Enzymes

Serkan SAYINER, DVM PhD. Assist. Prof.
Near East University, Faculty of Veterinary Medicine, Department of Biochemistry
serkan.sayiner@neu.edu.tr

Definition and History

- Most of the biochemical reactions are catalyzed by organic substances which have protein structure. These biological catalysts are called **enzymes**.
- **Enzymes are defined as biocatalytic substances that allow biochemical reactions to occur rapidly under normal conditions and constitute the basic character of living organism.**
- **Enzymes are molecules that catalyze a chemical reaction without breaking up or altering itself.**

Definition and History

- The enzyme word was first used by **Wilhelm Kühne** and means "fermented" by the Greek.

- Enzymes and their biochemistry are called "**Enzymology**".

- Today, it is still an important issue.

28 March 1837 – 10 June 1900

Definition and History

- The scientific discipline that includes studies and tests related to the monitoring of the treatment process and the monitoring of abnormal organ functions is called **CLINICAL ENZYMOLOGY**.
- Measuring enzyme activity in serum, plasma, urine or other body fluids can help physician to diagnose diseases, to make and differential diagnosis of diseases and also to bring out the prognosis.

ALP
ALT
AST
CK
GLDH
GGT
LDH
Amylase
Lipase
PLI
...

Structure

- **Except for a small group of catalytic RNA molecules, enzymes are in the protein structure.**

- Enzymes may contain only proteins in their structure, as well as a metal ion (Cofactor).
  - E.*g. GPx Se, Carbonic anhydrase Zn, Catalase and Cytochrome Fe, Tirozinaz Cu, xanthine oxidase Mo, Cytochrome c oxidase Cu.*

- The protein structure of the enzyme determines the substance to act on and the reaction to catalyze.

Terminology

- Often, enzymes need an activating additive to be effective.
- The enzyme, called protease, is called **apoenzyme**.
- The factor that activates apoenzymes is called **coenzyme/cofactor**.
- **A complete enzyme, the holoenzyme, is a apoenzyme with coenzyme.**

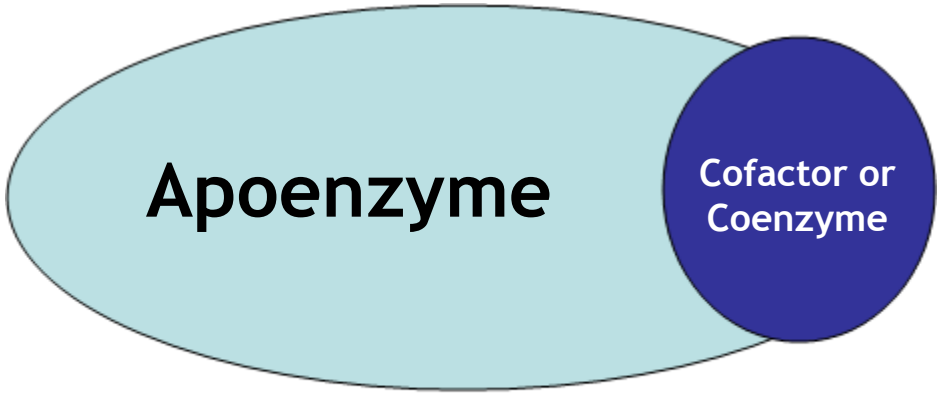
Terminology

- If a cofactor form an integral part of apoenzyme, then this cofactor is called a **prosthetic group.**
  - *E.g. Part of porphyrin peroxidase.*

- The pre forms, which consists of enzymes in inactive form, is called **preenzymes or proenzymes or zymogens.**
  - *E.g. Chymotrypsinogen is the zymogen of chymotrypsin.*

Terminology

- **The coenzyme or cofactor portion of the holoenzyme,**
  - One or more ***inorganic ions*** such as Fe2 +, Mg2 +, Mn2 +, Zn2 + for some enzymes;
  - For some enzymes it is an organic or metalloorganic complex molecule called ***coenzyme***.



