18

◉ Sheet ○ Slides

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Enzymes

Molecules don't function by themselves, proteins work with regards to other proteins. They don't function without interacting/binding with other molecules. ATP doesn't work without interacting with other proteins; such as myosin(for muscle contraction). It reacts with other proteins(enzymes) to transfer its phosphate group to another molecule or to use it as a source of energy.

- The function of nearly all proteins depends on their ability to bind other molecules (ligands).

Two properties of a protein characterize its interaction with ligands:

1-Affinity – the strength of binding between a protein and other molecules. (protein-protein, protein-small molecule, enzyme-substrate, ..etc)

2- Specificity – the ability of a protein to bind one molecule in preference to other molecules. So it can perform a specific function.

Proteins in the cell or in the blood interact with other molecules randomly at first, then they collide with each other and non-covalent interaction occurs (hydrogen bonds/electrostatic, etc). If the reaction is specific, then the interactions will be strong, otherwise, they will break.

- If non-covalent interactions are strong, then the affinity is high.

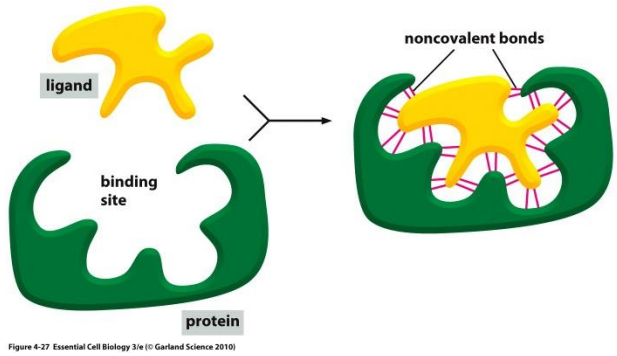


Figure 4-27 Essential Cell Biology 3/e (© Garland Science 2010)

What are enzymes?

Enzymes: specialized proteins that are able to conduct (catalyze) chemical reactions under biological conditions.

Example: H2+ O2→H2O

This reaction could happen after millions of years but with the presence of enzymes it would happen immediately- enzymes accelerate reactions.

Exception: Ribozymes!

They are enzymes but not proteins, they are Ribonucleic acid (RNA) molecules.

- Most enzymes have very specific functions converting **specific** substrates to the corresponding products.

Enzymes are catalysts.

- **They are usually used in small amounts relative to the reactants**. One enzyme can convert millions of substrates (in one second) to millions of products.
- **They modify and increase the rate of a reaction.**
- **At the end of the reaction, they undergone no change.** They aren't consumed in the reaction, but during the reaction the enzymes change a lot but they go back to their original structure at the end of the reaction (If enzymes don't change at all they will be useless), if enzymes at the end of the reaction are different then the beginning then they are considered as substrates.

How to express an enzymatic reaction?

*In enzymatic reactions: reactants = substrates

*** Simple expression of enzymatic reaction:**

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

E = free enzyme; S = free substrate, ES = enzyme-substrate complex; P = product of the reaction; EP = enzyme-product complex before the product is released

Simply, the enzyme binds to a substrate forming a complex (enzyme substrate complex(ES)), the enzyme changes the substrate into product forming (enzyme product complex(EP)), the product is released then we have product and the enzyme in its original conformation. In general, the reaction between enzyme and product doesn't exist , once the product is made, it's released immediately.

EP is not considered so the reaction is expressed as:

$$E + S \leftrightarrow ES \leftrightarrow E + P$$

What do enzymes do?

Enzymes accelerate reactions (range of 10^6 to 10^{14} (to 10^{20}).

Example: Catalase (10^8) & carbonic anhydrase (10^7)

Carbonic anhydrase is one of the fastest enzymes known.

One enzyme molecule hydrates 10^7 molecules of CO2 per second (versus 10^2 to 10^4 for uncatalyzed reactions)

Reaction Conditions	Activation Free Energy kJmol^{-1}	Activation Free Energy kcal mol^{-1}	Relative Rate
No catalyst	75.2	18.0	1
Platinum surface	48.9	11.7	2.77×10^4
Catalase	23.0	5.5	6.51×10^8

*You could notice that the rate of reaction and activation energy are inversely p.

Where does the reaction occur?

Each enzyme has a specific three-dimensional shape that includes the active site (a region where the biochemical reaction takes place).

•The active site contains a specialized amino acid sequence that facilitates the reaction.

• If a substance fits into the active site and binds to the enzyme, it is said to have affinity for the active site.

