8. ENZYMES

The substances in the protein structure that catalyze biochemical reactions are called enzymes. (Except for a small group of catalytic RNA molecules, all enzymes have protein structure). Enzymes are synthesized by living cells under biological conditions. However, they do not need to be in the cell to show activity. Because of their protein structure, the ordering of amino acids in proteins has a great importance in the acquisition of a certain conformation of the enzymes and of the three-dimensional structure. This three-dimensional structure designates its catalytic activity and its specificity.

The catalysis power of enzymes is quite high compared to other chemical catalysts. They can increase the reaction rate 10^6 -10^{16} times. In the reactions catalyzed by enzymes, by-product is not generated, and the reaction yield is 100%.

The compound interacted with the enzyme is called the **substrate.** Substrate binds only to a specific site called the active center, does not binds to the whole of the enzyme, and the biochemical reactions take place here. Active centers are composed of amino acid residues and have special geometry. Some of the amino acids involved in this section allow the substrate to be correctly attached to the active center. The active center has two zones. One of these is the binding site where the sustrate is bound; the other is the region of catalytic activity in which the substrate is modified.

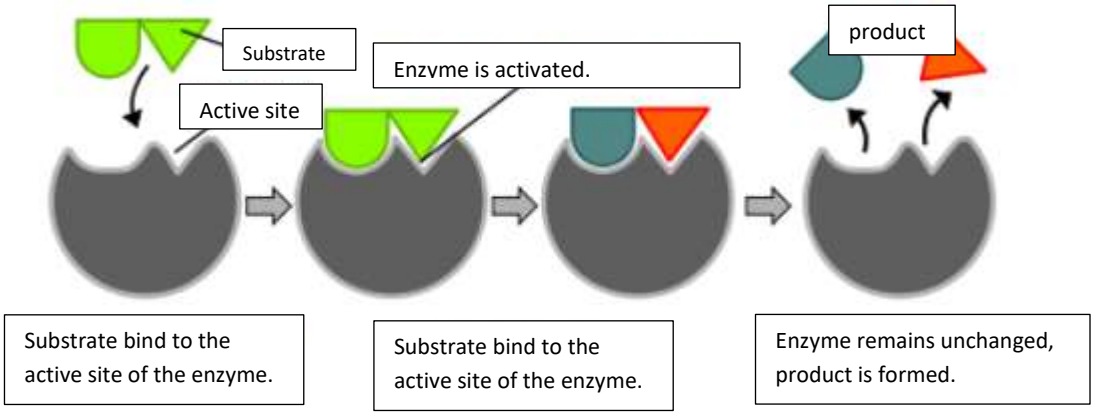


Figure 8.1. Enzyme substrate complex and product formation.

8.1. Michaelis-Menten Equation in Enzyme Kinetics

In 1913, Michaelis and Menten proposed a simple model covering the characteristics of enzyme-catalyzed reactions. With this model, the enzyme binds reversibly with the substrate, then releases

the enzyme to form an enzyme-substrate (ES) complex which is transformed into product. The model is as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + \ddot{U}$$

$k_{-2} \ll k_2$ $\quad V = k_2 . [ES]$

S: Substrate

E: Enzyme

ES: Enzyme-substrate complex

k_1, k_{-1}, k_2, k_{-2}: Speed constants

The Michaelis-Menten equation shows the change in the reaction rate with respect to the substrate concentration. In this correlation, the enzyme concentration ([E]) is constant and the reaction speed is determined by the substrate concentration ([S]). The result is a hyperbolic function and a the graph is a hyperbolic curve.

$$V = \frac{V_m . [S]}{K_m + [S]}$$

V: Reaction rate

V_m: Maximum speed

K_m: Michaelis-Menten constant

[S]: Substrate concentration

Michaelis-Menten equation

If the initial velocities are measured and plotted by varying the substrate concentration for a constant enzyme concentration, a graph similar to the curve in the figure is obtained.

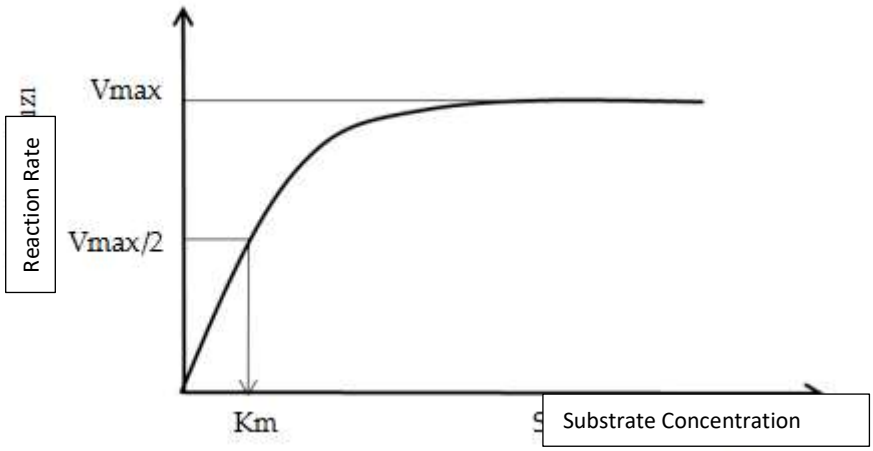


Figure 8.2. Michaelis-Menten chart

This graph shows the Km value from the substrate concentration corresponding to V_{max} / 2. The Km value is the substrate concentration required to fill half of the active center and is different for each enzyme. Same enzyme obtained from different sources has different Km values, as well as different substrates binded to enzyme obtained from one source has different Km values. pH and temperature also affect Km.

