Doctor 022

Biochemistry

Sheet no. 20

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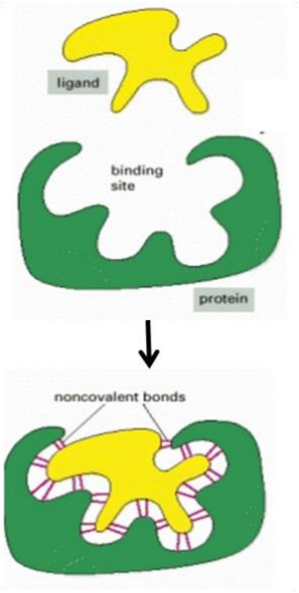
ENZYMES 1: characteristics and classification

General properties of proteins:



The function of proteins depends on their ability to bind other molecules (ligands). {hormone, peptides, lipids, other proteins}

Ligand: a substance that forms a complex with a biomolecule, usually via non-covalent interactions, to serve a biological purpose.

Two properties of a protein characterize its interaction with ligands:

1. **Affinity: the strength of binding between a protein and other molecules.**

An example of affinity is KD, which measures the rate at which the antibody dissociates from its target. Another example is P50, which measures the strength of interaction between {hemoglobin or myoglobin} and {oxygen}.

2. **Specificity: the ability of a protein to bind one molecule in preference to other molecules.**

***There are enzymes that interact with only one substrate, it's only involved in one reaction. On the other hand, there are non-specific interactions, for example: Albumin.**

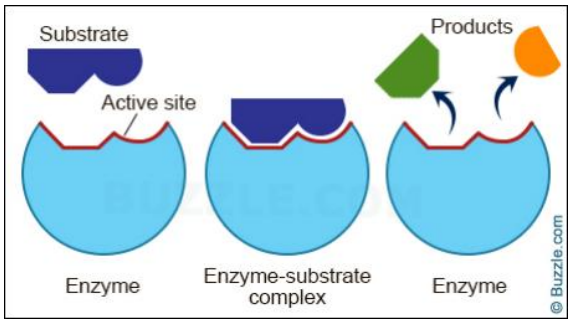
-Albumin binds to other proteins, hormones, drugs, and fats, and transports them through the blood. So, albumin is not specific at all, but it plays an important role in the transport of molecules.

***Remember: Albumin is a protein found in the blood plasma and it makes up about 60% of the proteins in the blood.**

***Physiologically, the interaction between two molecules is a non-covalent interaction, which is reversible, could be weak or strong (it depends on the number of interactions).**

What are enzymes?

❖ **Enzymes: Specialized <u>proteins</u> that conduct (catalyze) chemical reactions under biological conditions.**



***In general, all enzymes are proteins, except ribozymes (to be discussed).**

{So, they have primary structures, secondary structures, tertiary structures, and some of them have quaternary structures.}

In enzymatic reactions, reactants are known as substrates.

Most enzymes have very specific functions in converting specific substrates to products.

➢ **Enzymes are catalysts. (So, they speed up reactions.)**

- **They exist in small amounts relative to the reactants.**
- **They increase the rate of a reaction.**
- **At the end of the reaction, they do not change. (They change conformation during the reaction, then by end of the reaction, they go back to their normal conformation)**

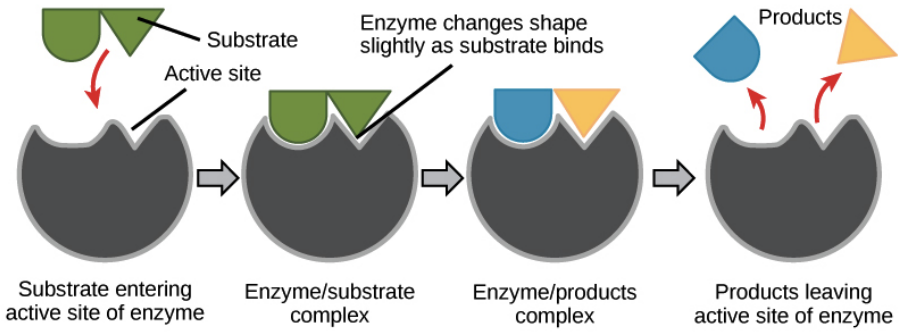
How can we express an enzymatic reaction?