• Binding of a substrate into the active site can be regulated by a **regulatory site.**

Regulatory site: It is the site that controls the rate of reaction by changing the structure of the active site.

For example:- Enzyme hexokinase (which break down glucose in order to generate ATP for metabolism) if there is a lot of ATP molecules they bind to regulatory site then they close the active site , so glucose can't go to the active site any more.

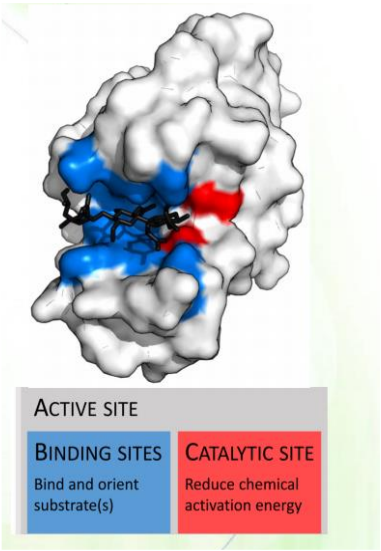
Catalytic group

Within the active site are two sub-sites, the binding site and the catalytic site.

The catalytic site contains residues (catalytic groups) that carry out the actual reaction.

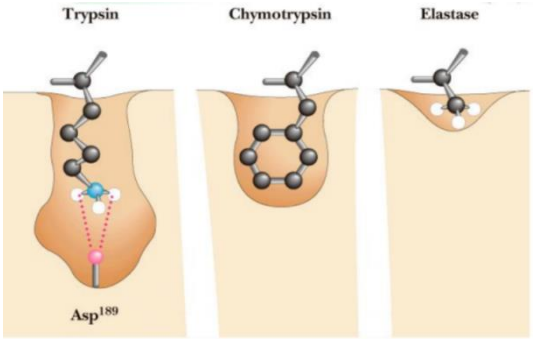
In some enzymes, the binding and catalytic sites are the same.

a funny example: we have two hands, one hand to hold the tomato (binding site) and the other one to dice it (catalytic site).



Binding specificity

The specificity and selectivity of enzymes is due to their precise interaction of active sites to their substrates and the degree of compatibility for this interaction.



Look at the figure- there are 3 ***proteases.*** (enzymes that break proteins into small peptides(proteolysis).

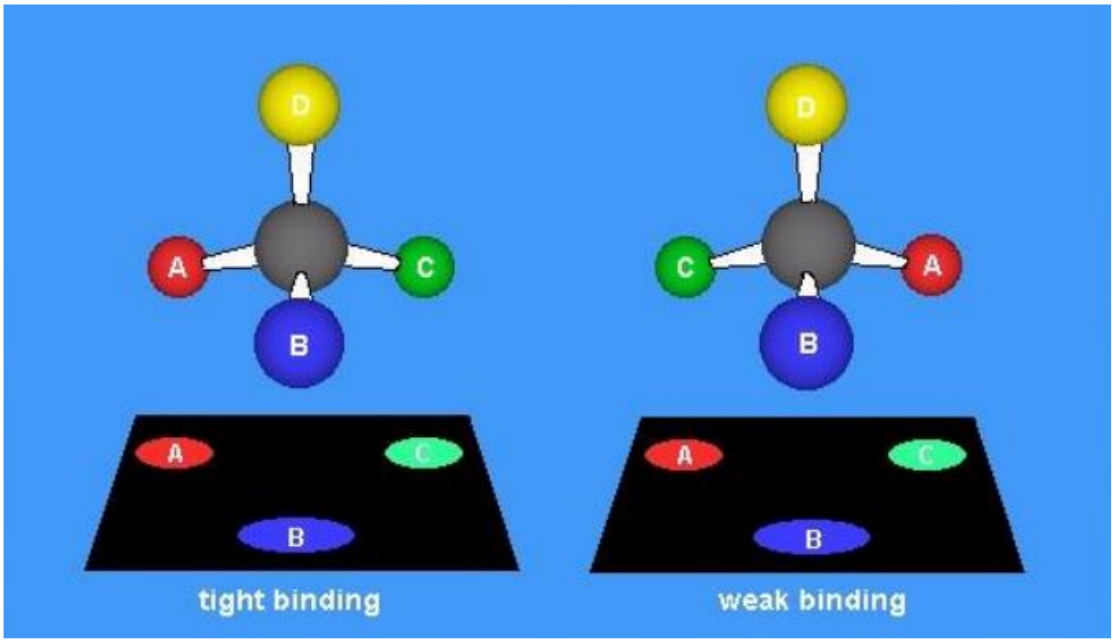
♣ Trypsin and chymotrypsin are digestive proteins that are secreted from the Pancreas and function in the intestine.

