Lecture : 18 (3rd After Mid)

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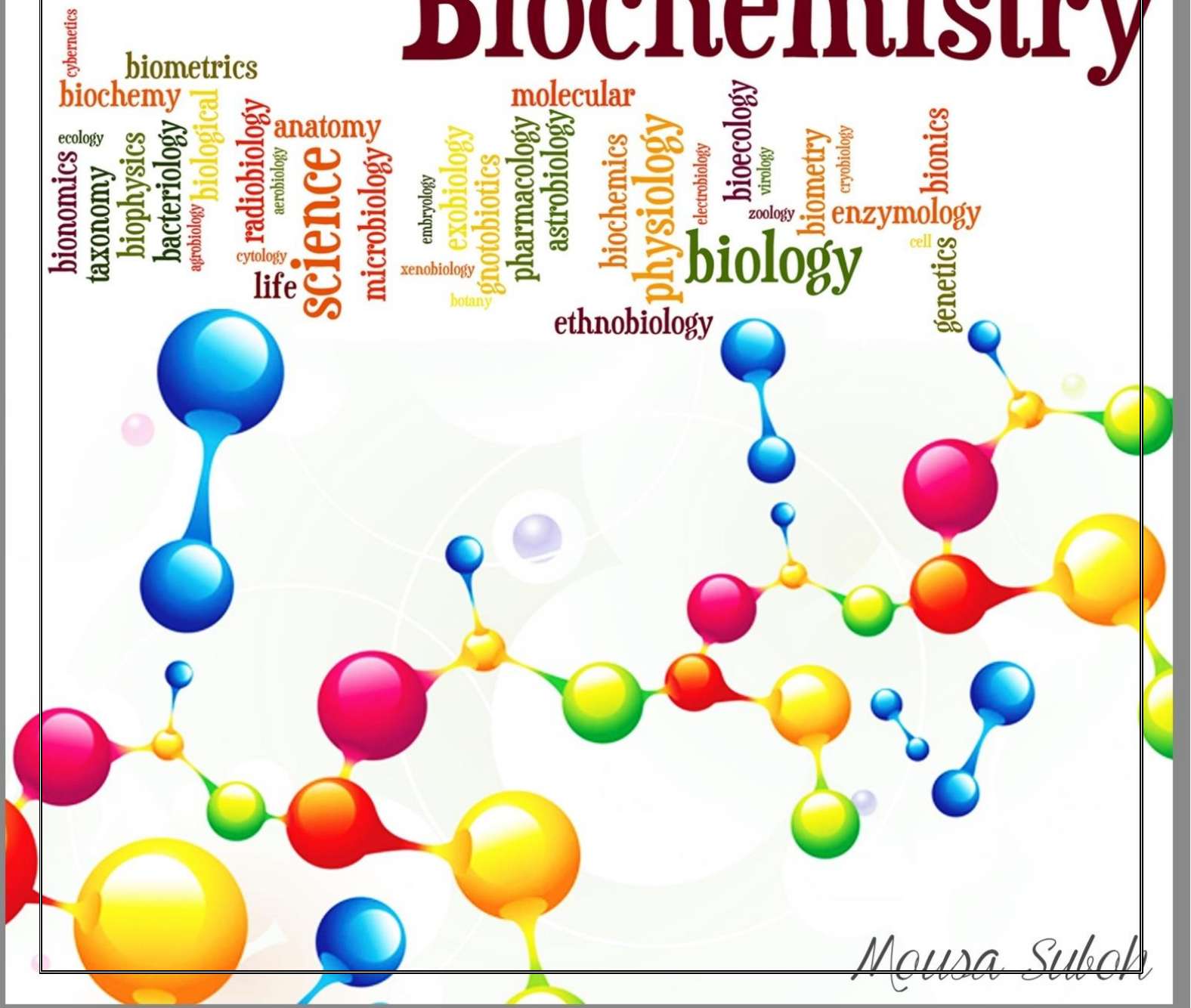
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

Biochemistry



Mousa Subeh

Enzyme Classification

Enzymes could be classified to six major classes according to their function :

1-***Oxidoreductases*** : at least one substrate gains electrons and becomes reduced, and another substrate loses electrons and becomes oxidized (transfer electrons from one molecule to another) also this type of enzymes Require coenzymes , it can be further sub-classified into four major subclasses :

I-Dehydrogenase : extract hydrogens from the substrate and add them to **nicotinamide adenine dinucleotide (NAD+)**(oxidized coenzyme) resulting in **(NADH)**(reduced coenzyme).

»Examples:

-lactate Dehydrogenase

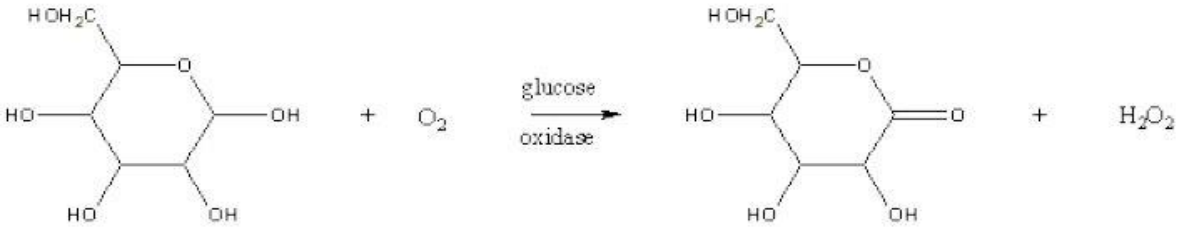
-alcohol Dehydrogenase

II-Oxidases : extract hydrogens (electrons as hydride anoine, H-) from the substrate and add them to molecular oxygen reducing it to hydrogen peroxide (product) .

-This enzyme needs a subsequent reaction which is catalase enzyme reaction to remove the toxicity of hydrogen peroxide by breaking it down to water and oxygen .

»Example:

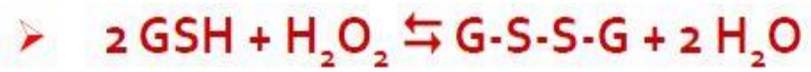
-glucose oxidase



III- Peroxidases: It oxidiases the substrates by using hydrogen peroxide as a substrate (not a product as in the case of oxidases).Water is also produced .

