Lecture : 21

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☐ Slide ■ Sheet

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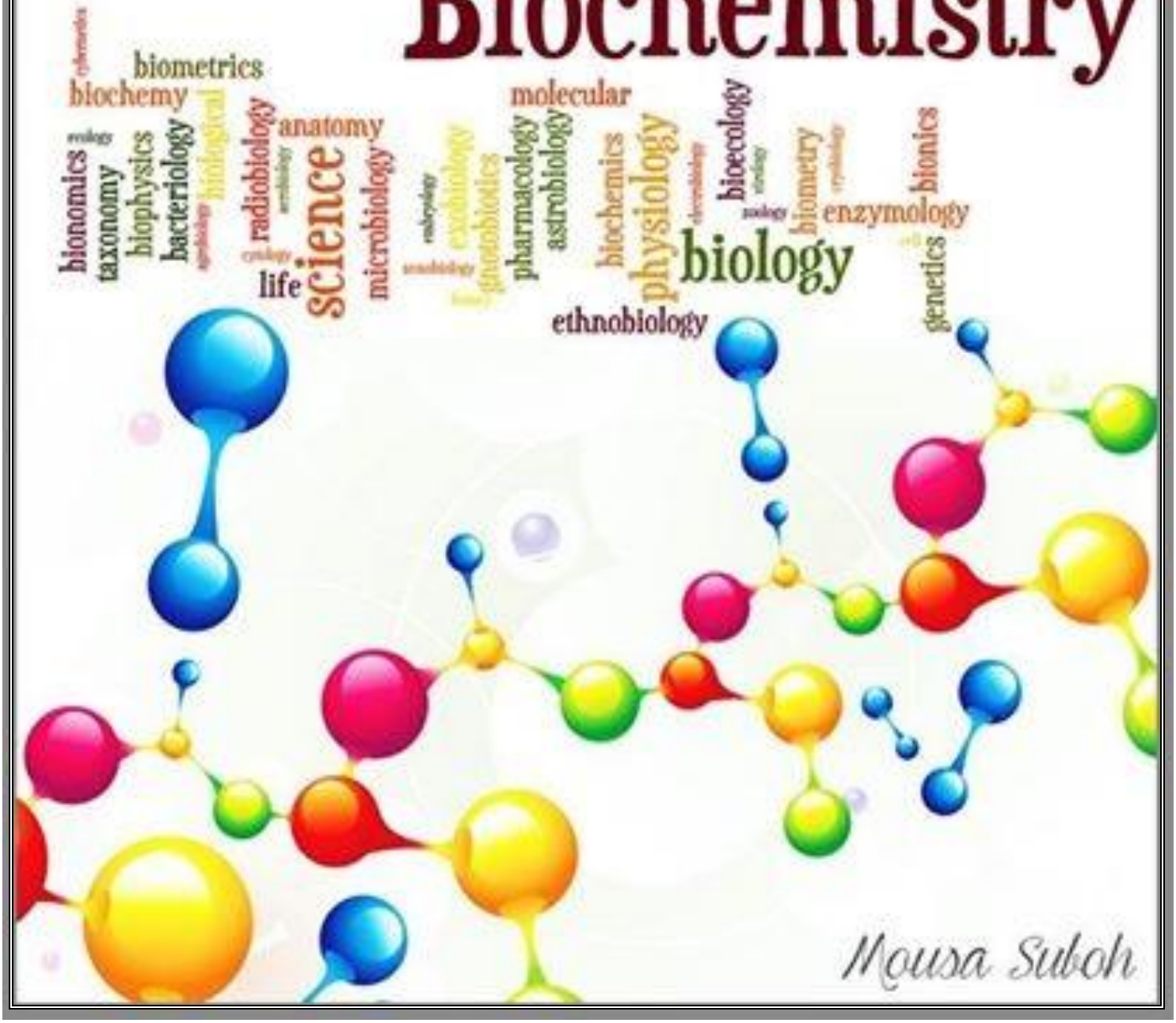
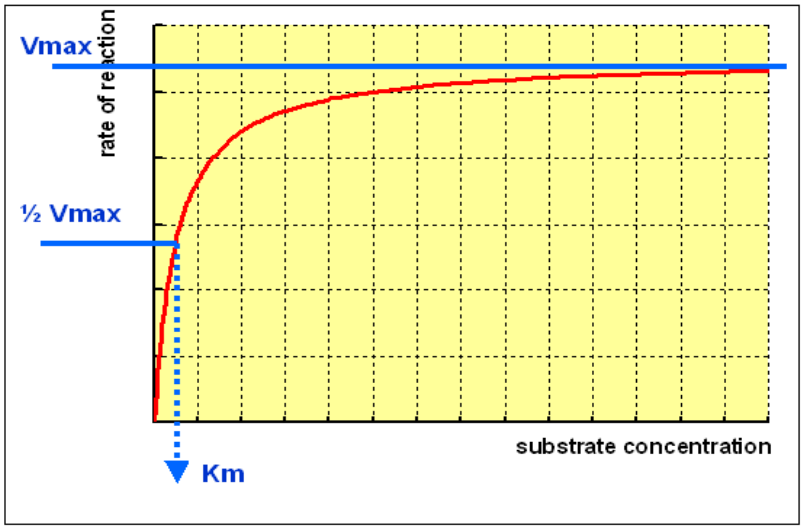
Biochemistry



