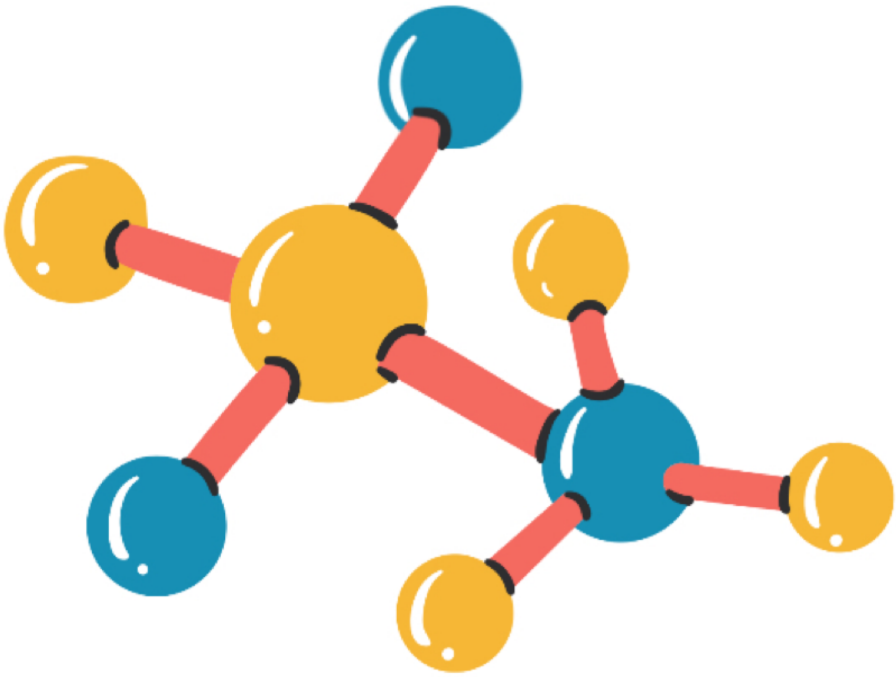
Biochemistry



Summer 2022

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Reversible Inhibitors:

What do we mean by reversible inhibitors?

Its effect can be undone, not permanent, they are characterized by rapid dissociation between the enzyme and that inhibitor (bind and leave).

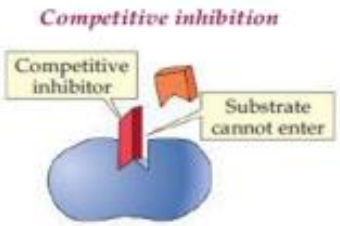
Usually, these inhibitors bind through non-covalent interactions & inhibitor maintains a reversible equilibrium with the enzyme.

Reversible inhibitors can be divided into two classes:

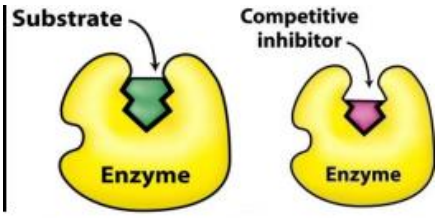
1-Competitive inhibitors 2- Non-Competitive inhibitors

❖Competitive inhibitors:

It competes with the substrate for the active site, and this means that the structure of the inhibitor and the substrate should look alike.



What determines whether the substrate or the inhibitor will bind to the active site?



1.The affinity 2. The concentration

If we have higher concentration of the substrate, it will eliminate the effect of the inhibitor even if the affinity is higher for the inhibitor.

If I can increase the substrate concentration a lot, we neglect the effect of the inhibitor then I can reach the V_{max} of the enzyme, accordingly in the presence of a competitive inhibitor you can still reach the V_{max} of the enzyme by increasing substrate concentration.

I reach the V_{max} in the presence of the inhibitor when the concentration of the substrate is very high.

**I have 10 active sites that can convert 10 substrates to 10 products per second, when I put an inhibitor; 7 active sites will bind to substrate and 3 will bind to inhibitor, increase the concentration of the substrate; 8 active sites will bind to substrate and 2 will bind to inhibitors and so on, until enzyme binds only with substrate again and there is no effect for the inhibitor.

