21

BIOCHEMISTRY

Faculty of medicine – JU2018

◉ Sheet ○ Slides

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In the previous lecture we have talked about isozymes and we said that isozymes (isoenzymes) are different enzymes but :

1- They catalyze the same reaction (they act on the same substrate(s) producing the same product(s).
2- They are produced by different genes that vary only slightly.
3- They can be regulated differently.
4- They can have different catalytic activities.
5- They are found in different tissues.

- And we said that (LDH) lactate dehydrogenase is a great example on isozymes .

LDH:

- It is made of 4 polypeptide chains (each one of them has its own active site).

- It is made of 1 or 2 types of polypeptides : **H** polypeptide/ M polypeptide, and we can have a combination of them e.g.[4H , 3H-1M, 2H-2M, 1H-3M, 4M]

- H stands for Heart : if we have 4H it is mainly found in heart.

- M stands for Muscles : if we have 4M it is mainly found in muscles.

☺ **LDH 1** : (4H) in the heart
Prefers converting lactate to pyruvate reaction
Why ??
The reason is that the heart can't function anaerobically.

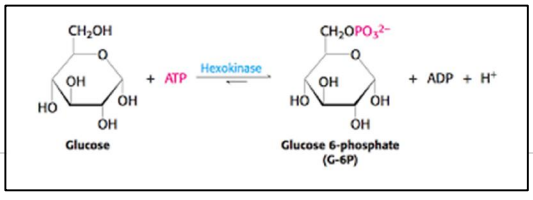
☺ **LDH 5** : (4M) in muscles
Prefers converting pyruvate to lactate reaction
Why ??
The reason is because muscles CAN function anaerobically .

ANOTHER EXAMPLE OF ISOZYMES :-

Hexokinase (hexokinase I) & **glucokinase** (hexokinase IV) :

☺ These 2 enzymes do the same reaction, they ***phosphorylate*** glucose to glucose-6-phosphate (Transferring phosphate group from adenosine triphosphate (ATP) to glucose to form glucose-6-phosphate)

☹ they differ in tissue distribution.



*Glucokinase	*Hexokinase
*Hexokinase 4	*Hexokinase 1
*liver's enzyme (found in liver)	*It is a RBC's enzyme (found in multiple tissues but we will focus on RBC's)

But what is the purpose for this distribution ??

Glucose-6-phosphate may then be metabolized in glycolysis, which generates energy in the form of ATP (in RBC) , or it can be converted to glycogen, a storage polymer of glucose (in the liver).*Keep in your mind that once glucose is converted to glucose-6-phosphate it becomes trapped inside the cell and can't cross the membrane, since phosphate group is large and carry **-ve** charge.*

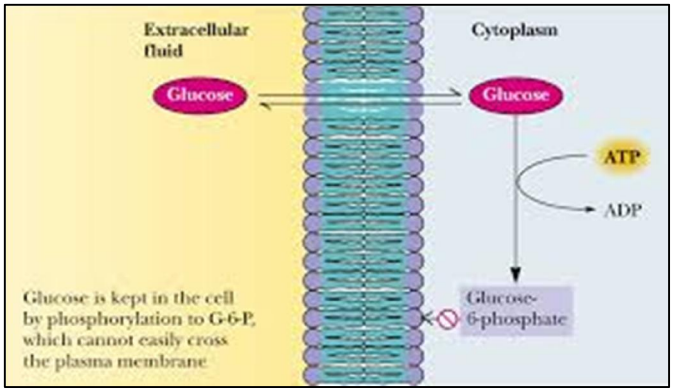
So , the distribution makes sense :

1- **Liver** : to balance glucose level in the blood , ensuring that all tissues have enough glucose and if there is excess glucose it stores it in the form of glycogen.
For more understanding ☺:
Glucokinase have a high level of social solidarity ,meaning that if it has glucose it doesn't *phosphorylate* it (phosphorylation: to store it) until checking on other tissues whether they need glucose or not. So, it provides other tissues with glucose before it uses it either as source of energy for the live cell itself or to store glucose as glycogen .

2- **On the other hand , RBC's** totally dependent on glucose metabolism to meet its needs for ATP (energy), [Because in RBC metabolism of glucose is the only source of energy, since RBC lack mitochondria], so once glucose is availabe it traps it by *phosphorylating* it.

