Doctor 022

Biochemistry

Sheet no. 21

Enzymes I: Characteristics and Classifications

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note: slides notes in purple, Dr. Mamoun’s explanation in black.

Revision

-Enzymes catalyze reactions by reducing the activation energy (energy needed to initiate the reaction) reaching the transitional state faster with the least possible energy amount.

-The transitional state is unstable and unfavourable so, the molecule requires more energy to keep itself intact away from breaking. (that’s why G is high during the transitional state)

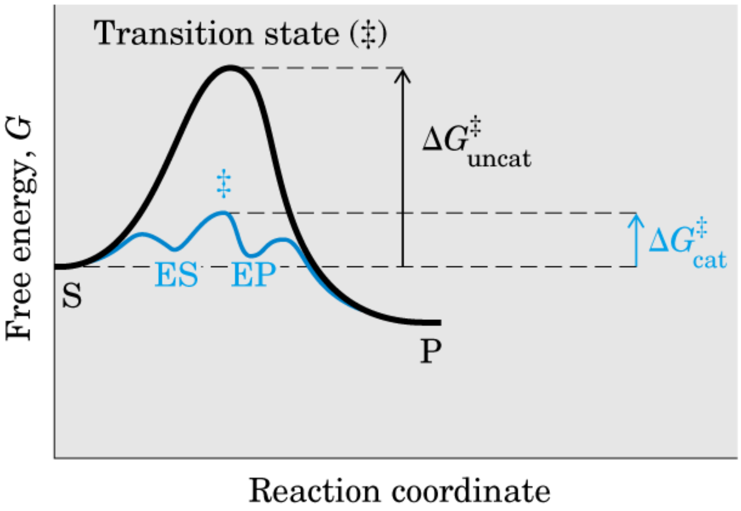
***G: free energy (potential energy)**

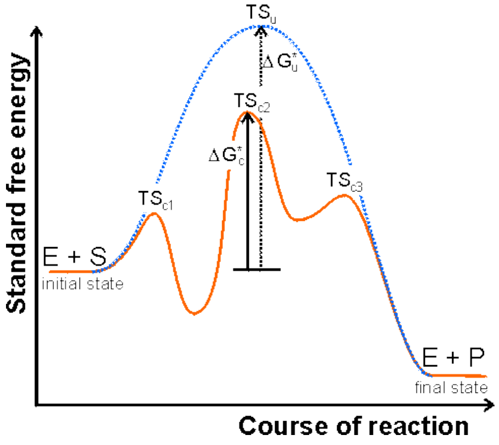
- **In any enzymatic reaction, there are several intermediates and transitional states. Every step has an intermediate that has its own G.**

-Substrates often undergo several transformations when associated with the enzyme and each form has its own free energy value (G).

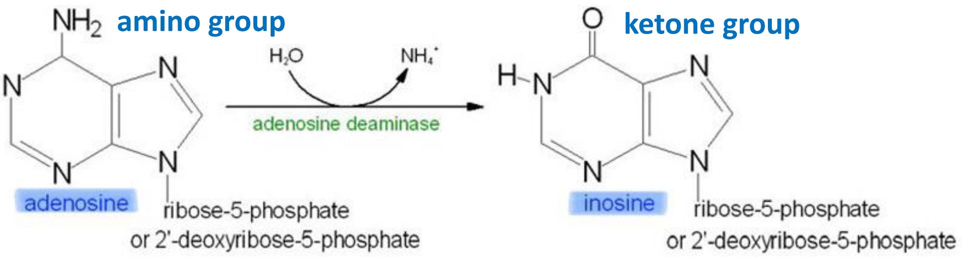
(Further explanation: For example when the enzyme interacts with the substrate it requires (G_1), an OH group is removed (G_2), and if the OH group is transferred from an R group in an amino acid in the active site to another R group (G_3), …etc)

- **G might be favourable (lower than $G_{substrate}$) or unfavourable (higher than $G_{substrate}$).**

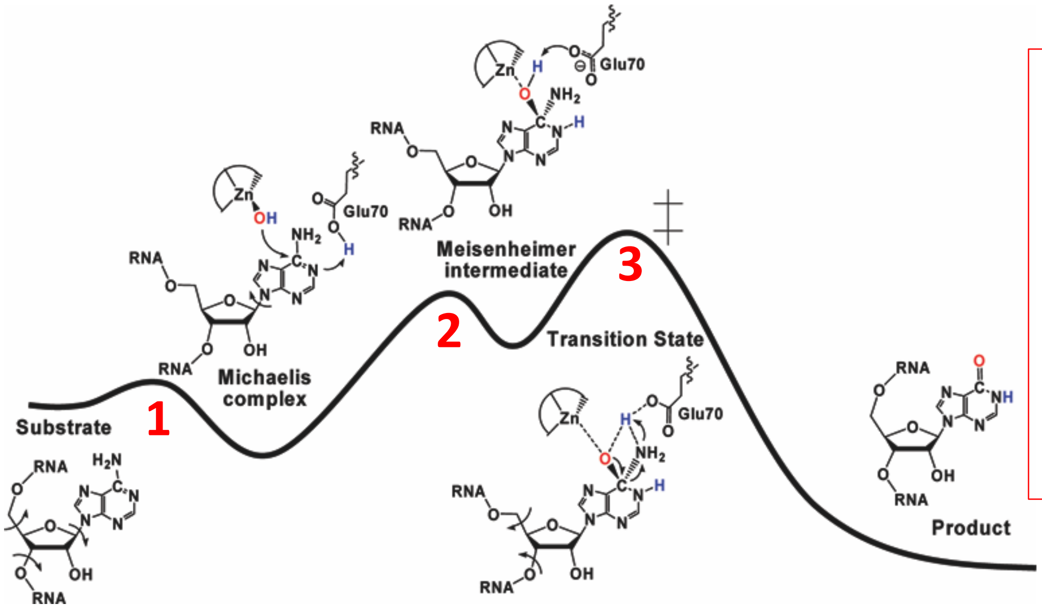




Example: *Adenosine Deaminase*



Deamination reaction



-Every peak (قمة) is a transitional state/ an intermediate.

-Every intermediate has its own G.

-In this example, there are 3 intermediates.

Q: How can we calculate the activation energy of a multi-step enzymatic reaction?

Activation energy = G(highest energy of a transitional state) − G(energy of substrates)

In the previous example: Activation energy = $G_3 - G_{substrate}$

**Important note: ΔG of the reaction doesn't change! Whether the reaction is chemical or enzymatic ΔG doesn't change. Because both $G_{substrates}$ and $G_{products}$ don't change. ($\Delta G = G_{products} - G_{substrates}$) Rather than the activation energy that is lower in the enzymatic reactions.

