Lecture :..17 (2-After Midterm)

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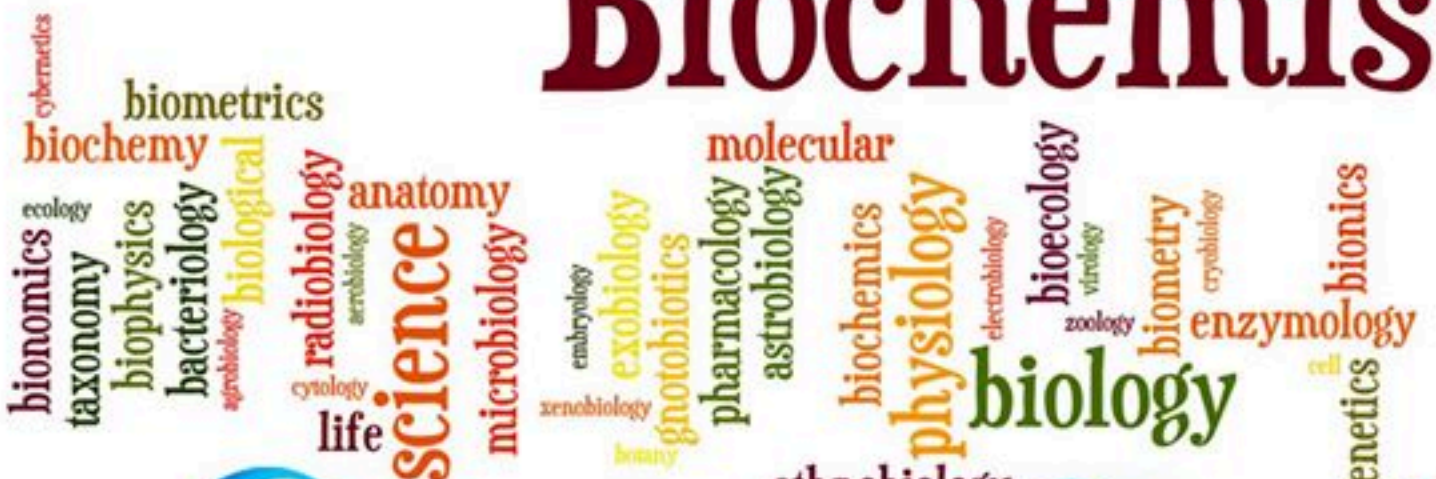
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

Biochemistry



Enzymes (2)

In the previous lecture we talked about the general properties of proteins, enzymes and their two features:

1- Affinity 2- specificity of ligands to proteins.

-Why do we name it ligand?

ligand comes from the verb "ligate", which means to bind/ unite, so the substrate that binds to the protein is named ligand.

- We also talked about the exception of enzymes that are not proteins (ribozymes), what the catalytic activity means, what rate means and how are they different from chemical catalysts. We also mentioned thermodynamics theory and how it explains enzymatic function, and kinetic theory which also explains enzymatic functions.

.Rate does not mean thermodynamics; rate *DOESN'Tmeans **ΔG, ΔH**, or **ΔS** -

- Enzymes decrease the activation energy without affecting the starting or final state resulting in fixed **ΔG** (they do not change ΔG).

- How to express an enzymatic reaction?
Enzymes bind to substrates forming enzyme-substrate complex (the first transitional state), and then moving to enzyme-product complex (the second transitional state), the reaction finishes by separating the enzyme-product complex into enzyme + product.

$$E + S \leftrightarrows ES \leftrightarrows EP \leftrightarrows E + P$$

- Active site:

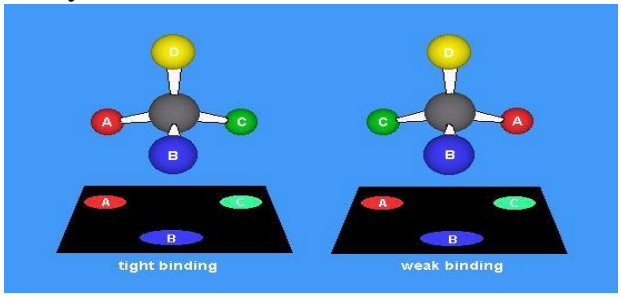
- Where ligand binds.
- Site of reaction.
- Where some regulation occurs.