Enzyme Kinetics

Vmax: The Maximal rate

- The rate of reaction when the $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$ enzyme is saturated with substrate.



-You can calculate it by the following equation:

Vmax = *k*2 [E] T

[E]T :Total enzyme concentration

- You can determine it in the graph by looking at the constant- linear state at the top.

Km : The Michaelis constant

-Describes the affinity of enzyme for the substrate

*The lower the Km the higher the affinity

- **Km is the concentration of substrate which permits the enzyme to achieve half of Vmax .**

- $K_M = \frac{k_{-1} + k_2}{k_1}$

Written by Baha Aldeen Alshraideh

Q-why is it constant at the top (in the graph) ?

Because, all the enzymes had been fully saturated, no matter how much we increase substrate it would not affect the rate of concentration.

Kcat (The turnover number) :

-Is the concentration of substrate molecules converted into product per unit time per concentration of enzymes.(the maximum number of molecules of substrate that an enzyme can convert to product per catalytic site per unit of time). -Wikipedian Definition-

-You can calculate it , by the following equation only If [E]T is known :

$$Kcat = Vmax / [E]T$$

-Another way to calculate the Turnover number is:

$$Kcat = \text{specific activity} \times \text{molecular weight of enzyme}$$

Example:

a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate

1) Kcat in (unit = min^{-1}) ?

2) How much time Each reaction takes to occur ?

1) Kcat=Vmax / [E]T

→ Kcat = 0.6 / 10^{-6}

→ Kcat = $6 \times 10^5\ s^{-1}$

Which means carbonic anhydrase can catalyze 6 x 10^5 reactions per second

Divide it by 60 to get it in minutes → $10^4\ min^{-1}$

You have 6 x 10^5 reactions per second, how much time each reaction would take?
1/ 6 x 10^5 = 1.7 μs
Each catalyzed reaction takes place in a time equal to 1/*k*2,which is 1.7 μs for carbonic anhydrase

Q-Why do we need KM ,KCAT , Vmax ?

To be able to define enzymes, classify them & make comparison between them.

Why do we need enzyme kinetic parameters? To say that this enzyme has a higher affinity to this substrate, this enzyme is more efficient than the other enzyme, if you want to synthesize an enzyme that is more efficient and potent than the normal enzyme? How would you do this? By knowing the parameters of the enzyme and then test your new enzyme and see if it's efficient or not.

Are there other kinetic parameters that people use in enzyme kinetics? Yes, one of them is the fractional

saturation, what does it mean? We test enzyme under their maximal velocity (Vmax), we take the Kcat and the Vmax conditions, we know the Km. So are enzymes saturated all the time or usually under physiological conditions? No. How much they are saturated? It's defined as the fractional saturation. When it's fully saturated you'll have the Vmax, when it's partially saturated you'll have a certain velocity. So the fractional saturation is defined by the velocity you have divided by the maximum velocity of the enzyme.

Under physiological conditions, enzymes are not fully saturated, the substrate concentration is not even reaching the Km, what is the Km of the enzyme? It's the concentration you need to reach 50% of the maximum velocity, when the substrate concentration is too low that it will not reach the concentration that will make the enzyme reaching 50% of Vmax, so when you divide the substratre concentration by Km you'll get 0.01 and 1.0 (it's usually low).

