Doctor 022

Biochemistry

Sheet no.23

Regulation of enzymes

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Enzymes regulation:

Enzymes aren't always active in our bodies; they are regulated to work or to be active in certain times and to stop in others.

We have different mechanisms of regulation:

Non-specific regulation: (it affects a large group of enzymes) (temperature, pH, diffusion, and expression) (it can be done using two mechanisms):

—>Localization (compartmentalization and complexing of enzymes)

—>Expression of isoenzymes

Regulation of enzymatic activity (enzyme specific mechanisms of regulation):

—>Inhibitors

—>Conformational changes

- ***Modulators**
- ***Reversible covalent modification**
- ***Irreversible covalent modification**
- ***Allostery**

TEMPERATURE:

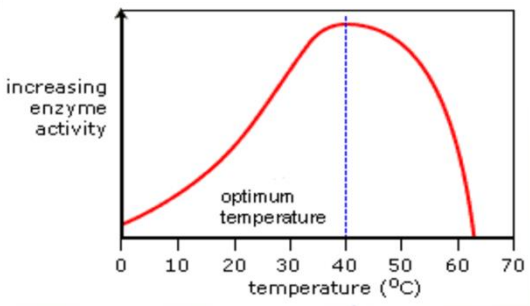
Reaction rates increase with temperature due to increased kinetic energy of the molecules resulting in more collisions between enzymes and substrates.

However, high temperatures lead to protein denaturation.

Each enzyme has an optimal temperature.

Temperature affects electrons kinetic energy (movement of electrons) which changes the stability of enzyme's structure by affecting Van Der Waals interactions for example.

Enzymes have variable sensitivities for temperature, for example human body temperature is about 37 but the enzymes activity increases when the temperature reaches 40, this is due to the increase in molecules kinetic energy which increases the probability of collisions, so the enzyme can find the substrate easier.



However excessive increase of temperature will lead to protein denaturation.

For thermophilic bacteria, the optimal temperature is as high as 72°C (it is also active at different temperatures).

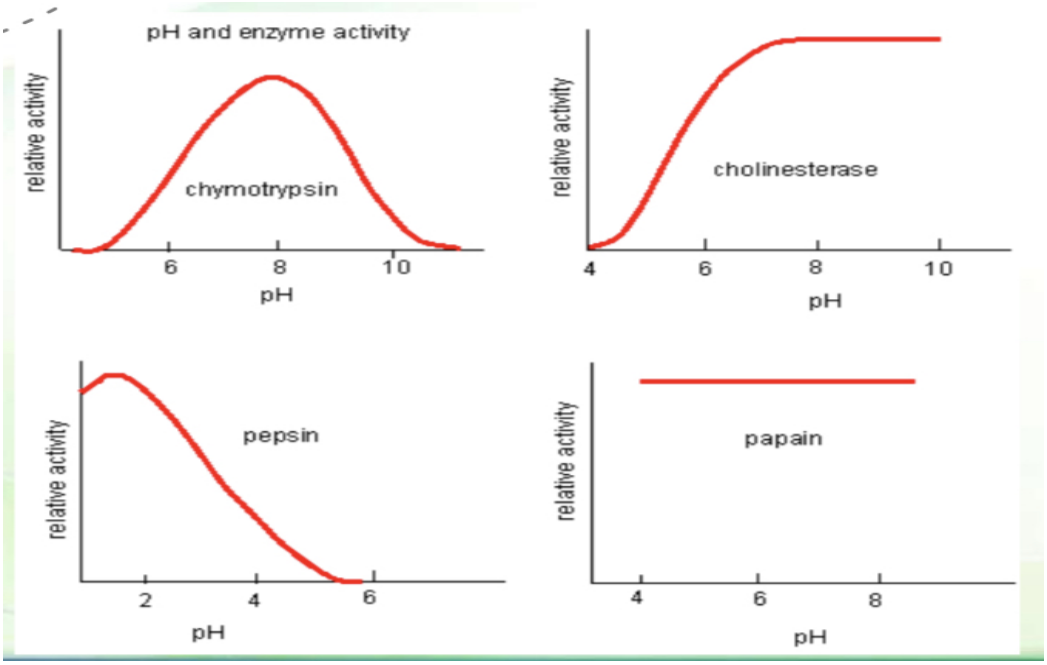
Such as TAQ POLYMERASE Which is a heat stable DNA synthesizing enzyme which we use in PCR.

PH:

pH alters the protonation state of the substrate and/or the enzyme and, hence, their binding.

The effect of pH is enzyme-dependent.

Most enzymes work well in physiological ph which is 7.2 (in cells, cytosol, mitochondria, etc) however others need a different ph such as:



-Chymotrypsin: a digestive enzyme in small intestine that is secreted by the pancreas, its optimal ph is 8 which is the ph in small intestine (acidic juice from the stomach is neutralized when it reaches the intestine by bicarbonate that is secreted by the gallbladder so we will get this PH which is almost 8).

-Cholinesterase: (in neurons) it is responsible for the degradation of the neurotransmitter acetylcholine, this enzyme functions at almost ph=7 and isn't affected if ph was high such as 10, but its activity will decrease under ph=7

-Pepsin: a digestive enzyme in stomach where the environment is very acidic, ph=2 which is the optimal ph of pepsin.

-papain: it is extracted from papaya fruit, this enzyme isn't affected by ph whatsoever.

Regulation of enzyme amount:

Three mechanisms:

1-Enzyme synthesis at the gene level (transcription and translation regulation)

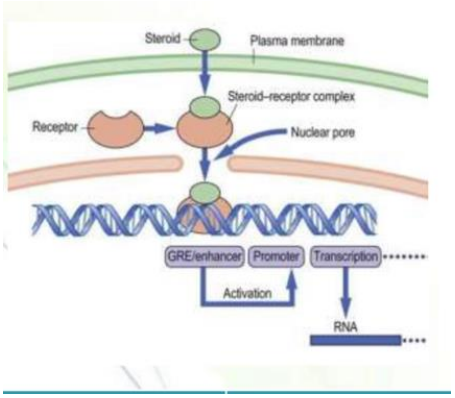
2- Enzyme degradation by proteases (turnover or recycling of amino acids after degradation)

3- Synthesis of isozymes

They are comparatively slow mechanisms for regulating enzyme concentration (hours-weeks)

Enzyme	Half-life (days)
Catalase	1.4 days
Glucokinase	1.2 days
Lactate dehydrogenase	
LDH1 (heart)	1.6
LDH5 (liver)	16
LDH5 (muscle)	31

Enzymes Stability differs between tissues.



Half-life: is the time required for protein to decrease by half.

Compartmentalization:

Compartmentalization reduces the area of diffusion of both enzyme and substrate increasing the probability that they collide. (Collision is a random process)

Examples about compartmentalization:

1) lysosomal enzymes: hydrolytic enzymes (proteases, glycosidases, lipases, saccharidases) are found inside lysosomes and facilitate degradation of proteins, carbs and lipids.

Smaller compartment increases the probability of collisions.

The ph of the lysosome (5.5) also contributes to the denaturation of proteins and sugars which facilitates the degradation of molecules.

2) fatty acid metabolism: (anabolism and catabolism)

Synthesis of fatty acids occurs in cytosol, whereas break-down is mitochondrial.

As opposite reactions occur in different locations, this ensures that only one of them will be occurring at a time.

Enzyme complexing:

It helps reducing diffusion area of enzyme and substrate.

Formation of a complex of multiple enzymes also reduces diffusion.

Example: Pyruvate dehydrogenase (mitochondria) is composed of 3 enzymes: decarboxylation, oxidation, & transfer of the acyl group to CoA.

Example about complexing: E: represents enzyme.

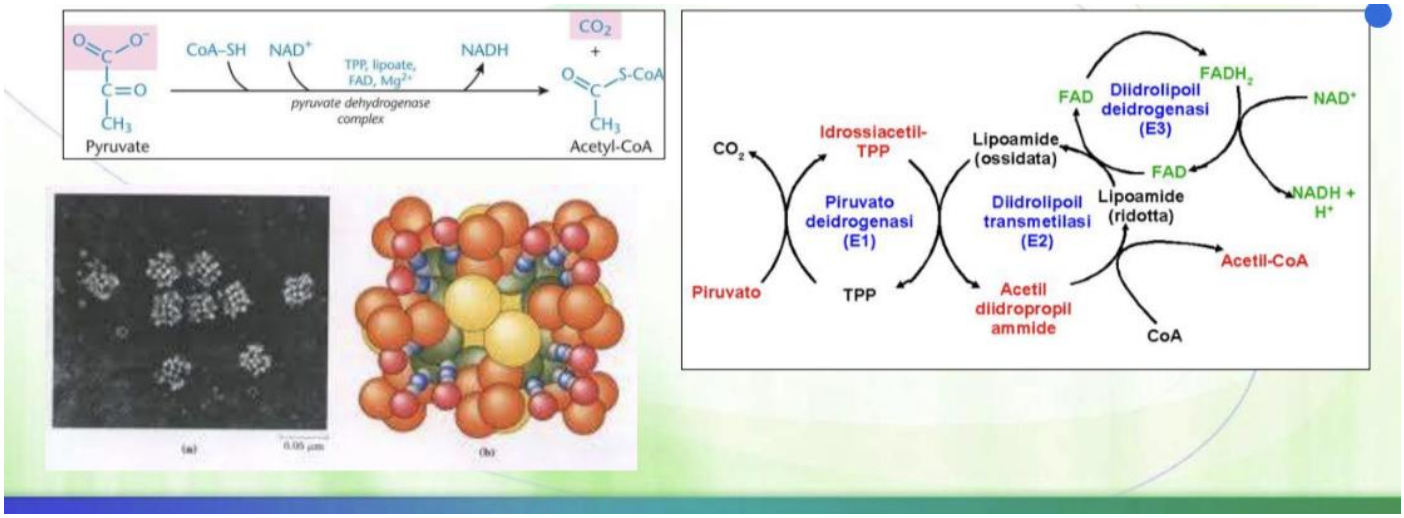
E1. E2. E3. (enzymes)

A ———> B ———> C———>D (substrates)

3 different reactions, substrate A meets with enzyme 1 (collision) turning it into Substrate B and releases it from the enzyme, then again B needs to meet up with E2, then C needs to meet up with E3, this increases the time of transformation due to random collision probability (we consume more time until the substrate finds the enzyme).

Enzymes complexing is putting E1, E2 and E3 in one complex as if it was a quaternary structure, E1 will turn A into B and then move it to E2 then E3. This increases the rate of reaction.

Example of complexing is pyruvate dehydrogenase (is very large enzyme with 60 polypeptide chains) in the mitochondria which turns pyruvate to acetylCoA by a chain of reactions facilitated by three different types of enzymes in a complex.



Isoenzymes (isozymes):

Isoenzymes are enzymes that can act on the same substrate(s) producing the same product(s).

They are produced by different genes that vary only slightly.

Often, various isozymes are present in different tissues of the body. They can be regulated differently.

They can have different catalytic activities.

they have different kinetics.

