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DEPARTMENT OF FOOD TECHNOLOGY

23FTT204- BIOCHEMISTRY & NUTRITION

UNIT V – ENZYMES

Mechanism of enzyme action; active site; Specificity.

- The mechanism of action of enzymes in a chemical reaction can occur by several modes; substrate binding, catalysis, substrate presentation, and allosteric modulation.
- But the most common mode of action of enzymes is by the binding of the substrate.
- An enzyme molecule has a specific active site to which its substrate binds and produces an enzyme-substrate complex.
- The reaction proceeds at the binding site to produce the products which remain associated briefly with the enzyme.
- The product is then liberated, and the enzyme molecule is freed in an active state to initiate another round of catalysis.

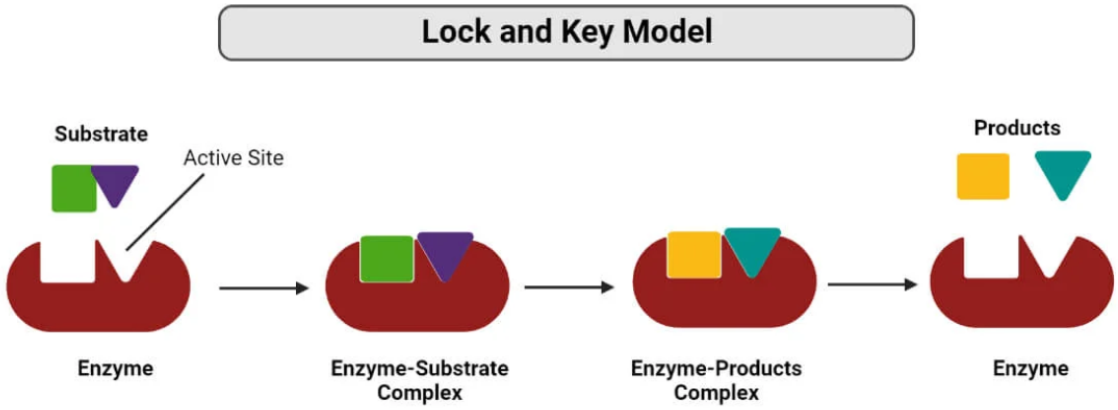
To describe the mechanism of action of enzymes to different models have been

proposed;

1. Lock and key hypothesis

- The lock and key model was proposed by Emil Fischer in 1898 and is also known as the template model.
- According to this model, the binding of the substrate and the enzyme takes place at the active site in a manner similar to the one where a key fits a lock and results in the formation of an enzyme-substrate complex.
- In fact, the enzyme-substrate binding depends on a reciprocal fit between the molecular structure of the enzyme and the substrate.
- The enzyme-substrate complex formed is highly unstable and almost immediately decomposes to produce the end products of the reaction and to regenerate the free enzyme.
- This process results in the release of energy which, in turn, raises the energy level of the substrate molecule, thus inducing the activated or transition state.
- In this activated state, some bonds of the substrate molecule are made susceptible to cleavage.

- This model, however, has few drawbacks as it cannot explain the stability of the transitional state of the enzyme and also the concept of the rigidity of the active site.



2. Induced fit hypothesis

- The induced fit hypothesis is a modified form of the lock and key hypothesis proposed by Koshland in 1958.
- According to this hypothesis, the enzyme molecule does not retain its original shape and structure.
- Instead, the contact of the substrate induces some configurational or geometrical changes in the active site of the enzyme molecule.
- As a result, the enzyme molecule is made to fit the configuration and active centers of the substrate completely.
- Meanwhile, other amino acid residues remain buried in the interior of the molecule.
- However, the sequence of events resulting in the conformational change might be different.
- Some enzymes might first undergo a conformational change, then bind the substrate.
- In an alternative pathway, the substrate may first be bound, and then a conformational change may occur in the active site.
- Thirdly, both the processes may co-occur with further isomerization to the final confirmation.

Active site of enzymes:

Enzymes are much larger than the substrate they act on, and thus there are some specific regions or sites on the enzyme for binding with the substrate, called active sites. Even in enzymes that differ widely in their properties, the active site present in their molecule possesses some common features;

1. The active site of an enzyme is a relatively small portion within an enzyme molecule.

2. The active site is a 3-dimensional entity made up of groups that come from different parts of the linear amino acid sequence.

3. The arrangement and orientation of atoms in the active site are well defined and highly specific, which is the cause of the marked specificity of the enzymes. However, in some cases, the active site changes its configuration in order to bind a substance.

4. The interactions or forces between the active site and the substrate molecule are relatively weak.

5. The active sites in the enzyme molecules are mostly present in grooves or crevices from where large quantities of water are excluded.

Enzyme-substrate complex:

1. The enzyme-substrate complex is a transitional molecule formed after the substrate binds with the enzyme.
2. The formation of the enzyme-substrate complex is important for several reasons.
3. The most important and notable reason is that the substrate binds with the enzyme temporarily and the enzyme is set free once the reaction is complete.
4. This allows a single enzyme molecule to be used millions of times, and thus, only a small amount of enzyme is required in each cell.
5. Another advantage of an enzyme-substrate complex is the reduction in the free energy (activation energy) required for the substrate to rise into the high-energy transition state.

Enzyme specificity:

Most enzymes are highly specific towards the substrate they act on. Enzyme specificity exists in a way that they may act on one specific type of substrate molecule or on a group of structurally related compounds or on only one of the two optical isomers of a compound or only one of the two geometrical isomers. Based on this, four patterns of enzyme specificity have been recognized;

1. Absolute specificity

Some enzymes are capable of acting on only one substrate, and an example of this is the enzyme urease that acts only on urea to produce ammonia and carbon-dioxide.

2. Group specificity

Other enzymes catalyze all reactions of a structurally related group of compounds. It is observed in lactic dehydrogenase (LDH) that catalyzes the interconversion of pyruvic acid and lactic acid along with a number of other structurally related compounds.

3. Optical specificity

Another important form of specificity is seen in some enzymes where a certain enzyme will react with only one of the two optical isomers of a compound. The oxidation of the D-amino acids to the corresponding keto acids by amino acid oxidase is an example of optical specificity. Among the enzymes that exhibit optical specificity, some might interconvert the two optical isomers of a compound. An example of this is alanine racemase that catalyzes the interconversion between L- and D-alanine.

4. Geometrical specificity

Geometrical specificity is observed in some enzymes exhibit specificity towards the cis and trans forms. An example of this is the enzyme fumarase that catalyzes the interconversion of fumaric and malic acids.