Holoenzyme

Terminology

- Sometimes there is a need for another enzyme in order for an enzyme to pass from the zymogen form to the active enzyme state. Such activating enzymes are called ***kinases***.
  - *E.g. Hexokinase and glucokinase, which phosphorylate the hexoses.*
- Some enzymes have multi-molecular variants that catalyze the same reactions. They are called ***isozymes/isoenzymes***.
  - They differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters, or different regulatory properties.

Terminology

- **The substance that the enzyme specifically acts is called the substrate.**
- **After an enzymatic reaction, the matter produced from substrate is called the product.**
- The enzymes released into the extracellular space that they will perform after they are made in a cell are called **exoenzymes**.
- **Endoenzymes** are the enzymes that remain in the cells they produce, that is, they do not release out of the cell and drive the catalytic effect inside the cell.

Locations of Enzymes

- **Enzymes are synthesized in the cell and the vast majority are used for intracellular purposes.**

- Enzymes such as pepsin, chymotrypsin and trypsin in the digestive system are released from the cells they synthesize.

- **The intracellular locations of the enzymes vary.**
  - **Mitochondria:** TCA cycle enzymes, Beta-oxidation enzymes
  - **Nucleus:** Pyrophosphorylases
  - **Erythrocyte:** Carbonic anhydrase
  - **Cytoplasm:** Glycolytic enzymes
  - **Blood:** ???

	Functional Plasma Enzymes	Non-functional Plasma Enzymes
Concentrationin plasma	Present in plasma in higher concentrations in comparison to tissues	Normally, present in plasma in very low concentration in comparison to tissues
Function	Have known functions	No known functions
The substrates	Their substrates are always present in the blood	Theri substrates are absent from the blood
Site of synthesis	Liver	Different organs e.g. liver, heart, brain and skeletal muscles
Effect of diseases	Decrease in liver diseases	Different enzymes increase in different organ diseases
Examples	Clotting factors e.g. Prothrombin, Lipoprotein lipase and pseudocholinesterase	ALT, AST, CK, LDH, ALP, GLDH, Amylase...

Specificity of Enzymes

- **Enzymes are highly specific.**
  - Specificity may vary.

- **Many enzymes act on only one substrate. They do not even affect the stereoisomer of a substrate.**
  - E.g. *Lactate dehydrogenase* acts only on L-lactate, *D-amino oxidase* acts only on D-form amino acids.

Specificity of Enzymes

- **Sometimes enzymes show group specificity.**
  - E.g. *Hexokinase* provides phosphorylation of hexoses.

- **Certain enzymes are absolutely specific for a substrate.**
  - E.g. If only glucose phosphorylates *glucokinase*.

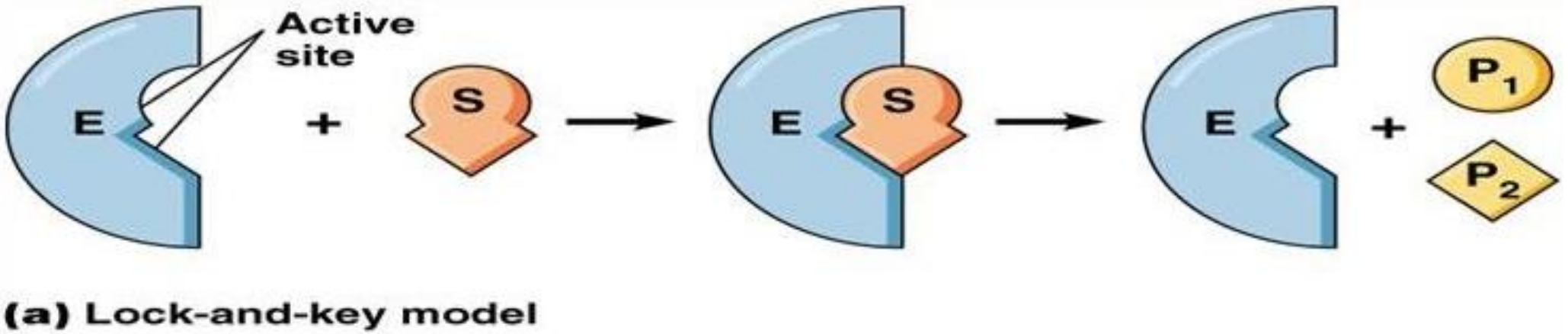
- **Some of the enzymes affect certain substances in certain classes.**
  - E.g. *Pepsin* is the effect of all the proteins.