- It's usually smaller than the size of the enzyme itself.
- Its shape is either fixed or changes upon reaction.
- Lining of the active site usually has multiple forms of secondary structure.
- The most important feature to mention is that it is pocket/canal shaped, lined by amino acids, and the R groups are projecting towards the pocket.
- Has a 3-D shape composed of different amino acids not in sequence. This is how the variability of active site occurs.
- The substrate binds to the active site by weak non-covalent attractions, ionic attractions, and hydrogen bonding.
  -Why do we need them to be weak? In order for the attraction to be reversible, to bind and dissociate.
  -Strong covalent interactions won't dissociate, stopping the enzyme from working, examples: poisons, fatal pesticides, nerve gases used in wars.

- Within the active site, sometimes there are two sub-sites:
1- Catalytic site - as the name implies it is for the catalysis, where the reaction occurs, usually polar.
2- binding site (recognition site) - where the substrate binds tightly, usually hydrophobic.

Often we find the catalytic site is the same as biding site, where same amino acids are responsible for binding and catalysis.

- Substrates bind the active site by at least on 3 points to insure the issue of chirality, if the enzyme has two or one binding site it won't be able to work only on D or L isomers.



-As we mentioned, the active site is usually small compared to the whole enzyme, why do we need the rest of enzyme?

1- To stabilize the structure of the active site.
2- To create more flexible modification of the active site due to the other amino acids.
3- To provide the space for other materials to bind in order to regulate the enzymatic function. (Regulatory sites)

- How do enzymes work?

(A) Key and lock theory:

the shape of the substrate is a 100% fit to the active site, but this theory failed and cannot be applied to all active site because some enzymes work on several substrates and due to the newer techniques in visualizing the protein's motion during a reaction which proved that the structure of proteins in general and enzymes specifically are flexible and not that rigid, there is always rotation around bonds and they can move a little bit, eg: hemoglobin and myoglobin.

(B) Induced-fit theory:

As the name implies that the perfect fit is after binding (induced), the substrates bind at first due to a certain degree of complementarity with the active site; however this complementarity will be much higher after binding.

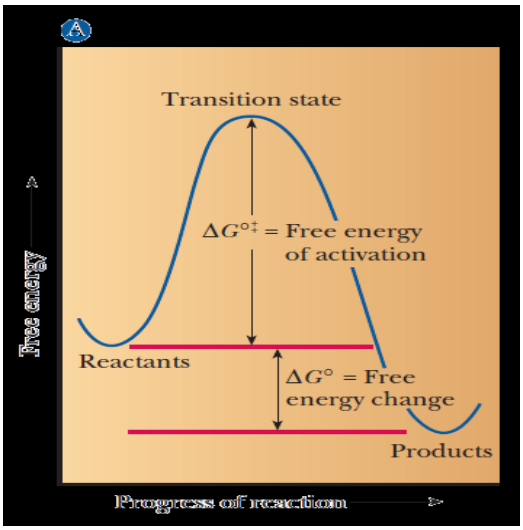
Example: the enzyme glucokinase, kinase means adding a phosphate group to the substrate, and from the name we can know that the substrate is glucose forming glucose-6-phosphate.
-The most common donor of phosphate in our body is ATP.

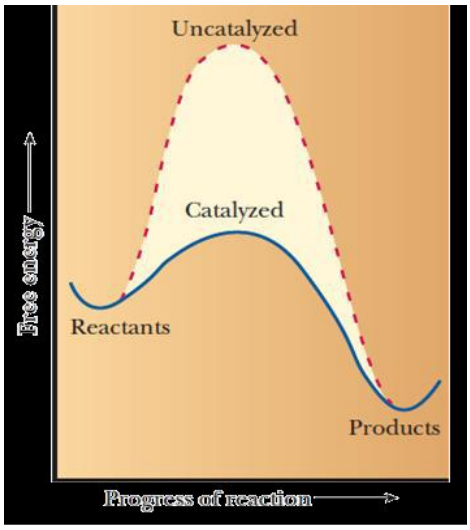
Glucokinase changes the active site's shape before and after binding with phosphate, why?
1- To insure better binding, reaction, and enclosing (fit).
2- To modulate the reaction; glucokinase binds glucose and ATP. When it changes shape it provides a better binding site for ATP.