* Trypsin: Cleaves peptide chains mainly at the carboxyl side of positively charged amino acids like: lysine and arginine, because the active site contains aspartate (negatively charged amino acid).
*Chymotrypsin: Cleaves peptide chains on the C-terminal of AA which are aromatic like: phenylalanine, so the active site is more hydrophobic to fit the ring structure.
* Elastase: It cleaves peptide chains after Glycine (C-Terminus), so the active site must be small to fit the substrate.
Enzymes have different active sites and that's what make them specific.

Features of active site 1

-Binding occurs at least three points.

- So Chirality is important.



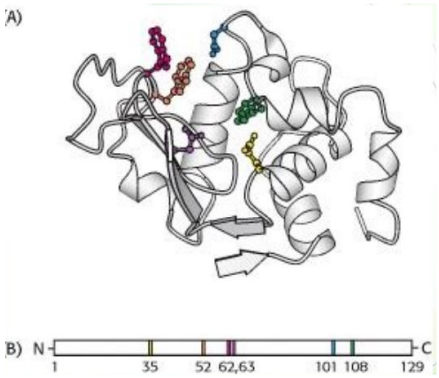
♣ The reaction between enzymes and substrate is specific because reaction occurs at least on 3 binding sites.
♣ Groups that are chiral, mirror images and non-superimposable are different.

Features of active site 2

It is a three-dimensional pocket or cleft formed by groups that come from different parts of the primary structure usually forming a domain made of multiple secondary structures.

♣ Amino acids, which form the active site, are not necessarily located next to each other in the primary structure.

♣ In the primary structure, amino acids of the active site are far away from each other, and when the protein folds all of them will get close to each other.

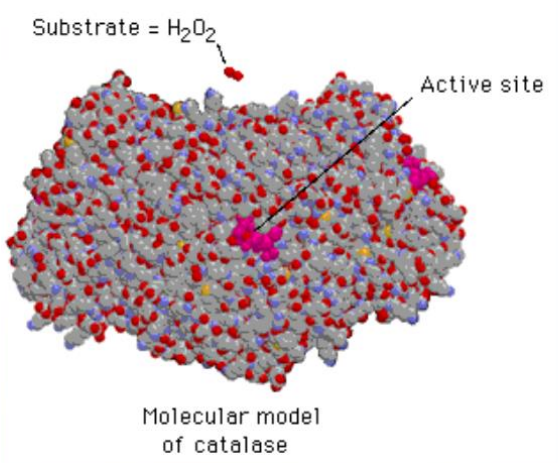


Features of active site 3

It is small relative to the total structure of an enzyme. The “extra” amino acids create the 3D active site.

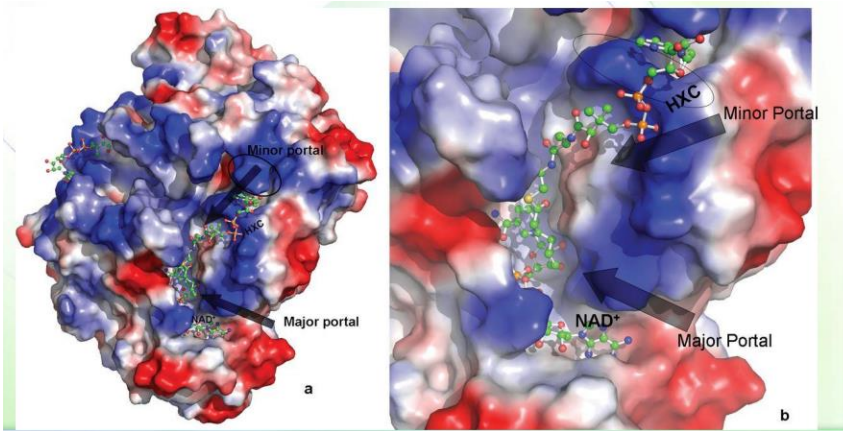
Active sites function, but can’t be formed without the help of the surrounding amino acids.

•In many proteins, the remaining amino acids may make up regulatory sites. When a regulator (whether it’s an activator or inhibitor) affects the enzyme, those amino acids move until the final structure of the active site changes completely.