-The activation energy corresponds to the complex with the highest energy.

-The energy of activation does not enter into the final ΔG calculation for a reaction.

How do enzymes catalyze reactions?

-We know that they catalyze the reaction by reducing the activation energy but how do they do that?

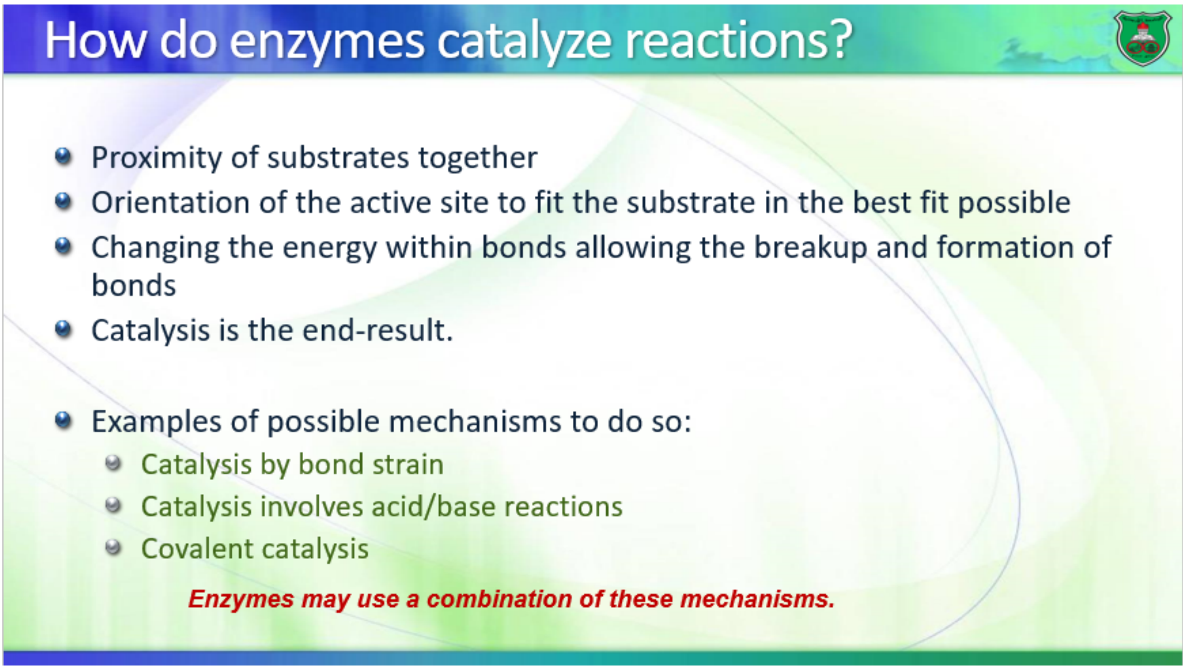
There are different mechanisms.

1. One of the most important mechanisms is *Proximity of substrates together*. They bring substrates together in a proper orientation/ proper angle in the active site (e.g. Glucokinase gathers the glucose and ATP proximately)

The doctor said: الإنزيم بجمّع راسين بالحلال

2. Enzymes work by altering the energy within bonds, causing them to become strained and easier to break. This weakening of bonds allows for the formation of new bonds, resulting in catalysis.

Catalysis is the end-result.



How do enzymes catalyze reactions?

- Proximity of substrates together
- Orientation of the active site to fit the substrate in the best fit possible
- Changing the energy within bonds allowing the breakup and formation of bonds
- Catalysis is the end-result.

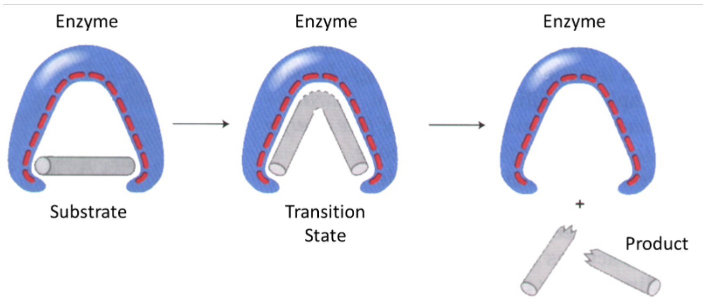
- Examples of possible mechanisms to do so:
  - Catalysis by bond strain
  - Catalysis involves acid/base reactions
  - Covalent catalysis

Enzymes may use a combination of these mechanisms.

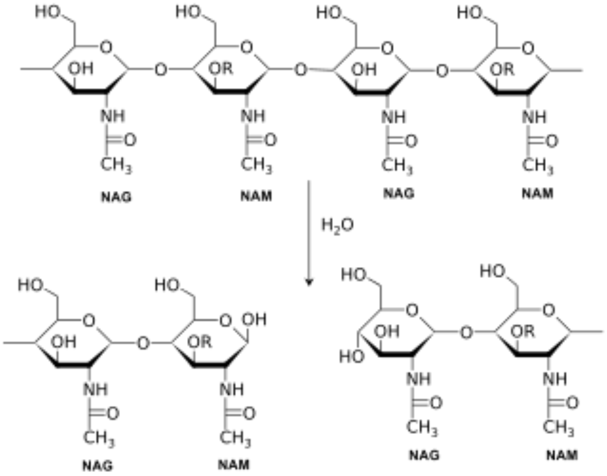
Examples of possible mechanisms :

1 . Catalysis by bond strain

-The induced structural rearrangements produce strained substrate bonds, which more easily attain the transition state. The new conformation makes the substrate bonds “vulnerable”.(ضعيف)



Bending of substrate → cleavage of glycosidic bond



Example: Lysozyme

Lysozyme functions by hydrolyzing glycosidic bonds in sugars. It binds to the substrate and induces strain by bends it, leading to an unfavorable orientation of the glycosidic bond. This strain makes the bond more vulnerable and facilitates its breakage.

The substrate is distorted from the typical 'chair' hexose ring into the 'sofa' conformation, which is similar in shape to the transition state.

Stable substrate (favourable bonds) → Unstable intermediate (unfavourable bonds)

2. Catalysis involving acid/base reactions

-Oxidation/reduction reactions (donating/ accepting H^+).

The R groups of amino acids act as donors (acids) or acceptors (bases) of protons. Enzymes that use this mechanism must have reactive R groups (are willing to donate or accept protons) in their active sites.