Simple expression of enzymatic reaction:

E + S ⇆ ES ⇆ EP ⇆ E + P **For simplicity: E + S ⇆ ES ⇆ E + P**

E = free enzyme; S = free substrate, ES = enzyme-substrate complex,

P = product of the reaction, and EP = enzyme-product complex before the product is released



What do enzymes do?

- **Enzymes accelerate reactions (usually within a range of 10^6 to 10^{14}).**

Example: Catalase (10^8) & carbonic anhydrase (10^6)

- ✓ **Carbonic anhydrase:** $CO_2 + H_2O \overset{\text{Carbonic anhydrase}}{\rightleftharpoons} H_2CO_3$

***One enzyme molecule hydrates 10^6 molecules of CO2 per second (versus 1 per 10 seconds for uncatalyzed reactions).**

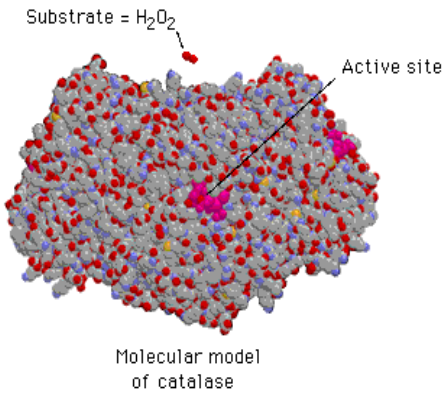
- ✓ **Catalase:** $2\,H_2O_2 \overset{\text{Catalase}}{\rightleftharpoons} 2\,H_2O + O_2\,(g)$

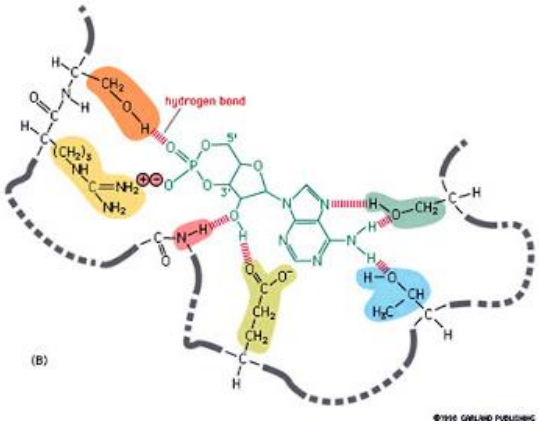
Reaction Conditions	Relative Rate
No catalyst	1
Platinum surface	2.77×10^4
Catalase	6.51×10^8

*Note: Platinum surface is not an enzyme, but the substrate binds with the surface and it is catalysed by changing its structure.

Where does the reaction occur?

- **Each enzyme has a specific three-dimensional shape called the active site (a region where the biochemical reactions take place).**



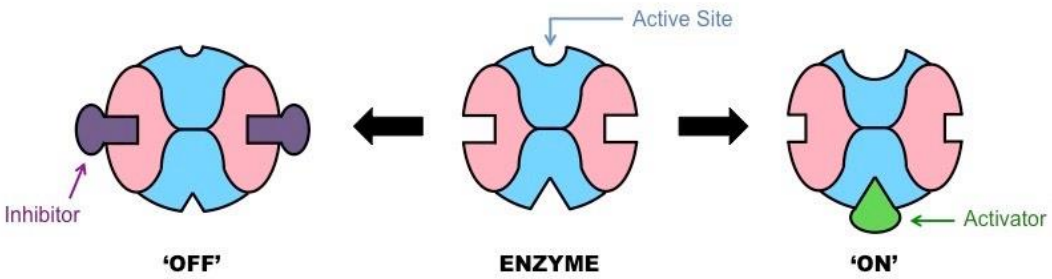


The active site contains a specialized amino acid sequence that facilitates the reaction.