According to this, if we draw Michaelis Menten plot for the following 2 experiments, so that:

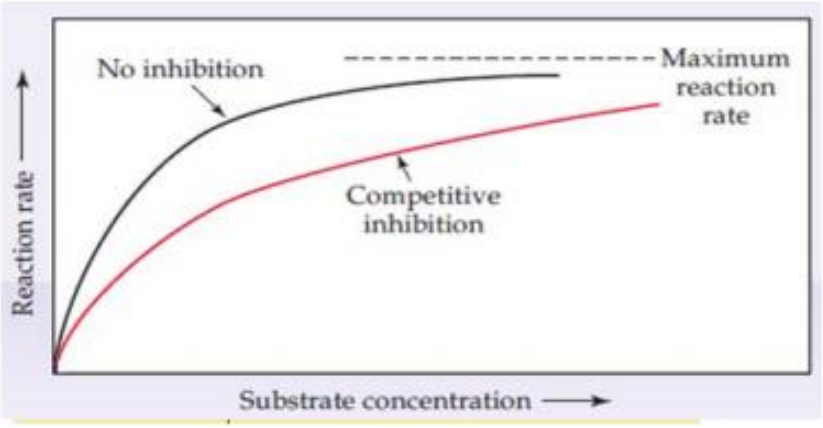
X-axis: substrate concentration

Y-axis: reaction rate.

Experiment 1 (black curve): a reaction without competitive inhibitor and I put the substrate and I observed the V_{max} and the km.

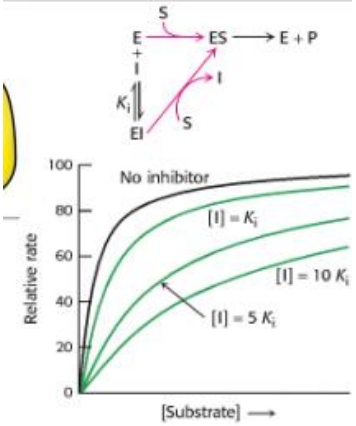
Experiment 2 (red curve): I put an inhibitor and I kept increasing the substance concentration, I achieved the same V_{max} as experiment 1 but when we compare the km values, in experiment 2 I need much higher concentration of substrate to reach V_{max}, so km will be higher in experiment 2.

*I need higher concentration to reach the V_{max}.



V_{max} value is not changed in the presence of competitive inhibitors, but the km value increased.

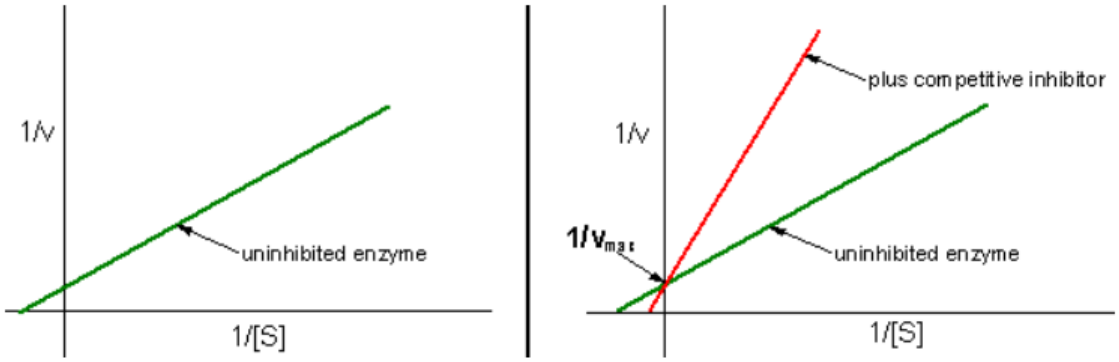
When I add the competitive inhibitor, the plot will shift to the right meaning that I need higher substrate concentration to reach the same V_{max} value and km value is shifted to the right (higher).



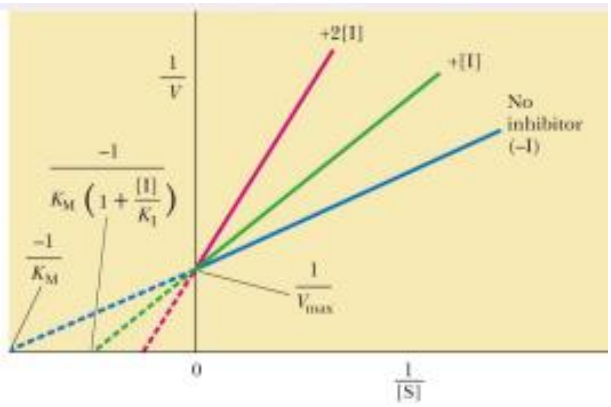
- The double-reciprocal plots are highly useful for distinguishing among these inhibitors.

If we draw Lineweaver-Burk plot (1/V Vs. 1/[S]) for the same experiments, we can get more accurate values of V_{max} using this plot.