Examples:

- Histidine at physiological pH

pka of R group of Histidine is close to the physiological pH:

pka of R group of individual histidine = 6

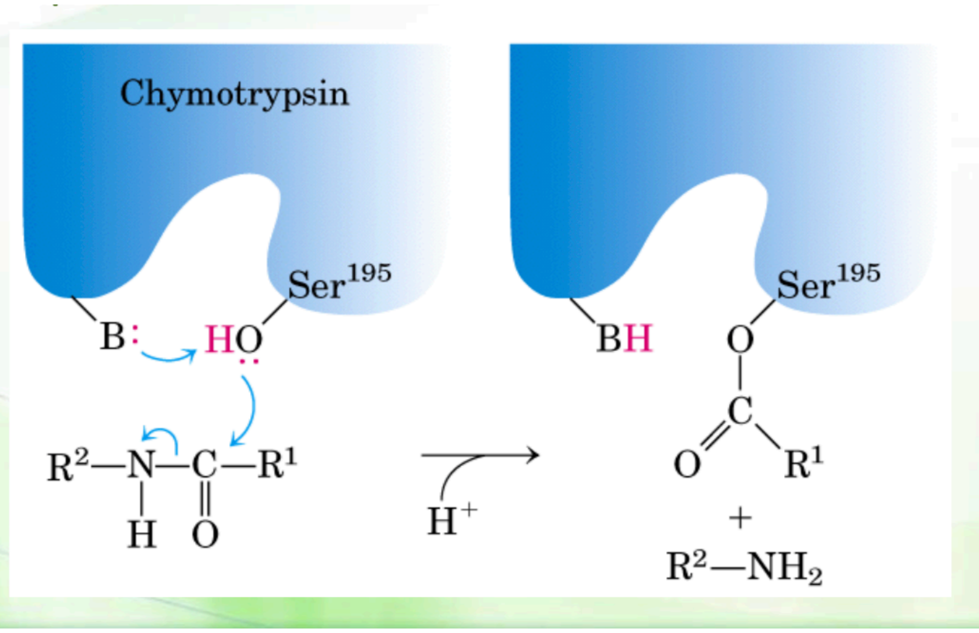
pka of R group of histidine residue = 7.3

(it’s affected by the R groups surrounding it)

So, Histidine can accept or donate H^+ at physiological pH.

- Serine in serine proteases
- Aspartate
- Glutamate
- Threonine

Proteases mechanism:



An example of proteases is Chymotrypsin, it breaks peptide bonds by donating a proton from a serine residue, inducing the cleavage of peptide bond and bonding to the proton instead.

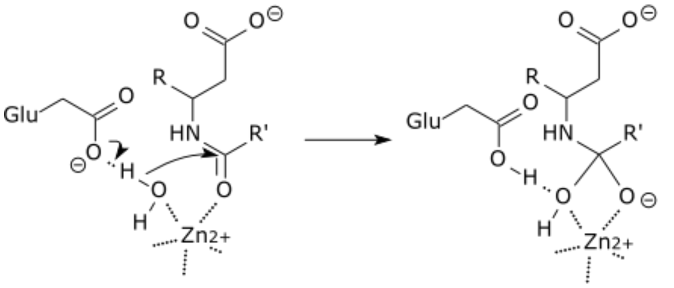
3. Covalent catalysis

This mechanism is used by Proteases. Proteases break the peptide bonds between amino acids in a substrate and make covalent bonds with those amino acids.

The Result = covalent interaction between the peptide chain and the active site of the enzyme and the peptide bond is broken.

-A covalent intermediate forms between the enzyme or coenzyme and the substrate.

- **Examples of this mechanism is proteolysis by Serine Proteases, which include digestive enzymes (trypsin, chymotrypsin, and elastase).**



Enzyme substrate complex

Tetrahedral intermediate

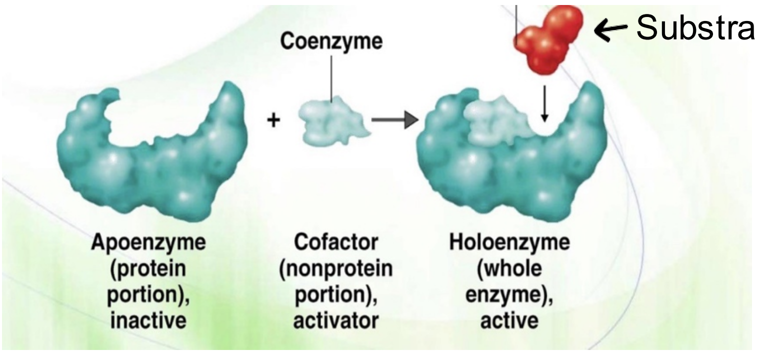
Enzyme Classification (structure)

There are 2 types of enzymes :

1) simple (only enzyme) (apoenzyme)

2) complex (conjugated) (holoenzyme) : enzyme which is associated with a coenzyme.

Coenzyme: non-protein group like Metal or organic molecule (heme)



Naming of enzymes

In general, enzymes end with the suffix (-ase). (like sugar with (-ose))
Most other enzymes are named for their substrates and for the type of the reactions they catalyze, with the suffix “ase” added.

ATPase breaks down ATP.
Elastase breaks down elastin.
Esterase breaks down ester group.
ATP synthase synthesizes ATP.

Some enzymes have common names Examples:
the proteolytic enzyme **trypsin**

Enzyme classes according to function

Enzymes are classified into six major groups:

1) Oxidoreductases: oxidation and reduction reactions.
2) transferases: they transfer molecule to another molecule.
3) Hydrolases: using water to break bonds.
4) lyases: break bonds or reform bonds without using water.
5) Isomerases: change from isomer to another isomer or molecule to its isomer such as glucose and fructose both of them are isomers that enzyme can change glucose to fructose or vice verse, as we talked about it in Benedict test.
6)Ligases: connect things

1 .Oxidoreductases:

Oxidoreductases are the largest class. They catalyze oxidation/reduction reactions involving the transfer of hydrogen atoms or electrons.

