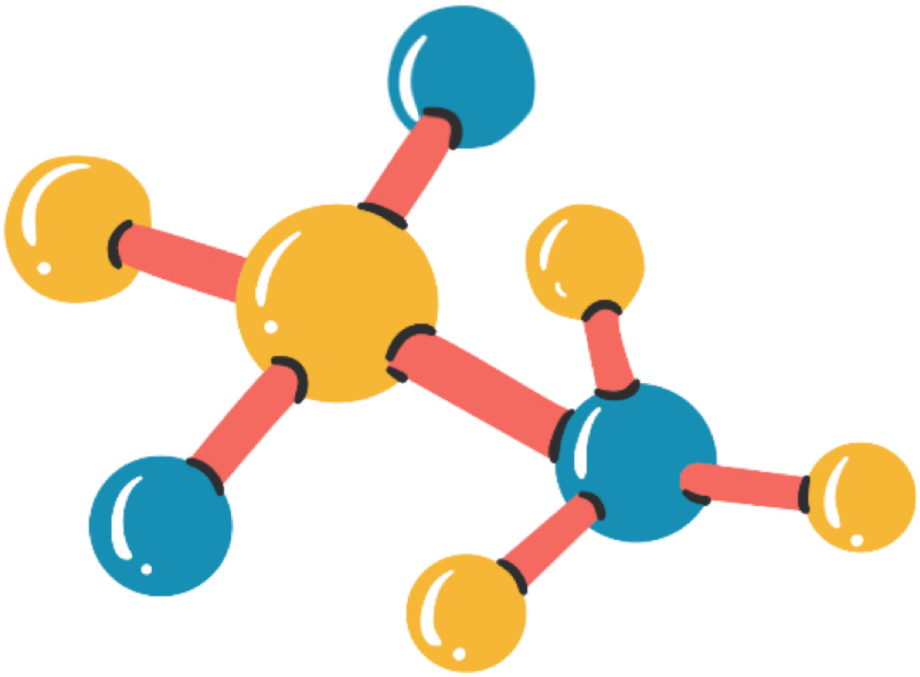
Biochemistry



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Writer: Doctor 2020
Corrector: Doctor 2020
Doctor : Dr.Nafeth AbuTarboush

Enzymes: -

- 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:
  - **Affinity: the strength of binding between a protein and other molecule**. (It's not specific for proteins only, it's the strength of binding between any two molecules.)
  - **Specificity: the ability of a protein to bind one molecule in preference to other molecules.**
- The specificity is a scale meaning that the **more binding** to different molecules → **less specificity**, the **less binding** to different molecules → **more specificity**.
- The more the specificity to a certain molecule the more the affinity **but isn't not a must all the time.**

Are enzymes important?

- **In the human body, almost every metabolic process involves the use of enzymes so without enzymes we can't live.**
  - The enzymes were exploited and produced in a massive amount so they can be enough to use in industry.

In the tea leaves there are polyphenols, these polyphenols have a cyclic (ring) structure that can withstand free radicals much better than aliphatic structures, so they work as antioxidant in a better way compared to aliphatic structure, polyphenols present in a high concentration in tea leaves.

- **What is the differences between green tea and black tea although they are produced from the same tea leaves?**
  - There are enzymes in tea leaves called **polyphenol oxidases which oxidize phenols and convert them into tannins** which impart the darker color and characteristic flavors but this enzyme can't convert the polyphenols without oxygen, so we crush the tea leaves and expose them to the oxygen in air and we will get black tea.