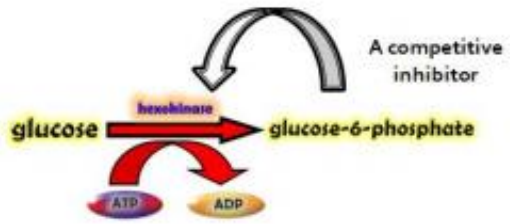
*Y-intercept: is$(1/V_{max})$ * X-intercept: is (-1/km)



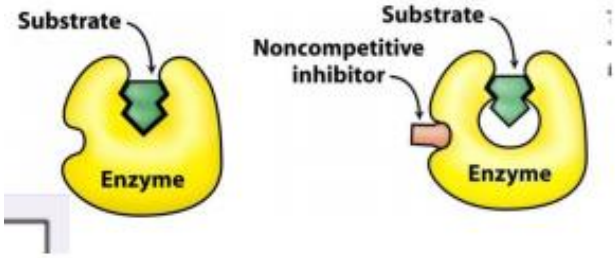
When there is a competitive inhibitor the slope increases, (the slope is km/ V_{max} that's why it increases, km gets higher, and V_{max} remains the same). And the Y-intercept will remain the same (because it equals $1/V_{max}$ and V_{max} will not change) while the X-intercept will change (because it equals -1/km and km increased; it will be closer to zero).



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



❖Non-Competitive inhibitors:



The inhibitor binds to a place other than the active site, inducing a conformational change in the enzyme, and this changes the geometry (configuration) of the active site so now its efficiency is less.

Now, does increasing the concentration of the substrate affect the action of the enzyme? No, because it's non-competitive, the substrate is not competing with the inhibitor over the active site, each one binds to a different place so increasing the concentration is useless.

لما نشتري سيارة جديدة، بتكون الكفاءة أعلى ما يمكن وبتقدر توصل لأعلى سرعة ممكنة للسيارة، بس بعد مرور وقت السيارة رح تتعب و تقل كفاءتها وتصير أعلى سرعة ممكن إنها توصلها أقل من أعلى سرعة كانت توصلها وهي جديدة، مع إنك بالوقتين (زمان وهلأ) بتحتاج تدعس نفس الدعسة.

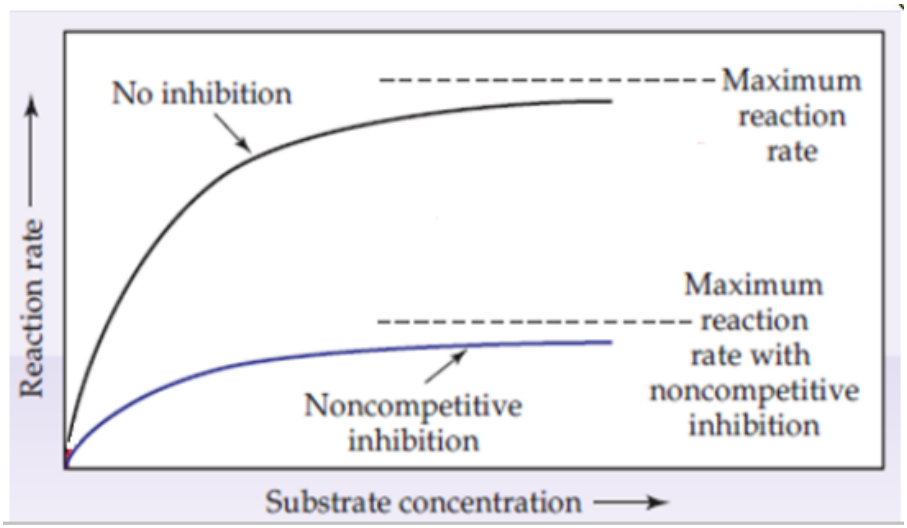
We have 10 enzymes that can convert 10 substrates to 10 products per second, when adding inhibitor, the geometry of the active site will change but it still can bind to the substrate but it's less efficient

and it still can convert 10 substrates into 10 products, but it will take 2 seconds, so in the presence of the non-competitive inhibitor, for sure I will have a new V_{max} which is less, because the capability of the active site of changing the substrate into product per unit of time is less, but how many molecules should I bind to the active site of the enzymes so as to get the new V max ? 10.

So, less velocity (for example 50%) will be achieved by having the same number of the molecules how much the new Km? it is the substrate needed to achieve 50% of the new maximum velocity.

<u>The needed amount of the substrate is the same so Km is the same in both situations</u>.

Michaelis-Menten plot:



when there is a non-competitive inhibitor, the curve will be lower (lower V_{max}).