They can be divided into 4 subclasses:

- Dehydrogenases
- Oxidases
- Peroxidases
- Oxygenases

IMP

You should know the definition of the class and what does it do Reactions

and enzymes → no need to memorize (just examples)

Type of questions:

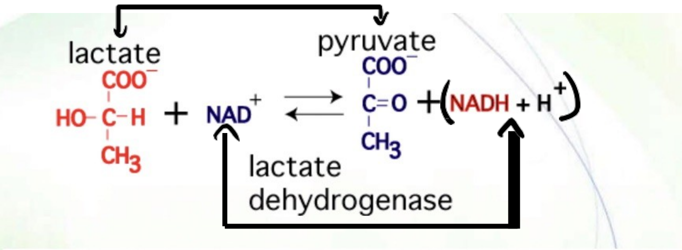
- this enzyme can catalyst which reaction(you can solve it depending on the criteria and conditions)
- what type of enzyme can do this reaction(depending on the substrate and product)
- this enzyme belong to which class (from the name of the enzyme)

1a - Dehydrogenases (removal of water or proton (H))

Dehydrogenases transfer electrons in the form of hydride ions (H-) or hydrogen atoms using an electron-transferring coenzyme(electron carriers), such as (NAD+/NADH/FADH2/FAD/FMN/FMNH2).(these are oxidizing agents)

Lactate dehydrogenase:

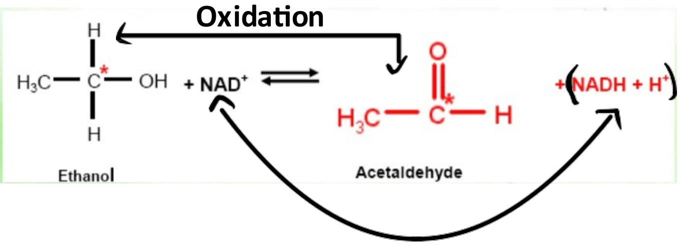
To change lactate to pyruvate



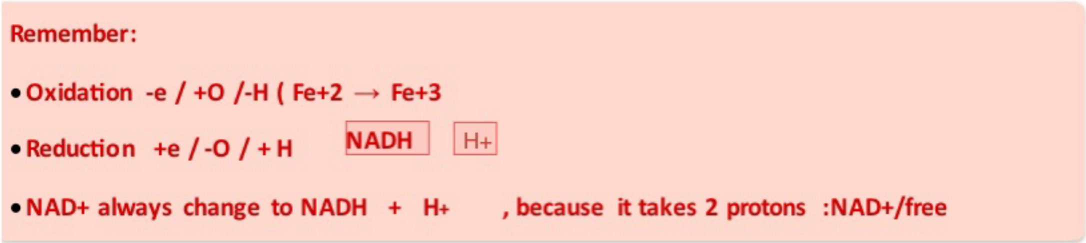
Oxidation

Alcohol dehydrogenase:

To change ethanol to acetaldehyde



Oxidation



Remember:

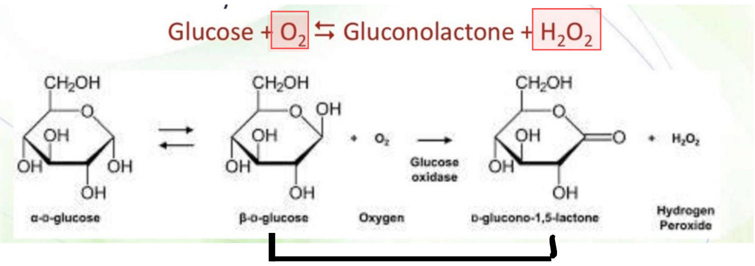
- Oxidation -e / +O /-H (Fe+2 → Fe+3
- Reduction +e / -O / + H NADH H+
- NAD+ always change to NADH + H+ , because it takes 2 protons :NAD+/free

1b - Oxidases

Oxidases catalyze hydrogen transfer from the substrate to molecular oxygen producing hydrogen peroxide as a by-product.

Glucose oxidase catalyzes this reaction:

We take the electrons from glucose and give it to O2 to become H2O2



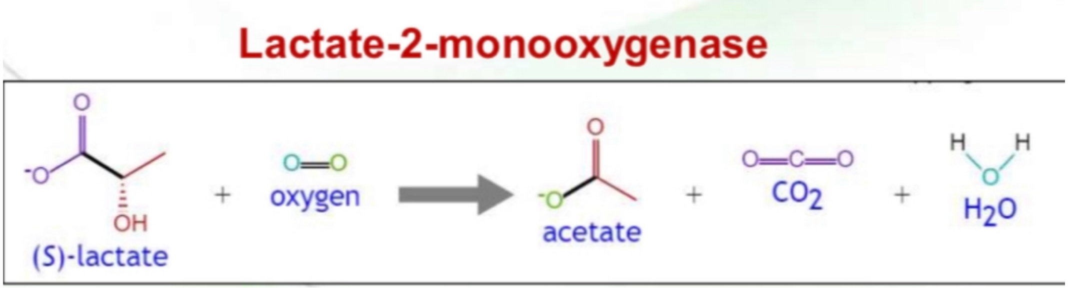
Oxidation

In this reaction the substrate must be O2 and the product must beH2O2(hydrogen peroxide).

1c - Oxygenases:

- **Oxygenases catalyze substrate oxidation by molecular oxygen through introducing oxygen into the substrate.**

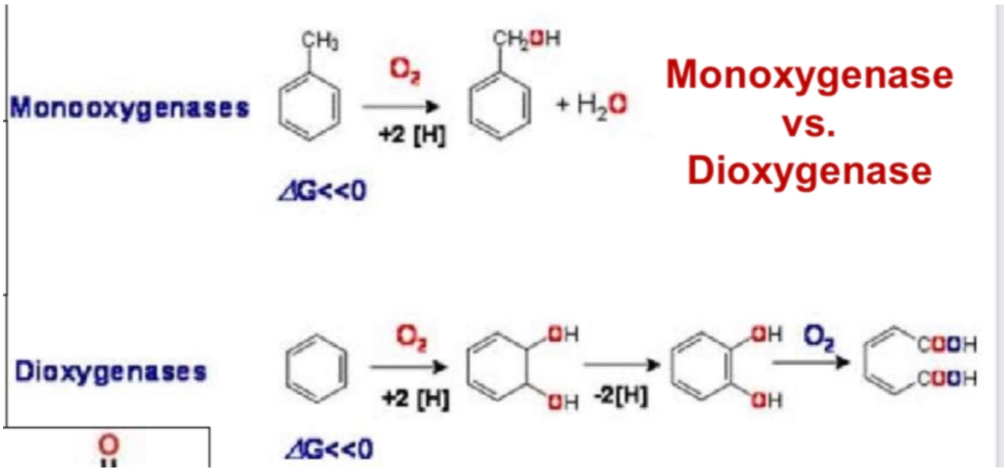
We should have a substrate and an oxygen however in this case the oxygen will be transferred to the substrate.