Enzyme Catalysis

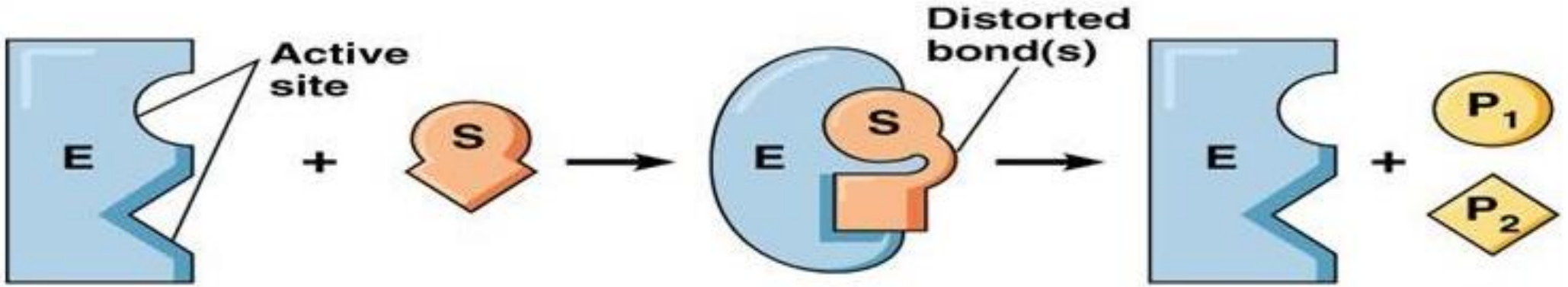
- **The function of an enzyme as a catalyst is to increase the rate of a reaction by reducing the activation energy.**
  - The distinguishing feature of an enzyme-catalyzed reaction is that it occurs in a pocket called the active center on the enzyme.
  - The active center is the specific region on the enzyme molecule, which has the substrate binding property; Substrate and the enzyme-substrate complex is formed, and the product liberates by separating the enzyme from the enzyme-product complex formed by the transformation of the substrate into the product.

Enzyme Catalysis

- For the active site, two models have been proposed that explain the enzyme-substrate binding.
  1. **In Emil Fischer's key-lock model**, it is assumed that the substrate and the active site of the enzyme are predetermined to match each other.
  2. **According to Daniel Koshland's adaptation model**, the active center is flexible; In the presence of the substrate, with a change in the structure of the protein in the tertiary structure, it undergoes a formal change that binds the enzyme substrate in the most appropriate form suitable for catalysis.



(a) Lock-and-key model



(b) Induced-fit model

Classification of Enzymes

1. **Oxidoreductases:** Enzymes related to biological oxidation.
   i. Dehydrogenases (Reductases): Takes Substrate H in the presence of a suitable H receptor.
   ii. Oxidases: H has oxygen as a receptor.
2. **Transferases:** It is any one of a class of enzymes that enact the transfer of specific functional groups
   i. Those who transfer 1 C groups
   ii. Those who transfer aldehyde or ketone groups
   iii. Those who transfer noble groups
   iv. Those who transfer sugar groups
   v. Other transferases