When the substrate concentration is low and you look at the Michaelis equation

Specificity constant : =(Kcat/ Km)

It has two terms, Kcat and Km, it's a ratio, the higher the ratio, it means that either Kcat is too high or Km is very low, when Kcat becomes very high? When the enzyme is highly efficient, and when Km becomes very low? When

the affinity is very high between the substrate and the enzyme (the enzyme is very specific to its substrate) so the higher the ratio the higher the efficiency, the higher the ratio also, the higher the specificity.

determines the relative rate of the reaction at low [S]

By neglecting [s]in **the addition process only**, because it's too small

We get

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{k_{cat}[E_T][S]}{K_M + [S]} \rightarrow V = (k_{cat}/K_M)[E][S]$$

** The benefit of specificity constant, that its used as an indicator for :-

Enzyme's substrate specificity	• The higher the ratio, the higher the specificity
Enzyme's catalytic efficiency	• The higher the ratio, the more efficient the enzyme

Q-Using specificity constant is better than using Kcat or Km, Why?

Kcat values vary over a range of nearly 3000
KM values vary over a range of nearly 300
(Kcat/KM), the range is only 4

Reaction rate; measures the concentration of substrate consumed (or product produced) per unit time (mol/{L.s} or M/s)

Enzyme activity; measures the number of moles of substrate consumed (or product produced) per unit time (mol/s)

Enzyme activity = rate of reaction × reaction volum

So how reaction rate and enzyme activity differ from each other?

Reaction rate measures concentration, enzyme activity measures number of moles

Specific activity; measures moles of substrate converted per unit time per unit mass of enzyme (mol/{s.g})

Purification processes (if you want to know if your enzyme is pure or not you'll measure the specific activity of the enzyme)

Specific activity = Enzyme activity / mass of enzyme

- **Turnover number**; measures moles of substrate converted per

unit time per moles of enzyme (min^-1 or s ^-1)

Turnover number = specific activity × molecular weight of enzyme

Be careful, The reaction rate concentration is mol/L , but the Enzyme activity concentration is **only moles without the volume (L)

Sample calculations:

A solution contains initially **25.0×10^{-4} mol L^{-1}** of peptide substrate and **1.50 µg chymotrypsin** in **2.5 mL** volume. **After 10 minutes, 18.6×10^{-4} mol L^{-1}** of peptide substrate remain. Molar mass of chymotrypsin is **25,000 g mol^{-1}**.

peptide substrate consumed = 6.4×10^{-4} mol L^{-1} in 10 minutes
Rate of reaction = **6.4×10^{-5} mol L^{-1} min^{-1}**

Enzyme activity = 6.4×10^{-5} mol L^{-1} min^{-1} x 2.5×10^{-3} L
(rate × volume) = **1.6×10^{-7} mol min^{-1}**

Specific activity = 1.6×10^{-7} mol min^{-1} / 1.50 µg
(activity / mass) = **1.1×10^{-7} mol μg^{-1} min^{-1}**

Turnover number = 1.1×10^{-7} mol μg^{-1} min^{-1} x 25,000 g mol^{-1} x 10^{6} µg g^{-1}
(sp. act. × molar mass) = 2.7×10^{3} min^{-1} = **45 s^{-1}**

The disadvantage of Michealis-Menten equation & Lineweaver-Burk or double – reciprocal plot:

Q- Experimentally it is too difficult to determine Vmax, ,Why?

Because, whenever we increase the substrate concentration, the velocity will keep increasing, unless we add a large amount of substrate until it stops increasing, & that's not efficient because it's costly and time consuming.