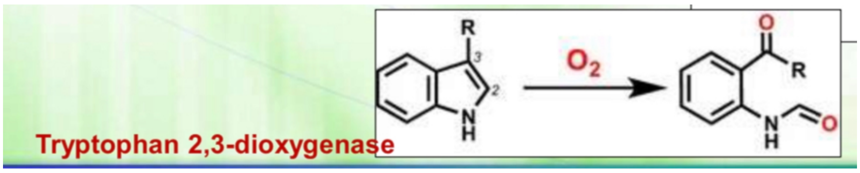

Lactate-2-monooxygenase

There are 2 types:

1- monooxygenase —> one atom will be added to the substrate and the other will be added to water

2- dioxygenase: both atoms are added to the substrate




Tryptophan 2,3-dioxygenase

The reduced product is water, not H_2O_2

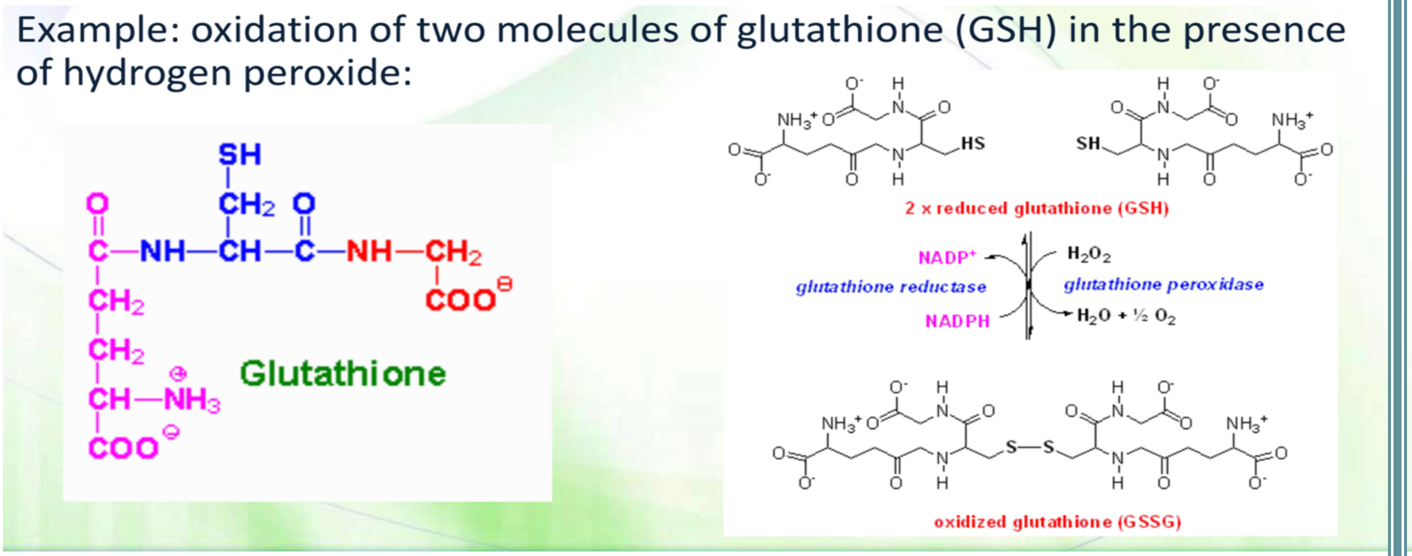
Oxidases and Oxygenases may have similar substrates but the difference between them:

Oxidase by-product—> H2O2

Oxygenase by-product —> H2O

1d - Peroxidases:

Transforms H_2O_2 to H_2O. H_2O_2 is toxic.
Peroxidases catalyze oxidation of a substrate by hydrogen peroxide



Example: oxidation of two molecules of glutathione (GSH) in the presence of hydrogen peroxide:

Glutathione is a tripeptide (glutamate + cystine + glycine), it reacts with the reactive oxygen species such as H_2O_2
How? It reduces the H_2O_2 and transforms it into H_2O, glutathione is then oxidized forming an oxidized glutathione (2 molecules linked by a disulfide bond)

Glutathione peroxidase is one of the important enzymes that protect us from the reactive oxygen species, people with a defect in glutathione or glutathione peroxidase or reductase are susceptible to hemolysis of RBC (death of RBC)

The oxidized glutathione must be converted back to 2 molecules of glutathione to again help in protection (cycle)
You need NADP and NADPH (electron carrier)

Note : the reaction needs 2 molecules of glutathione

Glutathione peroxidase —> oxidoreductase
Glutathione reductase —> dehydrogenase, why? Because of the involvement of an electron carriers (NADP, NAD, FAD, FMN) , enzymes that use electron carriers are dehydrogenases . Important

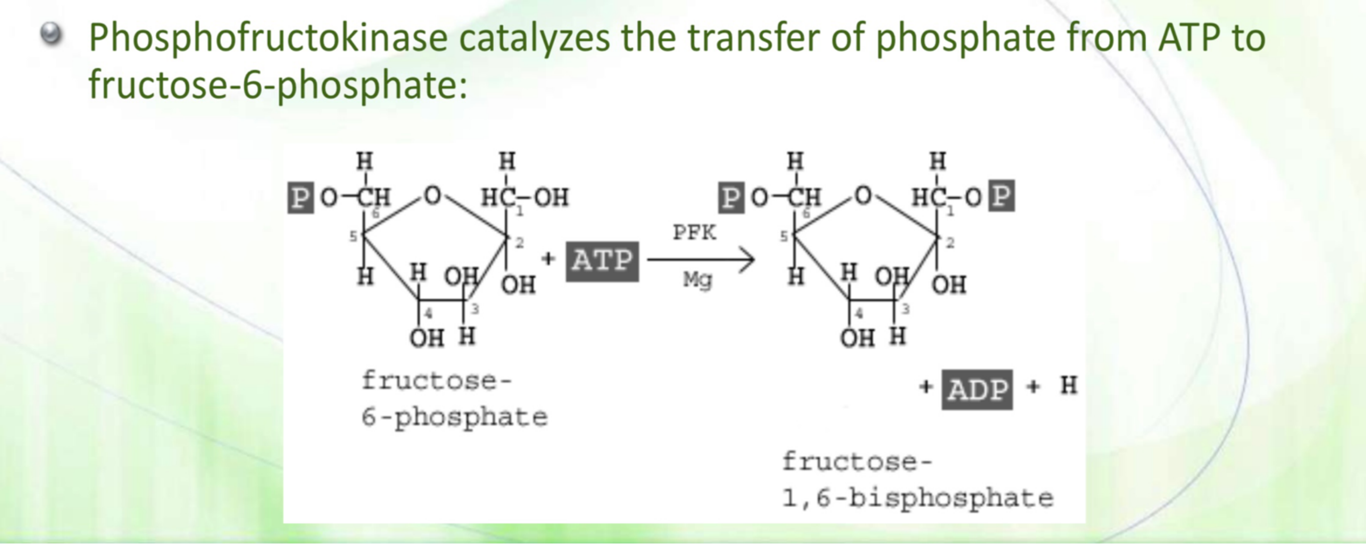
2 . Transferases :

These enzymes transfer a functional group (C, N, P, or S) from one substrate to an acceptor molecule.