Features of active site 4

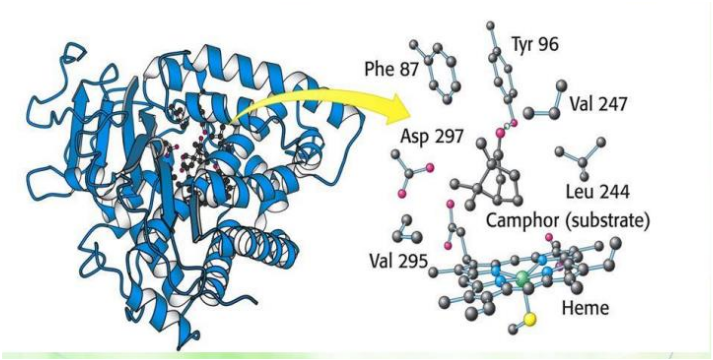
The active site is internal, not on the surface of the protein. So the substrate needs to go through a canal (cleft or crevice) to reach the active site.
The reaction takes place inside the enzyme, It contains nonpolar as well as polar residues.
The reaction doesn't occur on the surface because for example lactase is susceptible to disrupting factors (e.g. water) because it interacts with polar amino acids, so it disrupts the reaction between the substrate and the enzyme. Water is usually excluded from active sites unless it is part of the reaction.



Features of active site 5

Substrates bind to enzymes by multiple weak attractions (Non-covalent interactions). Usually, occur on the surface of the enzyme or the entrance of the active site, the substrate enters the active

site and the reaction occurs. So, non-covalent interactions are important to determine the function of the enzyme, if covalent interactions occur and these covalent bonds don't break before the end of the reaction, we lose the enzyme (that's how toxins and poisons work). In some reactions covalent bonds are formed but they break before the end of the reaction (Covalent catalysis).



How do substrates fit into the active site of enzymes?

1-Lock-and-key model (old)

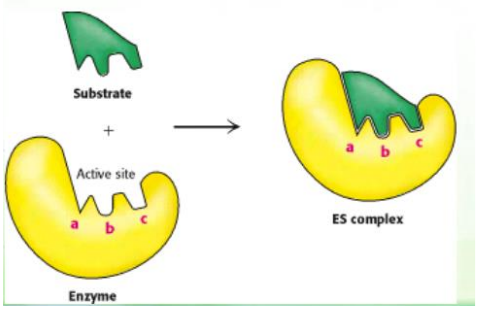
The structure of the molecule is complementary to the shape of the active site.

Here, the substrate fits directly into the active site (substrate doesn't change, and the enzyme doesn't change). Not really!!

-Proteins are dynamic in nature.

-Some enzymes can bind different substrates.

-Some enzymes catalyze multi-substrate reactions.



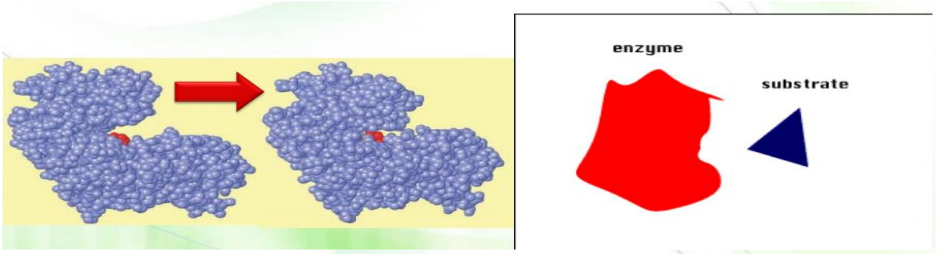
2-Induced fit model

More realistic model, the active site changes its shape according to the substrate.

Enzymes are flexible and active sites can be modified by binding of substrate.

♣ This model takes into consideration that enzymes are flexible and that the shape of active sites can be markedly modified by the binding of substrate. When the substrate first collides with the enzyme, the structure of the active site changes, so it fits the substrate better (it's dynamic). this noncovalent interaction may change the active site as well

** We must remember: the positive and negative charges of amino acid affect the shape of proteins (enzymes), repulsion of the same and attraction of the different.



How do enzymes accelerate reactions?

Every enzymatic reaction requires energy. This energy is stored in the bonds of the substrates.

H_2O has a covalent bond, if we break it, it will produce energy.

There are two forms of energy:

Potential - capacity to do work (stored)

Kinetic - energy of motion

Potential energy is more important in the study of biological or chemical systems. Molecules have their own potential energy stored in the bonds connecting atoms in molecules. It is known as **free energy or G (for Josiah Gibbs)**. It is the energy that is available for reactions.