- **Note in both green and black tea there are enzymes but in black tea the enzymes work much better because we exposed them to oxygen.**

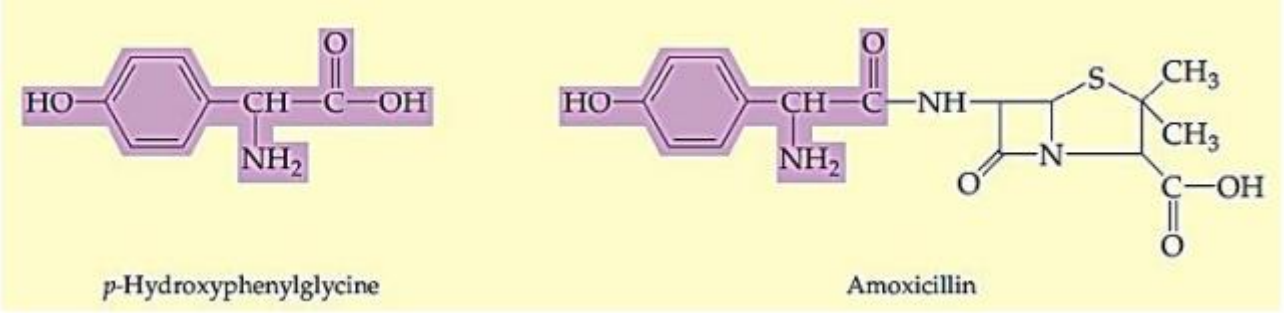
- **Why these candies are always sweet and, cohesive and connected to each other in a certain structure?**

  - Because we use corn syrup, this corn syrup is being made through enzymes and its available and cheap, it looks like honey but we don't use honey because honey is very expensive due to its natural occurring.

- **Why chocolate is hard from outside and soft from inside?**

  - Because there are enzymes called **sucrase** (yeast enzyme) which **breaks up the sucrose** into its monomers, these enzymes present inside the chocolate and gives it the soft structure.

- ✓ Production of Amoxicillin depends on the use of enzymes to connect the **Hydroxyphenylglycine** into the other structure and build up the **Amoxicillin**.
- ✓ Amoxicillin is the most widely used antibiotics in the whole world.



- Enzymes also can be used on meat to break down proteins and make it more soluble which will result in a very soft structure.
- The washing liquid contains enzymes to break down lipids.
- We also use enzymes in researches and experiments.

Wherever you look in this life you will find enzymes.

Enzymes: The Biological Catalysts

What are enzymes?

- **(They are specialized proteins mostly, small amounts, they accelerate the reaction and on the end of the reaction they come back to their original shape, and they are not being consumed during the reaction, no change).**
  - Enzymes should change their structure/conformation during the reaction and this what makes them catalyze / speed the chemical reaction and after they get finished they get back to their original shape allowing them to catalyze another reaction.
  - **Ribozymes are RNA molecules that can function as enzymes, so they are the exception from the above definition** (because they are not protein enzymes).
  - Because enzymes can be used again and again, thus we don't need a high concentration of them.

❖ When we define the enzymes we say that they are biological catalysts (they work in biological systems or they are produced in them), accordingly there are other type of catalysts which are named **chemical catalysts** (they have the ability to catalyze reactions outside biological systems and they aren't produced in them).

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} 2\,H_2O + O_2\,(g) \qquad CO_2 + H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} H_2CO_3$$

❖ This is the **Carbonic Anhydrases** enzyme that produce **Carbonic acid** from **Water** and **CO_2** which is the buffering system of the blood, and it works in both directions.

❖ **Catalase** enzyme has the ability of breaking down **Hydrogen peroxide H_2O_2**(toxin material) into water and oxygen, this reaction can occur by its self but it needs a huge amount of time.

Reaction Conditions	Activation Free Energy		Relative Rate
	$kJmol^{-1}$	$kcal\ mol^{-1}$	
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

If you compare this reaction without Catalyst, chemical catalysts, and enzymes you will notice that **chemical catalysts** speed up the reaction about **4 folds** but the **enzymes** about **8 folds.**

- **Enzymes are the most efficient catalysts ever known.**
- **They speed up the reaction usually in the range of 10^6 to 10^{14} and it can reach 10^{20}**
- **Non-enzymatic catalysts (chemical catalysts) speed up the reaction usually in the range (10^2 to 10^4).**
- **The actions of enzymes are fine-tuned by regulatory processes.**
- **Examples: catalase (10^8) & carbonic anhydrases (10^7).**

How to express an enzymatic reaction?