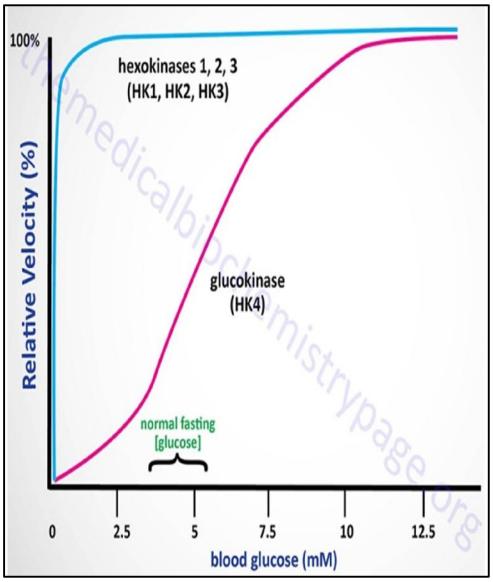
-Liver has a low efficiency enzyme (glucokinase) it only phosphorylates glucose (trapping it) when it makes sure that glucose is not needed by any other tissue, it doesn't waste ATP (used in phosphorylation) on nothing .

-RBC have high efficiency enzyme (hexokinase) binds to it very quickly and phosphorylates it instantaneously trapping it.



A- ***Their catalytic activity*** *:*

Enzyme's name	Km and affinity	plot of the Michaelis-Menten equation
Glucokinase	High k_m – 10 mM	Slightly sigmoidal
Hexokinase (1,2,3)	Low k_m- 0.1 mM	Highly hyperbolic



So, from the graph we can see that :

When you wake up in the morning (low level of blood glucose) what is active is the hexokinase not glucokinase. In the liver glucokinase isn't active , it doesn't phosphorylate glucose and it doesn't store glucose. But RBC and muscles require hexokinase to be active ,so that they can produce energy . Then when we eat lunch and dinner and the level of glucose is high ,both enzymes are active specially glucokinase ;because now there is enough glucose and it can be ***phosphorylated*** and stored .

B- ***Their regulation*** :
Hexokinase and glucokinase differ in their regulation:

☺ **Hexokinase** :

it has low k_m -0.1 mM (high affinity) .**This makes sense ,how?** means even at low levels of glucose Hexokinase is still active .

INHIBITION :

This enzyme is inhibited by glucose-6-phosphate (the product inhibits the enzyme). That's true ,why ??

Well RBCs aren't selfish they don't consume all glucose in blood . They need high level of glucose-6-phosphate and they need hexokinase to be active but, if there is enough glucose-6-phosphate they keep some glucose for other tissues .

☺ **Glucokinase** :
it has high k_m-10mM (low affinity) . This makes sense , how ?
That is only when the glucose level is high it binds to glucokinase in the liver and it is phosphorylated and then it's stored .

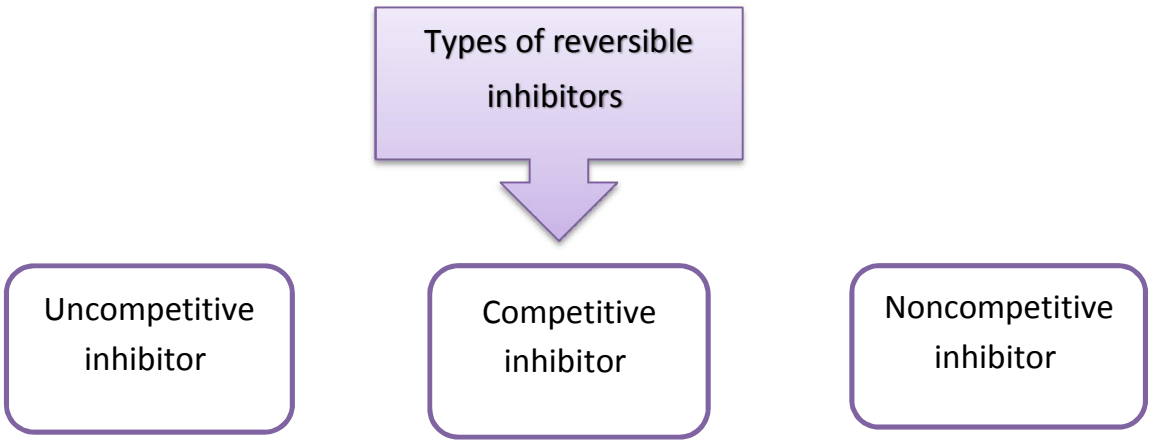
INHIBITION:
It isn't inhibited by glucose-6-phosphte ,why?
If there are high levels of glucose it needs to be phosphorylated in liver cells *(for glucose to not go to waste, it is stored in the form of glycogen)*which means as a result also high levels of glucose-6-phosphate. That's why Glucokinase shouldn't be inhibited by glucose-6-phosphate to be able to store more and more glucose as it level goes up.