»Example:

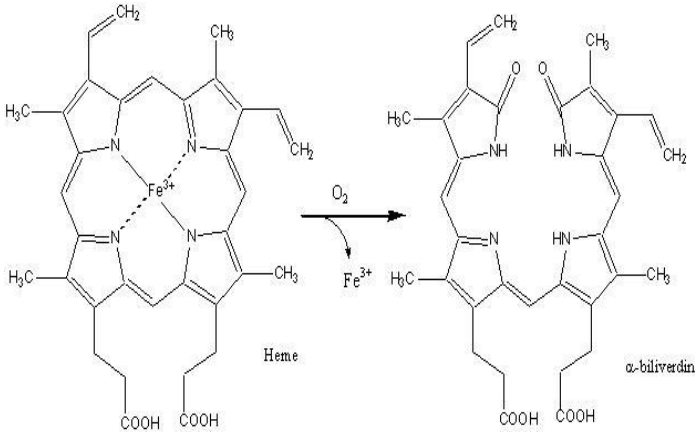
-glutathione peroxidase



IV-Oxygenases : It can oxidize the substrate by transferring oxygen from molecular oxygen to the substrate .

→Monooxygenase: It can add one oxygen atom to the substrate from molecular O2, and the other oxygen will be reduced to water.

→dioxygenase : It can add two oxygen atoms to the substrate.



»Examples:
-heme Monooxygenase
-heme dioxygenase

Q) What is the difference between oxygenases and oxidases?

oxidases don't introduce the oxygen to the substrate itself, oxygenases do. Oxidases extract the Hydrogen from the substrate and introduce it to molecular oxygen as mentioned before .

2- Transferases: transfer a functional group(C,N,P,S,O) from one molecule (substrate) to an acceptor molecule .

Subclasses:-

I-Kinases: they transfer phosphate group from ATP to the substrate, the most common donor for phosphate is ATP .

»Example:

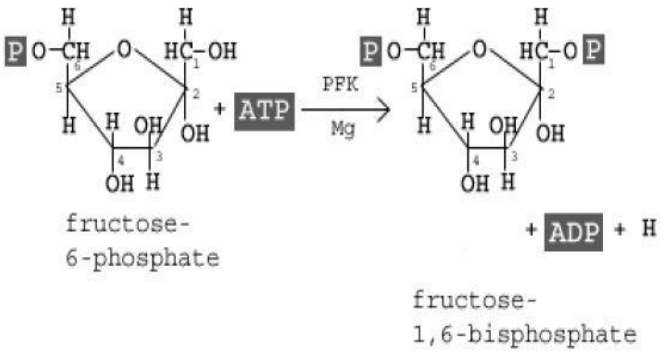
-phosphofructokinase(PFK): the most important enzyme in the process of glycolysis (carbohydrate metabolism), as it is the rate limiting enzyme .Adds phosphate from ATP to fructose-6-Phosphate,to produce fructose 1,6 bisphosphate + ADP .

*Any enzyme including kinase in it's name, will always be a transferase.

**The doctor said that you will see enzymes in the exam that you should be able to recognize their functions, even though you are not very familiar with them.

In conclusion , all kinases have the same function and you will be asked about the things you are supposed to know about them.

➢ Fructose 6-P + ATP ↔ F 1,6 bisphosphate + ADP



II-Transaminase (amino transferase) : It moves an amine group from an amino acid (substrate) to a keto acid (another substrate) **.{amino acid↔keto acid}**

-An amino acid contains an amine{NH2} group and a keto acid contains a keto{C=O} group. In transamination , the NH2 group of one molecule will be exchanged with the {C=O} of the other molecule . the amino acid becomes a keto acid , the keto acid becomes and amino acid .

*In the amino acid metabolism , there will be a conversion from amino acid to keto acid , then from keto acid to another amino acid by transferring the amine group.

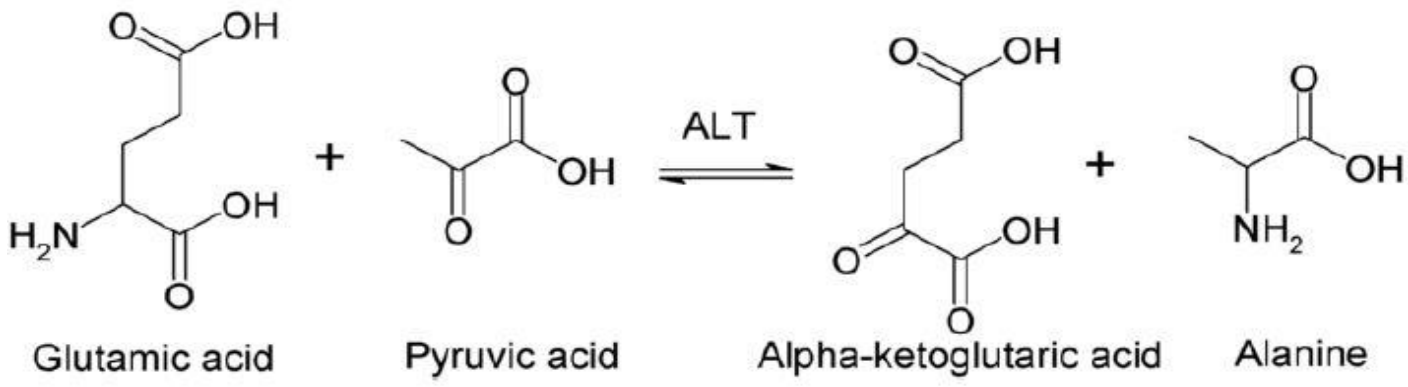
»Examples on some of these interconversions:-

1-pyruvic acid (3 carbons) is a keto acid , when we give the pyruvic acid an amine group, it will be an amino acid (alanine) .**{alanine ↔pyruvate}**

*pyruvate is used in energy metabolism, so proteins (amino acids) could aid in the process of energy metabolism (proteins→energy).

2 -Aspartate is an amino acid, and if we remove the amino group, the aspartate will be converted to oxaloacetate (4 carbons ,In kreps or citric acid cycle).And if we add amine group to oxaloacetate it will be aspartate.

{ Aspartate↔ oxaloacetate}



3 -glutamate if we remove the amine group it will be converted to alpha-ketoglutarate (one of the kreps cycle intermediates) .And if we add the amine group back to alpha-ketoglutarate it will be converted to glutamate .**{ glutamate ↔ alpha-ketoglutarate}**

*Carboxylic acid protonated→ends with the suffix (-ic acid) {pyruvic acid)

* Carboxylic acid deprotonated→ends with the suffix(-ate){ pyruvate}

»Examples on the enzymes that catalyze the interconversion:

-ALT :ALnine Transaminase.

-AST:ASpartate Transaminase.

**** Clinical note*** (using enzymes for medical diagnosis) :if the doctors doubted that the patient has a liver problem , they (the doctors) will order a liver function test for liver enzymes (ALT,AST).

Q) What is the idea behind using enzymes for medical diagnosis?

High concentration of diagnostic enzymes(enzymes that have high concentration within certain cells and low concentration in other) in the blood, indicates that tissue or cellular damage occurred resulting in the release of intracellular components into blood.