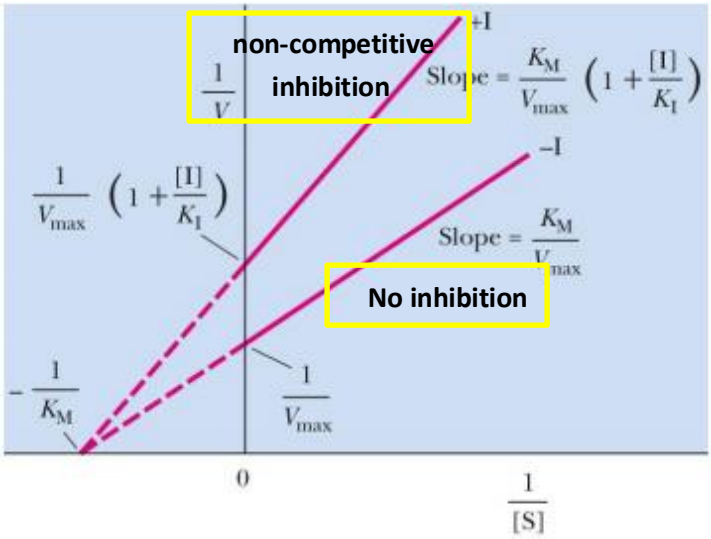
Km stays the same (substrate concentration to reach half of V_{max} will be the same in both cases).

Example for further understanding only:

In this figure assume that the old V_{max} = 100. Km = 2 for example (remember Km = substrate needed to reach half the maximum velocity). The new V_{max} = 40, To achieve V = 20 (half the V_{max}),

Km = 2 (stays the same).

Lineweaver-Burk plot:



The intercept with the X-axis won't change (km stays the same), and since the V_{max} decreased the intercept with Y-axis will be higher ($1/V_{max}$).

The slope will increase. (slope= km/V_{max} , km stayed the same while V_{max} decreased).

- 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} will change while Km will stay the same.
- Can we reach V_{max}? No.

Regulation through conformational changes

Another way of regulating the enzymatic function, is to change geometry of the enzyme itself (conformational change) so you can control the enzymatic catalysis, enzyme action and enzyme processivity. There is more than one way regulating the enzymatic reaction through playing in the conformation of the enzyme.

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

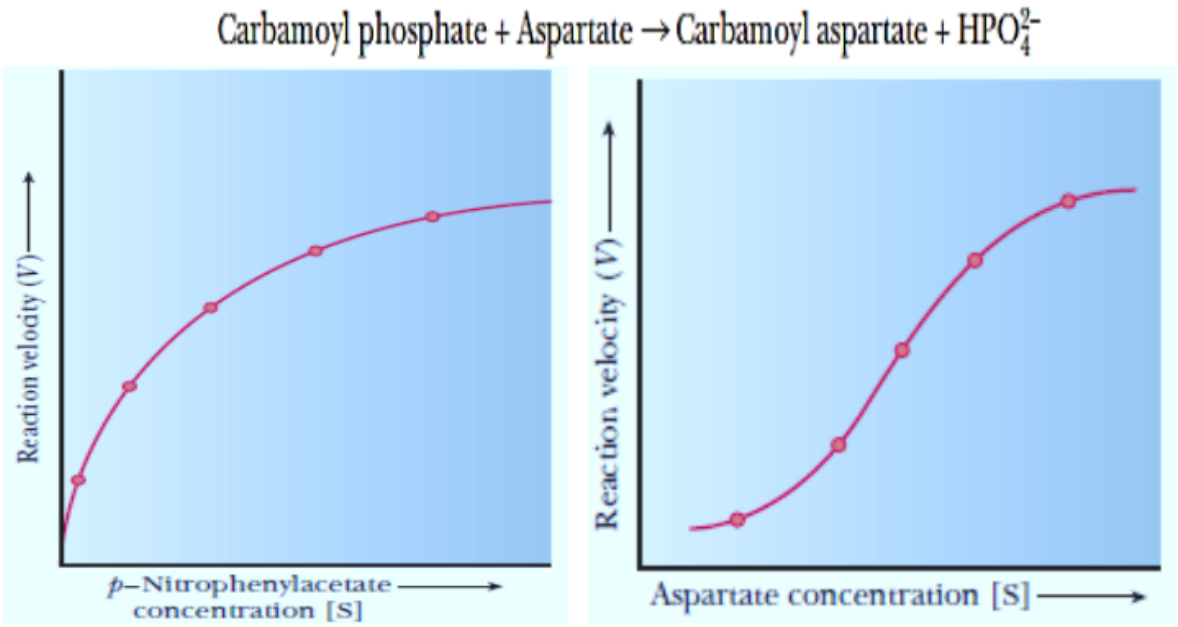
D. Proteolytic cleavage

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

As we said before when Michaelis-Menten studied huge number of enzymes it appeared to them that we have two classes of enzymes according to their behavior: certain enzymes gave a hyperbolic plot and we called them Michaelis-Menten enzymes (simple enzymes) because they follow the Michaelis-Menten kinetics and equation.

And other enzymes don't follow it and we called them allosteric enzymes and their plot is sigmoidal.

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



What does a sigmoidal plot mean?