✘ INHIBITORS :

1- Inhibitors are used to regulate enzyme's activity
2- Inhibitors can be either reversible or irreversible

A- *Reversible inhibitors* : all physiological inhibitors are reversible (binds to enzymes non-covalently which means can easily dissociate compared to covalent bond)
The inhibitor binds to the enzyme and inhibits it and then it can be released .

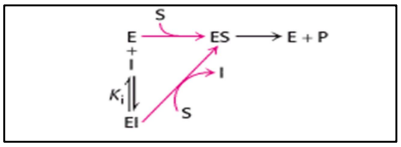
A- *Irreversible inhibitors*: they are mainly synthetic .e.g : drugs (binds to the enzyme covalently ***or*** with very strong and very high affinity non-covalent interactions) The inhibitor binds to the enzyme inhibiting it completely and they aren't released (Covalent binding).



1- Competitive inhibitors :

☺ compete with the substrate for the active site. So, we have a competition between the substrate and the inhibitor, if there is a high concentration of the inhibitors it wins the competition (meaning that it makes it hard for the substrate to bind to the active site) ,but if we increase the substrate concentration it can overcome inhibition and eventually, we reach Vmax.

Let's have a look at this figure: The enzyme can either binds to the *substrate* or to the *inhibitor*. So, we can have a complex of (enzyme + substrate ES) or a complex of (enzyme + inhibitor EI)



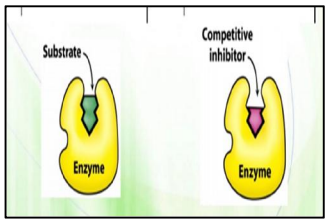
☺ **from michaelis-menten equation's graph :**

Same Vmax

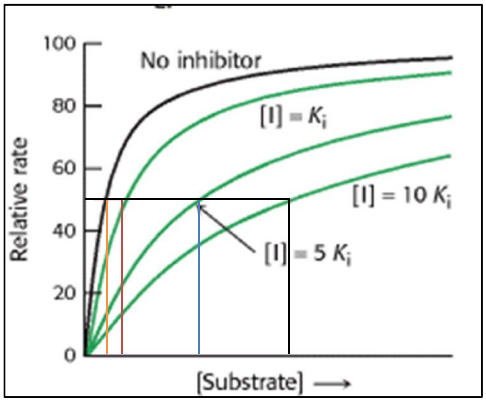
higher Km (lowering the affinity)

HOW ? If we add inhibitor at certain concentrations (while increasing the substrate concentration) what happens is eventually we will get to (Vmax). If we add more inhibitor it makes it hard for the substrate to bind to the active site, if we add more inhibitor it makes it even harder for the substrate to bind to the active site , but if we increase the substrate's concentration to really high level ,eventually we should reach Vmax; because the substrate will win the competition and it will bind to the active site.

But K_m will increase (affinity is decreased) ,because in fact some active sites are blocked by the inhibitor.



We can calculate Vmax using :

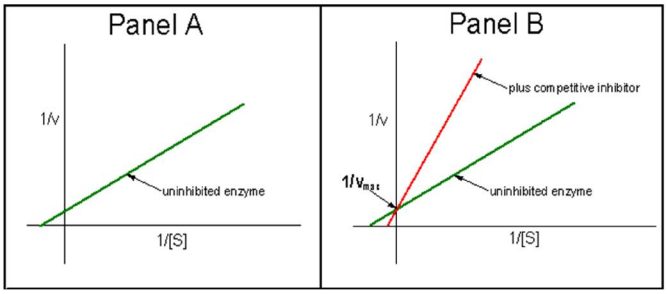


1- Michaelis-Menten equation

It is hard to calculate Km since we can't reach Vmax. Notice different K_m values.