Other examples of functional groups OH, aldehyde, ketones

Example: Kinases (the transferred group is a phosphate)

Usually, the phosphate is taken from a high energy molecule such as ATP, GTP, CTP, etc.

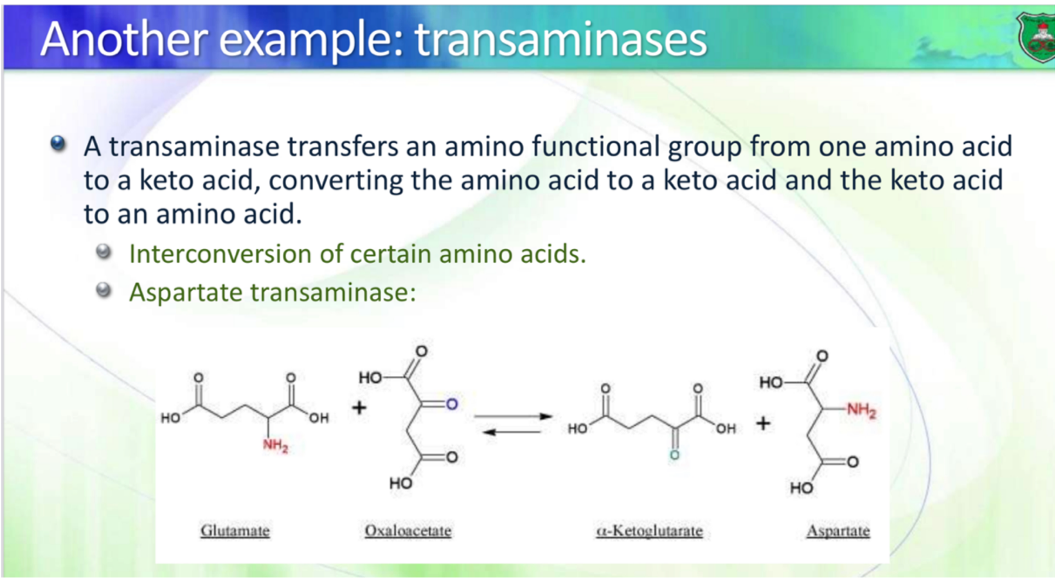


- Phosphofructokinase catalyzes the transfer of phosphate from ATP to fructose-6-phosphate:

EXTRA: what is the difference between fructose bisphosphate and fructose diphosphate?

Bisphosphate —> 2 phosphate groups on different carbons

Diphosphate —> 2 phosphate groups on the same carbon



Another example: transaminases

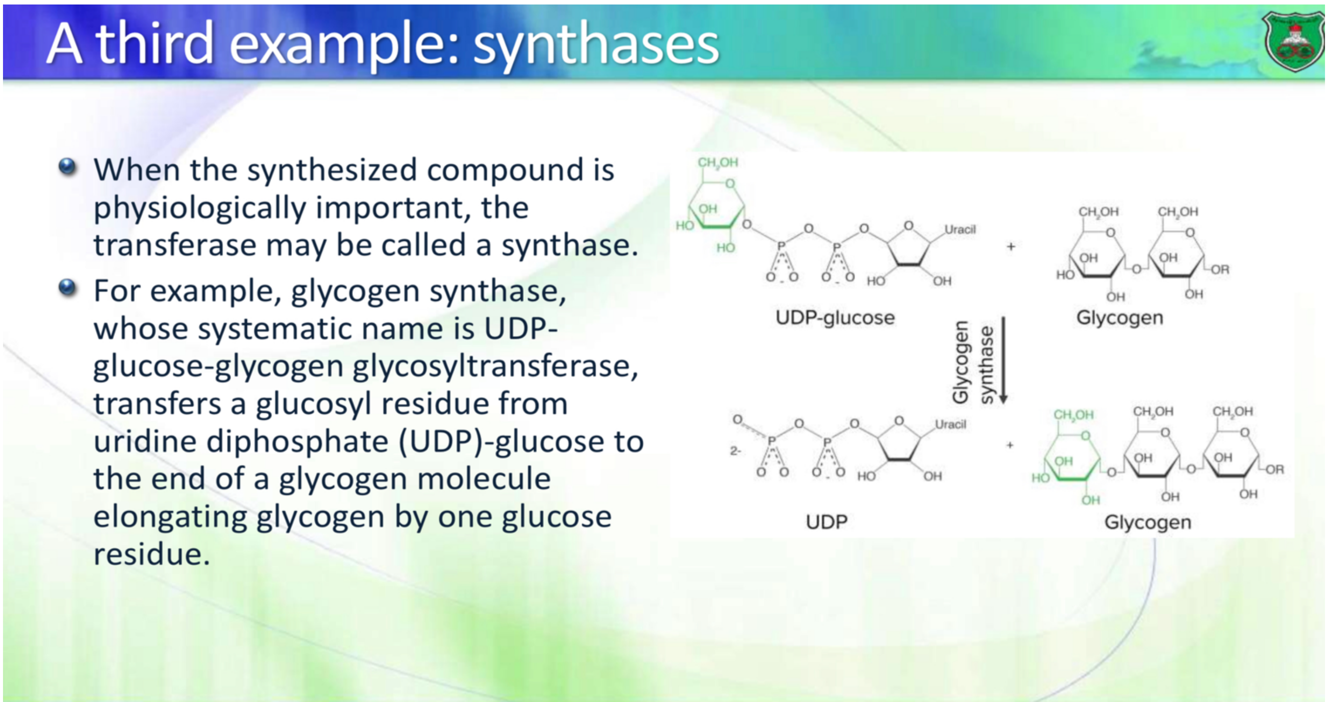
- A transaminase transfers an amino functional group from one amino acid to a keto acid, converting the amino acid to a keto acid and the keto acid to an amino acid.
  - Interconversion of certain amino acids.
  - Aspartate transaminase:

Transaminases are trasnferases that transfer an amine group from an amino acid to a keto acid
The general formula :

Amino acid + keto acid $\rightleftharpoons$ keto acid + amino acid

Δ G = zero

The reaction Is in equilibrium and reversible
An example is Aspartate transaminase,
Why is it important? Depending on the body needs I can convert Glutamate to other important amino acids
For example: transferring an amino group to oxaloacetate gives me aspartate



A third example: synthases

- When the synthesized compound is physiologically important, the transferase may be called a synthase.
- For example, glycogen synthase, whose systematic name is UDP-glucose-glycogen glycosyltransferase, transfers a glucosyl residue from uridine diphosphate (UDP)-glucose to the end of a glycogen molecule elongating glycogen by one glucose residue.