In this example, the cleft/canal introduced by the active site is filled with water, enzymes are in an aqueous solvent, so when the shape of active site changes, water would be excluded because water can interfere with the reaction.

- How do enzymes work from the energy aspect?

By reducing the activation energy (energy needed to reach transitional, unstable state). The transitional state has a much better fit than the substrate to the active site.





- Enzymes provide a place to bring the reactants closer that's why we call the first effect that enzymes do: **proximity effect.**

After the initial binding, the substrate will orient itself (**orientation effect**) within the active site in the best binding fit, this is induced by the amino acids that constitute the active site.

Example: a hydrophobic pocket site will bind with the hydrophobic part of the substrate (puts it in the best place).

The second thing the active site does is orientation effect, after that it will catalyze the reaction; this is called the **catalytic effect** (induced by **polar amino acids**)

The last effect is the **energy effect;** which means after binding it will change the energy within bonds within substrates (making it

weaker which means higher energy). Changing in energy of the substrates makes the bonding weaker.

- To sum up with, the enzyme works by four effects:
1- Proximity effect.
2- Orientation effect.
3- Catalytic effect.
4- Energy effect.

- Let's take a quick example:
Chymotrypsin: a protease enzyme that is found in the intestine, secreted by pancreas to pancreatic duct then bile duct reaching the duodenum.
Chymotrypsin works on basic pH, inside the active site we have a hydrophobic pocket, and also we have polar and charged amino acids that help in the catalytic effect. We will talk about the details of its working mechanism later on.

- How to name enzymes?
**note:* whenever you see the suffix (-ase) then it's definitely an enzyme.

Usually enzymes are named according to their substrates, followed by the type of reaction, ending with (-ase), for example: as we mentioned earlier, glucokinase. (Substrate ⟶ glucose, type of reaction ⟶ kin**ase**; adding phosphate)
In this type of naming, two enzymes cannot share the same name.

Some enzymes have common names that provide little information about the reactions that they catalyze, eg: trypsin, pepsin, chymotrypsin.

Another way of naming is **EC-numbering:**
Each enzyme has four digits; the first digit is for the major enzyme class, the second digit for the sub-class and further sub classification.

**note: we are required to memorize the first digit with its 6-types. These 6-major classes are numbered from 1 to 6, so in the exam we should be able to recognize for example number 3 stands for what.*

What does this method of naming depend on?

To answer this question, take this enzyme for example: tripeptide aminopeptidase

"EC 3.4.11.4"

Number 3 means it belongs ~~to~~→ Hydrolases (enzymes which break off their substrates through the addition of water).

All macromolecules break down by the addition of water because they are all made by a condensation reaction.

EC 3.4: hydrolases that act on peptide bonds

EC 3.4.11: hydrolases that cleave off the amino-terminal amino acid polypeptide

EC 3.4.11.4: cleave off the amino-terminal end from a tripeptide.

In this way of naming, two enzymes can share the same number although they are different, because it describes a reaction and not specific for the substrate.

How do we divide enzymes? First, we should group it according to common features; secondly we classify them according to differences between them.

So, enzymes are classified according to:

1- Function. 2- Structure (simple or conjugated)

Simple enzymes can catalyze the reaction depending only on their amino acid sequence, whereas conjugated enzymes should have a non-protein part that binds to them in order to work.

In conjugated enzymes, if the non-protein part (co-factor) is bound to the enzyme it's named: **holoenzyme.** If the co-factor is out the enzyme, it's named: **apoenzyme.**

** Note: the apoenzyme is not functioning, the holoenzyme is functioning.*

-Enzyme classification according to function: (6-major types)

1- Oxidoreductases.

2- Transferases.

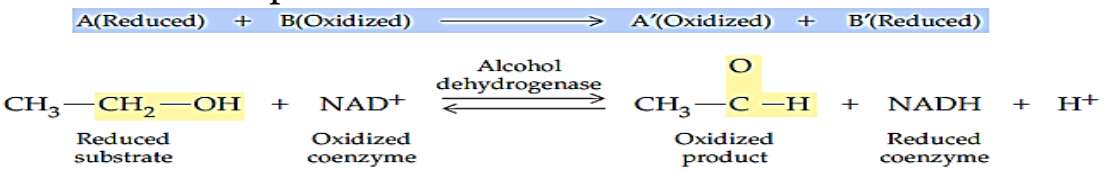
3- Hydrolases.