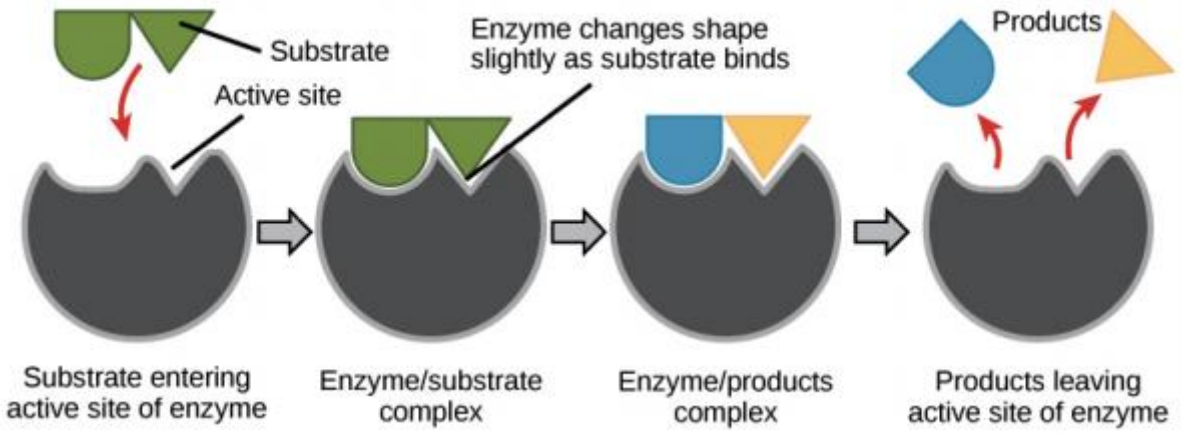
- **In enzymatic reactions, reactants are known as substrates that bind to enzymes.**
- **We can simply express an enzymatic reaction using this formula:**

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

Or

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

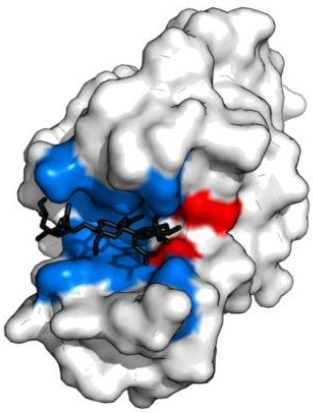
Where E is the free enzyme; S is the free substrate, ES is the enzyme-substrate complex; P is the product of the reaction; and EP is the enzyme-product complex before the product is released.

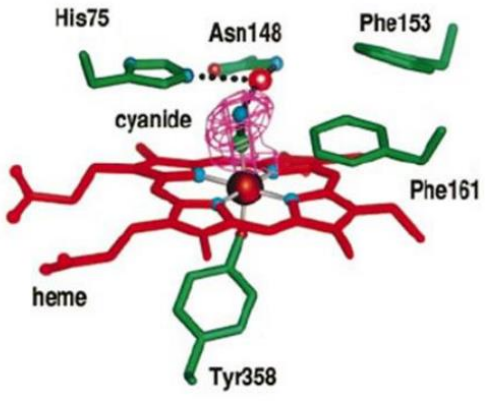


So enzyme plus substrate produce a new structure called enzyme substrate complex, and there will be a change on this complex to produce enzyme product complex, and this structure (product complex) will release the product, and the enzyme will get back to its original shape.