That this enzyme has two states (high affinity, low affinity), this enzyme has a cooperative behavior, and since it has cooperative behavior it's structure should be quaternary, it should have more than one subunit.

All enzymes that have a sigmoidal plot we call them allosteric.

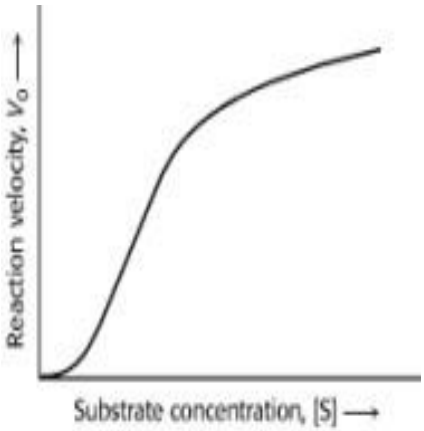
❖Allosteric regulation:

- Allosteric enzymes have quaternary structure with multiple subunits. (Allosteric means controlling the enzyme from far place).
- These enzymes have regulatory and catalytic subunits. Binding of something on regulatory subunit will change behavior of the other subunit.

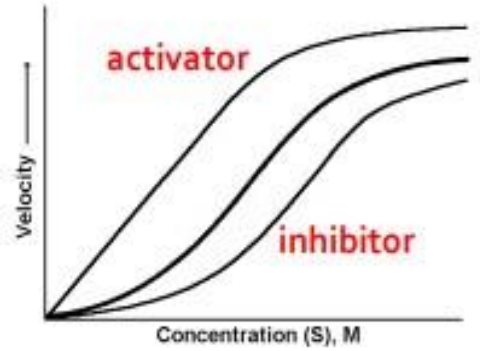
  - Catalytic subunits are responsible of the reaction.
  - Regulatory subunits are responsible of binding materials which affects the reaction either negatively (inhibitors) or positively (activators), these materials are called effectors (modifiers).

If the effector material is the same material that binds to the active site, then we will call it homotropic effector (such as O_2 in hemoglobin) and when it's not the same we call it heterotopic.

  - Allosteric enzymes have sigmoidal plot (S curve).



- When an inhibitor binds to regulatory subunit and affect the catalytic subunit the sigmoidal curve will shift to the right (more sigmoidal, less hyperbolic).
- While binding of activators will shift the curve to the left (less sigmoidal, more hyperbolic).



However, allosteric enzymes plot will never become hyperbolic (it will only become more hyperbolic less sigmoidal, and when zooming you will see the delay in the x-axis).

V_{max} in allosteric enzymes will stay the same, in the presence of inhibitor initial velocity will be less and we will need more substrate concentration to reach V_{max}, while in the presence of activators we can reach V_{max} with lower substrate concentration, so V_{max} value is not changing.

The substrate concentration at half of the Vmax is called

($K_{0.5}$).

What about K_m??

$K_{0.5}$ is the same as K_m but we don't use K_m because its specific for Michaelis-Menten kinetics and it doesn't apply on allosteric enzymes so just for the naming to be right we don't use K_m.

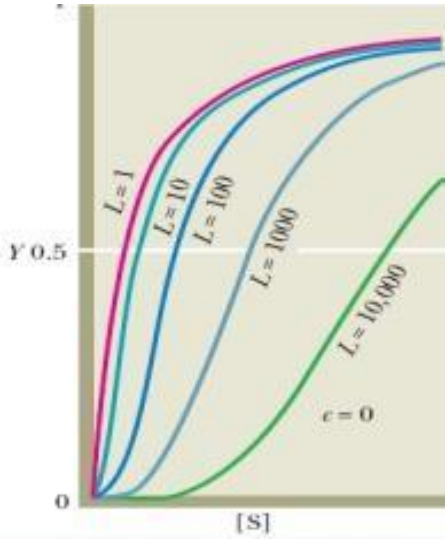
In the presence of inhibitor $K_{0.5}$ will increase, while activators will decrease it, in both cases V_{max} stays the same.

Allosteric enzymes are proteins in their nature, so they exist in two states:

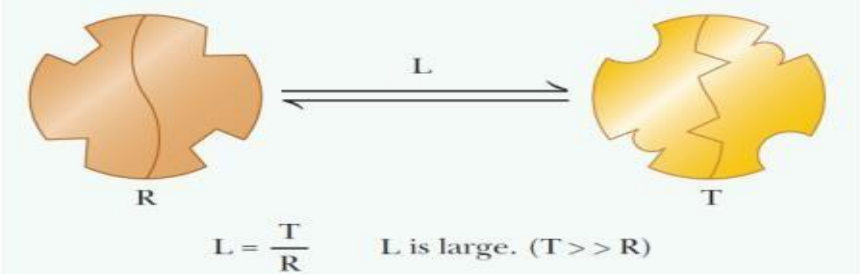
1) R state: it means relaxed; this state has high affinity toward its substrates.