Glycogen synthase is a transferase that transfers a glucose to the end of the glycogen making it longer

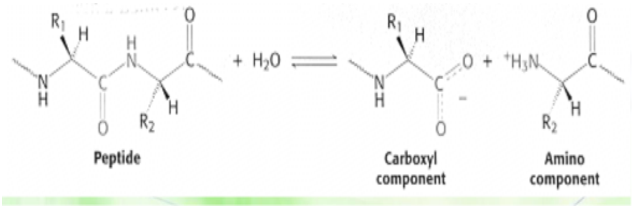
3.Hydrolases

They use water to break bonds by giving H+ for a part and OH- to the other.

They catalyze cleavage reactions using water across the bond in the form of OH– and H+ to being broken or the fragment condensations (in other words, hydrolysis).

Proteases (breaking peptide bonds), esterases (breaking ester bonds), lipases (breaking down lipids), glycosidases (breaking down di- tri- poly-saccharides), and phosphatases (remove phosphate groups) . Hydrolases are named depending on the type of bond cleaved

Example: proteases that catalyze proteolysis(hydrolysis of peptide bonds within proteins)

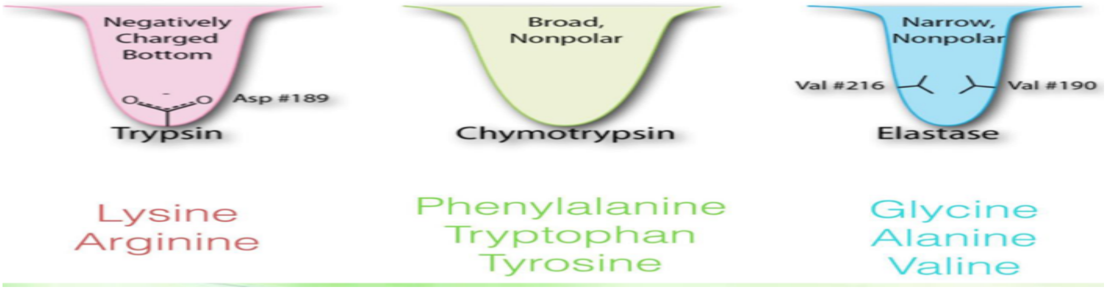


Another specific example of hydrolytic enzymes is digestive enzymes which are really specific enzymes and differ in their degree of substrate specificity

They don't break any peptide bond, they break peptide bonds with specific amino acids

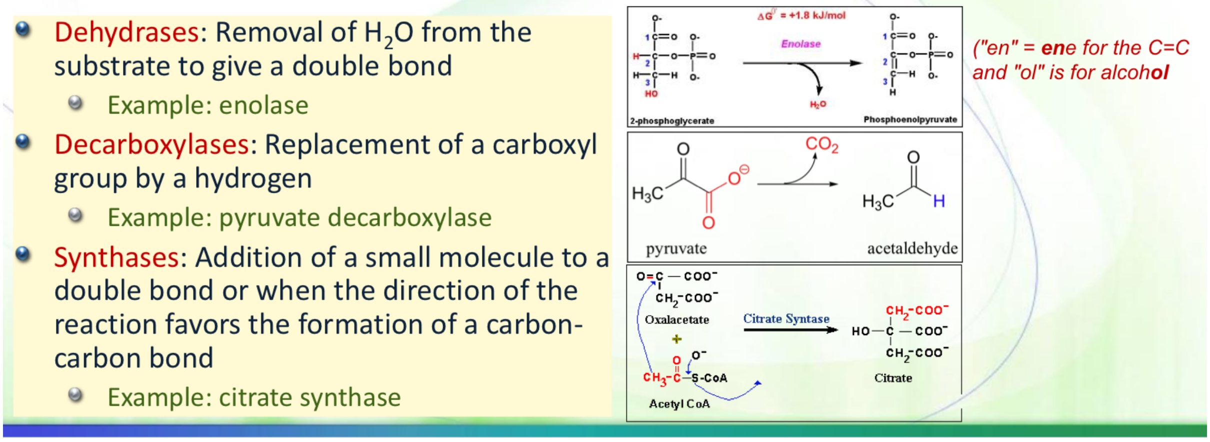
-Trypsin breaks up peptide bonds on the carboxyl side of only Lys and Arg. Chymotrypsin hydrolyzes peptide bonds involving bulky aromatic amino acids.

-Elastase hydrolyzes peptide bonds involving small, uncharged groups such as Ala, Val, or Gly



4.Lyases

They cleave C-C,C-O,C-N and others <u>without using water or oxidation</u>, which is the main difference from hydrolases, leaving double bonds or rings, or conversely adding groups to double bonds without hydrolysis.



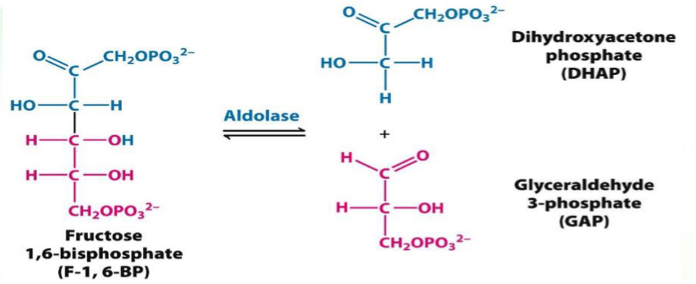
- Dehydrases: Removal of H_2O from the substrate to give a double bond
  - Example: enolase
- Decarboxylases: Replacement of a carboxyl group by a hydrogen
  - Example: pyruvate decarboxylase
- Synthases: Addition of a small molecule to a double bond or when the direction of the reaction favors the formation of a carbon-carbon bond
  - Example: citrate synthase

*("en" = **en**e for the C=C and "ol" is for alcoh**ol***

Another example of lyases are aldolases

Aldolase breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate

Dihydroxyacetone and glyceraldehydes-3-phosphate are isomers and the enzyme that convert one of them to the other is an isomerase

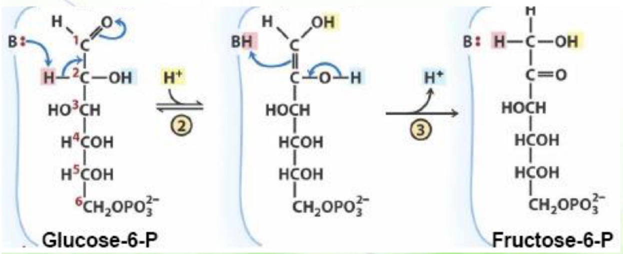


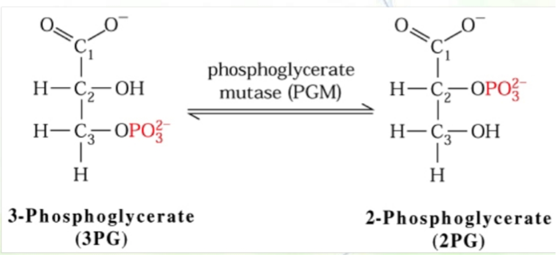
5.Isomerases

These enzymes catalyze intramolecular rearrangements

That means that they convert a molecule to its isomer

Enzymes that rearrange the bond structure of a compound are called isomerases, whereas enzymes that catalyze the movement of phosphate from one atom to another are called mutases





What happened in the above picture is:

Phosphoglucoisomerase isomerizes glucose-6-phosphate to fructose-6-phosphate.