بيشتغل على المثلث اكتر من الدائرة

و الدائرة بتعمل activation

المثلث اكتر من الدائرة

المثلث بيعمل inhibition

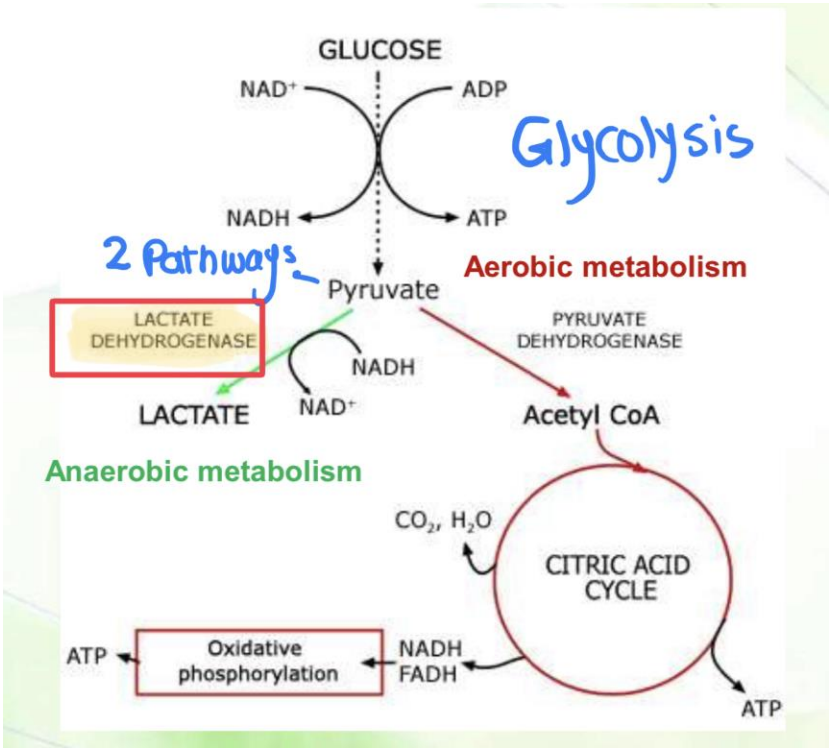
تركيزهم نفس الشي

It is regulated by neither triangle nor circle.

Examples of Isozymes are glucokinase and hexokinase.

Glucose + ATP —> Glucose-6-phosphate + ADP

Same reactions in different tissues, one in muscles and the other in the liver, they differ in kinetics and regulation.



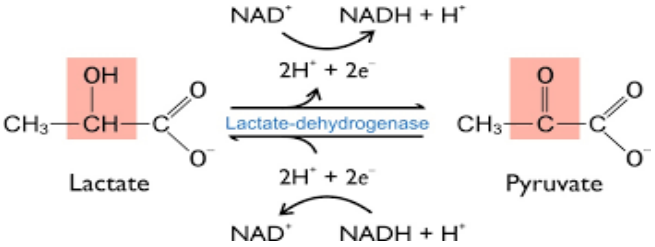
Lactate dehydrogenases (LDH)

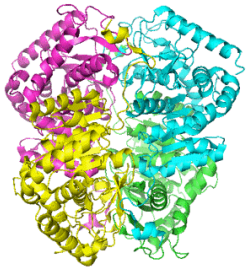
LDH is a tetrameric enzyme **(4 polypeptides)** composed of a combination of one or two protein subunits (**that come from different genes**): H (heart) and M (skeletal muscle).

These subunits combine in various ways leading to 5 distinct isozymes (LDH1-5) with different combinations of the M and H subunits.

The all H isozyme is characteristic of that from heart tissue, and the all M isozyme is typically found in skeletal muscle and liver.

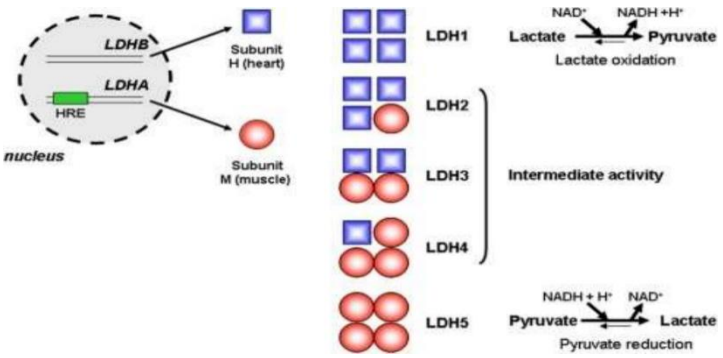
They have different genes, different distribution, different regulation and same reaction.





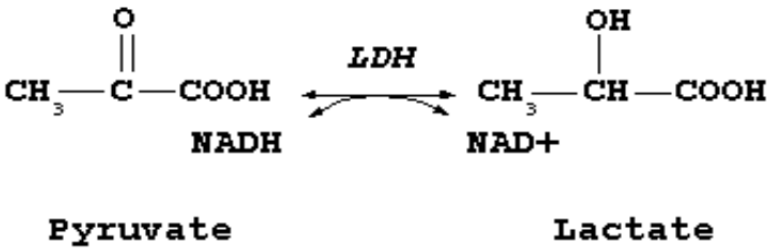
Although the five isoforms catalyse the same reaction, they differ in their primary structure (slightly), kinetic properties (such as the preference to react with pyruvate or lactate), tissue distribution, affinity to the substrate, regulation, and isoelectric point.
The M subunit has a net charge of (-6) and higher affinity towards pyruvate, thus converting pyruvate to lactate (and NADH to NAD+).
The H subunit has a net charge of (+1) and a higher affinity towards lactate, resulting in a preferential conversion of lactate to pyruvate (and NAD^+ to NADH)

Skeletal muscles prefer anaerobic pathway (LDH5 will convert pyruvate into lactate, and then lactate will transfer to blood to go to the heart, where LDH1 will convert lactate into pyruvate using aerobic metabolism, and this indicates that there is a difference between isozymes in the preference towards either pyruvate or lactate).



Function of isozymes:

Muscles can function anaerobically, but heart tissue cannot.
Whereas the all-M isozyme (M4) functions anaerobically and catalyzes the reduction of pyruvate into lactate, the all-H enzyme (H4) functions aerobically and catalyzes the reverse reaction.

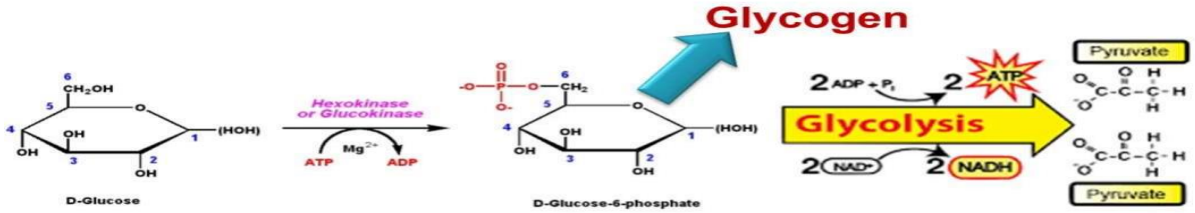


Regulation of LDH (At the level of enzymes and kinetics):

LOW KM=HIGH AFFINITY
H4 LDH has a low Km for lactate, high Km for pyruvate, and is inhibited by high levels of pyruvate (in order to control the mechanism)
The H4 isoenzyme favors (lactate to pyruvate).
H=heart so we need aerobic pathway→pyruvate.
The M4 LDH enzyme has a high Km for pyruvate and is not inhibited by pyruvate. (Pyruvate to lactate)
M=skeletal muscle→ anaerobic→lactate
M4 LDH is always active even at high levels of pyruvate in skeletal muscles ensuring that pyruvate is always funneled to anaerobic metabolism.

Hexokinase vs glucokinase

Hexokinase and glucokinase (hexokinase IV) are allosteric isozymes that catalyse:
Glucose → Glucose-6-Phosphate
Glucokinase is a liver (and pancreatic) enzyme (in the picture below it will produce glycogen), whereas hexokinase is found in RBCs (and skeletal muscle) (in the picture below, it is the enzyme that will undergo glycolysis).
The purpose of liver glucose is to store glucose (in the form of glycogen) and balance glucose levels in the blood.
The purpose of glucose in skeletal muscle and RBC is to produce energy.



Biological significance (of glucose 6-phosphate)

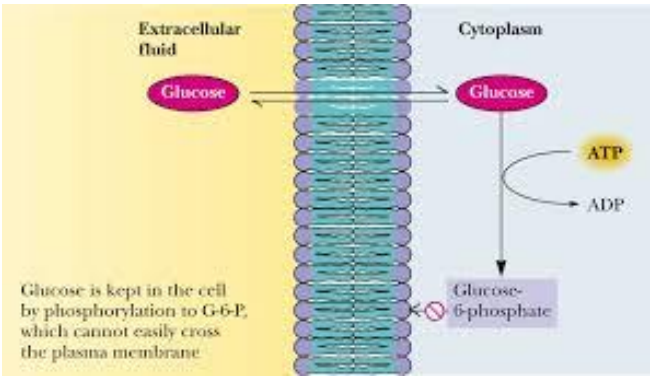
Note: once glucose is phosphorylated, it cannot cross plasma membrane out of cells.

Liver: low efficiency enzyme to provide glucose to other organs.

In the liver we need the process of phosphorylation to be slow… that will give the glucose the time needed to leave to different cells.

RBC and skeletal muscles: high efficiency enzyme to trap glucose.

Here we need the process of phosphorylation to be fast to transform glucose into pyruvate.



Regulation of hexokinase and glucokinase:

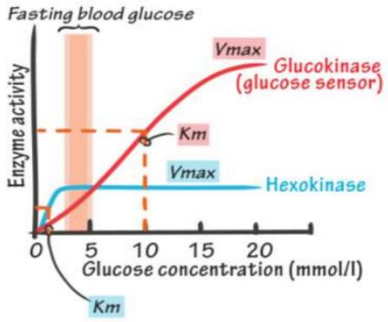
Note Vmax and Km values (low - 0.1 mM for hexokinase) and (high - 10 mM for glucokinase)

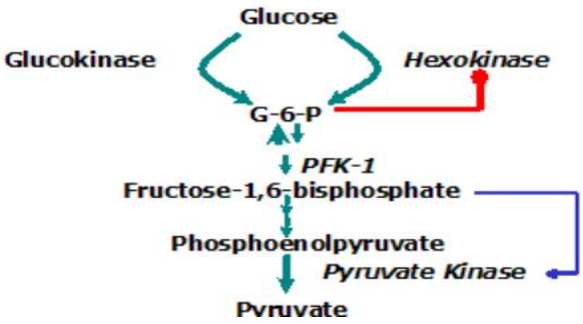
-Regulation:

Hexokinase is inhibited by glucose-6-phosphate, but glucokinase is not.

Glucokinase is activated by insulin and inhibited by glucagon.

in starvation→ glucagon will be released→inhibit glucokinase→glucose will go to cells.





-Significance:

At fasting state, glucose is not stored.

At well-fed state, RBCs and skeletal muscles do not consume all glucose in blood, and liver can convert excess glucose in glycogen for storage.

Further explanation for the point above:

-Hexokinase binds glucose with high affinity, but it has low V_{max} and it is also inhibited by glucose-6-phosphate, which means that not all glucose in skeletal muscles will be bound to it.