*Some examples for using enzymes for medical diagnosis:

-*liver enzymes(ALT,AST):*enzymes that have high concentration only in liver cells. So when there is a problem in liver, the liver cells will die due to necrosis,releasing liver cells contents(ALT,AST) To the blood. Then the doctors will take a blood sample and examine it for liver enzymes. If the concentration of these liver enzymes is high(higher than normal levels) ,that is an indication for necrotic (dying) liver cells .

-*cardiac enzymes*:for people who have angina(جلطة, ذبحة صدرية), there will be a high concentration of cardiac enzymes(AST,Creatine phosphate,Creatine phosphokinase,Lactate dehydrogenase)

-*bone problems* (bone cancer).

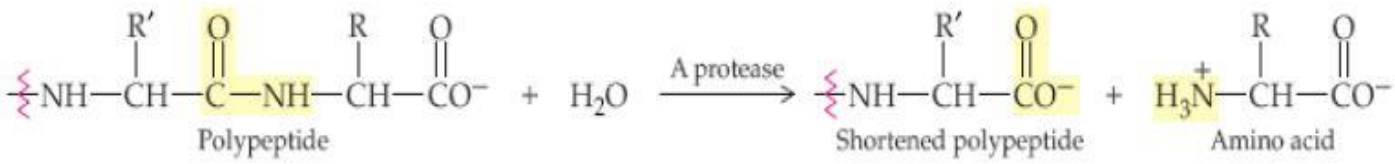
-*brain cancer*.

3-Hydrolases:cleaves their substrate using water. The names depend on the type of bond cleaved.

»Examples for Hydrolases :

-*Peptidases*: the cleaved bond is peptide bond in short polypeptides chains.

-*Proteases*: the cleaved (hydrolysed) bond is peptide bond in long and complex polypeptide chains (proteins).



-*Esterases*: the cleaved bond is ester bond.

-*Lipases*: they cleave the lipids.

-*Glycosidases*: the cleaved bond is glycosidic bonds in complex sugars.

-*Phosphatases*: the cleaved bond is phosphoester bond.

-*Nucleases*: they cleave nucleic acids.

* . Condensation(dehydration) reaction is the opposite for hydrolysis reaction

**The peptide bond consists from carbonyl group and nitrogen.In the process of hydrolysis the (OH) will be added to the carbonyl group and hydrogen will be added to the nitrogen.

»Examples for *Proteases*:

-**Trypsin**: is quite specific ,splits the peptide bond only on the carboxyl side of (LYS,ARG).

-**Pepsin**: degrades food proteins into peptides .

-**chymotrypsin** :recognizes the aromatic structure (Tyrosin,Phenylalanine ,Tryptophane) And then breaks the peptide bond towards the carbonyl group(towards the free carboxyl group).

-**Thrombin** :recognizes the ARG-GLY bond and catalyzes the hydrolysis of it.

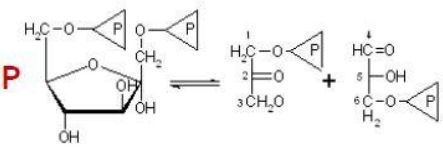
* Pepsin , chymotrypsin and Trypsin are the three principal protein-degrading , or proteolytic, enzymes in the digestive system.

4-Lyases:addition or removal of function groups from their substrates associated with the removal or the formation of a double bond between C-C ,C-O ,C-N which means that it doesn't break down the substrate itself .

»Examples :

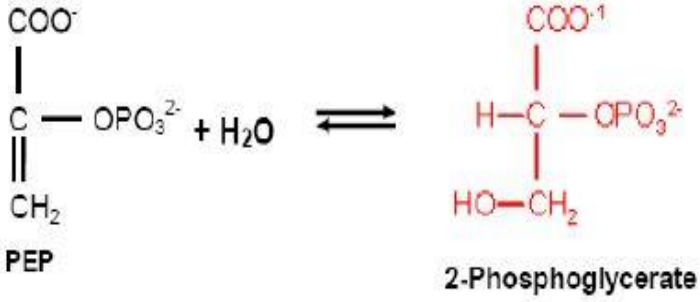
-**Aldolases** : converts fructose 1,6 bisphosphate into dihydroxyacetone phosphate (DHAP) and glyceraldehydes 3-phosphate.

➢ F 1,6 bisphosphate ⇆ DHAP + GAP



- **anolase:** converts phosphoenol pyruvate(PEP) to 2-phosphoglycerate.

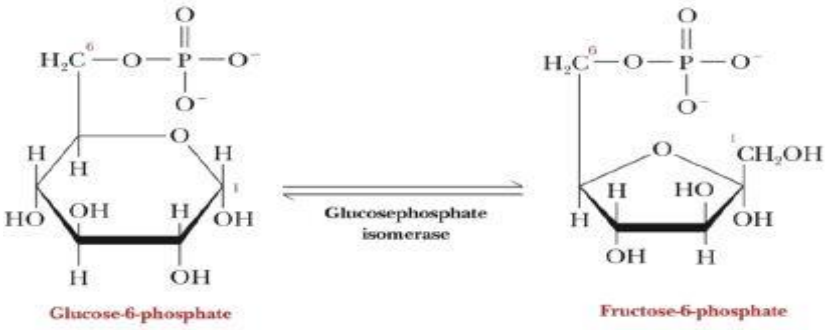
*The last step(material) in glycolysis before the formation of pyruvate is phosphoenol pyruvate.



5-Isomerases:they convert a molecule from one isomer to another (intramolecular rearrangements),(one substrate, one product).

»Example:

***glucose-6-phosphate isomerase**: isomerizes glucose-6- phosphate to fructose -6- phosphate .

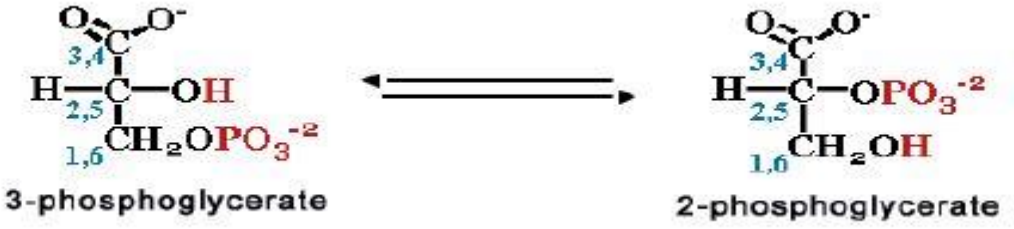


-**Mutases**:is an enzyme of the isomerase class.

»Examples:

*__Phosphoglycerate mutase__ :converts 3- phosphoglycerate into 2- phosphoglycerate.