Active site of enzymes: -

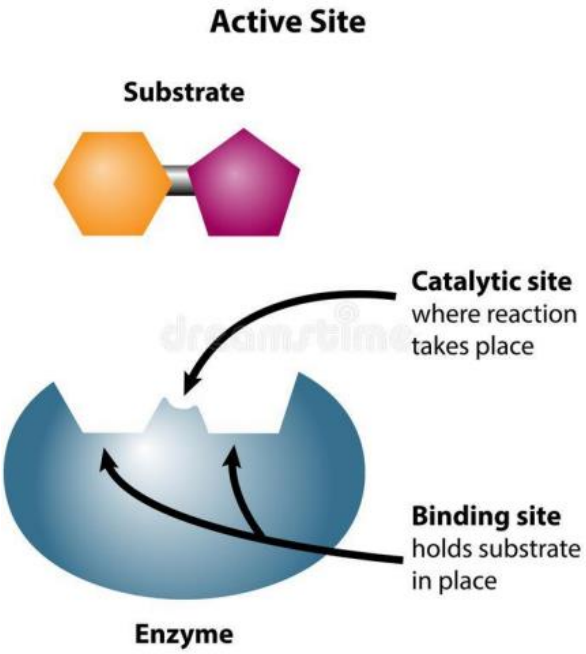
- Where does the reaction occur in the enzymes?
  - **In the active site within the enzyme which is a specific three-dimensional shape which includes a region where the biochemical reaction takes place.**
  - **The active site lined with a specialized amino acids that facilitate the reaction. (These amino acids aren't in a sequence they come from different parts of the protein (enzyme) to create this 3D space (Active site)).**



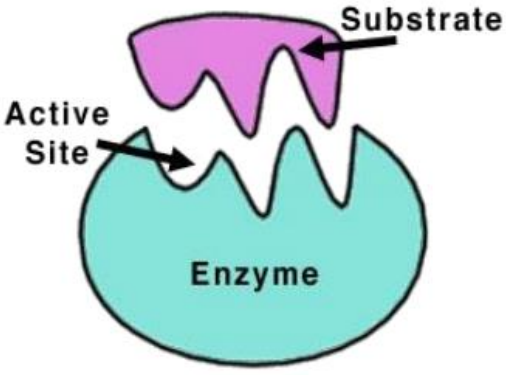


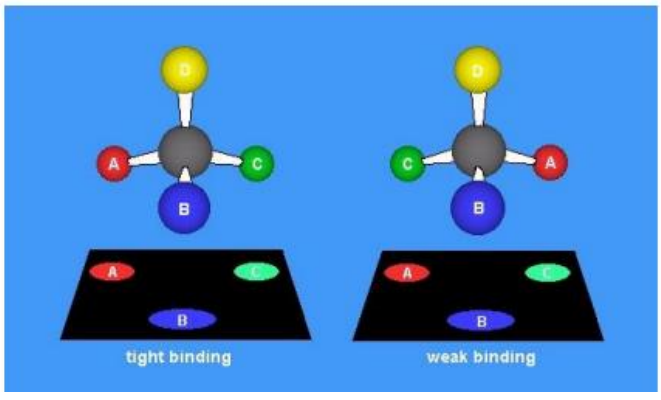
- **The specific amino acids inside the active site determine the specificity of the enzyme toward a certain substrate, accordingly the side chains / R groups of these amino acids are projecting to the space of active site itself, so they are able to bind the substrate.**

- **When the substrate come to the active site there will be two actions in that substrate: 1) it has to bind to the active site. 2) then the catalysis process is done.**
- **If the active site is large then it will have two sub-sites, the binding site and the catalytic site.**
- **When the size of the active site is small then the binding & catalytic sites are the same.**



  - **Binding site: binds substrate through ionic, H-bonding or other electrostatic forces, or hydrophobic interactions.**
  - **Catalytic site: contains the catalytic groups.**





- **You will never find an active site on the surface of the protein / enzyme it's always embedded inside the protein its look like canal, pocket, clefts or crevices.**

  - Why active site must be inside the protein not on the surface?
  - To enclose the substrate as the reaction can start and this doesn't work when the active site on the surface.

- **Water is usually excluded after binding unless it participates in the reaction**
- **Substrates are bound to enzymes by multiple weak interactions (non covalent interactions such as: electrostatic, hydrogen, van der Waals, & hydrophobic).**

➢ Why substrate should bind non covalently to the enzyme?

- Because substrate after the reaction -it will be product -should be released, but if it binds covalently it won't get out of the enzyme and the enzyme will be destroyed.