*For clarification:

-In general the enzyme is a large molecule, it has a site inside, it is called the active site, where the substrate binds and the reaction takes place, then the product is produced. So the enzyme catalyzes the reaction in the active site.

-Enzymes are proteins (consist of amino acids), what happens is that the substrate enters the active site, and the R groups of the amino acids in the active site interact (via non-covalent interactions) with the substrate, and change its shape, at the end of the process we get a product.



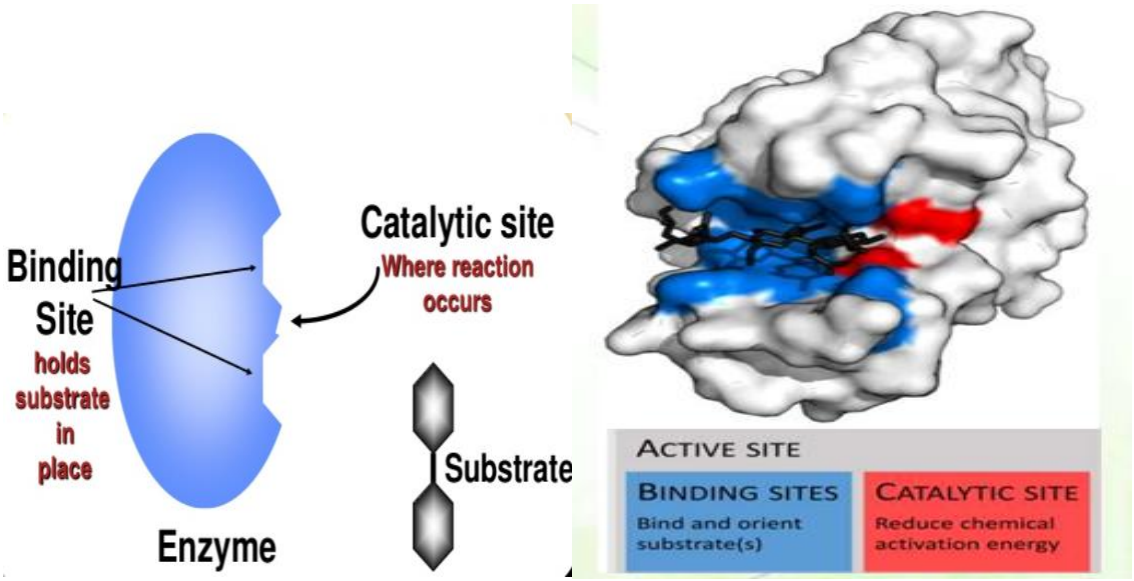
Binding of a substrate into the active site can be regulated by a *regulatory site*.

-Some enzymes have additional sites called the regulatory sites (that turn on /off) the reaction or (increase/decrease) the efficiency of the enzyme, depending on the type of regulator (effector).

➢ Catalytic and binding groups:

- ✓ Within the active site are two sub-sites: the binding and catalytic sites.
- ✓ The catalytic site contains amino acid residues (catalytic group) that carry out the actual reaction.
- ✓ In some enzymes, the binding and catalytic sites are the same.

*Catalytic site is the site that catalyzes the reaction, while Binding site : is the site where R-groups of amino acids bind to the substrate.