-- And because we can't major Vmax Experimentally we can't major Km –

$$v = \frac{Vmax\ S}{K_m + S}$$

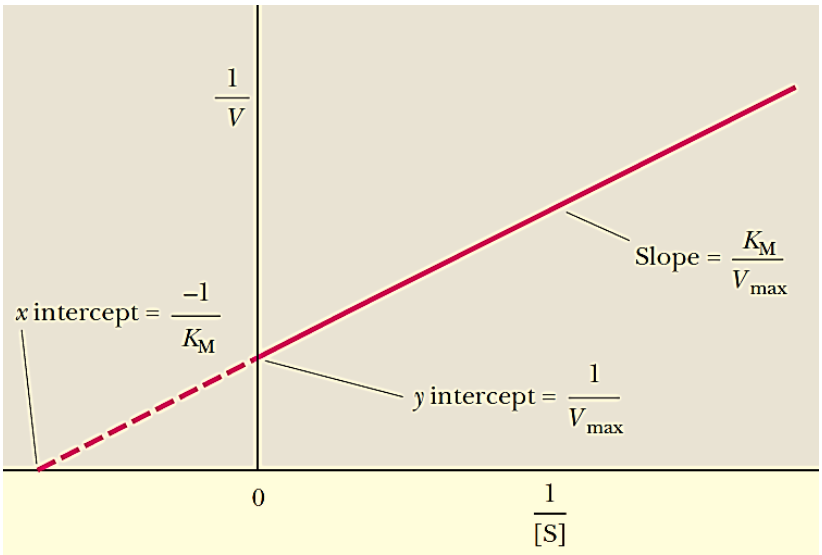
***** This equation Is a non-linear equation so, it's hard to draw it on a graph .***

Lineweaver-Burk equation:

$$\frac{1}{v} = \left[\frac{K_m(1)}{V_{max}[S]} + \frac{1}{V_{max}}\right]$$

Q-How did they come up with this equation?

In a simple way by dividing 1 over the V equation & that led to have a linear equation can be shown on the graph easily.



-Lineweaver-Burk plot: A plot of 1/v0 versus 1/[S] (double reciprocal plot), yields a straight line with an :-

- Y-intercept of 1/Vmax
-X-intercept of -1/*K*M
-slope of *K*M/Vmax

*Now a days scientist don't use this equation, because of technology.

** But still, they teach it in text books and slides.

Example one :-

A biochemist obtains the following set of data for an enzyme that is Known to follow Michaelis-Menten Kinetics. Approximately ,Vmax of this enzyme is___ & Km is ___?

Substrate concentration	**initial velocity**
1	49
2	96
8	349
50	621
100	676
1000	689
5000	699

A-5000 & 699 B-699 & 5000 C-621 & 50 D- 94 & 1 E-700 & 8

Answer : E

Explanation: you can solve it by using many equations but it's a difficult way and it takes a lot of time

You should think of another way, look where the velocity is not changing although we are increasing the substrate concentration it's 700. Km = the substrate concentration when Vmax is halfed so we have Vmax which is 700 it's half is 350 we look at the substrate concentration is 8. So the answer is 700&8

Example two :-

You are working on the enzyme "medicine"which has a molecular weight of 50,000 g/mol. You have used 10 micrograms of the enzyme in the experiment and the results show that the enzyme converts 9.6 micromole per min at 25 c . the turn-over number (Kcat) for the enzyme is :

A- 9.6 S^-1 B- 48 S^-1 C- 800 S^-1

D- 960 S^-1

Answer: C

Enzyme regulation

1- Isosymes:-

Q-What does "Isosymes" mean ?

-They are enzymes that catalyze the same catalytic reaction.

-They are a little bit different in their amino acid sequence. You are changing 4 amino acids in the whole enzyme structure.

-That means you need to have different codon and different gene to synthesize the different isosymes.

Q-Why do you need Isosymes (many enzymes catalyzing the same reaction)?

If you change one amino acid in the active site you'll change the affinity, which means you will change the Km, Vmax and Kcat of the enzyme (you are changing the parameters of the enzymes). The second thing: you can play with the regulation of the enzyme, if a material (inhibitor or activator) binds to the structure of the enzyme and you change an amino acid at that binding site, then the material can't bind or it'll bind with lower affinity to enzyme and the effect of this inhibitor or activator on this enzyme is totally different and this is what happens in isoenzymes.

Examples on Isosymes:- hexokinases

It has 4 types, we'll talk about 2 types: I and IV

Hexokinase I is found in RBCs while type IV which is called glucokinase is found in liver and pancreas. Small change in amino acids affects in the Km, for type one it's 0.05 mM, while it's (the Km) in type four 5 Mm which is a hundred folds increase in the Km, which means that type I is more specific for its substrate than the other type (liver and pancreatic one)

Q-Why do RBC's need a much higher affinity to bind with glucose in comparison with liver?

In RBC it's essential for them to take glucose all the time, to make them stay alive and maintain their function which is carrying oxygen, so if the blood glucose dropped below the normal fasting levels it will stay alive and not affected by the drop.