Classification of Enzymes

3. **Hydrolases:** Substrate causes hydrolysis by adding water.
   i. Simple esterase
   ii. Lipase: Fats are fatty acids and glycerol parts.
   iii. Phosphatase: Phosphoric acid esters are disintegrated into their constituent constituents.
   iv. Cholinesterase: They hydrolyze the esters of the choline.
   v. Peptide hydrolase: Peptide bond breaks down.
   vi. Nuclease: Nucleic acids hydrolyze the constituents themselves.
   vii. Enzymes affecting carbohydrates: Amylase, Cellulase, Inulinase, Glycosidase.
   viii. Enzymes that cleave carbon-nitrogen bonds and separate amino-containing groups: urease, asparaginase, glutaminase, nucleotide deaminase, arginase

Enzimlerin Sınıflandırılması

4. **Lyases:** They catalyzes the breaking of various chemical bonds by means other than hydrolysis and oxidation, often forming a new double bond or a new ring structure.
   i. Decarboxylases
   ii. Carbonic anhydrase: Breaks carbonic acid to H_2O and CO_2.
   iii. Dehydratases
   iv. Cysteine lyase
5. **Isomerases:** Convert a molecule from one isomer to another.
   i. Racemases and epimerases: Converts L- to D- or α- to β-.
   ii. Cis-Trans isomerases: Catalyze the isomerization of cis-trans isomers.
   iii. Intramolecular oxidoreductases: These isomerases invert stereochemistry at the target chiral carbon. E.g. interconverting Aldoses and Ketoses.
   iv. Intramolecular transferases: Catalyze the transfer of functional groups from one part of a molecule to another.
   v. Intramolecular lyases: Catalyze "reactions in which a group can be regarded as eliminated from one part of a molecule, leaving a double bond, while remaining covalently attached to the molecule.
6. **Ligases:** Enzymes that catalyze joining of C-O, C-S, C-N, etc.

Enzyme Kinetics

- **Enzyme kinetics** refers to the relationship between the changes in experimental parameters and the rates of enzymatic reactions.
- **The rate of an enzymatic reaction is expressed in terms of the amount of substrate converts to the product per unit time (1 minute or 1 second) with the effect of enzyme.**
  - Optimal pH, 25 °C temperature, and the number of substrate molecules converted to product by a single enzyme molecule at the concentration of the saturating substrate are denoted by the number of enzymatic conversions and are briefly denoted by the kcat symbol.