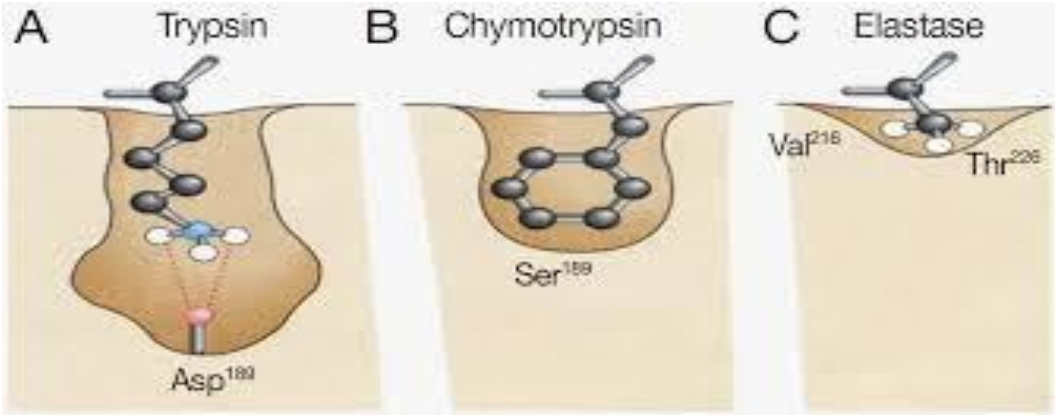
➢ Binding specificiy:

- 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.

-Enzymes are very specific, some enzymes may only bind a single substrate , such as catalase or carbonic anhydrase. Other enzymes can catalyze the same type of reaction with different substrates, such as Hexokinase (which

phosphorylates hexoses {sugers that have 6 carbons}). So, it is specific in terms of the type of reaction, but it has less specificity in terms of substrate (can bind to more than one substrate, but it isn't specific for all sugers, just {hexoses}).

***The specificity depends on the type of amino acid(s) that's found on the active site of the enzyme. (amino acids of the substrate must fit the two sub-sites: the binding and catalytic sites).**



Note: don't memorize the amino acids of the active site that interact with the amino acids of the substrate for each one.

- **Trypsin, Chymotrypsin and Elastase are proteases (specific enzymes that degrade proteins). These enzymes are secreted by the pancreas into the intestine to digest proteins.**

1- Trypsin: when the protein has (Lys or Arg) (aliphatic and positevly charged amino acids) in its structure, the active site of the enzyme interacts with the R groups of these amino acids, then breaks down the peptide bond between (Lys or Arg) and the next amino acid in the polypeptide chain.

***The active site of tyrpsin has (Asp) amino acid which is negatively charged, the attraction will occur between Asp and (Lys or Arg).**

2- Chymotrypsin: when the protein has (Phe, Tyr or Trp) (hydrophobic amino acids that have ring structures {aromatic rings} in their R groups).

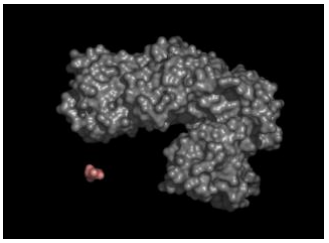
The active site of chymotrypsin has (Ser) which is a hydrophilic amino acid that has an OH group at the end of the R chain that makes it reactive.

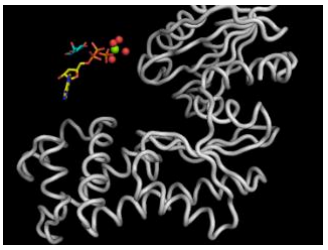
3-Elastase: when the protein has (Gly, Ala or Val) (amino acids that have Non-polar and relatively small R groups in their structure), so the active site will be small. The active site of elastase has (Thr) amino acid .

Features of active site:

1-It is internal relative to the enzyme and looks like a canal

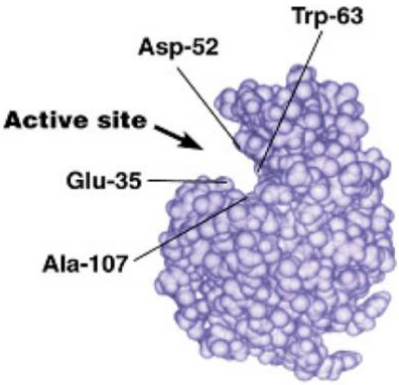
So, it doesn't exist on the surface because we need to isolate the active site from anything random that could bind to it, nothing could pass except the substrate that fits into the active site. the amino acids that exist on the gate work as a guard that interacts with the substrate allowing it to reach and bind to the active site, but if there is any repulsion no interaction will happen so it will not enter.