2- Lineweaver plot (it's linear)

By extending the line to the left (it's hypothetical because we can never have negative concentration of a substrate), eventually we will hit a point on the y-axis which is 1/Vmax (Vmax is constant)



In the presence of an inhibitor (the red line) Vmax didn't change, so the line will cross the same point on the y-axis, but K_m will change, it will increase.

NOTES FOR UNDERSTANDING :

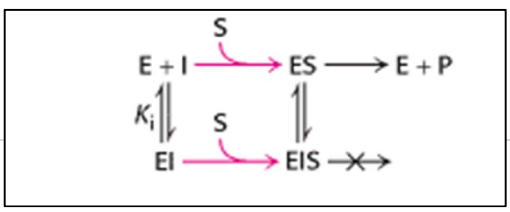
10 > 1 BUT. $\frac{1}{10} < \frac{1}{1}$

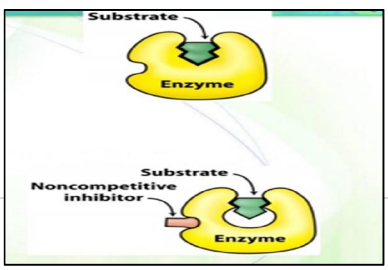
So, to interpret the value of Vmax and K_m just FLIP the concentration and notice the NEGATIVE charge. Example: if the line crosses the x-axis at (-10) what it the value of K_m?

$K_m = \frac{1}{10}$

2- Non-competitive inhibitors :

☺ Non-competitive inhibitors bind to E or ES complex at different site than the catalytic site (it binds to the regulatory site), and they don't affect the substrate binding to the active site, there is no competition between the inhibitor and the substrate. But it will affect the enzyme activity.





☺ **let's have a look at this figure** :

The enzyme can bind to the substrate without binding to the inhibitor forming (Enzyme+ substrate ES) complex , and it can bind to the substrate while binding to the inhibitor forming (enzyme + substrate + inhibitor ESI) complex , but it can't form a product .

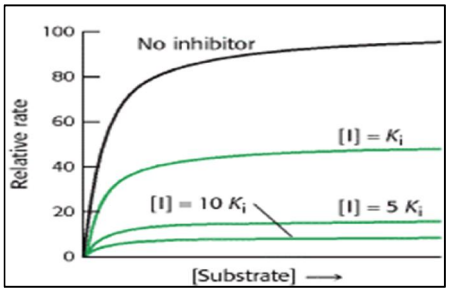
from Michaelis-Menten equation's graph :

☝Same K_m

✌ decreasing Vmax

How ??

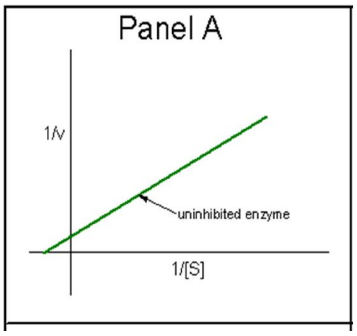
- **K_m** (affinity) won't be affected? because whether the enzyme binds to the inhibitor or not the substrate can still bind to the enzyme (remember :the inhibitor binds to the regulatory site not the catalytic site of the enzyme)
- **Vmax** is getting lower as we increase the inhibitor concentration, no matter how much substrate we add because the enzyme is inhibited (the same fraction of enzyme is inhibited), even though the interaction between the inhibitor and the enzyme is reversible; when the inhibitor molecule dissociates from one enzyme molecule it will bind to another, so the same fraction of enzyme molecules remains inhibited.