-glucokinase has lower affinity towards glucose, which allows other tissues to take their own needs of glucose before phosphorylating it and trapping it in the liver, and glucokinase has high V_{max} and is not inhibited by glucose-6-phosphate which indicates that it will continue adding glucose on the preexisting glycogen.

-when glucose concentration is low, hexokinases will do their jobs and glycogen will not be synthesized. And when the concentration is high, hexokinase will work with its V_{max} and glucokinase will do its job also.

Regulation of enzymatic activity:

Inhibitors:

Enzyme inhibition can be either reversible or irreversible.

All physiological inhibitors are reversible

Artificial inhibitors can be either reversible or irreversible.

Reversible inhibitors rapidly dissociate from enzymes (e.g. non-covalent binding).

- **They can be: Competitive, noncompetitive, or uncompetitive inhibition.**

An irreversible inhibitor is tightly bound (e.g. covalently) to the enzyme.

- **Lower concentration of active enzyme.**

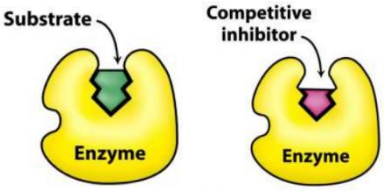
Competitive inhibition:

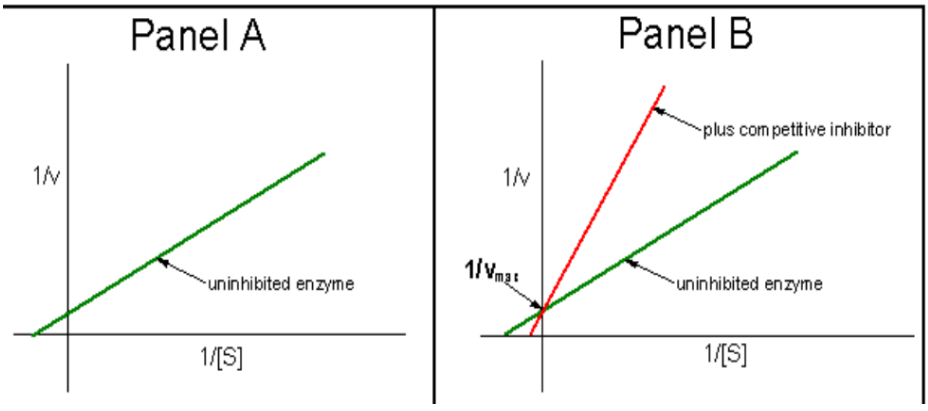
Competitive inhibitors compete with the substrate for the active site.

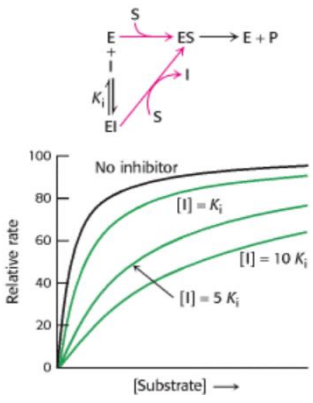
Increasing substrate concentration can overcome inhibition.

Same Vmax, but higher Km

If the ratio between the substrate and the inhibitor 1:1→that means 50% of the substrate will bind and 50% of the inhibitor will bind to the enzyme… when increasing the substrate concentration that will lead the substrate to win in this competition.



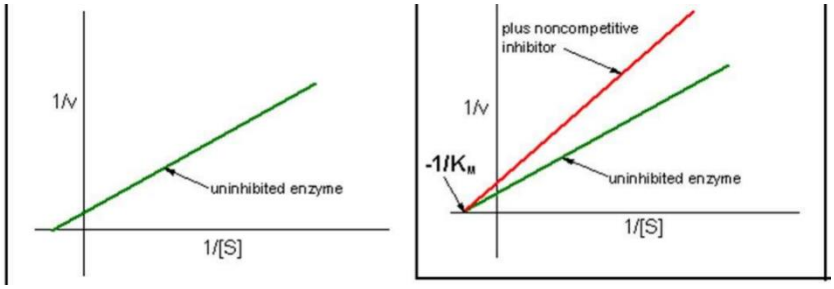


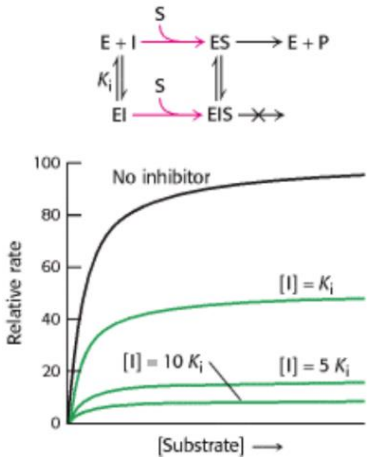


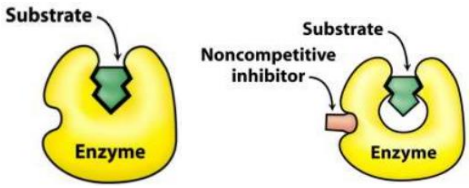
V_{max} will not change because we can reach it with a high substrate concentration, but Km will be higher, because we need more molecules to reach higher velocity, so as Km increases the affinity of the enzyme towards the substrate decreases.

Noncompetitive inhibition

Noncompetitive inhibitors bind E or ES complex at a site other than the catalytic site. Substrate can bind to the enzyme-inhibitor complex, but ESI cannot form a product. Lower Vmax, but same KM {because the enzyme is saturated by the substrate but it cannot finish the reaction}





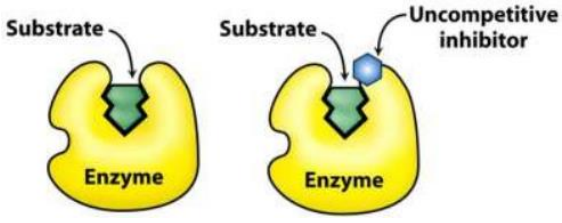


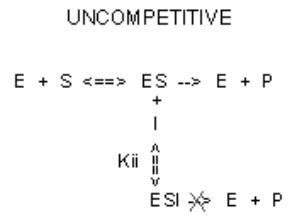
An advice: put numbers and notice the change in Km and V_{max} after the inhibition using the previous diagrams.

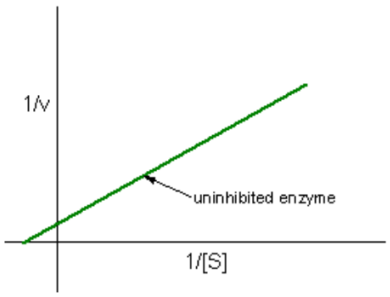
Uncompetitive inhibition

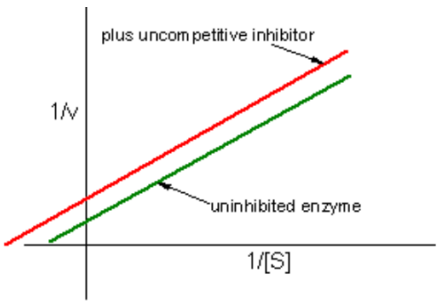
Uncompetitive inhibitors bind to the enzyme substrate complex. only reducing both V_{MAX} and Km. (increase the affinity and reduce V_{max}).

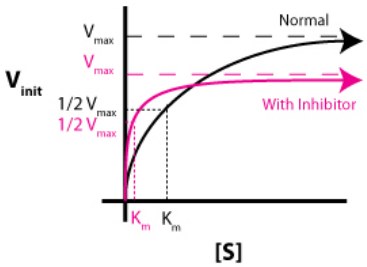
-note: after binding of the inhibitor the enzyme will not finish the reaction so V_{max} will be reduced











Mechanism-based inhibitors

- **Irreversible inhibitors**
  Mechanism-based inhibitor mimic or participate in an intermediate step of the catalytic reaction.

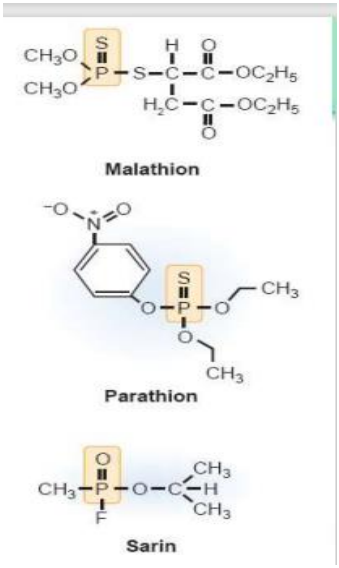
We can divide irreversible inhibitors into several types:

- **Covalent inhibitors**
- **Transition state analogs**
- **Heavy metals**

The idea of irreversible inhibitors is that they bind to enzymes covalently and have a very very very low possibility to dissociate from the enzyme (very high binding affinity) we can say that we reduced the concentration of the enzyme (free enzyme).

- **Covalent inhibitors**
  They form covalent or extremely tight bonds with active site amino acids.
  Example: diisopropyl fluorophosphate (DFP) is an organophosphate

This organophosphate has many types like:



- **The nerve gas sarin**

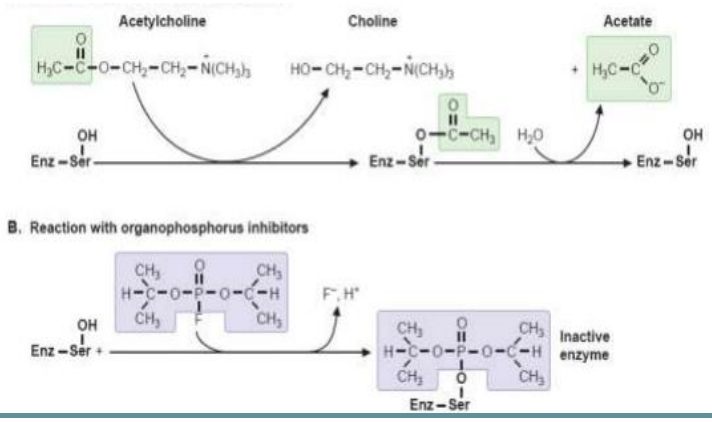
  Sarin gas (بستعملوه بالحروب وبعمل تشنجات).

- **The insecticides malathion & parathion.**

  Malathion, Parathion (مبيدات حشرات).

- **DFP inhibits acetylcholinesterase preventing the degradation of the neurotransmitter acetylcholine.**

The idea is: these organophosphates inhibit acetylcholinesterase (acetylcholinesterase breaks down acetylcholine —a neurotransmitter— to choline) so when acetylcholinesterase is inhibited, acetylcholine continues to stay in the CNS and this causes spasms (تشنجات).



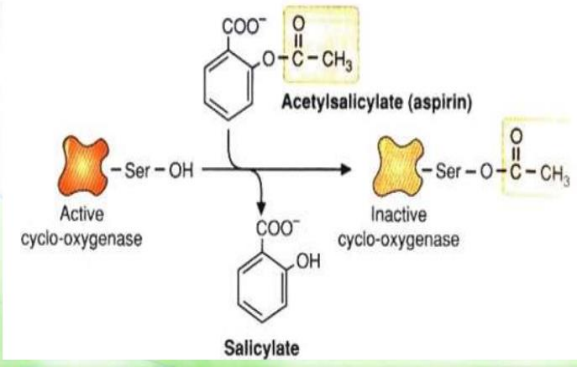
DFP also inhibits other enzymes that use serine (ex. serine proteases), but that is not lethal.