Phosphoglycerate mutase transfers a phosphate group from carbon number 3 to carbon number 2 of phosphorylated glycerate

6.Ligases

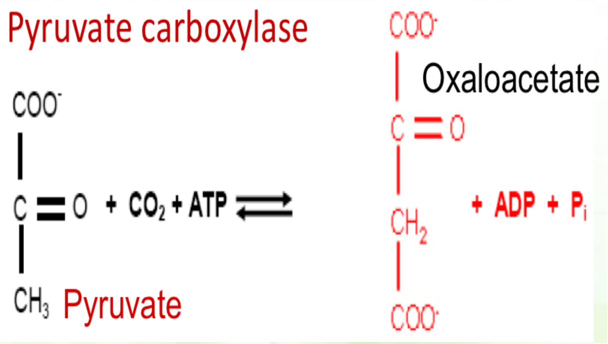
Enzymes that connect two molecules together and usually when we connect them we need energy and this energy comes most probably in most reactions from ATP or in other types may be used like GTP for example

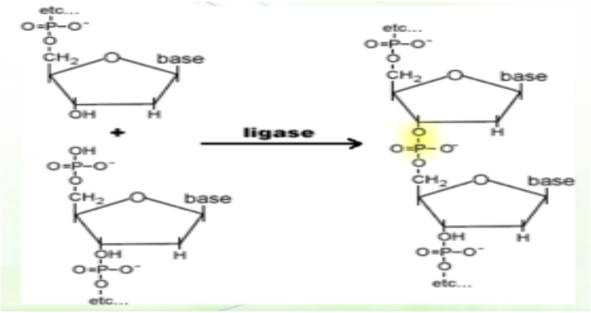
Ligases join C-C, C-O, C-N, C-S, and C-halogen bonds.

The reaction is usually accompanied by the consumption of a high-energy compound such as ATP and other nucleoside triphosphates.

Synthetases (ligases)derive the energy from the cleavage of high-energy phosphate bonds while synthases are lyases and use a different source of energy.

Ligases use ATPs energy to do its job without transferring a phosphate group to the substrate, unlike kinases that give energy to the reaction by transferring phosphate groups to the substrate





***Tip from Dr. Mamoun : practice classifying enzymes based on reactions at home, it is important for the exam.**

Somethings are just different

We said previously that enzymes in general are proteins, but sometimes enzymes can be something than proteins (really different) like:

Abzymes

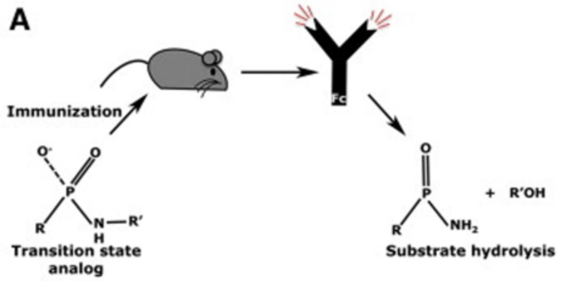
- **Abzymes are antibodies acting as enzymes**
- **They are produced against transition-state analogs**
- **How? a host animal is injected with a transition state analogue. The animal makes antibodies against it (binding with high affinity at specific binding points mimicking an enzyme's active site surrounding a transition state**

Since antigen fits an antibody just like substrate fits an enzyme, scientists thought of a way to utilise antibodies as enzymes, the mechanism is:

i. **They injected a mouse with transition state organic analog (or substrate)**
ii. **The mouse immune system reacts by producing antibodies for this substrate**
iii. **They collected those antibodies (now the antibodies are ready to be used as enzymes), then injected them into human body**
iv. **The mission was successful and the antibodies caught the transition state substrate and converted it into another type of molecules**

v. **This method is widely used in treating cocaine addicts, by converting the cocaine in their blood into another chemical molecule**

Abzymes with activity similar to cocaine esterase, which degrades cocaine, have been developed against analogs of its transition-state complex. Monthly injections are used to treat addicted individuals by destroying cocaine in the blood and, thereby, decreasing their dependence on cocaine.

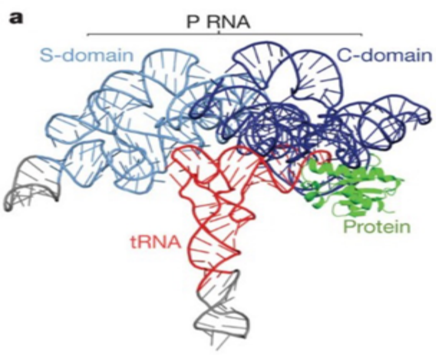


Ribozymes

- **Most enzymes are proteins, but RNA molecules can act as enzymes, too**
- **Ribozymes are enzymes made of both protein and RNA**
- **For some, catalysis is preformed by RNA** *(EXAMPLES INCLUDE THOSE INVOLVED IN RNA SPLICING AND PROTEIN SYNTHESIS IN RIBOSOMES)*
- **The catalytic efficiency of RNAs is less than that of protein enzymes, but can be enhanced and stabilised by the presence of protein subunits**

You have studied in molecular biology that ribosomes are proteins with some RNA material, actually the RNA is the one how plays the enzyme role not the protein as other enzymes
Ribozyme caught the attention of the scientists because they thought it could be the precursor of evolution:

i. **It could act as genetic material like DNA (DNA could not be the precursor because it needs protein)**
ii. **It could function as enzyme (proteins or enzymes could not be the precursor because they need DNA to be synthesised).**
iii. **iii. This leads the scientists to consider the RNA as a special support for permeative viruses .and this leads them to consider the RNA as a precursor (beginning point) of the life والله أعلم**



THE END

***Trust in your abilities**