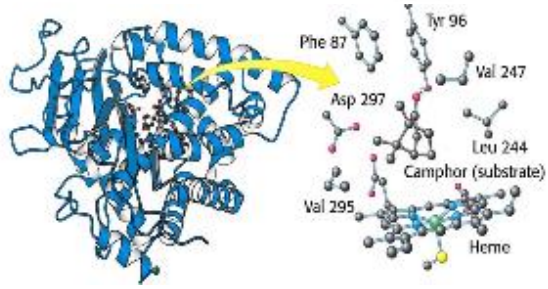




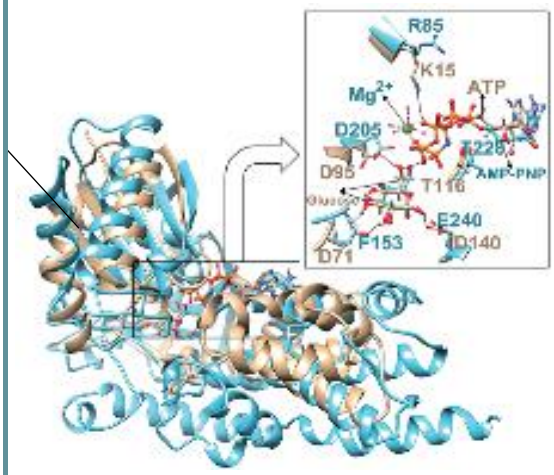
2-It is a three-dimensional pocket formed by amino acid groups that come from different parts of the primary structure usually forming a domain.

Just like any other protein, it will be folded (in this picture we could see that the active site contains Trp-63 (the amino acid number 63 in the primary structure) next to Asp-52 (the amino acid number 52 in the primary structure) and so on, these amino acids are from different parts of the primary structure but after folding they become next to each other.





lysozyme



Hexokinase

3- The amino acid residues can be nonpolar and polar.

- ✓ **Water is usually excluded unless it is a part of the reaction. (Because it is reactive).**
- ✓ **Substrates bind to enzymes by multiple weak attractions.**
  ***It depends on the substrate (to be polar or nonpolar) but in general the amino acids that do catalysis should be reactive (polar), the active site could contain nonpolar amino acids to help in the interaction with the substrate (non polar ones will be internal).**

***Enzymes always interact with their substrate by non-covalent interactions,**

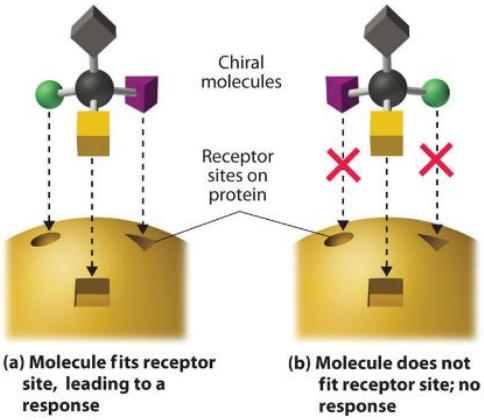
but during the process of catalyzing the reaction; the amino acid residues of the enzyme could form covalent bonds with the substrate itself, The doctor used the analogy of the black box, he said that enzymes always interact with their substrate via non-covalent interactions, but during the catalyzing process itself, you can consider that the substrate is trapped in a black box with a lot of things & stuff happening to it, like forming covalent bonds with the amino acid residues of the active site of the enzyme.

In short, enzymes interaction with their substrate==> non-covalent interactions, but during the catalyzing process {black box} ==> covalent bonds between the enzyme & the substrate could be formed.

4- Binding of substrates to active sites occurs at, at least, three points.

-Chirality is important, hence specificity.

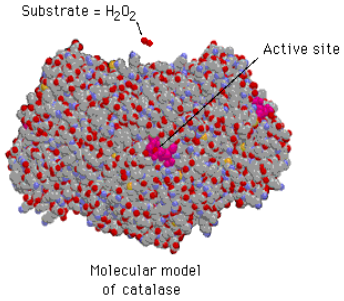
***The chirality means that the molecule with its mirror image are two different molecules (as an example, sugars that are found naturally in our body and our enzymes can handle are in the D conformation and not L, and the amino acids that are in the L conformation.)**



5- It is small relative to the total structure of an enzyme.