The normal fasting level of glucose in blood is 5mM

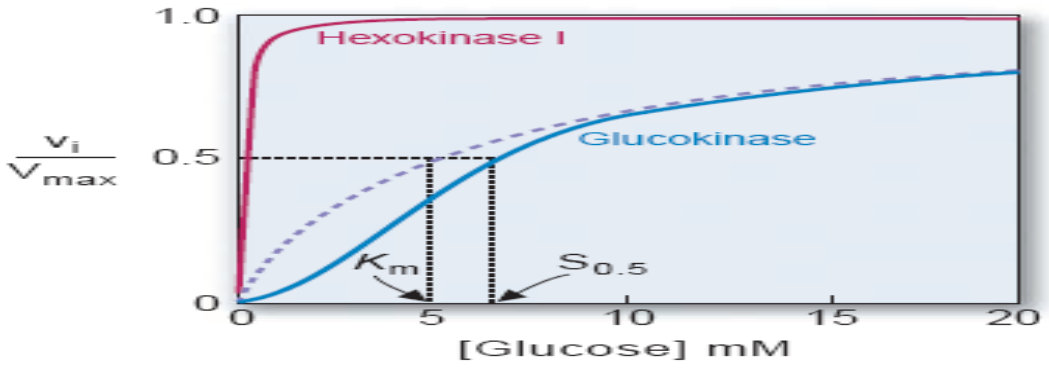
When the Km of the hexokinase in RBCs is 0.05mM which is a hundred folds less than what is present in the blood which means all the time RBCs can obtain glucose from the blood and catalyze the reactions.

The second thing: If the glucose level in the blood goes below the fasting level (5mM), still RBCs can obtain

glucose because it's 100 hundred folds less in Km. When you are at the fasting level, why liver needs glucose? For glycogen storage, do you need to store glucose when it's deficient or when it's in excess? The answer is it'll be in excess, so if it goes above 5mM, which is the Km of the glucokinase (IV), you'll start storing it, if it's not you won't store it.

Note: in Hexokinase, the product which is glucose-6-phosphate works as competitive inhibitor for the enzyme, in glucokinase which is hexokinase, a small change in amino acids won't allow the product to bind the enzyme so it won't work as competitive inhibitor,so the enzyme will keep working all the time which mean if you have more and more glucose the cells in the liver will keep taking glucose from the blood.

The pancreas works as a sensor for glucose in the blood, it'll start secreting insulin when glucose level is high, so glucose will enter cells. If glucose level was below 5mM in the blood, we don't need glucose to enter cells, so insulin won't be released, that's why the glucokinase is present in liver and pancreas.



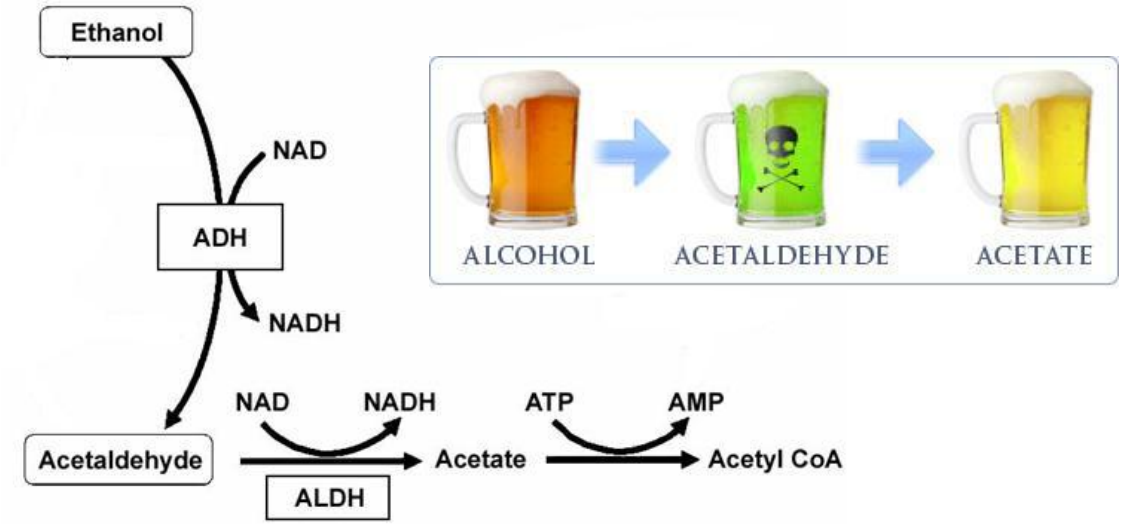
Another example it's lactate dehydrogenase, it converts pyruvat to lactate and lactate to pyruvate in a reversible manner. We have 5 different isozymes, we'll take about 2, it's a tetramer, composed of 4 polypeptide chains: if they are all blue we call it H form referring to h Heart, if they *are all red we call it M referring to the Muscles, and there are different combinations between them, you have 3 blue and 1 red, or 2 blue and 2 red and so on, this is for tissue localization, each one works in a tissue, comparison between the Heart and the muscles lactate dehydrogenases, both of them are lactate dehydrogenases, however because their parameters are different they'll be different in their function. In the heart, you do not need lactate, instead you need pyruvate to go in the aerobic respiration. In the muscles, you can't produce lactate, so anaerobically, you can consume lactate, so how to make the enzyme behaves differently in different tissues? The one in the heart has low Km for pyruvate and the one in the muscles has higher Km for pyruvate, this is how they're different through a little change in their amino acids sequence.