2) T state: it means tight; this state has low affinity for substrate.

- L ratio is defined as (T/R), this ratio is kept high (T is more than R to control the enzymatic action and to keep it regulated).



While increasing L ratio the shape of the plot will be more sigmoidal (it will shift more and more to the right).

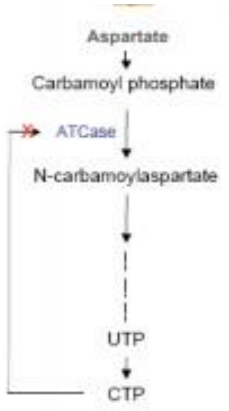


Proteins and enzymes that are composed of multiple subunits follow two models in changing their structure:

1) Concerted model: all subunits change their structure at the same time.
2) Sequential model: changing of one subunit structure changes the structure of the next one and so on.

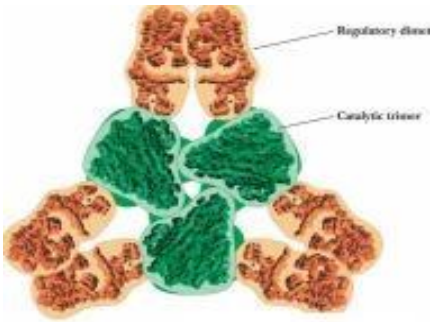
➢ Some proteins and enzymes follow both models, such as hemoglobin.

We will talk about ATCase (aspartate transcarbamoylase) which is an allosteric enzyme.



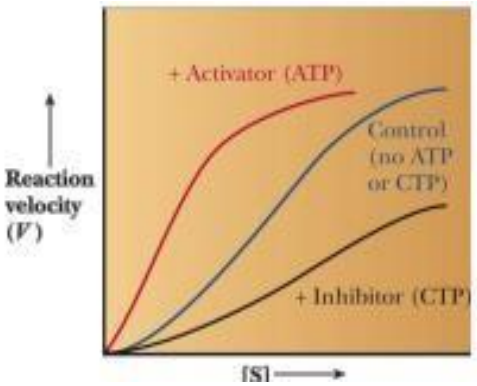
- It transfers carbamoyl group to aspartate (Aspartate is the substrate as the name of ATCase implies) and the end results of the reaction are UTP & CTP.
- UTP & CTP are used in DNA and RNA synthesis.

☐ This allosteric enzyme is composed of 12 subunits (6 catalytic (3 over another 3 subunits) in green & 6 regulatory in brown)



The uninhibited enzyme is shown in blue.

In the presence of the inhibitor which is CTP (it's a heterotopic inhibitor because the substrate is aspartate) the shape becomes more sigmoidal.



The activator of this enzyme is ATP, so once you have a lot of ATP it activates ATCase to make more nitrogenous bases (CTP & UTP) to combine with the other nitrogen bases to make DNA and RNA.

☐ Remember: ATP is not used as energy provider only; it's also used in synthesizing DNA & RNA.

- CTP makes feedback inhibition when it increases above physiological limits, because logically it should inhibit the enzyme from making more UTP & CTP because we have enough.

- Once we have more ATP and we don't have UTP & CTP the enzyme will work to form more of them.

Experiment was made by separating regulatory subunits from catalytic subunits in an allosteric enzyme, so what happened? It became simple enzyme, so Michaelis-Menten equation can be applied on it, and the result of its plot will be hyperbolic.

❖Conformational Changes from Covalent Modification:

Another way of regulating enzymatic function through playing with conformational changes is the covalent modification.

Covalent modification happens by binding materials with the enzyme covalently (not in the active side), thus changing its conformation, accordingly active site conformation will change, resulting in altering enzymatic activity.

Phosphorylation is the most common covalent modification that happens on enzymes, it follows the general mechanism (Illustrated above), but does it activate or inhibit enzymes?