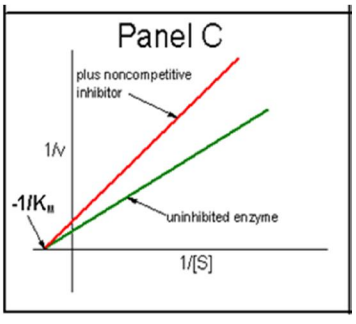


lineweaver plot :

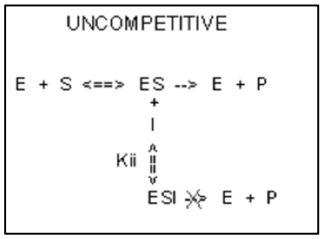
-These 2 lines will cross the (x-axis) at the same point which is K_m , but they will hit the (y-axis) at different points which represent Vmax values.

-Vmax is lower for the enzyme with the inhibitor than the enzyme without the inhibitor .





3-Uncompetitive inhibitor :



*They are similar to non-competitive inhibitors but they have different mechanism

*Uncompetitive inhibitors bind to the enzyme-substrate complex only.

☺ from Michaelis-Menten equation's graph :

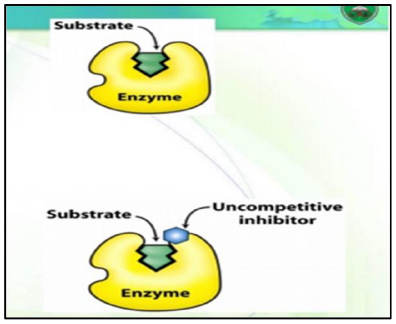
Vmax decreases

K_m decreases (affinity increases)

How ??

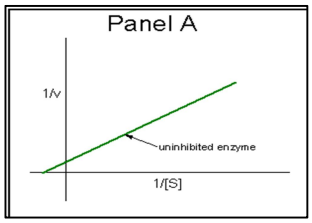
- K_m decreases (affinity increases) because we always will have the substrate bound to the enzyme .
- Vmax decreases because once the inhibitor binds to the (enzyme+substrate) complex , there won't be a reaction (no catalysis takes place), so Vmax is reduced .

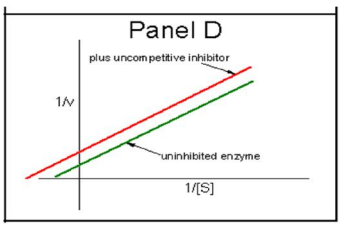
☺ lineweaver plot :



*These 2 lines hit the (x-axis) and the (y-axis) at different points .

*The enzyme without the inhibitor (enzyme+ substrate ES) complex will have **HIGHER** Vmax and Higher K_m (lower affinity) than the enzyme with the inhibitor (enzyme + substrate +inhibitor ESI) complex .





concerning types of reversible inhibitors you have to know :

1- Their mechanism of action .
2- How they affect Vmax and Km .
3- Interpret from the plot of medhaelis-menten equation and lineweaver plot wht type of inhibitor we are talking about .

Mechanism-based inhibitors (Irreversible inhibitor):

Irreversible inhibitors are mechanism-based inhibitors, which means that they **mimic** or participate in an intermediate step of the catalytic reaction

Irreversible inhibitors:

1-Covalent inhibitors 2- Transition state analogs 3- Heavy metals
-these inhibitors in general bind tightly to the active site or to enzymes, and they do that mainly covalently.

- irreversible inhibitors decrease the concentration of active enzyme(so overall what they do that that they decrease Vmax)

Mechanism of irreversible inhibitors:

1-Covalent inhibitors:

They form covalent or extremely tight bonds with active site amino acids.

Example: 1- diisopropyl fluorophosphate (DFP) 2-Aspirin

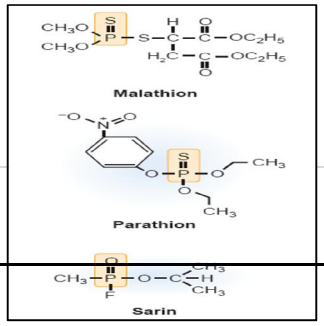
DFP includes :
a. The nerve gas sarin (used in wars and it causes spasm and shaking)
b. The insecticides(مبيدات) malathion & parathion.

