Subject :	**Kinetics2, Enzyme regulations**
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Number :	16

Kinetics

*** What are the enzyme parameters?**

- Properties of the enzyme related to its activity, such as:

V_{max}, enzyme activity (=rate of reaction × reaction volume), specificity constant ($=k_{cat}/K_M$), K_{cat} ($=V_{max}/[E]$ = the turnover number = specific activity × molecular weight of enzyme).

<u>Disadvantage of Michaelis-Menten equation</u>

- ✓ The reaction is "a matter of probability"; which means that a lot of experiments have to be done, with additional costs, efforts and manpower.
- ✓ Drawing the slope is another obstacle "in the past"; since it is not linear and results with reduced accuracy.
- ✓ Determining Km is not accurate since large amounts of substrate needed to know the Vmax and therefore the Km

<u>The solution:</u> turn the equation upside down! (take reciprocal of the whole equation)

- ✓ This results with a linear equation, which is easier to deal with:
- ✓ 1/V is plotted on the Y-axis while 1/S is plotted on the X-axis

$$v = \frac{V_{max} \cdot [S]}{[S] + K_m}$$

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

where: $1/V_{max}$ is the y intercept
K_m/V_{max} is the slope

- ✓ Linear equations need 2 experiments to have the curve drawn

This method is still being taught, but it is not as essential as it was in the past; because of the use of the computers, which make drawing curves an accurate and easy step.

Enzyme Regulations

Enzymes control all metabolic pathways and processes in the body, which makes their regulation an essential thing.

Modes of regulation

These modes are specific for certain enzymes, except for "non-special regulators", which can apply to all enzymes.

1- **Isozymes**

* They are enzymes which catalyze the same reaction of the "regulated enzyme"

* They are not structurally identical to each other; because having the same amino acid sequence means having the same tertiary structure, and so the same function. So they are different in several amino acid due a slightly different in the gene expression, and so have different parameters "ex. K_m"

Tissues and organs of the body are different, but have the same types of enzymes working in them, so they have to have different functions in every tissue, which is achieved by changing the parameters.

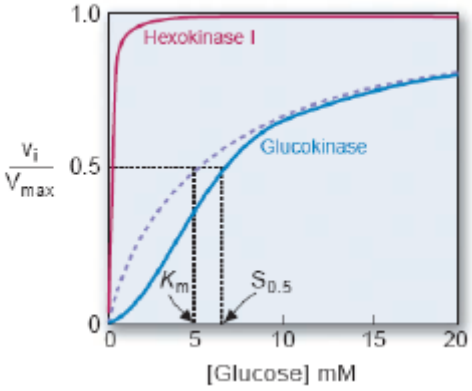
Examples:

- ✓ Hexokinases: group of enzymes, which **transfer** a phosphate group "phosphorylate" to hexoses "glucose". This allows "trapping" glucose inside the cell, so it can be used by the cell.
  Hexokinase 4 is also called glucokinase "liver, pancreas". Hexokinase 1 presents in RBCs.

  <u>Functional differences:</u>

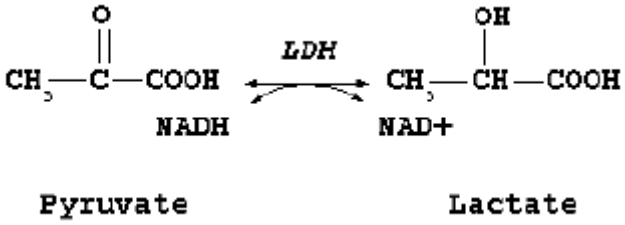
  RBCs: they need glucose for energy in high amounts, because in RBCs there are no Mitochondria, so the cell gets its energy only by glycolysis. When RBCs have enough glucose, its phosphorylation has to be stopped; since there is no need to keep trapping glucose inside the cell.

  Liver and Pancreas: Liver stores glucose in the form of glycogen, and the Pancreas monitors glucose levels in the blood, and releases glucagon when glucose levels drops, and releases insulin when glucose is plenty. So these organs have to trap glucose constantly.



Hexokinase 1 "in RBCs" has a K_m=0.1 mM, whereas HK4 in the Liver and Pancreas has a K_m=10 mM "100 folds", which means that HK1 has higher affinity for glucose, as fasting glucose level is 5-7 mM, which makes glucose enter the cells. High K_m of hepatic glucokinase promotes storage of glucose, and Pancreas works as a sensor.