Enzyme Activity

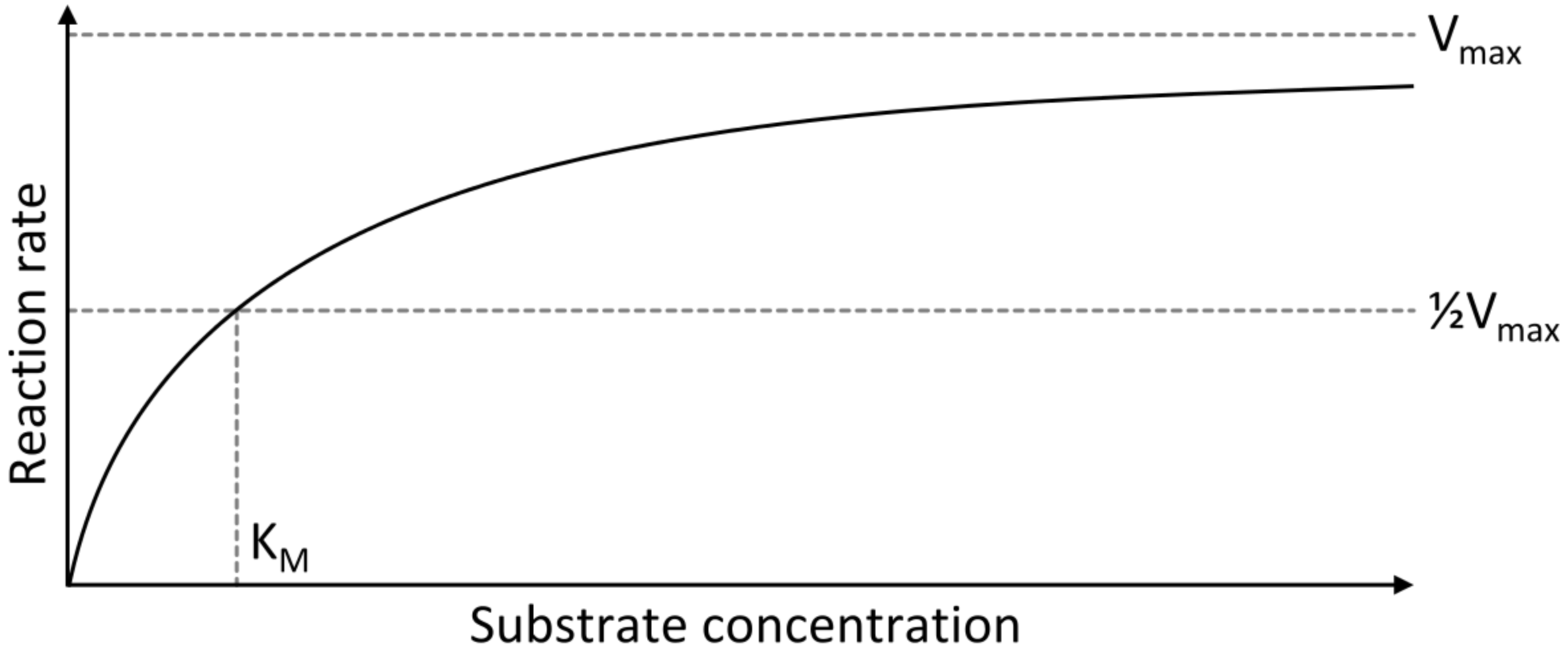
- **The rate of an enzymatic reaction is related to the the activity of the enzyme.**
  - The activity of an enzyme is the expression of the rate of the enzymatic reaction catalyzed by that enzyme by the amount of substrate converted to the product at a certain time under optimal conditions by the action of the enzyme.
  - An enzyme that has more activity transforms more substrate molecules into a product in a given time.
  - **The most commonly used enzyme activity unit is IU, but the SI unit is katal (kat).**
    - **1 katal = 1 mol/second; 1 IU = 1 µmol/min; 1 IU = 16.67 nanokatal**