- **The "extra" amino acids create the 3D active site.**

The remaining amino acids may make up regulatory sites.

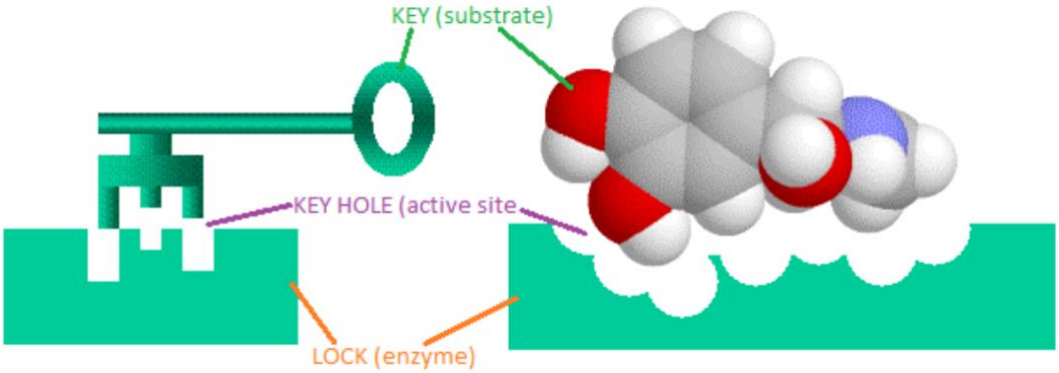


How do substrates fit into the active site of enzymes?

What is the shape of the active site? we have 2 theories: Lock-and-key model and induced fit model.

- **Lock-and-key model (old).**

Here, the substrate fits directly into the active site, as the key of the door, during the reaction the substrate binds to the active site, the active site can then change, after the reaction ends it returns to its default state.



❖ **Induced fit model.**

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

(in order for the substrate to fit into the active site, the active site must change its structure), so we conclude that the substrate and the active site before modification are not complementary to each other , the modification can be done by the amino acids in the enzyme , example : aspartate in the active site of the enzeyme moves toward lysine in the substrate.

Glucokinase is the king of both!

A kinase specific for glucose only , works as lock-and-key and induced fit.

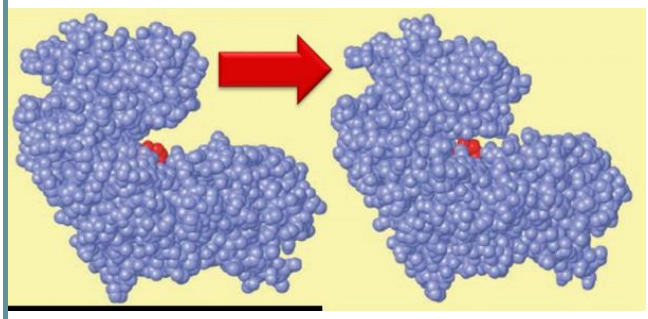
- **As an example for enzyme has the both models : is {Glucokinase which phosphorylates the glucose suger at carbon 6 .}**

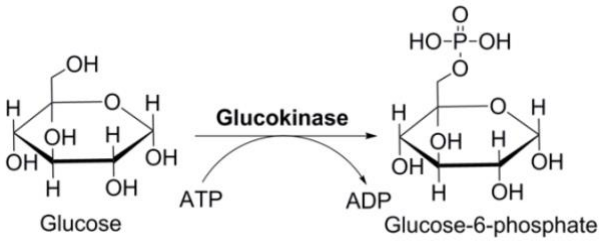
- **Glucose binds the active site of glucokinase (tightly) as a lock and key resulting in the induced fit.**

- **The induced fit changes the conformation of the whole enzyme closing the cleft, and improving the binding for ATP, the other substrate. (we get the phosphate group that is added to glucose from ATP).**

- **Thus, the multiple interactions between the substrate and the enzyme in the catalytic site serve both for substrate recognition and for initiating the next stage of the reaction.**

So, glucose binds as lock-and-key , while ATP as induced fit.