♣ In biochemistry, we are dealing with potential energy, which is the energy that exists in the bonds connecting atoms of the molecules [substrates].

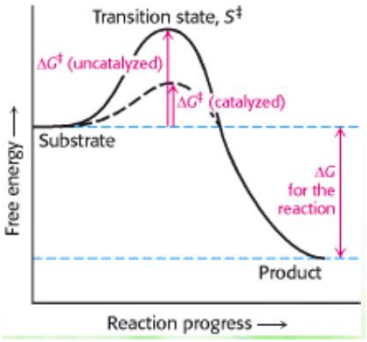
Free energy change:

The difference between the free energy values between reactants(substrates) and products (free- energy change ΔG)

$\Delta G = G_{products} - G_{reactants}$

*substrates: reactants in enzymatic reactions.

ΔG accounts for the equilibrium of the reaction and enzymes accelerate how quickly this equilibrium is reached.



What does it mean?

$$\Delta G = G \text{ products} - G \text{ reactants}$$

If ΔG is negative, **G** products is less than **G** reactants, energy is not needed to drive the reaction, but released, making the forward reaction (from left to right) spontaneous (the reaction is called exergonic).

If ΔG is positive, **G** product is more than **G** reactants, an input of energy is needed, making the reaction not spontaneous (the reaction is called endergonic).

- The reverse reaction is exergonic and, thus, spontaneous.

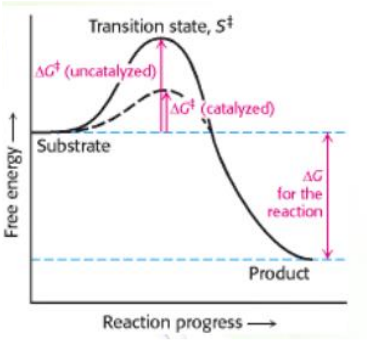
If ΔG is zero, both forward and reverse reactions occur at equal rates; the reaction is at equilibrium.

What do enzymes do?

*any enzymatic reaction whether endergonic or exergonic goes through a transition state (ES) that has higher free energy than both Substrates or Products. Transition State is an unstable molecule, and it isn't a one state but multiple states.

*The difference in free energy of the transition state and the substrate is called the ***activation energy.***

*Enzymes lower the activation energy, or, in other words, enzymes facilitate the formation of the transition state at a lower energy. ΔG of reactants or products never change!



*At the highest energy level, the substrate configuration is most unstable and is most tightly bound to the enzyme.

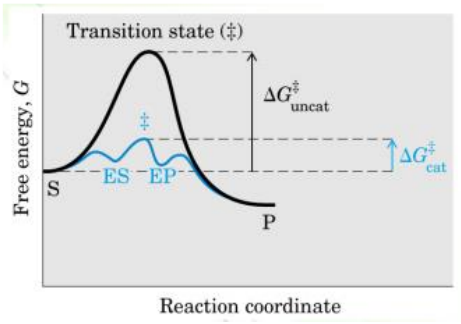
*The bonds or the electronic configuration are maximally strained.

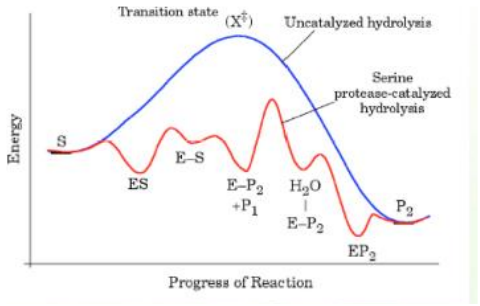
Alternative pathways

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

●The activation energy (EA) corresponds to the complex with the highest energy.

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

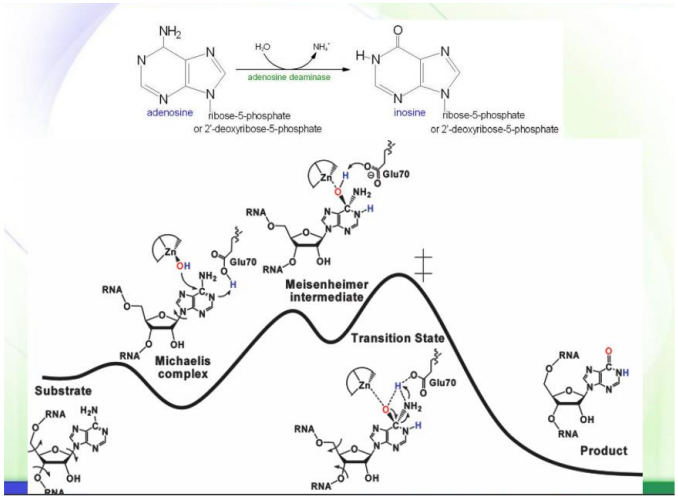




To calculate (EA)

It can be calculated by the difference between largest value of G in transition state and G of the substrate.