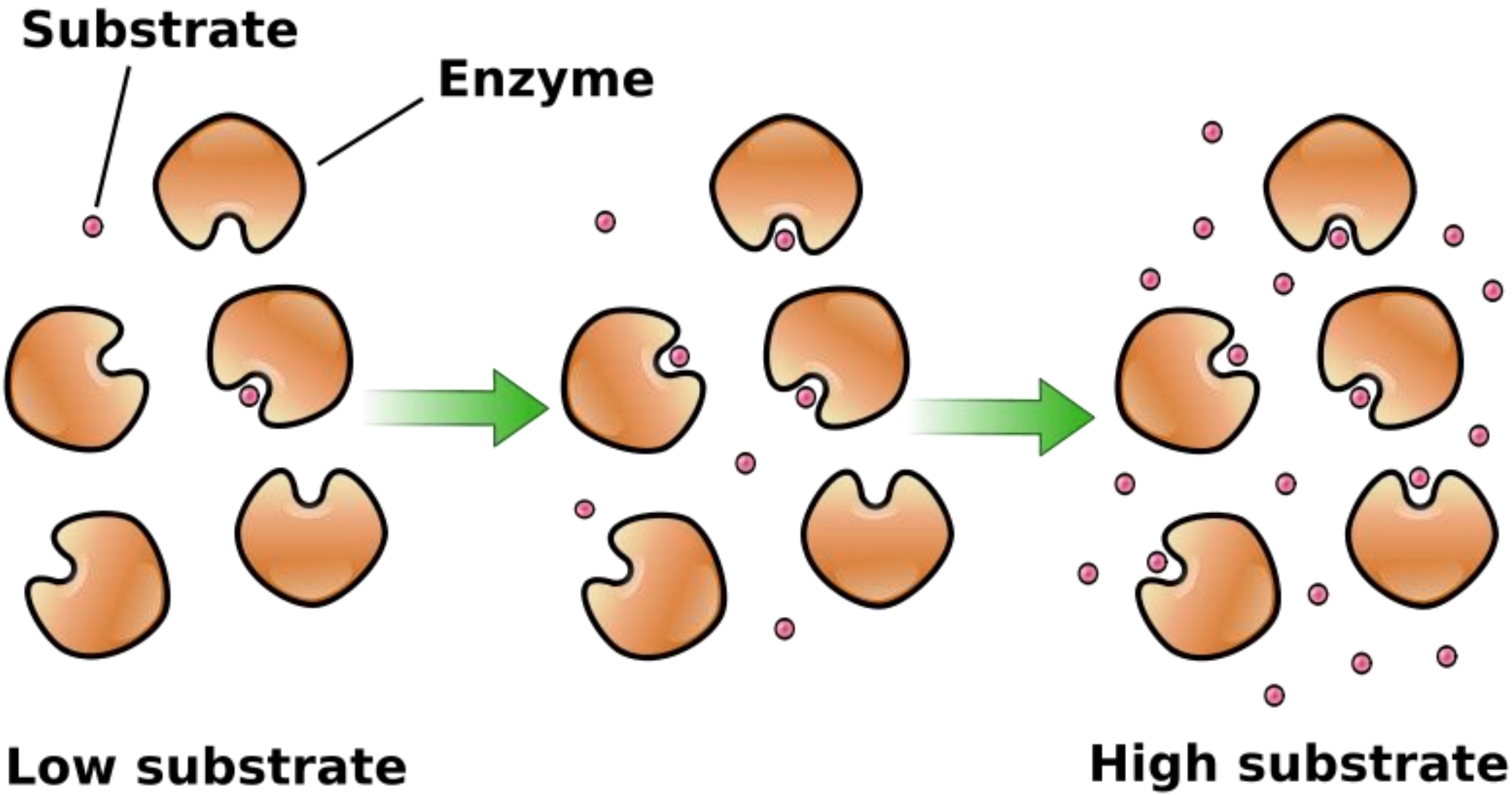
Factors affecting enzyme activity

1. Enzyme Concentration
2. Substrate Concentration
3. Hydrogen Ion Concentration (pH)
4. Temperature
5. Reaction Products
6. Light and Other Physical Factors
7. Time
8. Structure and Concentration of Ions
9. Allosteric Effect or Regulation
10. Effects of Hormones and Other Biochemical Factors

Factors affecting enzyme reactions

1. **Enzyme Concentration:** Under optimal conditions in which the substrate is abundant, the rate of enzyme reaction is directly proportional to the enzyme concentration.
2. **Substrate Concentration:** If the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity.
3. **Hydrogen Ion Concentration (pH):** Enzymes are affected by changes in pH. The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH. It vary between 5-9.


Vmax
½Vmax
Reaction rate
KM
Substrate concentration


Substrate
Enzyme
Low substrate
High substrate

Factors affecting enzyme reactions

4. **Temperature:** As in other chemical reactions, an increase in temperature accelerates the enzymatic reaction to a certain temperature in a reaction catalyzed by the enzyme.
   - Generally every 10ºC increases the enzymatic reaction by 2 times. However, when it reaches a certain temperature (45ºC) it causes the reaction to slow down due to denaturation.
5. **Reaction Products:** The rate of an enzyme reaction decreases with time due to the increase in reaction products. Because the enzyme reaction is backwards.

Factors affecting enzyme reactions

6. **Light and Other Physical Factors:** The activities of enzymes can be enhanced or inhibited by the effect of light. Red and blue light enhance the activity of salivary amylase and some other enzymes. UV rays act in the opposite direction.