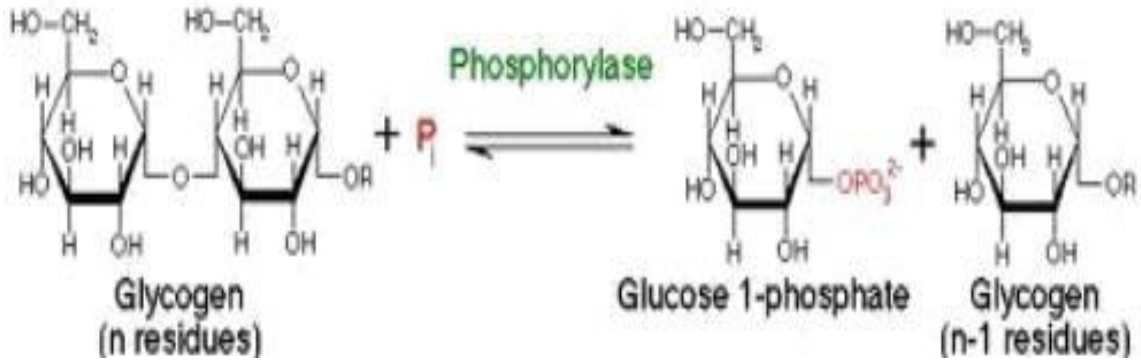
- ✓ It activates some and inactivate others, why? Because enzymes in pathways are different from each other, and you know that there are metabolic and anabolic reactions in the cells, and it doesn't make sense to have both reactions at the same time, so phosphorylation activates some enzymes and inhibits others according to the cell needs.

  For example, when we want to build up glucose in any cell, phosphorylation activates enzymes that are responsible of building glucose up and inhibits other enzymes that destroy it.

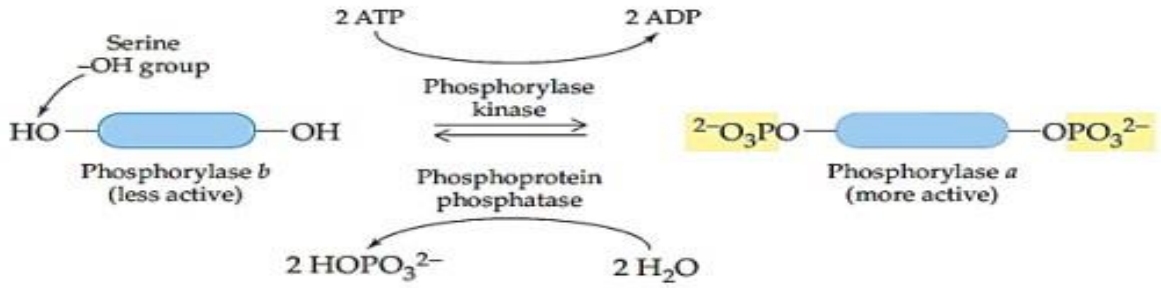
Why phosphorylation is effective in controlling enzymatic action?

- Because the phosphate is big, bulky and carries negative charge so it can change the conformation it can change the activity rapidly
- Often causes highly amplified effects.
- Adds two negative charges: new electrostatic interactions and accordingly 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. (It can change the activity rapidly).

- Rapid and transient regulation of enzyme activity – REVERSIBLE.
- Phosphorylation happens on specific places in proteins, as it needs oxygen to bind to it, so it works specifically on (Ser, Thr, Tyr)
- You should know that kinases (transferases) add phosphate, while phosphatases (hydrolases) remove it.
- An example on enzymes that make phosphorylation is glycogen phosphorylase.
  - As its name implies, its substrate is glycogen, and its function is phosphorylating glycogen. So, it comes to the last glucose and phosphorylates it, then it goes to the next one and so on.
  - When we need glucose in blood, glycogen phosphorylases do thier function and release glucose to blood. Also, this enzyme releases glucose when epinephrine (adrenaline) concentration is high in blood when you are afraid.



- Glycogen phosphorylase has two serine residues in its structure where they can be phosphorylated, when they are phosphorylated, the enzyme becomes more active and we call it **phosphorylase a**, the unphosphorylated one in the less active form and it is called **phosphorylase b**.
- Glycogen phosphorylase kinase is the responsible enzyme of adding phosphate, while phosphatase is the responsible of removing it.

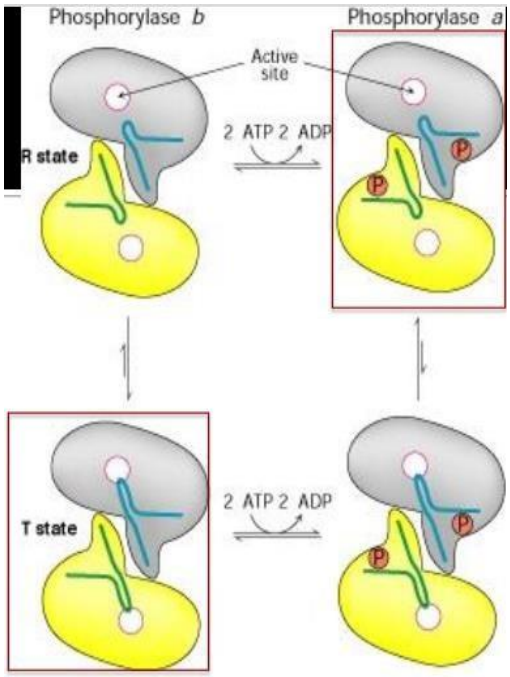


Both phosphorylase a and phosphorylase b exist at equilibrium between an active R-state and less active T-state.