How do enzymes accelerate reactions?

➢ Types of 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. (Enzymatic reactions).**
- **Molecules have their own potential energy stored in the bonds (because of the existence of electrons) connecting atoms in molecules.**
    - **It is known as free energy or G (for Josiah Gibbs).**
    - **It is the energy that is available for reactions.**

***When enzymatic reaction takes place, they rely on potential energy in the bonds. (G)**

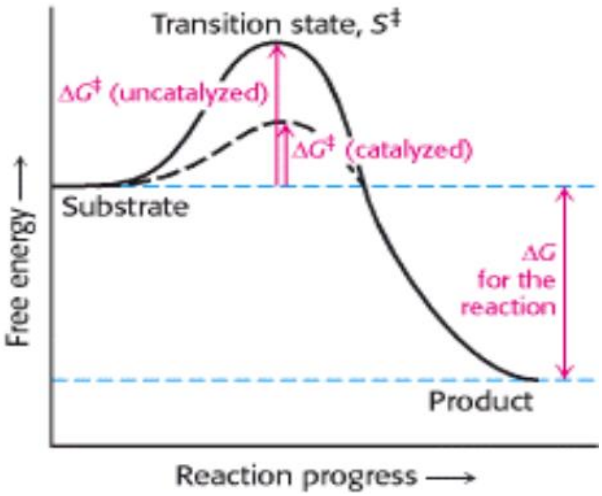
Free energy (G):

G: is the potential energy in bonds between 2 atoms or molecules.

The difference between the free energy values between reactants and products (free-energy change ▲G):

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

***Enzymes break bonds of reactants & make new bonds to produce the product, so there is a difference in G value between the reactant and the product.**



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

***Look at this figure, because $G_{product}$ is less than $G_{reactant}$ we conclude that there is a release of energy.**

The released energy could be any form of energy such as: sound, heat, etc.

What does it mean?

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

- **If ▲G 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 ▲G is positive, $G_{products}$ 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 ▲G is zero, both forward and reverse reactions occur at equal rates; the reaction is at equilibrium.**

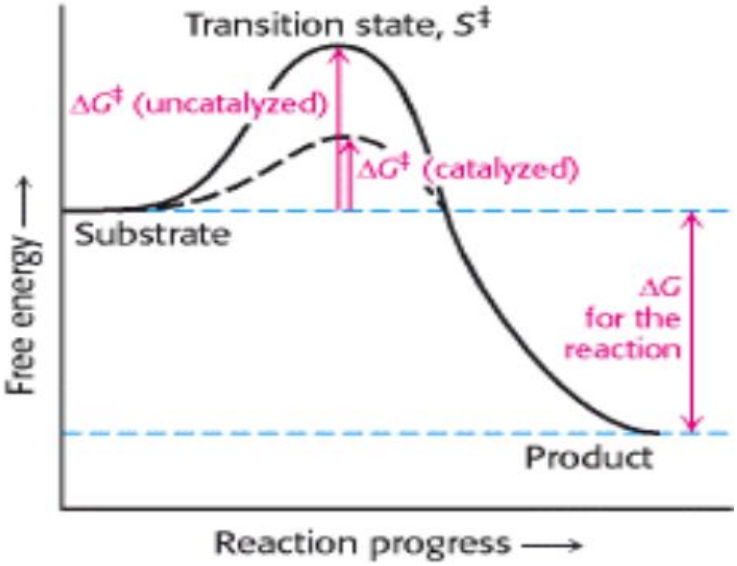
***All reactions need the activation energy, so the enzymes reduce this activation energy. (Look at catalysed plot in the figure above).**

What do enzymes do?

- **Any enzymatic reaction goes through a transition state (ES) that has a higher free energy than does either S or P.**
- **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.**

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

***The bonds are maximally strained.**



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