*4- Lyases.

*5- Isomerases

6- Ligases.

- Oxidoreductases: enzymes that do oxidation-reduction reactions. Whenever a material is oxidized there should be a reduced material, that's why in these reactions you must have at least two reactants and two products.



-Transferases : transfer of a functional group from one molecule to another, they don't add it, they transfer it. By definition, in these reactions, you must have at least two reactants and two products.

-Hydrolases: like proteases and glycolases, they introduce water to break down the substrate into at least two molecules.

-Isomerases: they convert one molecule to another in the form of isomers by changing the spatial arrangement of functional groups without changing the molecular formula. By definition, in this reaction you only have one reactant and one product.

-Lyases: they add or remove functional groups including water (hydrolases are specialized for water, whereas lyases might remove or add a functional group that could or could not be water) to double bonds causing them to become single bonds.

Ligases: enzymes that unite two materials together. By definition,- these reactions you *must have at least two reactants and ONE products. Ligation reactions require a source of energy most

commonly by ATP. (Ligation → anabolism which means it needs energy).

**Note: in lyases, you are adding a functional group to unsaturated bonds (double bonds), but in ligases you are adding them to saturated groups.*

_____.

Oxidoreductases happen by addition of oxygen, removal of hydrogen, or removal of electrons. For this major class, there are four sub-types:

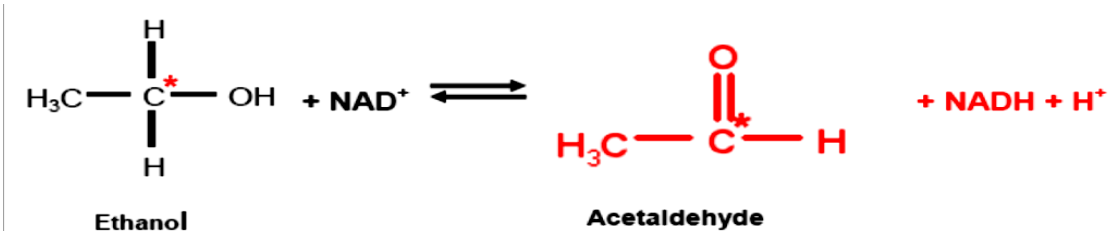
1) **Dehydrogenases**
2) **Oxidases**
3) **Peroxidases**
4) **Oxygenases**

-Dehydrogenases: take hydrogen out, and these hydrogen atoms are added to (NAD+), so in the exam whenever you read dehydrogenase you should know that one of the products is NADH.

-Lactate dehydrogenase: Lactate will be converted to Pyruvate.

Lactate + NAD+ → Pyruvate + NADH + H+

-Alcohol dehydrogenase: ethanol will be converted to aldehyde form.



-Oxidases: they also take hydrogen out (they extract electrons out in the form of hydrogens), but they add haydorgens to oxygen molecules forming hydrogen peroxide H2O2. (In all oxidases reactions H2O2 is definitely one of the products).

Example: diabetic chips, they have glucoseoxidase, through some steps you can finally know the percentage of sugar in the blood.

$$\beta\text{-D-glucose} + O_2 \leftrightarrows \text{gluconolactone} + H_2O_2$$

-Peroxidases: H2O2 is not in products like in oxidases, it's one of the reactants. They use H2O2 to oxidize materials, resulting in water.
Example: glutathione peroxidase.

$$2\,GSH + H_2O_2 \leftrightarrows G\text{-}S\text{-}S\text{-}G + 2\,H_2O$$

-**Oxygenases:** they have molecular oxygen in reactants, like oxidases, however it is not added to hydrogen, they introduce the oxygen itself to the substrate.

There are two types of oxygenases:
A- Monooxygenases; transfer one oxygen atom to the substrate, and reduce the other oxygen atom to water .
B-Dioxygenases, incorporate both atoms of molecular oxygen (O2) into the product(s) of the reaction

- They are found in our body in heme-metabolism, (heme monooxygenases, heme dioxygenases)

Note: please refer to the slides to check out the figures as we did not put a lot of them in this sheet because they will appear black after printing.