Another example: why people are different in their sensitivity to alcohols? When you consume alcohol (ethanol for example) it'll be converted to what is called acytaldehyde through the enzyme alcohol dehydrogenase, and acytaldehyde will be metabolized through aldehyde dehydrogenase to acetate and acetyl CoA, the material

which is responsible for alcohol toxicity is the acytaldehyde (causes dizziness)

-The affect of alcohol on people differs from one person to another.

-It may cause many symptoms such as **tachycardia.**



*People take up Alcohol as Ethanol, it goes to blood and it end up in the cells, then its converted to Acetaldehyde by ADH (Alcohol dehydrogenase) within the cells ,after that Acetaldehyde is converted to Acetate by ALDH(Aldehyde dehydrogenase).

****There is two types of ALDH:-**

- ➔ Mitochondrial: (low *K*m) –High affinity
- ➔ Cytosolic (high *K*m) – low affinity

Sensitive people have mutation in the mitochondrial one, which makes it less active & has a less affinity towards Acetaldehyde, and that will lead to the start of accumulation of Acetaldehyde in the cytosol and the escaping to the blood , causing the toxicity of Alcohols.

Inhibition:- it's either reversible or irreversible

Irreversible:- termed as mechanism baseded inhibition, why? Because it'll come to the active site and start doing covalent bonds with that active site, causing no dissociation from this inhibitor from the active site and at the end no reaction would occur.

It has something to do with the mechanism either from the initial binding or formation of transition state

Some inhibitors will bind at the beginning and cause irreversible inhibition for the enzyme through covalent bonds, others will mimic the shape of the transition state (we said before that the transition state has higher affinity to the active site that the substrate or product have), so if you design a drug or a toxin that can bind the enzyme with a higher affinity that the substrate and product finally no product is formed.

The first thing is heavy metals which are not specific however they can bind enzyme start downing bonds in the active site and then with no dissociation causing irreversible inhibition.

Covalent inhibitors:

-The lethal compound [DFP]: it's lethal compound

is an organophosphorus compound that served as a prototype for:

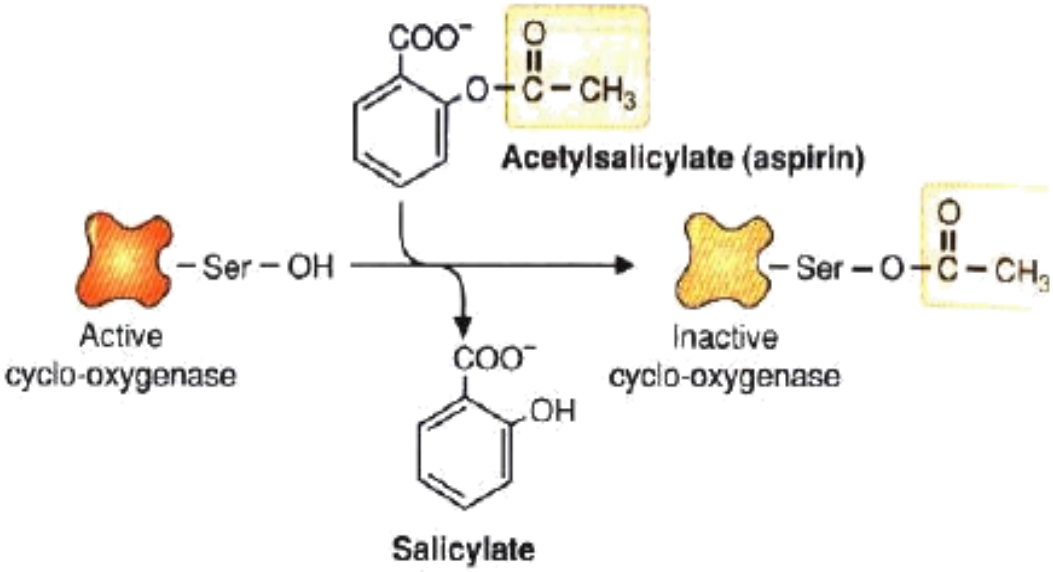
→The nerve gas sarin & it was used in the first time in Japan and lately in Syria

