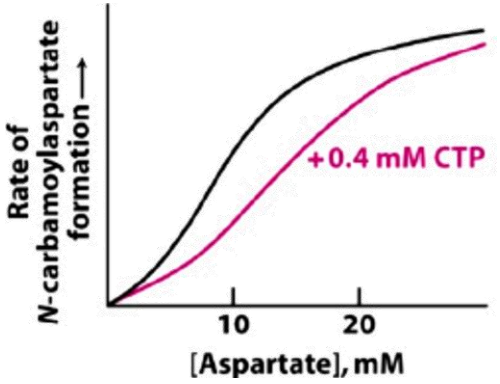
Regulation:
CTP acts as a feedback inhibitor. When CTP concentration is high, it binds the regulatory subunits and inhibits the enzyme. This is an example of a **heterotropic** effector.
ATP acts as an activator. When ATP concentration is high, it signals the need for more pyrimidines (CTP/UTP) for nucleic acid synthesis.
Kinetic effect on the allosteric plot:
CTP shifts the sigmoidal curve to the right (decreased activity).
ATP shifts the curve to the left (increased activity).

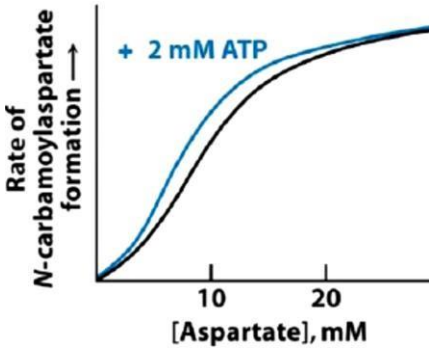




Structure
ATCase has 12 subunits:
6 catalytic subunits (green) – responsible for the chemical reaction.
6 regulatory subunits (brown) – responsible for allosteric regulation.

Experimental observation:
If the regulatory subunits are removed, the enzyme loses allosteric behavior and behaves as a simple enzyme, showing a hyperbolic (Michaelis–Menten) kinetics.





For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

رسالة من الفريق العلمي:

وَسَارِعُوٓاْ إِلَىٰ مَغۡفِرَةٖ مِّن رَّبِّكُمۡ وَجَنَّةٍ عَرۡضُهَا ٱلسَّمَٰوَٰتُ وَٱلۡأَرۡضُ أُعِدَّتۡ لِلۡمُتَّقِينَ (133)
ٱلَّذِينَ يُنفِقُونَ فِي ٱلسَّرَّآءِ وَٱلضَّرَّآءِ وَٱلۡكَٰظِمِينَ ٱلۡغَيۡظَ وَٱلۡعَافِينَ عَنِ ٱلنَّاسِۗ وَٱللَّهُ يُحِبُّ
ٱلۡمُحۡسِنِينَ (134)وَٱلَّذِينَ إِذَا فَعَلُواْ فَٰحِشَةً أَوۡ ظَلَمُوٓاْ أَنفُسَهُمۡ ذَكَرُواْ ٱللَّهَ فَٱسۡتَغۡفَرُواْ لِذُنُوبِهِمۡ
وَمَن يَغۡفِرُ ٱلذُّنُوبَ إِلَّا ٱللَّهُ وَلَمۡ يُصِرُّواْ عَلَىٰ مَا فَعَلُواْ وَهُمۡ يَعۡلَمُونَ (135)أُوْلَٰٓئِكَ جَزَآؤُهُم
مَّغۡفِرَةٞ مِّن رَّبِّهِمۡ وَجَنَّٰتٞ تَجۡرِي مِن تَحۡتِهَا ٱلۡأَنۡهَٰرُ خَٰلِدِينَ فِيهَاۚ وَنِعۡمَ أَجۡرُ ٱلۡعَٰمِلِينَ (136)
سورة آل عمران