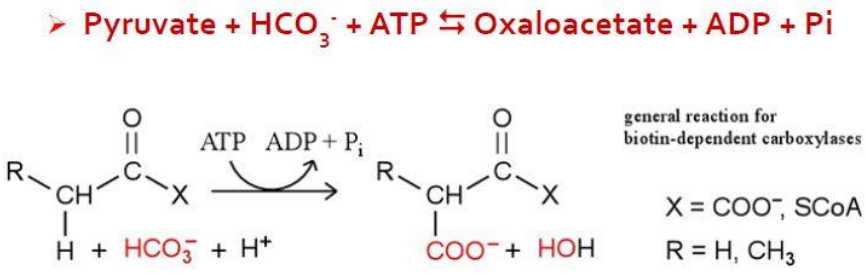
*__Phosphogluco mutase__ : converts glucose -6- phosphate into glucose -1- phosphate.



6-***Ligases***: (ligase means "to bind,to glue") catalyze the joining of two molecules by forming a new bond .

»Example:

-Pyruvate carboxylase (important in the citric acid cycle): this enzyme adds a carboxylic group to saturated carbon .The pyruvate (3 carbons)is converted to oxaloacetate(4 carbons, citric acid cycle intermediate) by adding one carbon (carboxylic group).



*Decrease in the concentration of the oxaloacetate in the cell will cause this rxn to take place to compensate for the decrease in oxaloacetate.

……..

Mechanism of action of enzymes

*_Chymotrypsin_ as an example.

**What happens specifically in the active site of _Chymotrypsin_ .

--}Chymotrypsin :

-Digestive enzyme.

-Member of serine proteases super family (the main amino acid in the active site that catalyze the rxn is serine).

*These group of enzymes are named for the main amino acid in the active site that catalyze the rxn .For example: Tyrosine proteases(the main aminoacid that catalyze the rxn in Tyr), serine proteases and Tyrosine kinases .

-Hydrolytic enzyme which breaks the peptide bonds at the aromatic ring structure amino acids (Phe , Tyr, Trp)

Q)Why does the rate in catalyzed rxn become faster ?
The rate is faster because Functional groups in the enzyme active site:
-Activate the attacking hydroxyl group on the serine side chain by HIS and ASP

-Stabilize the oxyanion transition-state complexes by H-bonds

-Form a covalent intermediate

-destabilize the leaving group

**All of these steps will be explained in details in this lecture

***The reaction takes place in two stages:
-Cleavage & formation of intermediate ,the peptide bond is broken.

-Hydrolysis of intermediate to release protein substrate and convert the enzyme back to it's original shape.

The first event of the catalytic action

Q) why the peptide bond can't be broken without an enzyme?

-The energy of (-OH) in water is not enough to reach Ea (activation energy).

-Too few (-OH) molecules colliding with the substrate at the right orientation.

*The uncatalyzed hydrolysis rxn (all hydrolytic process is spontaneous) can take place over long period of time (very slow),but the catalyzed rxn via Chymotrypsin takes place very fast.

**Enzymes can't change the favorability of the rxn.

Q)Why Chymotrypsin ?what is the difference between Chymotrypsin and any other enzyme?

The Chymotrypsin has serine(#195 amino acid in the active site), and serine has a hydroxyl group on the side chain which could be connected to the carbonyl carbon via the oxygen atom on the peptide bond (serine activate the carbonyl group on the peptide bond).

Then there will be an activation for water via Histidine residue, the (OH) in activated water will be given to the carbon and the serine will return to it's original shape.

The (OH) in water (initially) can't attack because the carbonyl group isn't activated, so we use the hydroxyl group on the serine which will activate the carbonyl group .And when the carbonyl group is activated it can bind the (OH) in water.

Q) Is serine activated at physiological PH(7.36-7.44)? Can serine attack the peptide bond at physiological PH ?

No, though serine is a (polar non-charged)amino acid, it doesn't have a charge for the attack, because the attack needs a full charge of electrons (strong nucleophile).And because the Pka for the (OH)group of the serine is 13,it will be protonated (not charged) at the physiological PH (7.36-7.44).

Q) How to make the (OH) on the serine side chain more nucleophilic(increase the negative charge of electrons on the oxygen atom)? How to activate the serine and make it able to attack?

The serine could be activated by making the oxygen has a negative charge and this could be done by taking the hydrogen atom from the hydroxyl group on the serine side chain to the nitrogen atom of imidazole ring in Histidine.

Q) How to take the hydrogen atom from the hydroxyl group on the serine side chain?

We need an internal process so that it can be repeated again and again. So there is an amino acid (Histidne) that acts as a base close to the serine (acid-base catalysis) and accept the Hydrogen atom from serine's hydroxyl group via the nitrogen atom which has a free lone pair of electrons .(lewis base)
-(HIS)acts as a base and (SER) acts as an acid .

Q)How to activate the Histidine ?

The Histidine is activated by the amino acid aspartate (negatively charged oxygen and this oxygen binds to the hydrogen on the Histidine, and this lead to decrease the total charge on Histidine and activate the nitrogen to attack the serine.

*ASP→HIS→SER(195) is a series of amino acids called the catalytic triad ,the ASP AND HIS cooperate in converting the (OH) on the serine into a better nucleophilic attacking group.

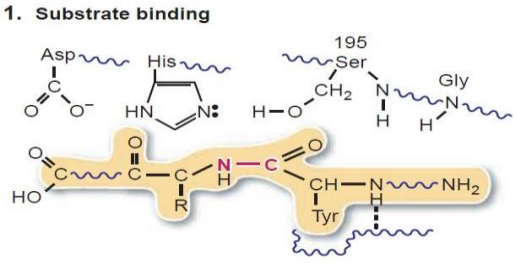
**After the (OH) attacks the carbonyl group , it forms an oxyanion intermediate (unstable),which has a full negative charge on the oxygen atom .The oxygen atom usually doesn't have a full negative charge , it has a partial negative charge .

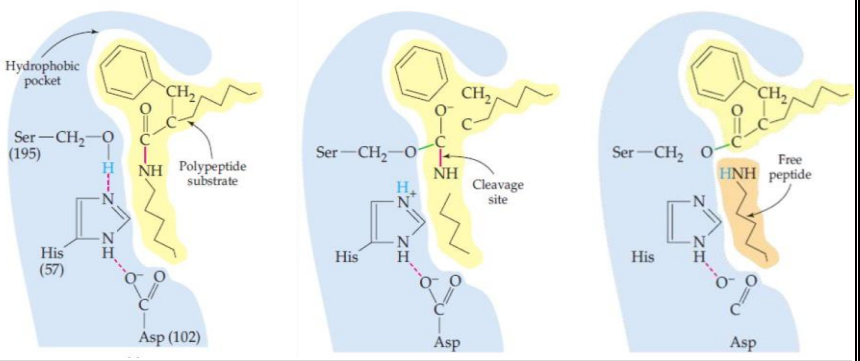
The enzymes do four major *effects (process)*on their substrate in the active site:

- **Proximity effect: Bring substrate(s) and catalytic sites together .**
- **Orientation effect: Hold substrate(s) at the exact distance and in the exact orientation necessary for reaction .**
- **Catalytic effect: Provide acidic, basic, or other types of groups required for catalysis .**
- **Energy effect: Lower the energy barrier by inducing strain in bonds in the substrate molecule.**

Q)what is the effects (process) that chymotrypsin active site do on the substrates?