➢ Are there any molecules that can bind covalently to the enzymes?

- Actually there are, toxins, poisons, and some drugs that are used to inhibit the activity of some enzymes.

- **Binding occurs at least at three points (chirality).**

➢ Why binding should occur at least at 3 points?

- To prevent the rotation and to be able to differentiate between isomers (L isomer and D isomer)
- Every isomer (L or D) has a specific enzyme.

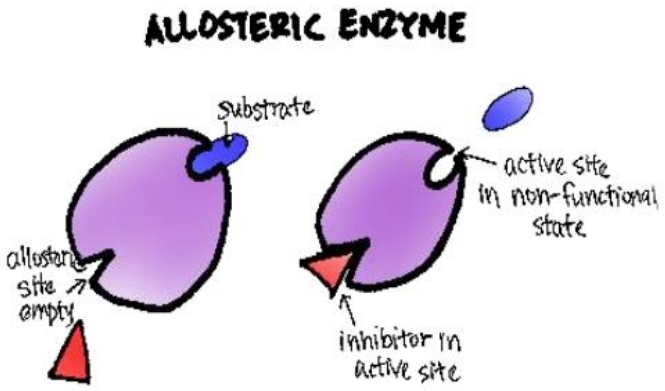
➢ The active site is a domain (part of a protein that can function on its own) so if we cut the active site it should work on its own, however it doesn't why is that?

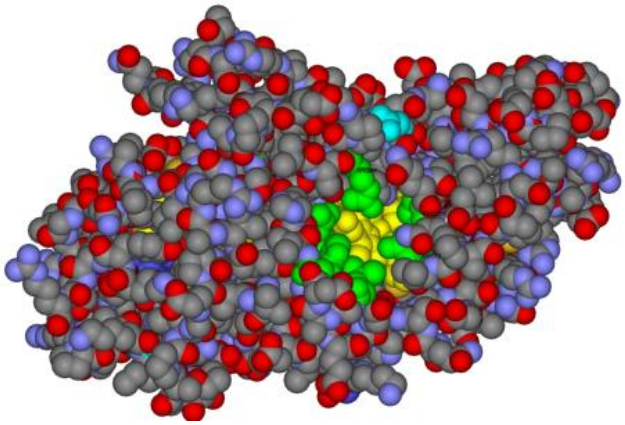
- Because the other parts of protein/enzyme are supporting the active site.

✓ Mostly size of the enzyme is large compared to the activating site, so the main function of the enzyme parts is to support the active site.

- Why we need a huge enzyme in compared to the active site?

1. To support the active site.
2. To have additional regulatory regions (activator and inhibitors).

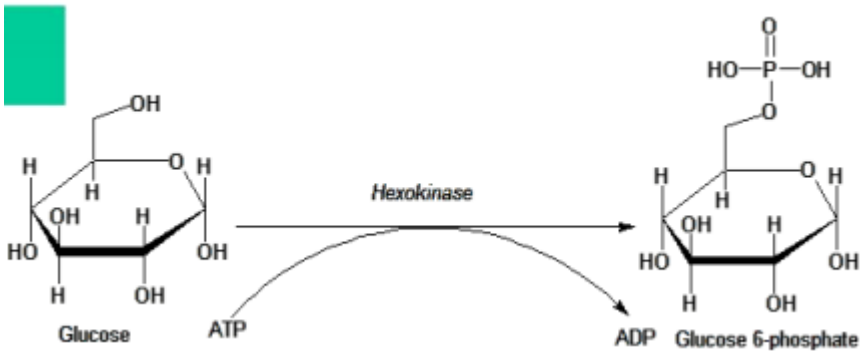




- **Active site forms by a groups from different parts of the amino acid sequence usually forming a domain made of multiple secondary structures**
- **Active site takes up a relatively small part of the total volume.**
- **The "extra" amino acids help create the three-dimensional active site & in many enzymes, may create regulatory sites.**

How do Enzymes work?