7. **Time:** When a reaction catalyzed by an enzyme, the rate decreases over time.
   - The reason for this is the fact that the products that come to the foreground combine to form a reaction in the opposite direction, the enzyme is inactivated over time, the substances that inhibit the reaction form, the depletion of the substrate.

Factors affecting enzyme reactions

8. **Structure and Concentration of Ions:** The rate of enzymatic reactions is considerably influential in the structure and concentration of ions present in the environment.

9. **Allosteric Effect or Regulation:** The end product of many enzyme reactions inhibits the enzyme's action by the feed-back mechanism.
   - Such an inhibition form is called feed-back inhibition, retro-inhibition, allosteric inhibition, or end-product inhibition. The first enzyme inhibited by the end product is also called the **allosteric enzyme**.

Factors affecting enzyme reactions

10. **Effect of Hormones and Other Biochemical Factors:** Hormones, amino acids and other substances can affect the state of the enzyme.
    - This change in enzyme structure can be brought about by various estrogenic, androgenic and some steroid hormones.
    - Steroid hormones also have the capacity to regulate the ability of cells to synthesize enzymes.

Inhibitors and Activators of Enzymatic Reactions

- **Inhibitors**
  - There are also substances which inhibit enzyme reactions. These substances are called **enzyme poisons**.
  - Certain well known matters, for example cyanide, arsenic and antimony, have strong toxic effects on cells.
  - **Competitive Inhibitors**
    - They compete with the substrate. Because the chemical structures are similar to the substrate.
    - In fact, the competitive degree measured as the reaction rate depends on the substrate concentration, the inhibitor concentration and the relative relevance of the enzyme and substrate to inhibition.

Inhibitors and Activators of Enzymatic Reactions

- **Non-competitive Inhibitors**
  - These enzymes act directly and unite with it.
  - Some of them affect the prosthetic group of the enzyme. Some non-competitive inhibitors also react with the protein portion of the enzyme.
  - This group contains especially heavy metals. Many of these enzymes react with the -SH groups of the protein moieties and degrade the functional activities of the enzymes.

- **Other Inhibitors**
  - Some proteins inhibit certain enzymes.
  - Globulins found in soy and legumes inhibit trypsin.
  - Not-easily digestible fats inhibit the digestion-of easily fats from being broken down by lipases.

Inhibitors and Activators of Enzymatic Reactions

- **Activators**
  - Enzyme activators are also called accelerators.
  - Many enzymes are found inactive form in cells or secretions. They need to be activated before they can catalyze specific reactions.
  - Some zymogens become auto-catalytically active, or an enzyme activates it by acting on the inactive form. These are mostly inorganic and sometimes organic salts.
  - For functions, enzymes that require the presence of free sulfhydryl groups in their molecules are activated by reducing agents such as KCN, H_2S, glutathione, or cysteine.

Coenzymes

- **The coenzyme is the active part of the holoenzyme and is directly incorporated into the enzyme catalysis.**

- Many vitamins, especially **the group B vitamins** or the organic compounds which they contain these vitamins, act as coenzymes.

- **Coenzymes are classified according to the groups they transfer.**

Coenzymes

- **Energetically Rich Triphosphates**
  - **Adenosine Triphosphate (ATP):** found in all cells. Energen is rich in 2 phosphates. It is an energy source for many reactions.
  - **Active Sulphate (3'-phosphoadenosine-5'-phosphosulfate = PAPS):** It is the most common coenzyme in sulfotransferase reactions.
  - **Uridine triphosphate (UTP):** Its main role is as substrate for the synthesis of RNA during transcription. UTP also has the role of a source of energy or an activator of substrates in metabolic reactions.
  - **Cytidine triphosphate (CTP):** CTP is a substrate in the synthesis of RNA. CTP is a coenzyme in metabolic reactions like the synthesis of glycerophospholipids and glycosylation of proteins.
  - **Guanosine triphosphate (GTP):** Act as a substrate for both the synthesis of RNA during the transcription process and of DNA during DNA replication. It also has the role of a source of energy or an activator of substrates in metabolic reaction.