Phosphorylase b is usually inactive because the equilibrium favors the T-state.

Phosphorylase a is usually active because the equilibrium favors the R-state.

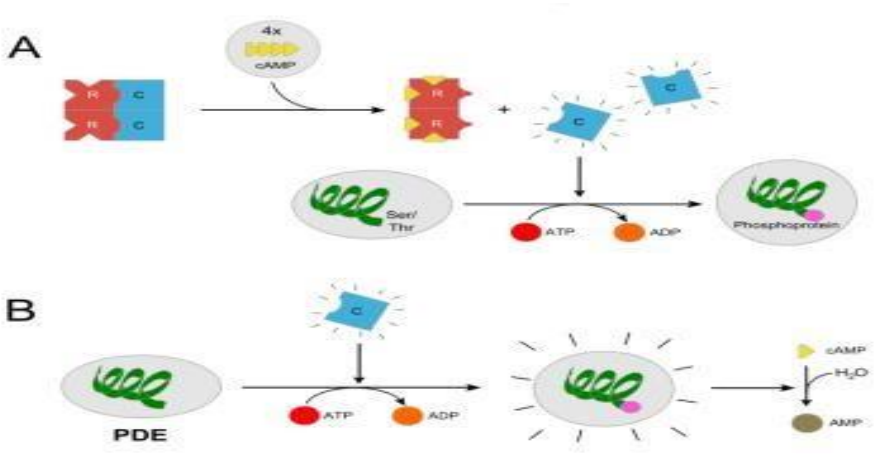
The transition of phosphorylase b between the T and R state is controlled by the energy charge of the muscle cell.



Protein kinase A (PKA): an enzyme that its activity is dependent on cyclic AMP (cAMP) which is a second messenger.

As always, we will tell you it's life story.

Hormone binds to a receptor causing changes in its structure→ affects on G protein → affects adenylate cyclase enzyme so it converts ATP to cAMP (2nd messenger) → cAMP binds to protein kinase A which is composed of 4 subunits, 2 catalytic, 2 regulatory (regulatory subunits bind to 4 cAMP) → when cAMP binds to regulatory subunits it causes conformational change which causes dissociation of catalytic subunits from regulatory subunits and now catalytic subunits become active → phosphorylates glycogen phosphorylase kinase which becomes more active → phosphorylates glycogen phosphorylase which becomes more active → phosphorylates glycogen → releasing glucose to the solution. (This is called phosphorylation cascade).

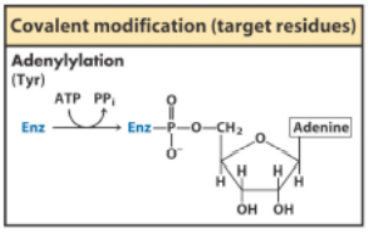


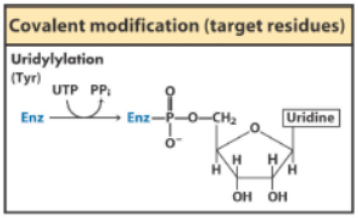
This signaling is stopped by many things, one of them is phosphodiesterase which breaks cAMP converting it to AMP. (Catalytic subunit is responsible of activating phosphodiesterase).

Other covalent modifiers are: -

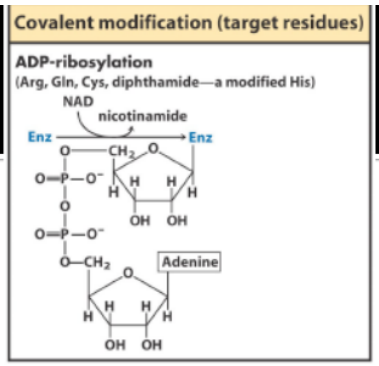
1. **Adenylate group**
2. **Uridylate group**
3. **ADP ribose**
4. **Methylation: masks a negative charge & add hydrophobicity on carboxylate side chains.**
5. **Acetylation: masks positive charges when added to lysine residues.**

- 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).

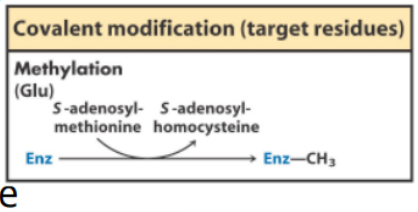




Other covalent modifiers



- ADP-ribosylation: inactivates key cellular enzymes
- Methylation: masks a negative charge & add hydrophobicity on carboxylate side chains
- Acetylation: masks positive charges when added to lysine



اللهم صل وسلم وبارك على سيدنا محمد وعلى آله وصحبه أجمعين

تم بحمد الله.