1+2)Proximity and orientation: the substrate enters the active site, the active site has a hydrophobic pocket which binds the aromatic amino acids (TYR,PHE,TRP) by the glycine residues via hydrophobic interaction. Then there will be a conformational change in the active site that will lead to move the serine in such way that the serine's oxygen become more close to the carbon in the substrate and the hydrogen more close to nitrogen in Histidine.

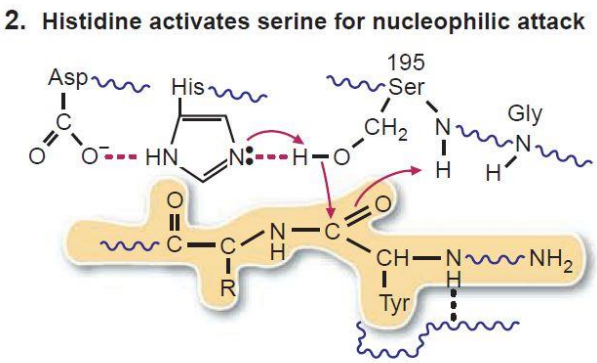


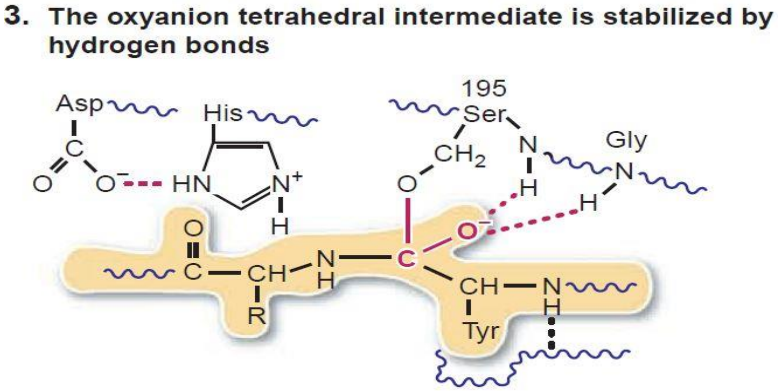


*The number of serine in the active site which is responsible for the catalytic action is 195.

3)Catalysis (acid-base catalysis, covalent catalysis):due to proximity of the hydrogen (in the O-H of the serine)to the lone pair of electrons of the histidine's Nitrogen atom, this will lead to transfer the (H) from serine to histidine making the oxygen atom more active. And the histidine becomes active because of the attraction between the aspartate's negative oxygen atom and the other Nitrogen atom in Histidine (series of bonds).

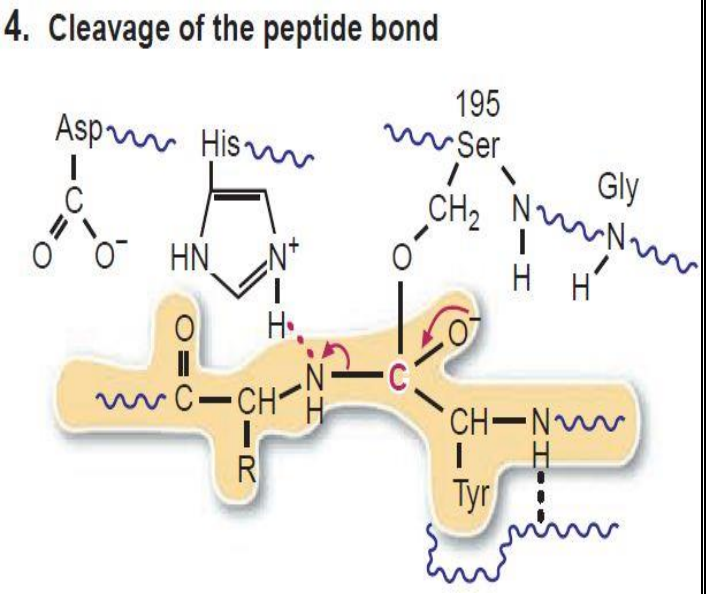
*When the Oxygen lose the(H) and become negative(active) it will attack the double bond at the carbon atom (nucleophilic attack).





4)Energy: after nucleophilic attack , the Oxygen will make a covalent bond with the carbon pleading to decrease the strength of other bonds around the carbon and the electrons of the double bond (half of them) will be moved to the Oxygen as a free lone pair of electrons. So the double bond will be converted to a single bond and the oxygen will have a negative charge .This will result in the formation of the first oxyanion tetrahedral transition state complex(unstable) and then will be stabilized by H-bonds with the hydrogens attached to the Nitrogen atoms in the Glycine residues. This will lead to stabilize the oxyanion tetrahedral transition state complex and lower it's energy level and increase the number of molecules that reach this energy level.

☆ **Covalent catalysis** :after the stabilization of the oxyanion intermediate ,the bond between the oxygen(of the serine) and the carbon becomes stronger (full covalent bond), and this will lead to weaken the other bonds especially the weakest bonds, the H-bond with the backbone of the enzyme. The lone pair of electrons(negative charge)of the oxygen will be moved back to form a double bond . The formation of the double bond will weaken the peptide bond (scissile bond; Pronounced {sis-il},capable of being cut or divided) and then break it.

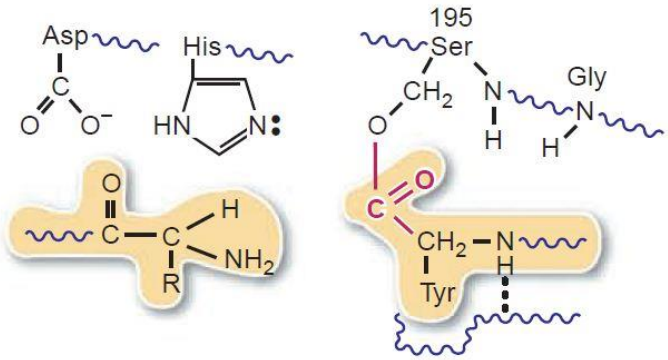


The second event of the catalytic action:

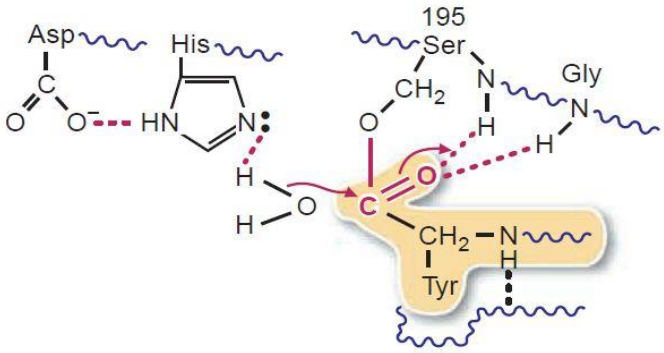
Hydrolysis of the intermediate to release the protein substrate and return the enzyme back to it's original shape.