Aspirin:

Another example of covalent inhibitors is aspirin.

Aspirin (acetylsalicylic acid) acetylates an active site serine of cyclooxygenase.

Aspirin acetylates serine that exists in the active site of COX and inhibits it without any release of aspirin from the active site.



NOTE: COX functions in converting arachidonic acid into prostaglandins (eicosanoids) that cause plattelet aggregation.

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

- ## Substrate and transition-State analogs

Suicide inhibitors

They are substrate analogs or transition state analogs, they are inhibitors that look like a substrate or a transition state so they bind to the active site then the reaction happens and stops before it is completed.

Why does the reaction stop?

لأنه الانزيم بكون مفكر حاله ارتبط بال Substrate

وببلش يضيف ويعدل عالمركب ,لكن بالأخير بيجي يضيف مجموعة بمكان معين بلاقي المكان مش موجود ف هون بعلق وببطل لا قادر يرجع خطوه لورا ولا قادر يقدم خطوه لقدام

لأنه الي ارتبط مش الsubstrate

Meaning that the substrate analog is a fake substrate.

They bind more tightly than substrates.

Why do we use transition state analog?

Because the Interaction between the transition state and the enzyme is very tight (high affinity).

Drugs cannot be designed that precisely mimic the transition state! (Highly unstable structure).

Examples on suicide inhibitors:

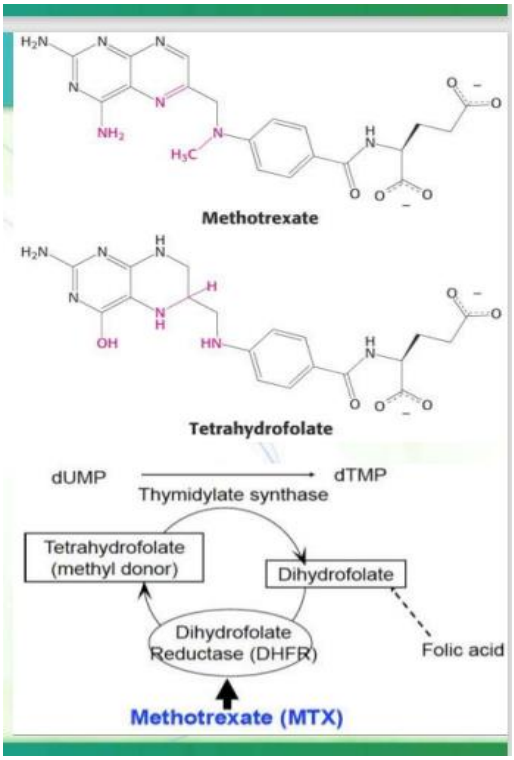
Methotrexate

Methotrexate is a synthetic inhibitor used to treat cancer.

And it can be used to treat inflammatory diseases such as rheumatoid arthritis.

Where can methotrexate be used in?

Methotrexate is useful to deal with cells that proliferate such as cancer and inflammatory cells, these cells always need a DNA synthesis so they need nucleotides such as dTMP (deoxy TMP), synthesis of dTMP is enzymatic and needs tetrahydrofolate (folate is a vitamin).



To complete the synthesis of dTMP:

Tetrahydrofolate must be converted to dihydrofolate, DHFR is the reason of transformation of dihydrofolate to tetrahydrofolate (reduction of dihydrofolate).

When we are forming dTMP, tetrahydrofolate must be converted into dihydrofolate to complete this reaction, and to move between these two forms we need dihydrofolate reductase.

Methotrexate is a structural analog of folate, a substrate for the enzyme dihydrofolate reductase, and a coenzyme for thymidylate kinase, both of which are responsible for the synthesis of nucleotides.

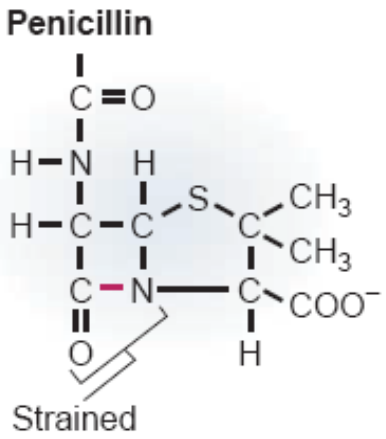
From the figure above, note that methotrexate looks like folate (looks like the substrate), what will happen?

This methotrexate binds with the active site of dihydrofolate reductase, more tightly than the natural substrate(dihydrofolate) (1000-fold more tightly), but the reaction isn't completed and there will be an inhibition for that enzyme, so there is no formation of

tetrahydrofolate (that contributes to the synthesis of dTMP), no dTMP and no DNA synthesis and no cell division.

Another example of substrate and transition state analog is:

Penicillin



The cell wall is the outer covering of the bacteria-containing peptidoglycan layer which is made up of peptides that are cross-linked by glycopeptidyl transpeptidase.

The idea behind it is: penicillin is an antibiotic that is used in stopping bacteria from growing up, enabling the body to get rid of bacteria.

Bacteria got a plasma membrane, cell wall.

This cell wall is peptidoglycan (sugar molecules with attachment to peptides), a cross linking happens between amino acid residues in those peptides, and this makes the cell wall coherent, helping bacteria to resist the immune system.

The way to get rid of bacteria is to prevent the synthesis of cell wall (reducing the bacterial resistance to the immune system).

The mechanism of action for penicillin (transition state analog) is that it prevents the cross linking of the peptides, so a weak cell wall will exist.

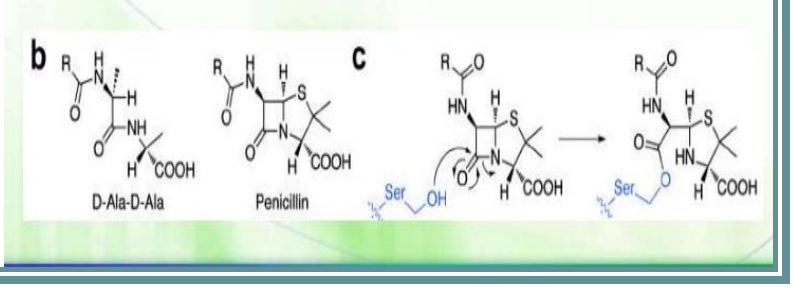
The cross linking happens between two alanine residues, and the penicillin is a transition state analog that looks like the two alanine's transition state. Penicillin contains a ring structure that is called β-lactam ring. The amide bond in the β-lactam ring of penicillin looks like the natural transition-state complex. The active site serine attacks the highly strained β -lactam ring.

What happens is that when there is a cross linking, the reaction begins then penicillin binds to serine that is found in the active site of the enzyme (the enzyme which makes the cross linking –glycopeptidyl transpeptidase—), then the reaction stops and penicillin is still bound to the active site.

ف زي كأنه التغى عمل الانزيم وجدار الخليه في خبر كان.

We have talked about:

1) Covalent inhibitors
2) Substrate analog, transition state analog
   Now we are going to discuss 3) heavy metal.



- ## Heavy metals

Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe) result in tight binding to a functional group in an enzyme.

Heavy metals bind to the enzymes very tightly and they can bind non-specifically, they can also bind to other sites that are not active causing a change in shape of the enzyme then the active site can't bind to the substrate.

- **Nonspecific inhibition at high doses.**

Mercury binds to reactive sulfhydryl groups away from the active site and affect the binding of substrates.

- **Unknown enzymes are inhibited in mercury toxicity.**

They can bind to many many enzymes leading to inactivation of the enzymes that's why mercury is toxic.

Lead replaces the normal functional metal in an enzyme such as calcium, iron, or zinc by an irreversible mechanism.

- **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.**

We'll see some enzymes that need zinc to bind to its active site, when there is a replacement of it, enzymes will be inactivated, specifically the enzymes that exist in the CNS, that's why lead is toxic.

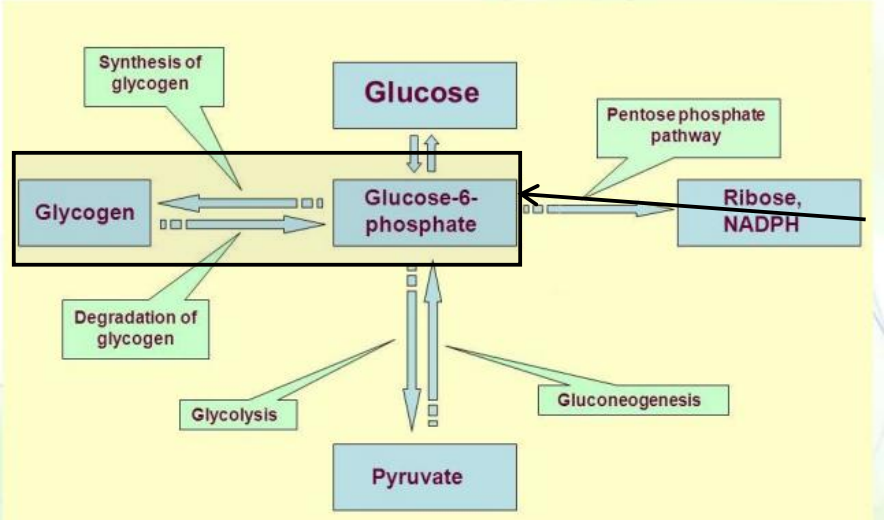
Now we will discuss some mechanisms that depend on the conformational change of the enzyme.

Regulation through conformational changes

The different fates of glucose

Now we are discussing how our bodies deal with glucose.

Glucose in liver cells, skeletal muscle cells, and red blood cells gets phosphorylated by hexokinase enzyme or glucokinase enzyme.



الدكتورحكى انه رح نركز على هذا ال Pathway

In skeletal muscle cells or red blood cells glucose 6-phosphate enters glycolysis and then becomes converted into pyruvate to synthesize energy.

In liver cells and skeletal muscle cells glucose 6-phosphate may not leave the cell because its phosphorylated and may be added to glycogen molecule.

In case peripheral cells need glucose, a removal of glucose from glycogen happens in the liver (in the form of glucose 6-phosphate) and then it will enter glycolysis phase.