- ✓ Lactate dehydrogenase

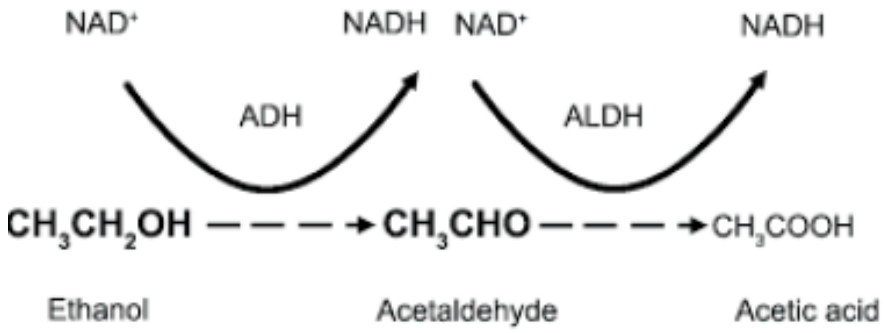


Lactic acid is produced by anaerobic respiration in skeletal muscles, causing fatigue. Lactate dehydrogenase is common between tissues, but has specific features in each one. It has 2 types of its subunits: M and H.

Functional Differences:

Heart muscle does not produce lactic acid because they do not respire anaerobically, even though it works all the time. However, skeletal muscles produce lactic acid. This functional difference is made possible by the small difference in the AA sequence of the enzyme in these two locations. In the heart, the enzyme has 4Hs, but in the skeletal muscles, it has 3Ms. K_m in the heart is so much higher than that in skeletal muscles, which means that the affinity in skeletal muscles is higher. In addition, pyruvate binding inhibits the enzyme in the heart "substrate inhibition", which makes the forward reaction inhibited, with the result the backward reaction is encouraged, turning all

lactate in the heart into pyruvate.



- ✓ **Aldehyde dehydrogenase (ALDH)**

People worldwide differ in their tolerance to alcohol toxic symptoms. Chinese and Japanese rapidly show such symptoms, which include Flushing response and Tachycardia.

Alcohol pathway: Alcohol is turned into acetaldehyde, which is then turned into acetic acid, by aldehyde dehydrogenase. This enzyme is present in 2 places in the cell; in the cytosol with high K_m value "low affinity", and in the mitochondria with high K_m value "high affinity", where the enzyme takes all the acetaldehyde inside.

Acetaldehyde is toxic, and responsible for the symptoms discussed. Chinese and Japanese have a mitochondrial ALDH which is not expressed or highly mutated. This leads to acetaldehyde being accumulated in the cytosol, and in the blood, spreading it all over the body.

2- **Inhibition:**

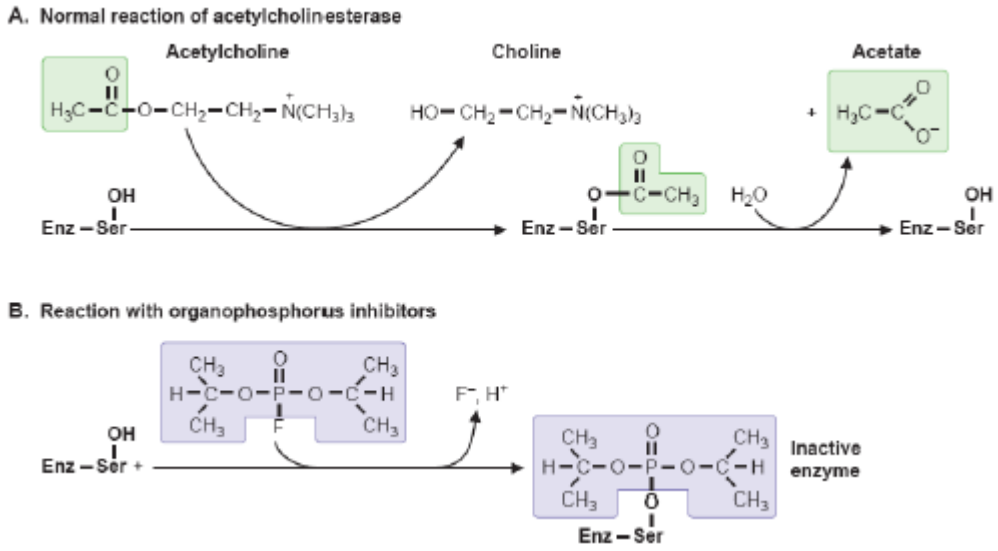
Inhibition can be reversible or irreversible. In this section, we are going to talk about irreversible inhibition mechanisms.
Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction. The term includes: Covalent inhibitors, Transition state analogs, Heavy metals.