-After the cleavage of the peptide bond ,the amino acid attached to the amine group could be released , this will lead to more space in the active site so water can get inside ,and make a Hydrogen bond with the free lone pair of Nitrogen (on HIS)(N----H−OH).So the covalent bond between H and OH in the water will be weakened, the OH now is activated and can attack the carbon atom (acid-base catalysis).

5. The covalent acyl–enzyme intermediate

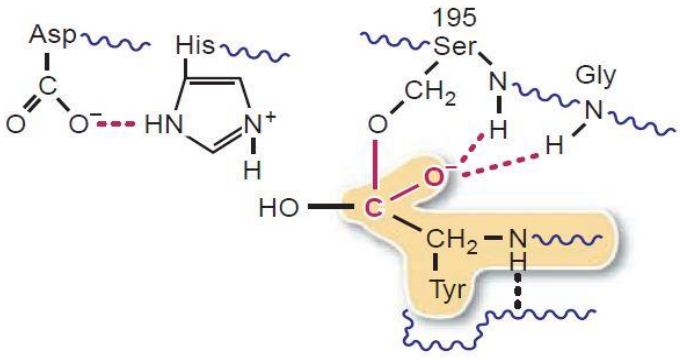


6. Water attacks the carbonyl carbon



-After this attack , there will be a formation of second oxyanion tetrahedral transition state complex(unstable).Electrons from the double bond move to the oxygen making it negative then there will be stabilization due to H-bond formation.

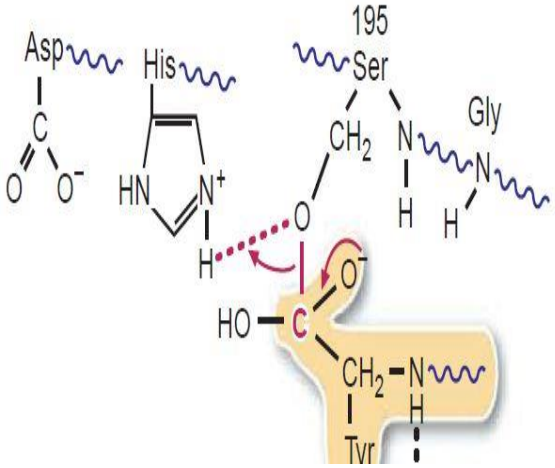
7. Second oxyanion tetrahedral intermediate



-The binding of the OH with the carbon will weaken the H-bonds and then there will be a double bond formation. The binding of the OH , double bond formation and the transfer of the H atom from Histidine to serine's oxygen (acid-base catalysis) will cause the full covalent bond between the carbon and the serine's oxygen to break.

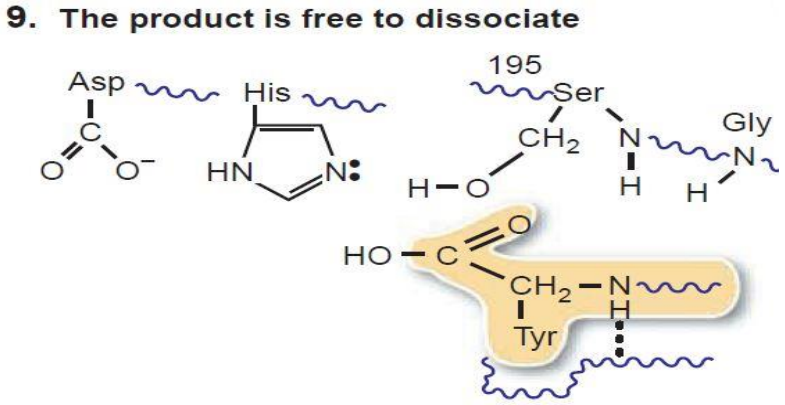
-Finally, the product dissociates and the serin and enzyme will return back to their original shape.

8. Acid catalysis breaks the acyl–enzyme covalent bond



Q) Why the Histidine can't activate the water before the cleavage of the peptide bond?

Because before the cleavage , the polypeptide backbone took the whole space in the active site ,so the water was excluded from the active site (no water to be activated from the first place).



➢ Energy diagram:

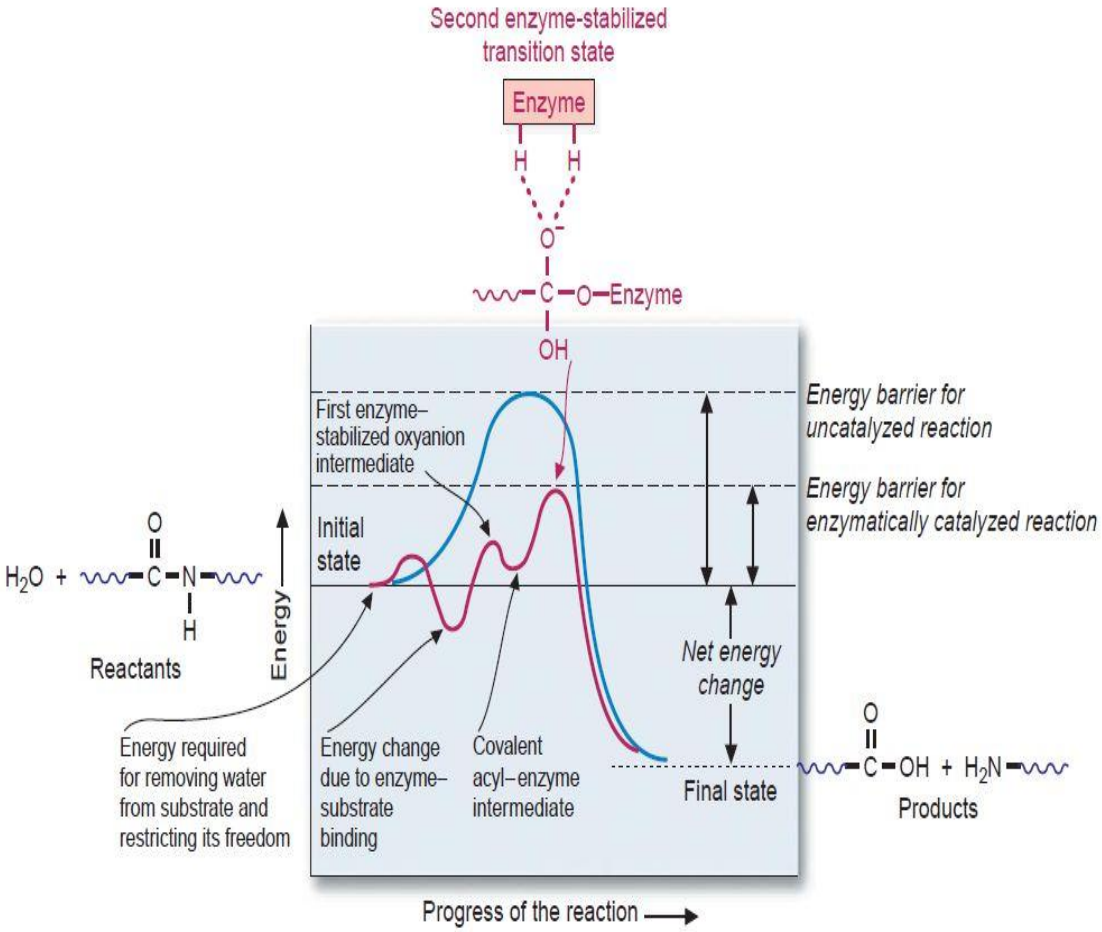


FIG. 8.10. A postulated energy diagram for the reaction catalyzed by chymotrypsin. In the presence of enzyme (*red*); in the absence of enzyme (*blue*). The energy barrier to the transition state is lowered in the enzyme-catalyzed reaction by the formation of additional bonds between the substrate and enzyme in the transition-state complex. The energy is provided by substrate binding to the enzyme. The enzyme does not, however, change the energy levels of the substrate or product.

: *Notes for the energy diagram*

.Formation of the intermediates →increase the energy-

. Stabilizing the intermediates by the formation of bonds → decrease the energy-

. Formation of bonds(H-bonds, covalent bonds) → decrease the energy-

* Formation of bonds —> GIVES energy. Because it's exothermic

*Breaking of bonds —> NEEDS energy. Because it's endothermic-

Intermediate: is the reaction product of each of these steps(in multistep rxn), except for- the last one, which forms the final product "قاع"

Transition state: state corresponding to the highest potential energy along this reaction- unstable),it can give either a product or a reactants)". "قمة

Uncatalyzed rxn : (blue line)

-One step rxn , One transition state , No intermediate.

-Higher Ea, slower rxn.

-spontaneous ,ΔG is negative , EXERGONIC.

****Catalyzed rxn***: (red line)

-Multistep rxn

- Three transition states (the three peaks) , the first one isn't a catalytic transition state because it's not from the mechanism of the rxn .

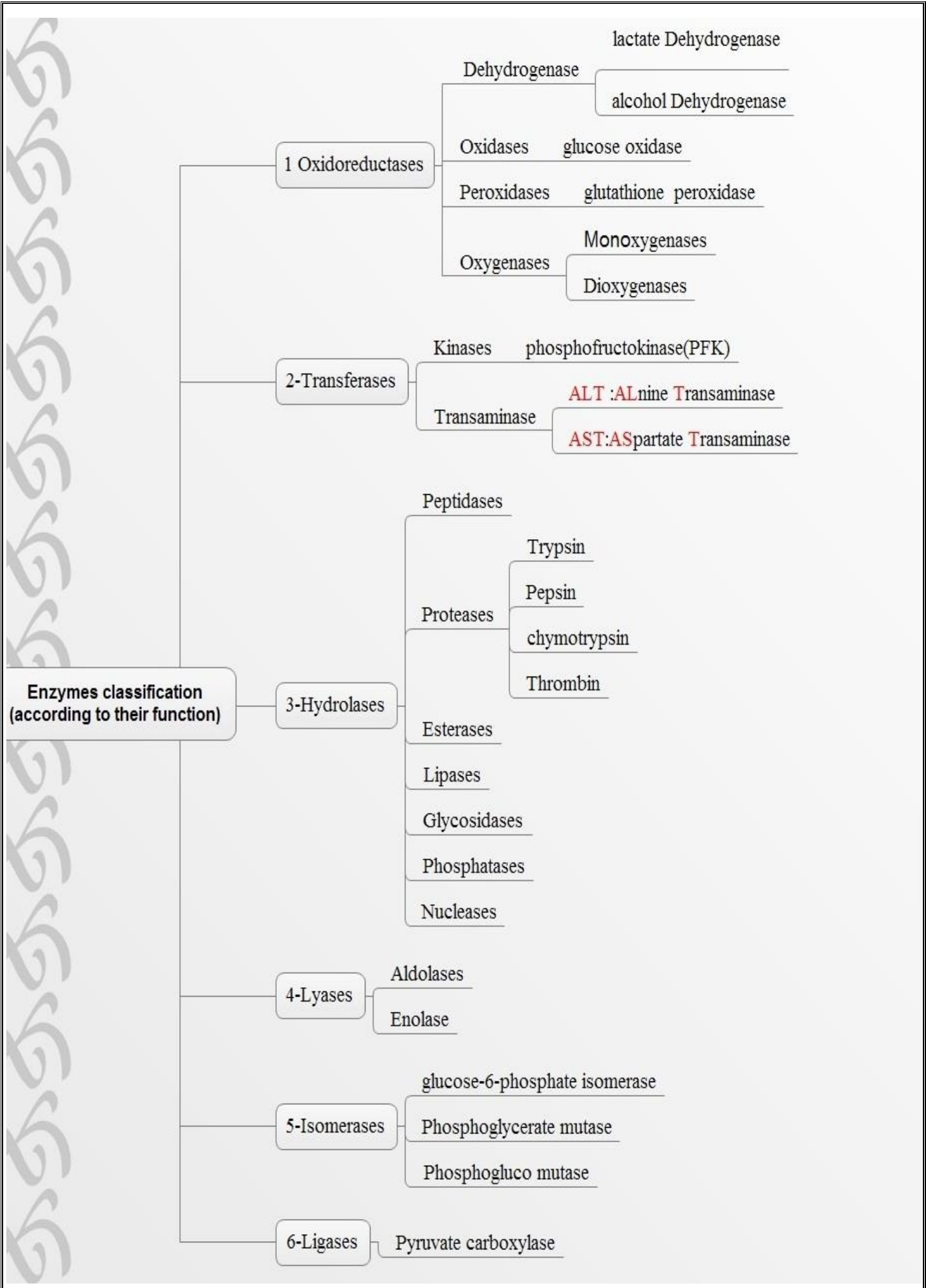
-Two intermediates that participate in the catalysis .

-The initial binding affects the bonds in the substance resulting in rising the energy, the stabilization and the right orientation lowers the energy .

-The second intermediate has higher energy than the first intermediate ;because the second one is attached to only OH and serine (less bonds, higher energy).The first intermediate is attached to serine and to other amino acid by peptide bond and that amino acid is attached to another amino acid and so on (more bonds ,less energy, more stable).