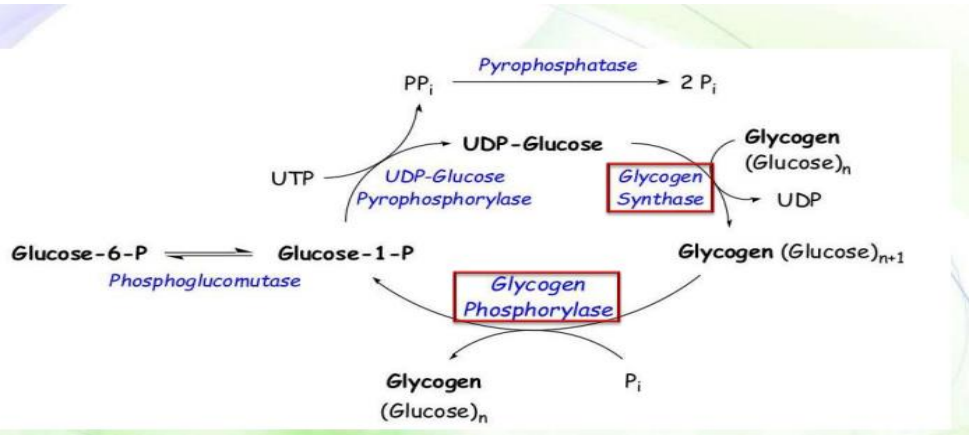
Metabolism of glycogen

Basically what happens is that glucose 6-p turns into glucose 1-p then turns into UDP-Glucose (don't go deep into these details Dr said) which is an activated molecule with a high energy, because when we add glucose to glycogen we need energy of course, then glucose is added to a pre-existing glycogen resulting in a glycogen with one more glucose molecule (by glycogen synthase enzyme), when our bodies need glucose, a signal is transduced (hormones) to the liver to release glucose from glycogen.

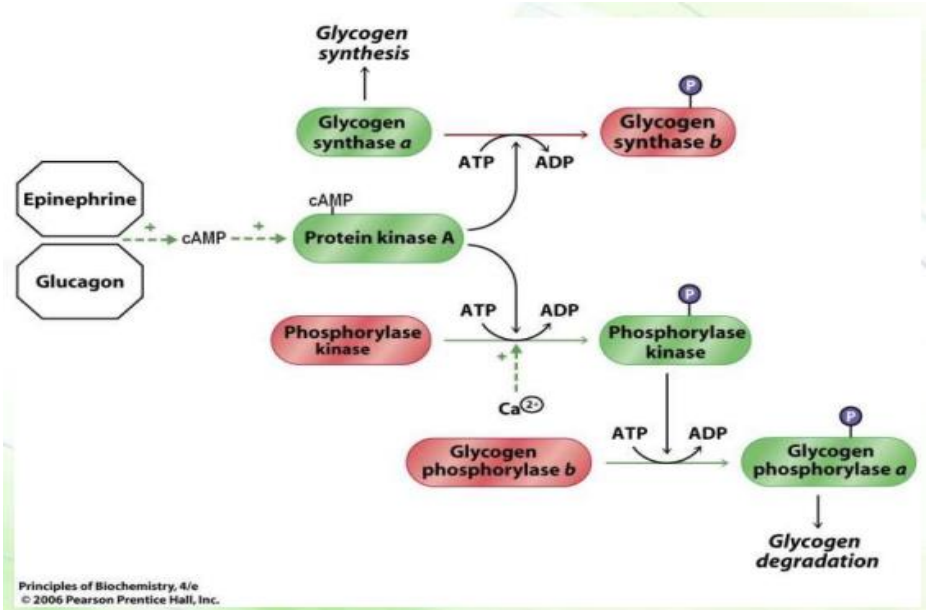
Releasing of glucose is caused by GLYCOGEN PHOSPHORYLASE which is an enzyme that removes a glucose molecule from glycogen in the form of glucose 1-p and then glucose 1-p turns into glucose 6-p.

In liver cells, glucose 6-p can turn into glucose then exit the cell, while in skeletal muscles the glucose is converted to glucose 6-p which enters the glycolytic pathway.

THE TWO RED SQUARED ENZYMES ARE REGULATED HORMONELY عليهم ركزوا و!!



Regulation by phosphorylation:



The release of glucose from glycogen is regulated by two hormones:

- **Epinephrine:**
  Skeletal muscle cells

-Glucagon:
Liver cells

But they do the same job, same mechanism, in case of starvation these hormones are released followed by releasing of glucose (degradation of glycogen).

The two hormones, mentioned above, bind to receptors on the cell surface activating the enzyme that synthesizes cyclic AMP, then cyclic AMP binds to protein kinase A activating it, when protein kinase A is activated (which function is adding a phosphate group) there will be an addition of phosphate group to glycogen synthase turning it to an inactivated glycogen synthase (the inactivated one here is the glycogen synthase which contains a P-group), So the addition of glucose to glycogen is inactivated, at the same time protein kinase A phosphorylates PHOSPHORYLASE KINASE.

Then the phosphorylated phosphorylase kinase phosphorylates the glycogen phosphorylase (which removes the glucose from glycogen) and becomes activated, and then glycogen degradation happens.

Summary: when we add a P group to glycogen synthase it becomes inactivated عشان نمنع ارتباط الغلوكوز بالغلايكوجين

When we add P group to glycogen phosphorylase it becomes activated عشان نقدر نشيل الغلوكوز من الغلايكوجين

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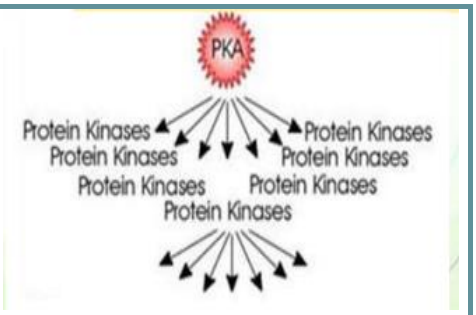
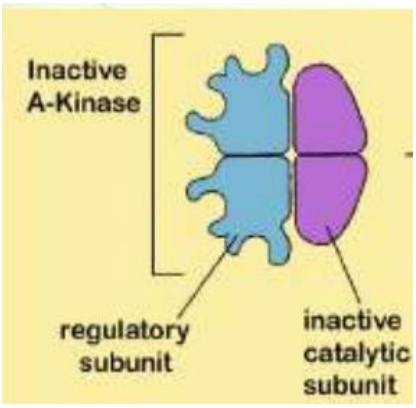
NOTICE THAT protein kinase A phosphorylates two enzymes, one is activated and the other is inactivated.



Inactivation is for glycogen synthesis pathway and activation is for glycogen degradation pathway.

NOTE: the effect of protein kinase A on the inactivation pathway is direct, while the effect on activation (degradation of glycogen) pathway is indirect (because it has multiple steps).

PKA-structure and regulation:



When inactive, protein kinase A (PKA), a serine/threonine kinase (Meaning that this enzyme phosphorylates serine and threonine residues), consists of four subunits (R2C2).

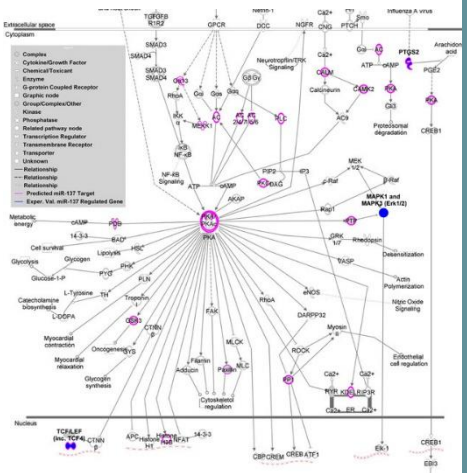
As we can see protein kinase A is tetrameric (with 4 subunits), two subunits contain the catalytic site (active site), and the other two subunits are regulatory subunits.

When the two regulatory subunits are attached to the two catalytic subunits this means that the enzyme is INACTIVE

Two regulatory (R) subunits with high affinity for cAMP.

Two catalytic (C) subunits.

PKA phosphorylates several enzymes that regulate different metabolic pathways (for example: glycogen phosphorylase kinase)

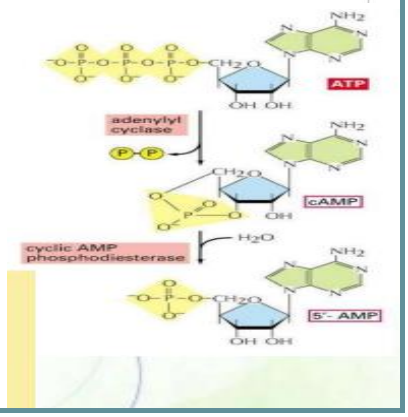


whenever PKA is activated, it phosphorylates many many many proteins including glycogen phosphorylase kinase and glycogen synthase.

The many targets of PKA

This picture is just showing you the huge number of proteins that proteins kinase A can phosphorylate (don't memorize anything from it).

cAMP and protein kinase A (PKA)



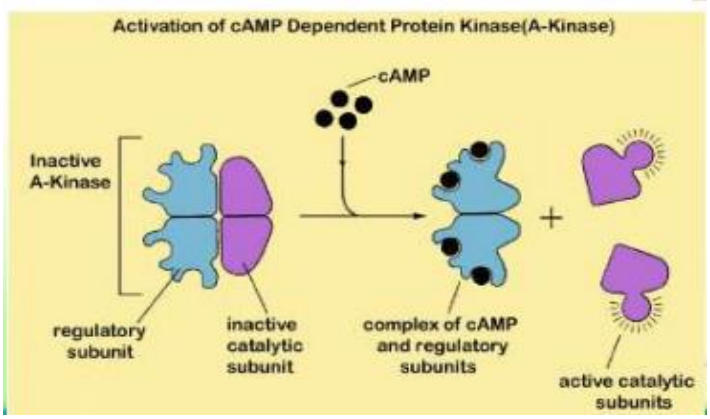
This shows that when glucagon or epinephrine binds to the receptors, an activation of adenylyl cyclase enzyme happens, adenylyl cyclase turns ATP into cyclic AMP then cyclic AMP binds to PKA (on the regulatory subunits) when the cyclic AMP binds to the regulatory subunits, the regulatory subunits are released and each catalytic subunit (which is active) moves alone and can phosphorylate proteins.

تذكر الطريقين الي اشتغلنا عليهم لما ثبطنا السيء وفعلنا المنيح .

Small-molecule modulators can have dramatic effects on enzymes.

For example, cAMP -which is structurally modified AMP- can activate protein kinase A (PKA).

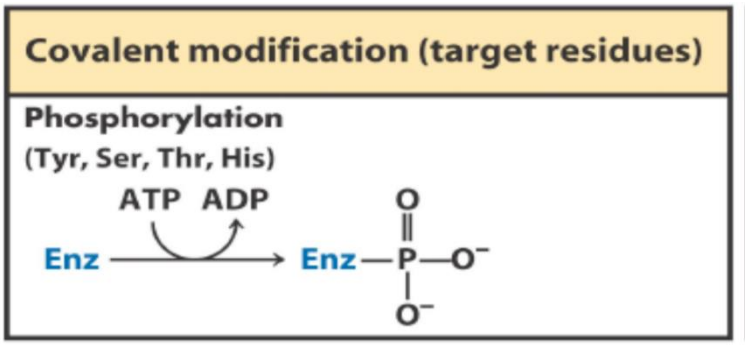
The binding of two molecules of cAMP to each regulatory subunit leads to the dissociation of R2C2 into an R2 subunit and two active C subunits.



Reversible covalent modification

The advantage of the phosphorylation

- The process of phosphorylation is rapid and transient.
- A most common mechanism is enzyme phosphorylation (the covalent addition of a phosphate group to one of its amino acid side chains) and they are usually serine, threonine, and tyrosine.