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

- **<u>Covalent inhibitors</u>:** Covalent or extremely tight bonds with active site amino acids. Amino acids are targeted by drugs & toxins.
  DFP is a huge organophosphorus compound that served as a prototype for manufacturing the nerve gas sarin, the insecticides malathion and parathion.

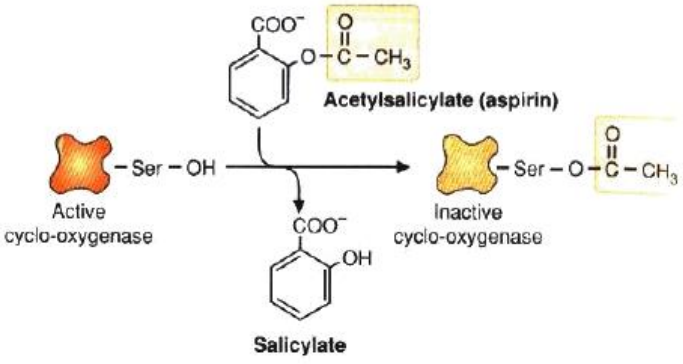
  You are supposed to know which enzyme these materials inhibit, and with what effect they result.

✓ **Sarin:**



Acetylcholine is released by the nerve, and leads to muscle contraction, and then is broken down by acetylcholineesterase into choline and acetate, which causes relaxation of the muscle. Serin, malathion and parathion all bind the active site of acetylcholineesterase, preventing relaxation, and resulting with full body fatigue and respiration arrest "caused by diaphragm tetanus".

✓ **Aspirin:**



COX enzyme is inhibited permanently by aspirin. This leads to enzyme loss, so

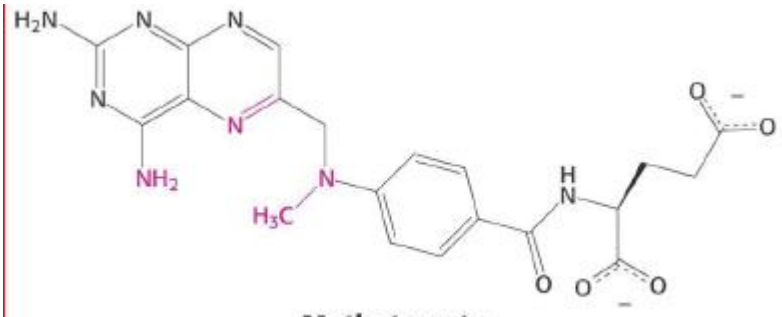
a long period has to be waited until the enzyme is renewed. COX is related to prostaglandins and thromboxane's production. So aspirin dose leads to stop what those compounds do of blood clotting, inflammation, swelling, etc.

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

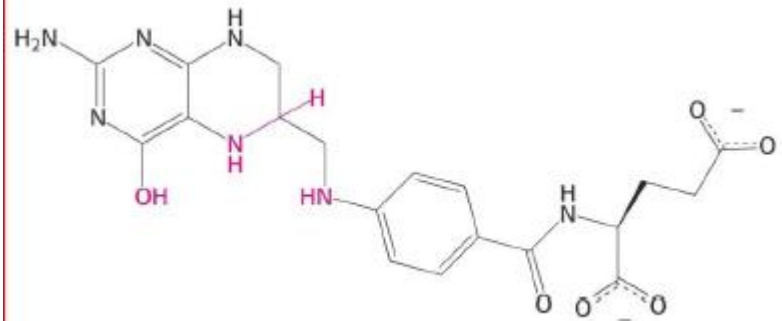
You've got to know that the transition state analog binds more tightly to the active site than does the substrate. So, the enzyme's affinity towards their binding will be higher than its binding to the substrate. Transition state analogs are synthesized materials which resemble a conformation similar to the transition state "transition state itself cannot be manufactured; it is highly unstable", and have modifications which stop the reaction. Those inhibitors are known as suicide inhibitors; since they bind to the enzyme so tightly, and the enzyme after that will not be able to perform its function anymore.

✓ **Methotrexate:**

It is a synthetic inhibitor, an anticancerous, and an analog of tetrahydrofolate "notice how similar they look to each other", which is the substrate of thymidylate synthase enzyme.