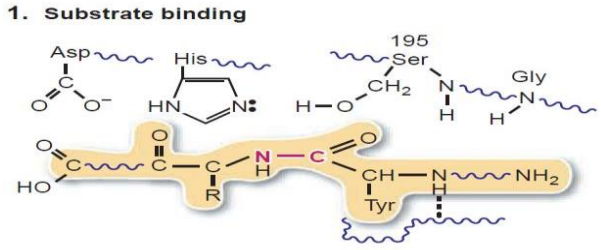
Q)How to know the role (function) for each amino acid in the active site ?

By site directed mutagenesis, they make mutations that change the amino acid with other amino acid which has the same shape, different function or different shape, same function. then they know the function of that amino acid .

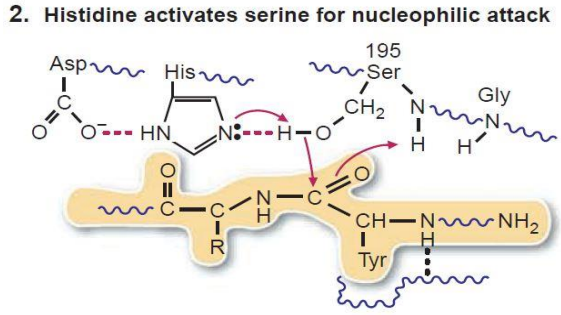

Enzymes classification (according to their function)
1 Oxidoreductases
Dehydrogenase
lactate Dehydrogenase
alcohol Dehydrogenase
Oxidases
glucose oxidase
Peroxidases
glutathione peroxidase
Oxygenases
Monoxygenases
Dioxygenases
2-Transferases
Kinases
phosphofructokinase(PFK)
Transaminase
ALT :ALnine Transaminase
AST:ASpartate Transaminase
3-Hydrolases
Peptidases
Proteases
Trypsin
Pepsin
chymotrypsin
Thrombin
Esterases
Lipases
Glycosidases
Phosphatases
Nucleases
4-Lyases
Aldolases
Enolase
5-Isomerases
glucose-6-phosphate isomerase
Phosphoglycerate mutase
Phosphogluco mutase
6-Ligases
Pyruvate carboxylase

The mechanism in brief :

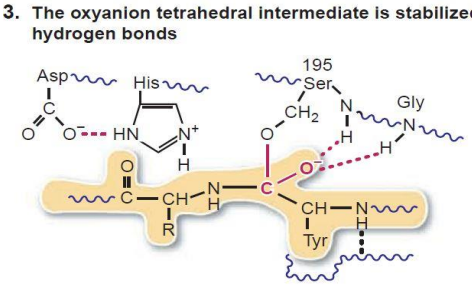
1: As the substrate protein binds to the active site, SER 195 and HIS are moved closer together and at the right orientation for the nitrogen electrons on HIS to attract the hydrogen of serine.



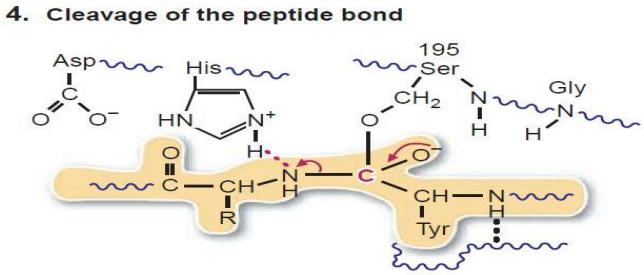
2: HIS serves as a general base catalyst because it abstracts a proton from the serine, increasing the nucleophilicity of the serine-oxygen, which attacks the carbonyl carbon on the peptide.



3: The electrons of the carbonyl group form the oxyanion intermediate. The oxyanion is stabilized by the N–H groups of serine 195 and glycine in the chymotrypsin peptide backbone.

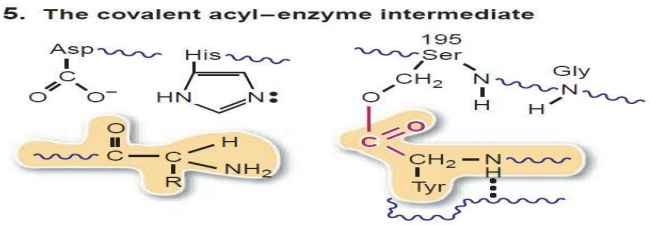


4: The amide nitrogen in the peptide bond is stabilized by interaction with the HIS proton.

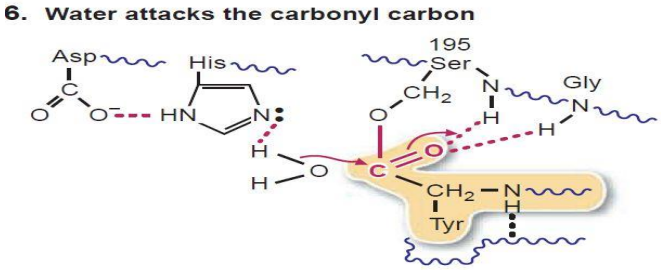


Here, the HIS acts as a general acid catalyst. Because the electrons of the carbon–nitrogen peptide bond withdraw into the nitrogen, the electrons of the carboxyanion return to the substrate carbonyl carbon, resulting in cleavage of the peptide bond.

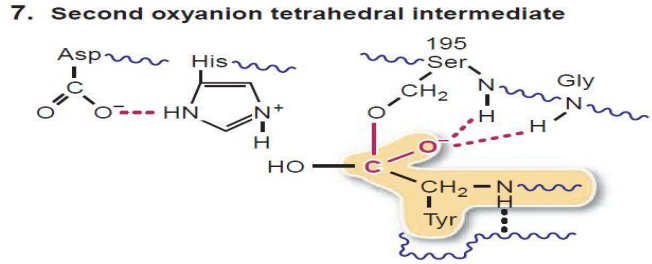
5: The cleavage of the peptide bond results in formation of the covalent acyl–enzyme intermediate, and the amide half of the cleaved protein dissociates.



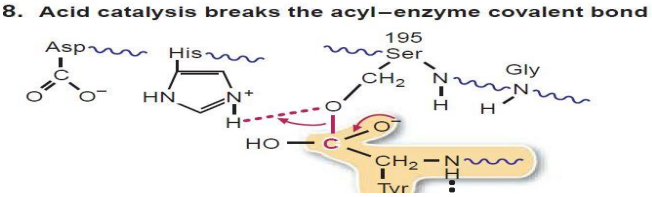
6: The nucleophilic attack by H_2O on the carbonyl carbon is activated by HIS, whose nitrogen electrons attract a proton from water.



7: The second oxyanion intermediate is formed. It is again stabilized by hydrogen bonds with the peptide backbone bonds of glycine and serine.



8: Because the HIS proton is donated to the electrons of the bond between the SER oxygen and the substrate carbonyl group, the electrons from the oxyanion return to the substrate carbon to form the carboxylic acid, and the acyl–enzyme bond is broken.



9: The enzyme, as it releases substrate, returns to its original state.