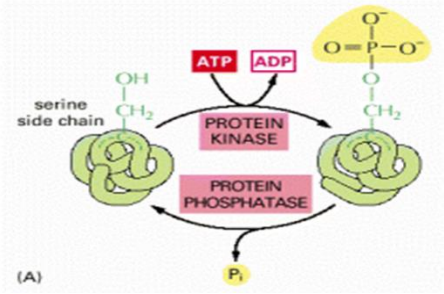


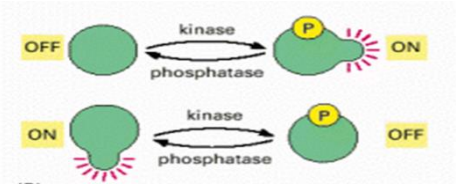
Enzymes catalysing (de)phosphorylation

Q: What are the enzymes that make the phosphorylation and dephosphorylation?

Ans: 1- Protein kinase which is a transferase and phosphorylates serine, threonine and tyrosine.

2-Protein phosphatase which is a hydrolase and dephosphorylates the residues.





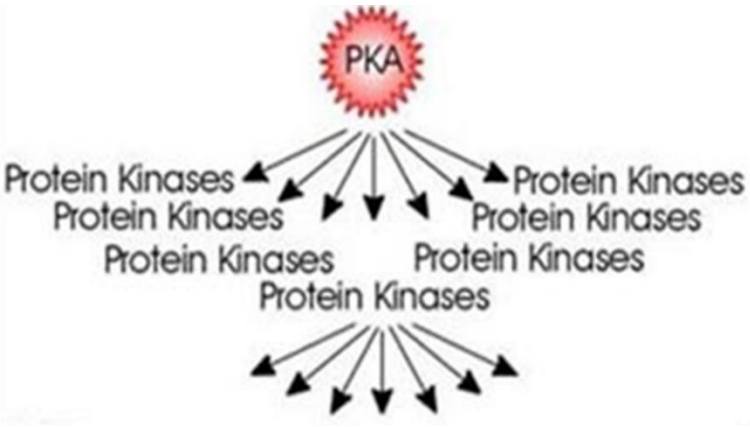
- ATP, mostly, is the phosphoryl donor in these reactions, which are catalyzed by protein kinases.
- The removal of phosphoryl groups (dephosphorylation) by hydrolysis is catalyzed by protein phosphatases.
- Note: dephosphorylation is not the reversal of phosphorylation.
- The addition or removal of a phosphate group to an enzyme may activate or inactivate these enzymes, it depends on the enzyme or protein and the best example is the glycogen metabolism:

the glycogen synthase —phosphorylation→ inactive

glycogen phosphorylase —phosphorylation→ active

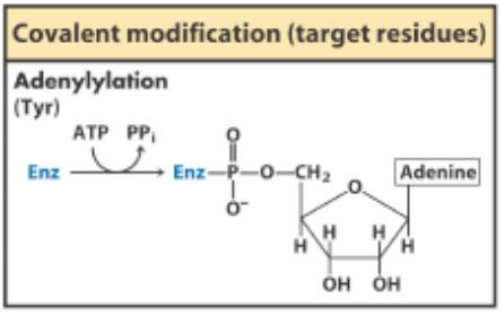
Why is phosphorylation effective?

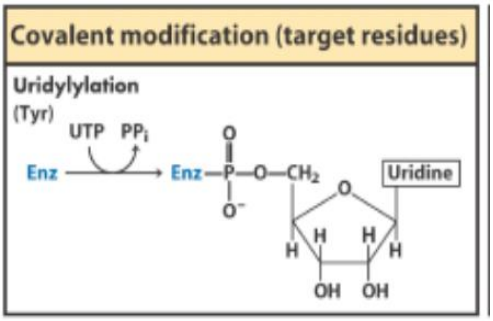
- Formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity and it can change in the bonds of the enzymes.
- It can happen in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects.
- Protein kinase, when phosphorylated, phosphorylates other enzymes. Amplifying the effect.



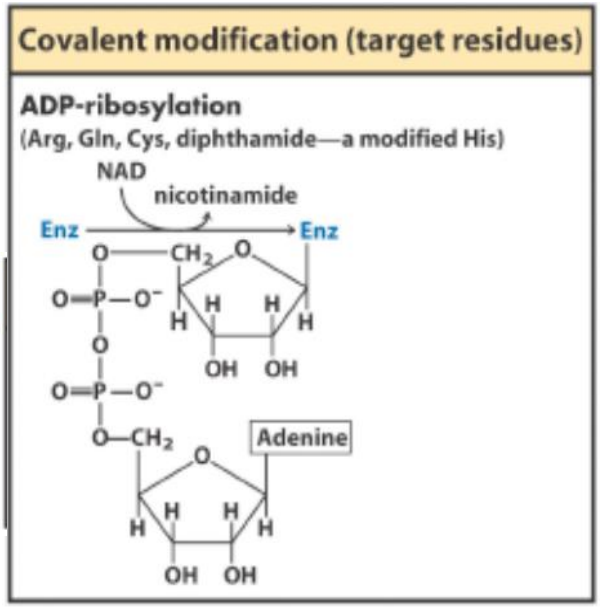
Others (The process of reversible modification for proteins can be in other ways):

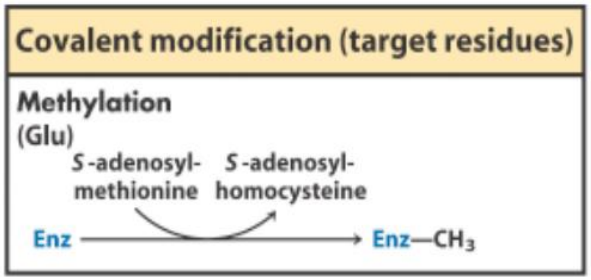
- **Adenylylation: (addition of adenylyl group). AMP is transferred to Tyr residues through phosphodiester linkage.**
- **The addition of bulky AMP inhibits cytosolic enzymes.**
- **Uridylylation: (addition of uridylyl group).**
- **ADP-ribosylation (addition of adenosine diphosphate ribosyl group) inactivates enzymes.**



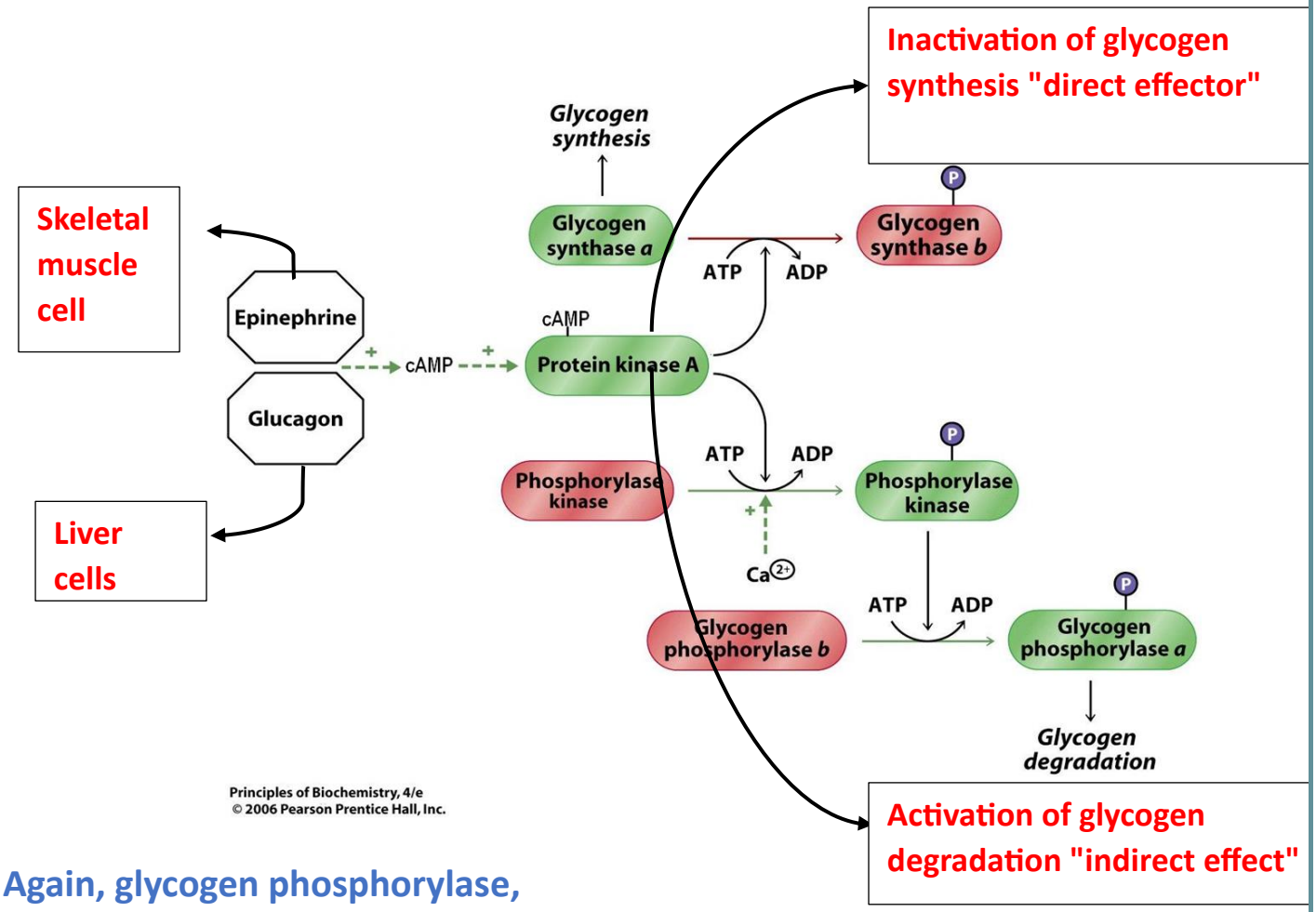


- **Methylation of carboxylate side chains masking negative charges.**
- **Acetylation (from acetyl Co) to lysine residues masking positive charges**
  for example, the histone molecules: it will be acetylated so the positive charges will vanish, which affects the relaxation of the DNA molecule and the activation of gene expression.





Phosphorylation cascade



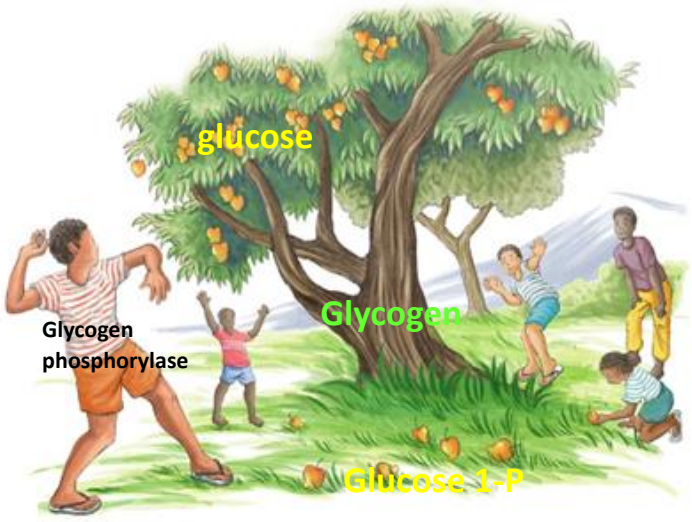
Again, glycogen phosphorylase,

The regulation of it doesn't depend only on phosphorylation but there are other factors that play a role in the regulation of glycogen phosphorylase.