→The insecticides malathion & parathion

All these compound are designed to bind covalently with the serine amino acid within the active site and not breaking away from it and that will lead to the inactivation of the enzyme.

-DFP and other related materials can also inhibit any serine proteases (chymotrypsin), so they can inactivate this enzyme irreversibly, however it's not lethal to inactivate chymotrypsin but it's lethat in activating other enzymes. Why they are lethal? Because they binds certain enzymes but which enzymes? Certain enzymes in the nervous system and serine containing enzymes in the active site they come and bind it like acetylcholinesterase, the idea that acetylcholine will be released from the nerves and it bounds with muscles as it binds to the muscles there will be contraction, acetylcholinesterase will come and break down acetylcholine causing relaxation in the muscles but if we inhibit acetylcholinesterase, acetyl choline will stay bound and causing compulsions to the muscles, consuming all the ATPs causing fatigue in the muscles. It's not lethal in the skeletal muscles but it's lethal in the diaphragm causing respiratory arrest.

-Aspirin (acetylsalicylic acid): it can inactivate the cyclooxyganse enzyme, how? Through causing a covalent bond between acetyl group and serine in the active site.



-Transition-state analog:

-Design a material that mimics the transition state of the enzyme, which has a higher binding affinity to the enzyme than the substrate or the product, however in cannot go in the reaction so you stop the enzyme's function, after binding with the transition state the enzyme cannot work after binding with it, we call these inhibitors suicide inhibitors (enzymes commit suicide after binding).

You cannot synthesize a material like transition state, why? Because it's very unstable

Example on Transition-state analog:

→**Penicillin:** an inhibitor which contains a peptide bond but its not a protein also it has a ring which is called **β-lactam ring.**

-Enzyme will bind in to it thinking that it's a protein.

-The peptide bond will be broken and on the other hand a covalent strong bond will be created between the enzyme and Penicillin.

-The enzyme will be inhibited and that will result in affecting the bacteria wall leading to its death.
Bacterail begin to synthesize an enzyme called **β-lactamase.**

→ **Allopurinol :**
- A drug used to treat gout (النقرص) The Kings disease, because they eat a lot of meat, which contains a lot of Nitrogen (amino acids).

- Guanine is converted to xanthine, & Adenine is converted to hypoxanthine then to xanthine .
-Xanthine can be oxidized by xanthine oxidase, creating uric acid.
- Uric acid it's not soluble so it gathers in joints, crystallizing there, causing an attacks of pain, most common places for the crystals are in the toe.

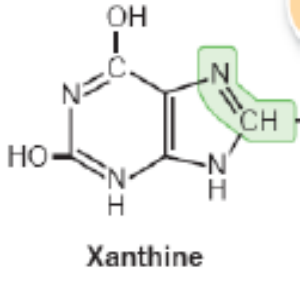
Q- What does Allopurinol do?

-Decreases urate production by inhibiting xanthine oxidase

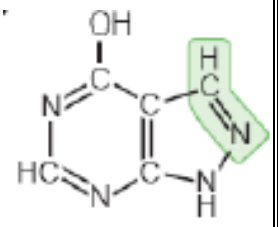
-The enzyme commits suicide by converting the drug to a transition state Analog .

Q- what is the difference between the Xanthine structure & Allopurinol Structure ?

They differ in the Carbon & Nitrogen atoms.



Xanthine



Allopurinol

Made by: Baha Aldeen Alshraideh

Pressure makes Diamonds