Example: Adenosine Deaminase



♣ This reaction undergoes different transition states, so we have different ΔGs.

♣ To calculate the activation energy:

- Activation Energy = [largest G of transition state – G of the substrate]

How do enzymes catalyze reactions?

1. Proximity of substrates together -to make the reaction faster.
2. Orientation of the active site to fit the substrate in the best fit possible. Enzymes put the substrates in the right angle/orientation, so the favorable interaction can occur.

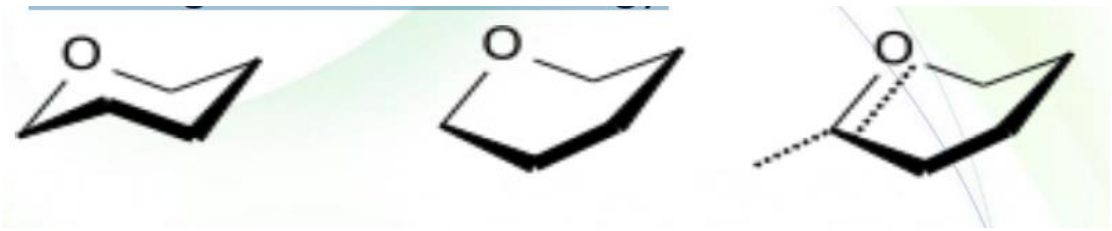
3. Changing the energy within bonds allowing the break up and formation of bonds. Enzymes strain the bond (they make it weak) so the reaction can happen.
4. Catalysis is the end result.

Examples of possible mechanisms to do so:

1. Catalysis by bond strain
2. Catalysis involves acid/base reactions
3. Covalent catalysis

Catalysis by bond strain:

In this form of catalysis, the induced structural rearrangements produce strained substrate bonds reducing the activation energy.



Example: lysozyme. The substrate, on binding, is distorted from the typical 'chair' hexose ring into the 'sofa' conformation, which is similar in shape to the transition state. (This enzyme reacts with the sugar, and it strains the bond/ changes the shape of the bond to make it easier to change to 'sofa' conformation.)

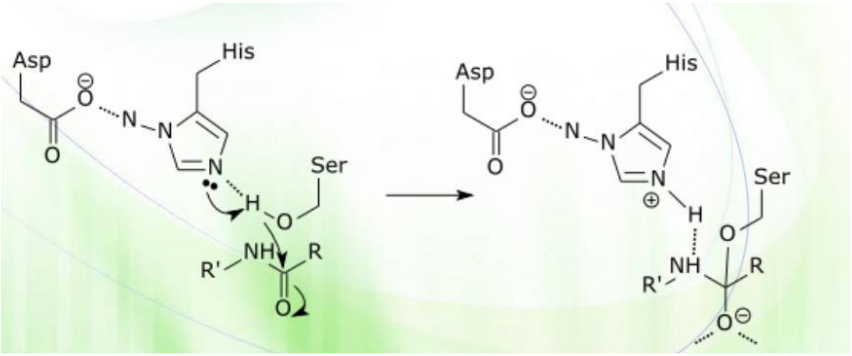
Catalysis involving proton donors (acids) and accepters (bases):

Enzymes facilitate the transfer of protons or electrons- they work as an acid/base. Usually we have polar amino acids like serine, glycine, arginine, etc.

The R groups of amino acids act as donors or acceptors of protons.

- Histidine is an excellent proton donor/acceptor at physiological PH.
- Example: - Serine proteases.

The enzyme doesn't change at the end, it only transfers electrons from one substance to another. (in this case it is a mediator)



Covalent and ionic catalysis: -

- A covalent intermediate forms between the enzyme or coenzyme and the substrate.
- Example: - proteolysis by serine proteases which include digestive enzymes.(trypsin , chymotrysin and elastase.)

*Trypsin cleaves the peptide bond between Lys or Arg and the next AA.

*This covalent bond is reversible and only present during the process of catalyzing the reaction.