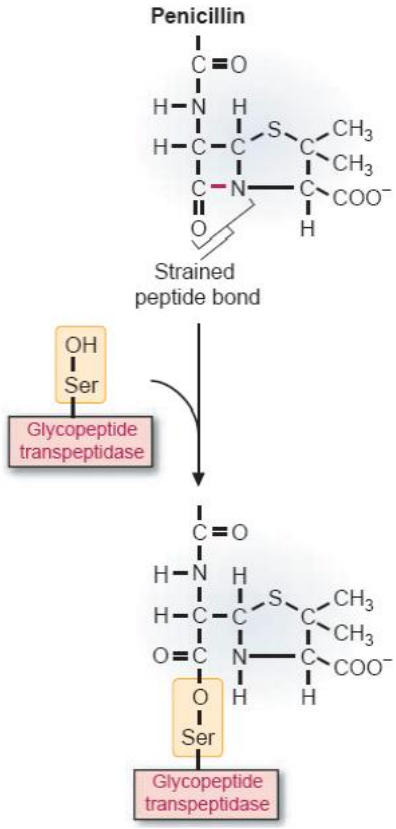
Methotrexate



Tetrahydrofolate

Folic acid is important for DNA and RNA formation, so when the enzyme is inhibited, no DNA and RNA can be synthesized, and so the cancerous cells will die.

✓ **Penicillin**

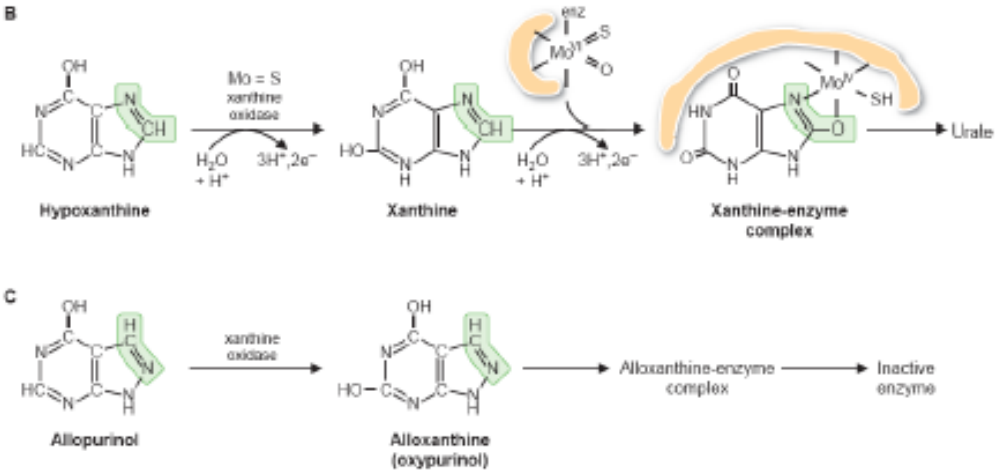


It is an antibiotic, and a transition-state analog to glycopeptidyl transferase or transpeptidase, which is important for bacterial cell wall formation, by breaking some peptide bonds. Penicillin inhibits the enzyme, resulting with no cell wall formation, and the bacteria die. Penicillin has a ring in its structure, which has a Nitrogen atom bound to a carbonyl group, which is similar to the peptide bond, and so it can bind the enzyme, which, after binding, will not be able to break free.

✓ **Allopurinol**

Gout is a disease accompanied by joints pain, and caused by eating a high meat diet, which contains huge amounts of proteins, which are rich in AAs, and AAs are the body source of Nitrogen. But the real cause of the disease is neither the proteins nor the amino acids. The Nitrogen is used to synthesize the nucleotides of DNA and RNA, which causes the accumulation of urate.

To stop that, Allopurinol decreases urate production by inhibiting xanthine oxidase. The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions. Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, a compound that binds very tightly to a molybdenum–sulfide complex in the active site.



✓ **Heavy Metals**

They may bind reversibly or irreversibly. They are relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity.

- Mercury: binds to so many enzymes, often at reactive sulfhydryl groups in the active site. It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity. It inhibits some of CNS enzymes.
- Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc. It reversibly inhibits heme groups, but irreversibly competes with Ca in the enzyme in the CNS. 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. Lead was common in the past in paintings manufactures, which made it dangerous for children, who may eat it when it deposits on the floor.

3- Conformation ---- to the next lecture
4- Amount ---- to the next lecture
5- Non-specifically ---- to the next lecture

Good Luck and sorry for any mistakes ☺