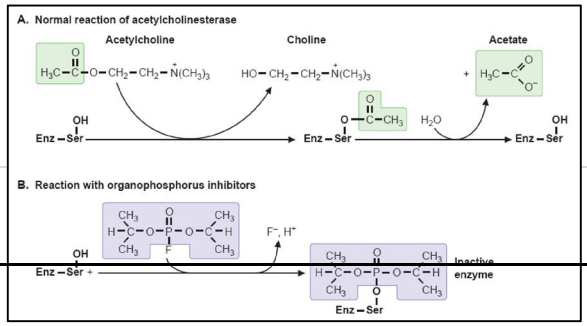
How does DFP work?
*They modify the active site of the enzyme acetylcholinesterase (an enzyme that exist in the central nervous system)
*The neurotransmitter acetylcholine is important for the normal function of the brain but it should not exist all the time around nerve cells to prevent the overexcitation (action potentials) so it has to be inactivated and that what *acetylcholinesterase* does, it degrades acetylcholine into choline and acetyl group so we do not have the signal of acetylcholine any more.

-Basically, what happens if someone ingests or inhales DFP that it goes to the active site of acetylcholinesterase and modifies the serine covalently

-When it binds to serine covalently the serine in the active site can not function and that kills the enzyme as if it is not existing

-as a result, the neurotransmitter acetylcholine stays in tissue for along time causing the signal to be amplified and causes spasm and shaking and it causes death
**DFP also inhibits other enzymes that use serine (ex. serine proteases), but not lethal (it does not cause the symptoms that were mentioned above)*



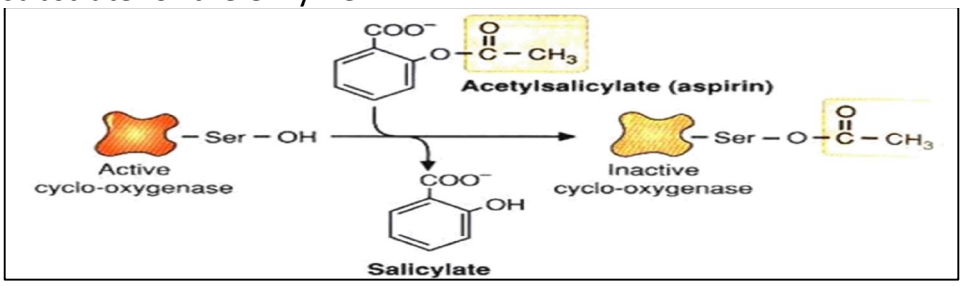


Another famous covalent inhibitor is **Aspirin** (acetylsalicylic acid)

How does it work?

-Aspirin (acetylsalicylic acid) acetylates(add an acetyl group) an active site serine of cyclooxygenase.

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



2-Substrate and transition state analogs :

Suicide inhibitors:

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

-Suicide inhibitors bind more tightly than substrates(they bind to the enzyme and hardly get released)

-some of ***Suicide inhibitors*** look like substrate , others look like the transition-state, so how do these inhibitors work ?

Basically, such an inhibitor binds to the enzyme (because it looks like either the substrate or the transition-state) and the enzyme starts the reaction by moving groups or electrons or any other modifications to the substrate(which is in this case is the inhibitor but the enzyme thinks it is the substrate) and then at a certain step when the enzyme wants to add a group or electrons to bind to a certain group on the substrate ,the enzyme discovers that the inhibitor does not have the group that can have the transfer of electrons or groups or.., *so the enzyme gets stuck ,meaning that the enzyme can not go backward or forward (the enzyme is killed).*

Examples:

1) Methotrexate

a. Methotrexate is a synthetic inhibitor used to treat cancer.it is also used in reducing diseases related to inflammation ,for example *Rheumatism and joint pain* .

b. it is a *chemotherapeutic agent*

c. it has severe side effects sometimes because it *affects* many cells

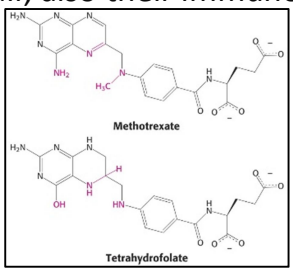
How does it function?

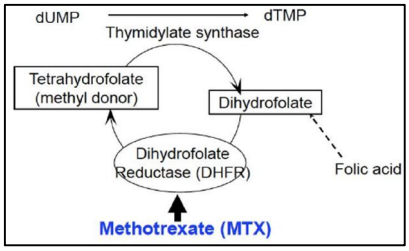
-It looks like cofactors (as you know enzymes need help by cofactors).