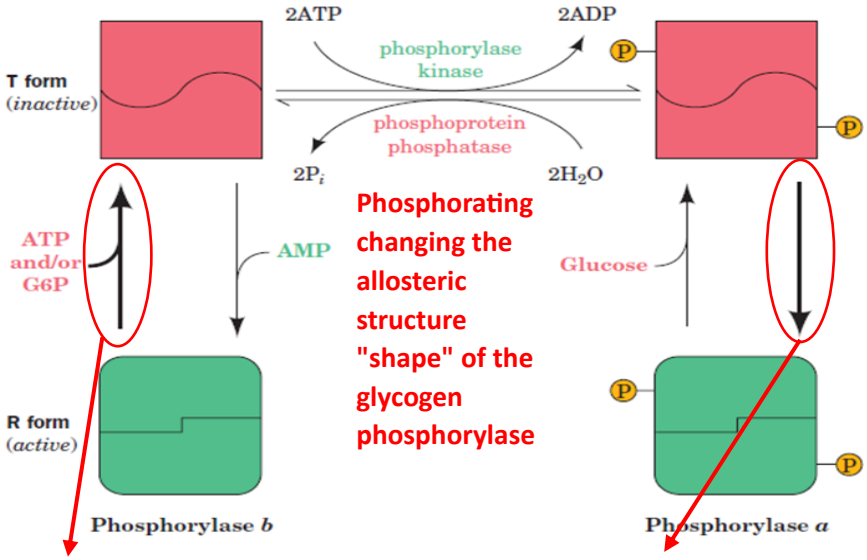
$$\text{glycogen}_{(n\ \text{residues})} + P_i \longrightarrow \text{glycogen}_{(n-1\ \text{residues})} + \text{glucose 1-P}$$

- Glycogen Phosphorylase catalyses the removal of glucose molecules from glycogen.
- Phosphorylation of a Ser residue away from the active site activates it.
- The enzyme exists in four forms:
  - T (inactive) or R (active) states
  - Phosphorylated (a) or dephosphorylated (b)

- *When phosphorylated, it is known as phosphorylase a.*
- *When dephosphorylated, it is known as phosphorylase b.*



Glycogen phosphorylases take a phosphate group and throw it on the glucose and the glucose-1-p will get out of the glycogen like the mechanism in the picture!!!

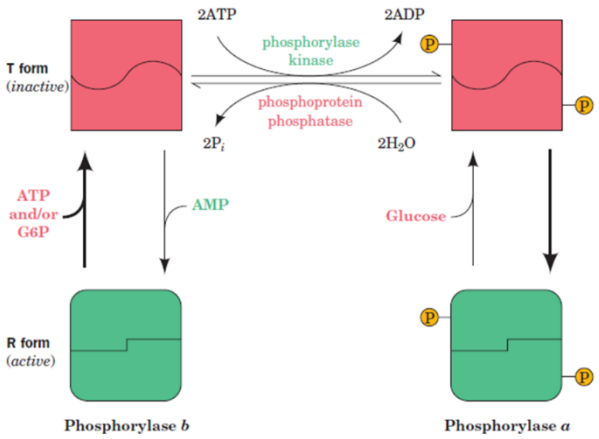


The equilibrium will go to make the T form more than the R form because of the absence of the phosphate group.

The equilibrium will go to make the R form more than the T form because of the phosphate group.

The two forms of each form

- Both phosphorylase a and phosphorylase b exist in equilibrium between an active R state and a less-active T state.
- Phosphorylase a is usually active because the equilibrium favors the R state.
- Phosphorylase b is usually inactive because the equilibrium favors the T state.



What are the regulators that regulate the glycogen phosphorylase with the phosphorylation?

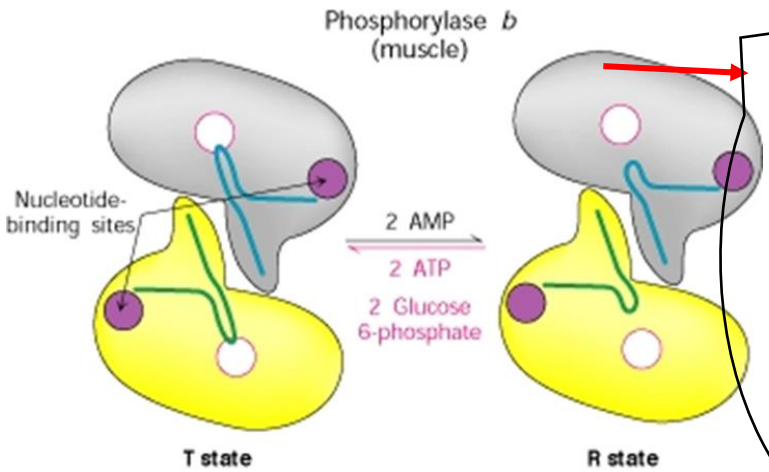
1) **The amount "the ratio" of ATP and AMP ((energy charge)).**
2) **The availability of glucose -6- phosphate.**
3) **High levels of glucose.**

- The transition of phosphorylase b between the T and the R state is controlled by the energy charge (ATP and AMP) of the muscle cell and the availability of glucose-6-phosphate.
- High levels of glucose drive the formation of the T state of phosphorylase a.

What do ATP and AMP do?

- Muscle phosphorylase b is active only in the presence of high concentrations of AMP, which binds to a nucleotide-binding site and stabilizes the conformation of phosphorylase b in the R state.
- ATP acts as a negative allosteric effector by competing with AMP and favoring the T state.

Glucose 6-phosphate also favors the T state of phosphorylase b, an example of feedback inhibition.



The two-phosphate groups (in ATP) can change the structure of the enzyme to make the T state more favorable than the R state.

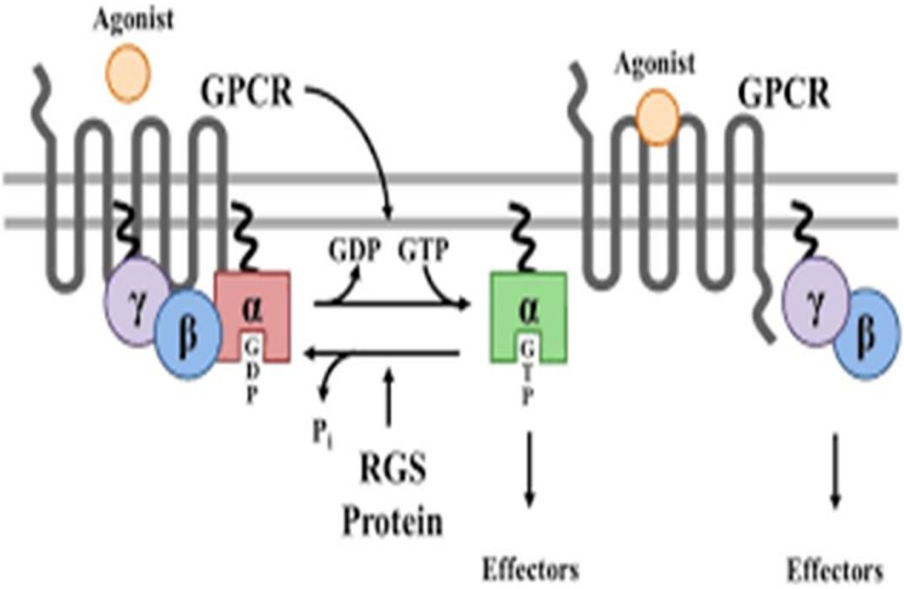
Note: if we increase the concentration of ATP, it will inhibit the enzyme and then it will not specially make the R state of glycogen phosphorylase b.

Large and small regulatory modulators

1) Trimeric large G proteins

❖ **Trimeric G proteins: a family of membrane-bound proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signalling factors through G protein-coupled receptors (GPCRs).**

- **When they bind GTP, they are 'on', and, when they bind GDP, they are 'off'.**
- **The α subunit binds to effectors stimulating or inhibiting them.**



The G-protein is a large protein structure with 3 subunits: alpha, beta, gamma and the alpha are "active" catalytic subunit ((not enzyme)), beta and gamma are regulatory. subunits.

G protein is not an enzyme, it is a modifier or modulator

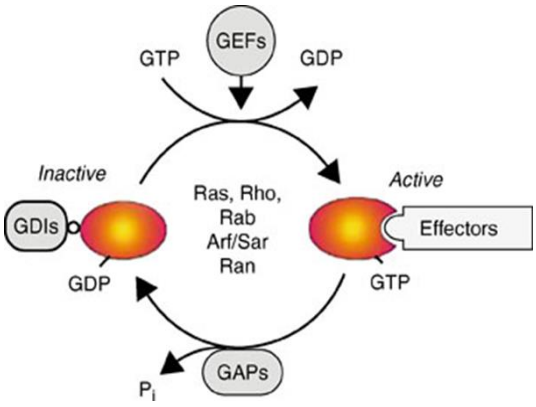
When the agonist binds to the G-protein then the agonist will change the structure of the GPCR and that will change the structure of G-protein, then the GDP will be removed from the alpha subunit and the GTP will replace it. The alpha subunit will be separated from the G-protein and will bind the effectors like adenylyl cyclase which converts ATP to cAMP that binds to the PKA in order to activate it. When we want to return everything back to the beginning, we hydrolyse the GTP to be GDP. In this case we have these reactions on the alpha subunit:

GDP→GTP (exchange reaction) GTP→GDP (hydrolysis reaction)

2) Small monomeric G proteins

- When GTP is bound, the conformation of the G protein allows it to bind target proteins, which are then activated or inhibited.
- The G protein hydrolyses a phosphate from GTP to form GDP, which changes the G protein conformation and causes it to dissociate from the target protein.
- GDP is exchanged for GTP, which reactivates the G protein.

The activity of many monomeric G proteins is regulated by:



1. GAPs [GTPase-activating proteins]

Activate the intrinsic enzymatic activity of the protein which is a power inside the protein itself, but it doesn't work on any protein except itself. (It enables protein to hydrolyse GTP).

2. GEFs [guanine nucleotide exchange factors]

They replace the GDP with a GTP

3. GDIs [GDP dissociation inhibitors]

Prevent the separation of GDP from the protein subunit ((such as Ras))

Irreversible covalent modification, Proteolytic activation (proteolytic modification of enzyme):

When covalent modification occurs, there is no point of return…and there must be other mechanisms to do enzyme regulation.

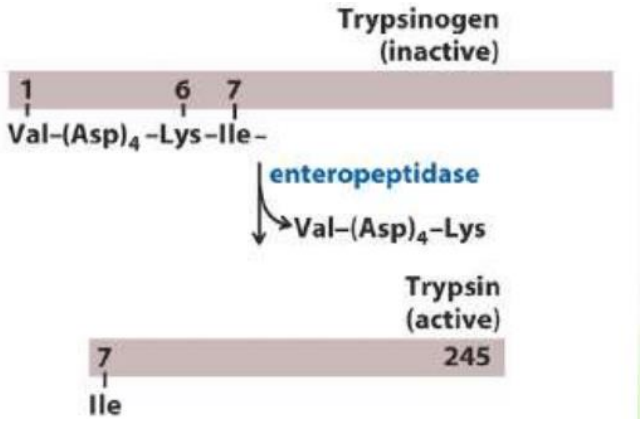
-Zymogens or proenzymes are inactive precursors of enzymes.