Coenzymes

- **Group-carrying Coenzymes**
  - **Pyridoxal phosphate (PLP):** The active form of vitamin B_6, is a coenzyme in a variety of enzymatic reactions. PLP acts as a coenzyme in all transamination reactions, and in certain decarboxylation, deamination, and racemization reactions of amino acids.
  - **Thiamin pyrophosphate (TPP):** A thiamine (vitamin B_1) derivative coenzyme. It is a coenzyme that is present in all living systems, in which it catalyzes several biochemical reactions. It is synthesized in the cytosol and is required in the cytosol for the activity of transketolase and in the mitochondria for the activity of pyruvate-, oxoglutarate- and branched chain keto acid dehydrogenases.

Coenzymes

- **Coenzyme A (CoA):** CoA biosynthesis requires cysteine, pantothenate (B_5 vit.), and ATP. It is notable for its role in the synthesis and oxidation of fatty acids, and the oxidation of pyruvate in the citric acid cycle.
- **Tetrahydrofolic acid:** A folic acid (B_9 vit.) derivative. It is a coenzyme in many reactions, especially in the synthesis (or anabolism) of amino acids and nucleic acids. It acts as a donor of a group with one carbon atom.
- **Biotin:** Also called vit. B_7 or vit. H. Biotin is a coenzyme for carboxylase enzymes, involved in the synthesis of fatty acids, isoleucine, and valine, and in gluconeogenesis.
- **Cobalamin (B_{12} vit.):** It has two coenzymes, Methylcobalamin and Adenosylcobalamin.
- **Lipoic Acid (α-lipoic acid-ALA):** Lipoic acid is coenzyme for at least five enzyme systems. Two of these are in the citric acid cycle.

Coenzymes

- **Coenzymes carrying hydrogen, electrons and oxygen**
  - **Nicotinamide Adenine Dinucleotide (NAD) and Nicotinamide Adenine Dinucleotide phosphate (NADP)**
    - They are coenzymes found in all living cells. It acts as an H^+ receptor for specific substrates.
    - In metabolism, NAD is involved in redox reactions, carrying electrons from one reaction to another.
      - E.g. *isocitric dehydrogenase, alpha-ketoglutaric dehydrogenase* and *malic dehydrogenase* needs NAD.
    - NADP is a coenzyme used in anabolic reactions, such as lipid and nucleic acid synthesis.
  - **Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN)**
    - FAD is involved in several important enzymatic reactions in metabolism.
    - FMN functions as prosthetic group of various oxidoreductases including NADH dehydrogenase as well as coenzyme in biological blue-light photo receptors.

Coenzymes

- FAD dehydrogenases also function in the conversion of D-amino acids to alpha-keto acids, aldehydes to carboxylic acids, xanthine to uric acid, reduced lipoic acid to oxidized lipoic acid.
- In general, the FAD in the respiratory chain transduces the electrons taken from the NAD dehydrogenases to the coenzyme Q.

- **Koenzim Q (Ubiquinone)**
  - It is found in animals, plants and microorganisms. It takes part in electron transport chain..

Coenzymes

- **Ferrous porphyrins**
  - It is the prosthetic group of oxygen or electron-transporting enzymes.
  - Cytochromes and cytochrome oxidases have the ability to receive and transmit electrons due to iron attached to the porphyrin ring system.

- **Copper as cofactor**
  - It is the functional part of many enzymes and forms the prosthetic group.

References

- Ası. T. 1999. Tablolarla Biyokimya, Cilt 1
- Sözbilir Bayşu N, Bayşu N. 2008. Biyokimya. Güneş Tıp Kitapevleri, Ankara

Question 1

- **Enzymes are …… structures except for a small group of catalytic RNA molecules.**

A. Lipid

B. Carbohydrate

C. Protein

D. Nucleic acid

E. Nitrogen base

Your questions?

Next topic;

Vitamins