-It is a structural analog of ***tetrahydrofolate***, tetrahydrofolate is the active form of folic acid (dihydrofolate) folic acid is reduced (adding H_2) by dihydrofolate reductase to be active and act as methyl donor to form *deoxy-thymine-mono-phosphate* which is required for DNA-replication.

-Methotrexate binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate(folic acid) and inhibits nucleotide base synthesis and that means that there is no ***DNA synthesis*** any more which is needed in all proliferating cells (including cancer cells)

- That's why Methotrexate affects all cells that can be regenerated like hair cells and layers of gastrointestinal system so cancer patients after taking methotrexate they become not able to eat because there are no cells that are renewed and they lose their hair, also their immune system could be affected.





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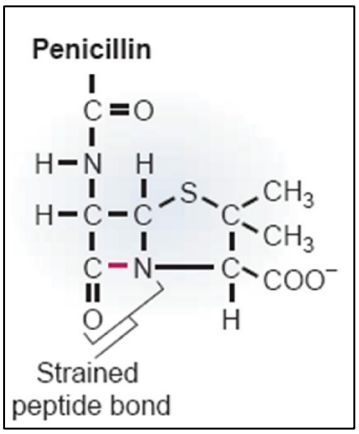
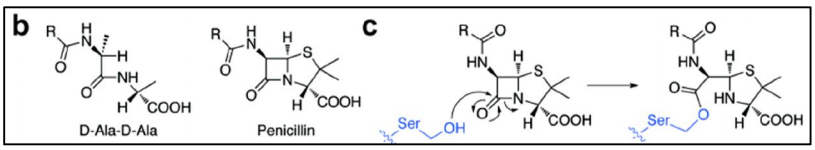
2)Penicillin

*It is a transition-state analog to glycopeptidyl transpeptidase, which is required for synthesis of the bacterial cell wall.



*The peptide bond in the β-lactam ring of penicillin looks like the natural transition-state complex

The active site serine of the enzyme attacks the *highly strained* β -lactam ring, so the enzyme starts the reaction (but Penicillin has a missing group which is required in the reaction), resulting in opening of the lactam. This reaction leads to irreversible covalent modification of the enzyme



3)Heavy Metals:

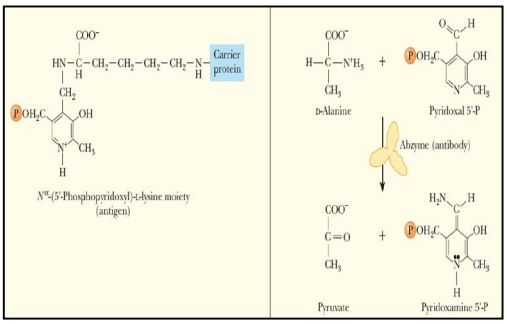
1- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe) result in tight binding to a functional group in an enzyme .
2- They do *Nonspecific* inhibition at high doses.
3- What they do is that they replace the metals which bind to metalloenzymes (enzymes that require metals to be in the active site in order to be active)

*Mercury binds to reactive sulfhydryl groups in the active site (sulfhydryl exists in cysteine, so all cysteine residues in the active sites would be modified)
-Unknown enzymes in mercury toxicity(any enzyme that contains cysteine)

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

Abzymes(transition state analog):

1-Abzymes are catalytic antibodies.
2-unlike regular antibodies, a single abzyme can inactive thousands of virus particles(it's an enzyme) while regular antibodies inactive one virus particle



So, an Abzyme is : An antibody produced against a transition-state analog & has catalytic activity similar to that of a naturally occurring enzyme.

- An abzyme is created by injecting a host animal with a transition-state analogue. The host animal makes antibodies to the foreign molecule, these antibodies(Abzymes) have specific binding points that mimic an enzyme surrounding a transition state.