- We will take hexokinase / glucokinase as an example, Kinase transfers a phosphate group from a molecule to another molecule (**phosphorylation**), hexo means that it works on a six membered ring sugar like glucose.

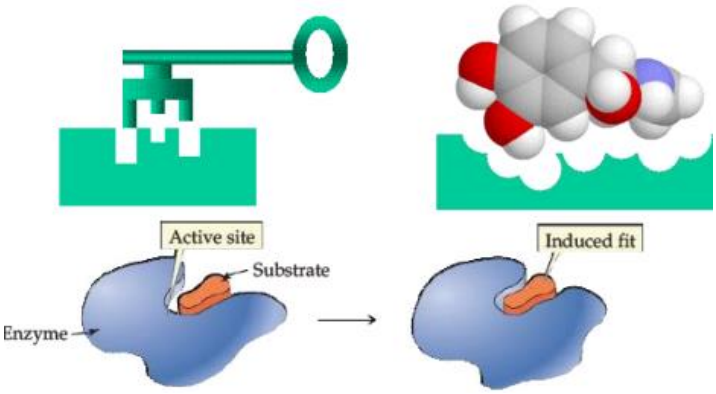


  - This enzyme takes phosphate group from a donor and it's usually ATP and adds it to a Carbon six of glucose and produces glucose 6-phosphate and releases ADP.

- **Two models have been introduced to know how enzymes work:**

Lock-and-key vs. Induced-fit model

- The old model is the **lock and key model** which defines that for each lock there is a specific key which can open it, this implies that for each enzyme there is a substrate that can bind to the active site and catalyze the reaction, with the advancement of science, scientists found two obstacles with that model:



- **There are enzymes that catalyze more than one substrate.**
- **The protein is dynamic in their nature not static so when the substrate bind they change the conformation.**

✓ **The new model is the induced fit model: this model implies that the complementarity of the active site and the substrate is not there from the start, and binding of the substrate is the induction for the conformational change and 100% complementarity to occur.**

Notes:

- **Binding leads to formation of transition-state.**
- **Usually, substrate binds by non-covalent interactions to the active site.**
- **The catalyzed reaction takes place at the active site, usually in several steps.**
- **Improving the binding site for ATP & excluding water (might interfere with the reaction).**

The end

TEST YOUR KNOWLEDGE

1- Which of the statements regarding enzymes is false?

a) Enzymes are proteins that function as catalysts.

b) Enzymes are specific.

c) Enzymes provide activation energy for reactions.

d) Enzyme activity can be regulated.

e) Enzymes may be used many times for a specific reaction.

Ans: C

2-The active site of an enzyme

a) remains rigid and does not change shape.

b) is found at the center of globular enzymes.

c) contains amino acids without sidechains.

d) None of the above choices are correct.

Ans:D

3-The transition state of a catalyzed reaction is

a) a highly-populated intermediate on the reaction pathway.

b) higher in energy than that of an uncatalyzed reaction.

c) lower in energy than that of an uncatalyzed reaction.

d) lower in energy than the reaction substrate.

Ans:C

4- 'lock and key' model of the enzyme action implies that

(A) The active site is complementary in shape to that of substance only after interaction.

(B) The active site is complementary in shape to that of substance

(C) Substrates change conformation prior to active site interaction

(D) The active site is flexible and adjusts to substrate

Ans:B

5-The relationship between an enzyme and a reactant molecule can best be described as:

a) a temporary association.

b) an association stabilized by a covalent bond.

c) one in which the enzyme is changed permanently.

d) a permanent mutual alteration of structure.

e) no complementary binding.

Ans:A

6-.This statement about enzymes is true:

(a) enzymes accelerate reactions by lowering activation energy

(b) enzymes are proteins whose three-dimensional form is key to their function

(c) enzymes do not alter the overall change in free energy for a reaction

(d) all of these

Ans:D

7-The nature of an enzyme is

(a) Lipid

(b) Vitamin

(c) Carbohydrate

(d) Protein

Ans: 😉

The End