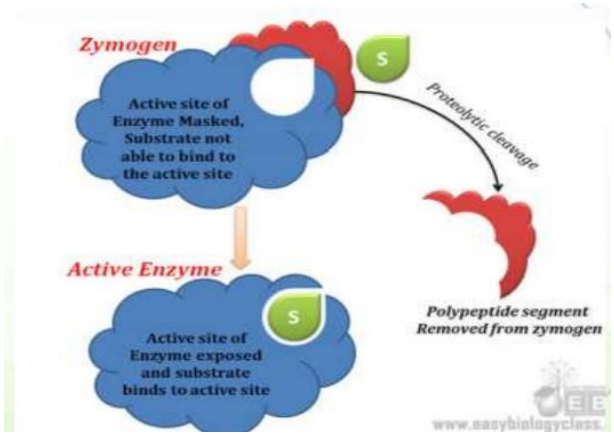
Red body: part of the enzyme which masks the active site (usually known as the pro region present at the N-terminus).

The activation is done by irreversibly removing this pro-peptide so, the substrate can bind on active site.

Examples: digestive enzymes such as Chymotrypsin, trypsin: in pancreatic cells. And Pepsin: in stomach cells. Chymotrypsin and trypsin are released inside the intestines and then they get activated when food is ingested to be digested.

(It is a fast mechanism that occurs to protect our cells in the pancreas and stomach to avoid inflammation if they are active inside them).





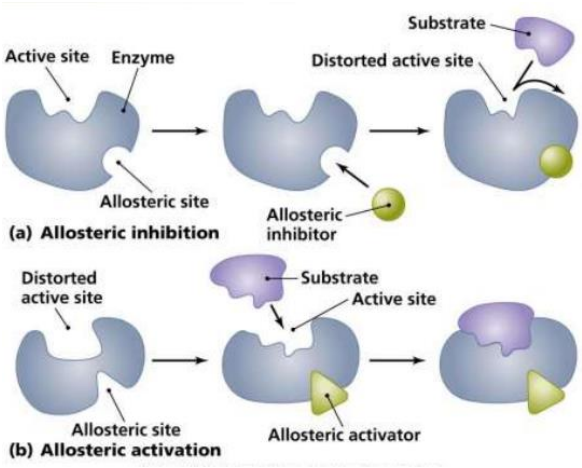
<u>Trypsinogen</u> (zymogen=inactive enzyme) is activated via removal of the first six amino acids at the N-terminus to be <u>trypsin</u> (active enzyme).

Trypsinogen → trypsin …. Chymotrypsinogen → chymotrypsin …. pepsinogen → pepsin ….

Proelastase → elastase …. Etc.

<u>Allosteric regulation:-</u>

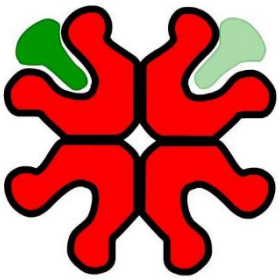
Allosteric enzymes are multi-subunit proteins:



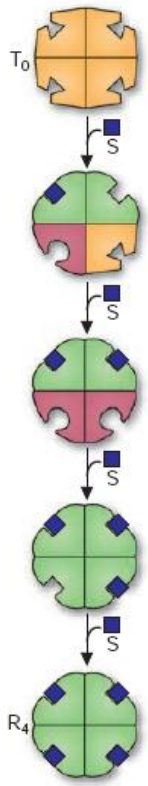
-One subunit contains the active site (catalytic subunit) and another containing the regulatory site (regulatory subunit).

-Multiple active sites can exist on multiple subunits.

What happens is that the binding of regulatory molecules (allosteric modifiers) triggers conformational changes in the active site via modifying non-covalent interactions to make it more active or less active.



Allosteric enzymes bind modifiers at the allosteric site, a site that is physically separate from the catalytic site.



A negative allosteric modifier (inhibitor) causes the enzyme to have less activity by making the active site not fit the substrate.

A positive allosteric modifier (activator) causes the enzyme to be more active by making the active site fit the substrate.

- **If the modifier is a molecule other than the substrate, then it is known as heterotropic.**
- **If the modifier is the same as the substrate, then it is called homotropic.**

The binding of the substrate causes the enzyme to become more active and binds to a second substrate at a different active site with more ease (This is called "positive cooperativity") - T to R conformation (like oxygen with hemoglobin).

There is also "negative cooperativity" when the binding to enzyme causes harder binding to a second substrate.

The Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes so, K0.5 (K half) is used instead of Km.

K half gives us an indication of affinity

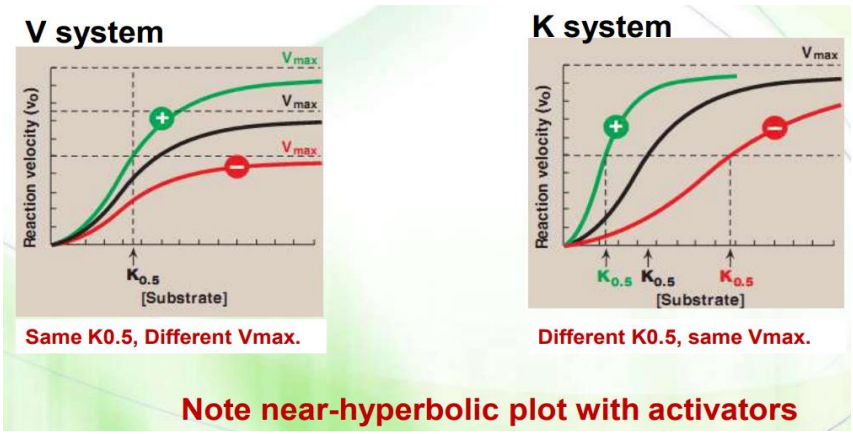
Black line: when enzymes do not have a modifier.

Green line: when enzymes have an (activator).

V-system → V max increases, k half remains constant.

K-system → V max remains constant, k half decreases.

Red line: when enzymes have an (inhibitor).



Allosteric inhibitors usually have a much stronger effect on enzyme velocity than competitive and noncompetitive inhibitors.

Allosteric enzymes are regulated by allosteric effectors that can be activators or inhibitors. (the enzymes that follow the Michaelis-Menten model are regulated by inhibitors, but allosteric enzymes have both activators and inhibitors)

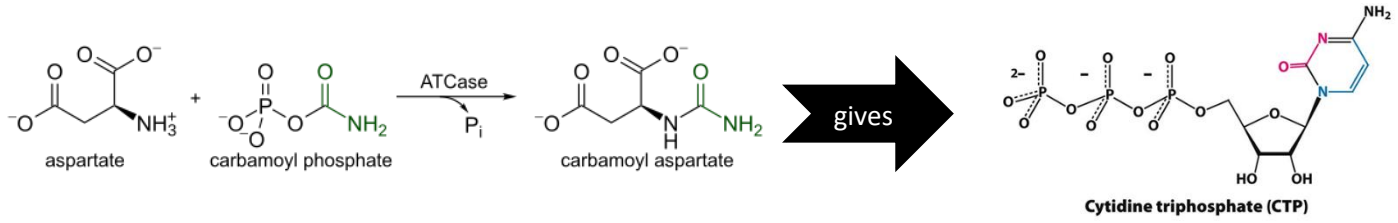
The allosteric effector does not need to have a similar structure as the substrate or product of the enzyme.

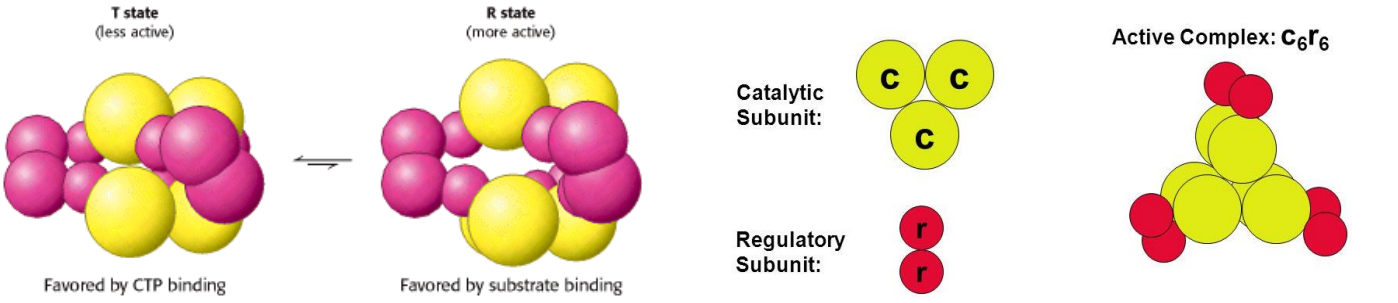
The effect of an allosteric effector is rapid occurring as soon as its concentration changes in the cell.

Aspartate transcarbamoylase (ATCase) catalyses the first step in the synthesis of pyrimidine nucleotides.

ATCase consists of 12 polypeptide chains: six catalytic subunits (two trimers) and six regulatory subunits (three dimers).

It exists in two forms: T state (less active) and R state (more active).





The effects of CTP on ATCase:

ATCase is inhibited by CTP, which is the end-product.

-inducing a major rearrangement of subunit positions

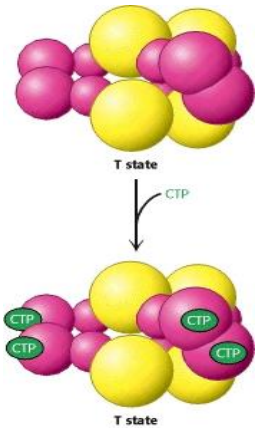
-stabilizing the T state of the enzyme.

-decreasing binding affinity for Asp (substrate) at active sites on catalytic subunits

-increasing K0.5 (K system)

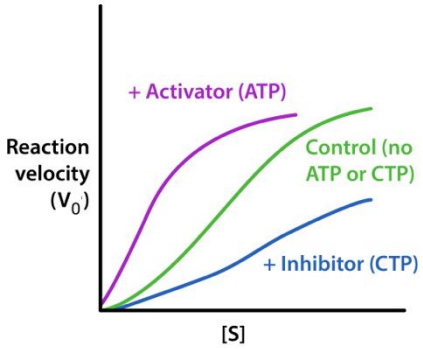
Note: a non-competitive inhibitor changes K0.5

On the other hand, ATP, a purine, heterotypically activates the enzyme in order to balance the rate of synthesis of purines and pyrimidines in cells.



Notes: the advantage of allosteric enzymes is that any change in the concentration of the substrate, modifier and inputs leads to a change in the activity of the enzyme itself. If we look at the plot and increase the activator concentration with the same substrate concentration, we will see that the curve will rise largely, which indicates the huge effect of the activator concentration.

Note: the doctor has focused very well in this lecture on the diagrams and what will happen if we change the variables of concentrations and constants and velocities, so please practice this topic well.



The End of Sheet 23