Another mechanism of regulation : ***Regulation through conformational changes***

We can regulate enzyme activity by changing its structure
These regulatory mechanisms include:

-*Allostery* (we talked about allosteric changes in the hemoglobin function)
-Covalent modulation
-Protein-protein interactions between regulatory and catalytic subunits or between two proteins
-Proteolytic cleavage

Allosteric enzymes:

Allo: means different / steric: means structure

*Allosteric protein exists in slightly different change (like the 10-angstrom movement of the heme group in hemoglobin that changes the structure of hemoglobin molecule by turning the peptide only a little bit)

*Allosteric proteins in general are multi-subunit proteins (dimer , trimer…)

- One subunit contains the active site (catalytic subunit) and another containing the regulatory site (regulatory subunit).
- Multiple active sites can exist on multiple subunits

The binding of regulatory molecules ***triggers*** conformational changes in the active site via modifying <u>non-covalent</u> interactions so the catalytic subunit will take the shape of either active or inactive enzyme depending whether the regulatory molecule is an activator or an inhibitor.

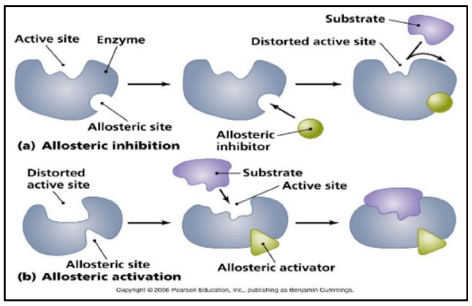
Allosteric modifiers:

1- Allosteric enzymes are regulated by different molecules (modifiers)
2- Modifiers bind to allosteric enzymes at the *allosteric site*, a site that is physically separate from the catalytic site

-modifiers can be activators or inhibitors

-A negative allosteric modifier (inhibitor) causes the enzyme to have less activity.

-A positive allosteric modifier (activator) causes the enzyme to be more active



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

-If the modifier is same as the substrate, then it called *homotropic*.(such as hemoglobin although it is not an enzyme, but it is an example of allosteric regulation that has a homotropic modifier which is oxygen)

-The changes that a modifier make are gradual(meaning there is a cooperativity which means that binding of a one molecule makes it easier for the substrate to bind ,and binding of two molecules makes easier and easier and so on.

-*positive cooperativity* is when 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

-There is also *negative cooperativity* (binding of the first modifier makes it hard to the substrate to bind and binding of the second molecule makes it even harder)

-also, in these enzymes we have the **T** (low affinity) and the R (high affinity) structures

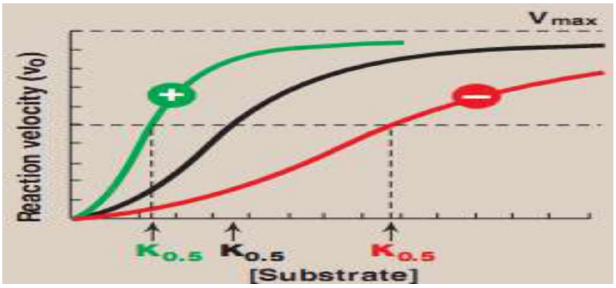
Types of allosteric enzymes

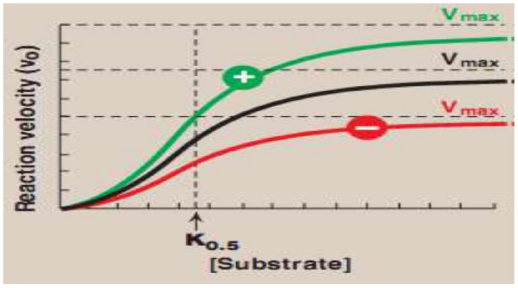
The Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes $k_{.5}$ is used instead of k_m. ($k_{.5}$ is different than K_m which is the Michaelis constant is reserved for non-allosteric enzymes)

The most important thing to notice that the shape of the plot is ***sigmoidal*** and not hyperbolic (such as hemoglobin) meaning that there is cooperativity

There are two systems :

1- V system : we have reduction on the v max but there is no change on K0.5 which is used as a reflection of the affinity of allosteric enzyme
2- K system: v max is still the same but $k_{.5}$ is different





NOTE: Doctor Mamoun put this massage on the E learning

Important correction:

In slide 36 of Lec13a: Enzyme activity: Regulation I", the slide is entitled "Types of allosteric enzymes", switch the names of the plots "K system" and "V system"
In the sheet they are switched according to this massage

GOOD LUCK