8.2. Factors Affecting Enzymatic Reaction

The rate of reactions catalyzed by an enzyme is affected by concentration of an enzyme and substrate, temperature, pH of the medium, time and reaction products.

8.2.1. Effect of enzyme concentration

In an environment where the substrate is abundant, the rate of enzymatic reaction is directly proportional to the enzyme concentration.

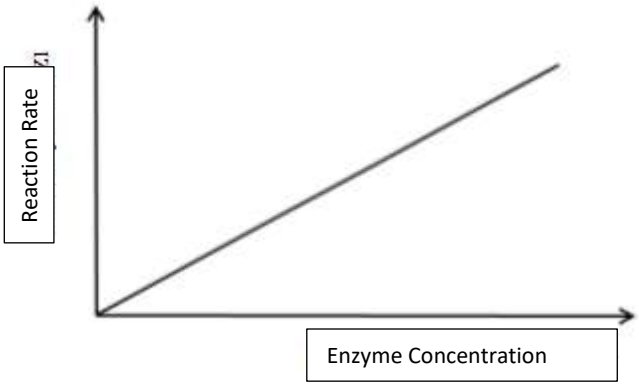


Figure 8.3. Effect of enzyme concentration

8.2.2. Effect of temperature

The speed of the enzyme reactions increases with temperature, but after a certain temperature the enzymes lose their activity as they are denatured and the reaction rate decreases.

Maximum temperature of the enzymatic reaction is called **optimum temperature**.

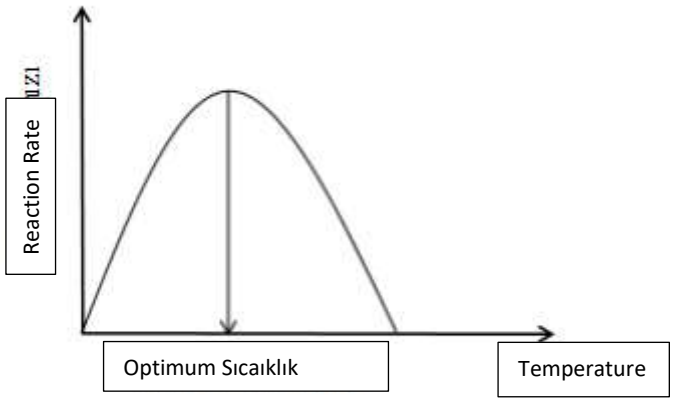


Figure 8.4. Effect of temperature

8.2.3. Effect of pH

Enzymes generally show the highest activity at a certain pH. This pH is called **the optimum pH** of the enzyme. Degree of ionization of the acidic or basic groups of amino acids and substrate in the active center of the enzyme determines the activity which depends on pH. In order to be the reaction rate maximum, these groups must be in a certain ionization state.

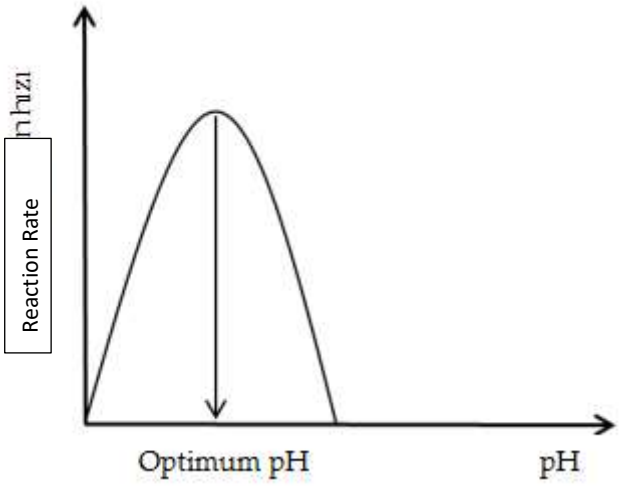


Figure 8.5. Effect of pH on enzyme activity

8.2.4. Effect of time

Initial rates are used to determine reaction rates which is predicated on the rate at which a substrate is expended, or the formation of a product.The change in decreasing or increasing substance concentration is monitored over time and the velocity is determined from the change in the unit time. The speed-time chart gives a line. If a curve is obtained, it is understood that the product is formed and thus the rate of reaction is reduced. Reduction of reaction rate is caused by the recombination of reaction products, the inactivity of the enzyme over time, the formation of reaction-inhibiting substances and depletion of the substrate.

8.3. Regulation of Enzyme Activity

The rate of enzyme-catalyzed reactions is reduced by certain substances called inhibitors. Inhibitors reduce the rate of reaction by inhibiting the formation of the enzyme-substrate (ES) complex. Via the inhibition study, information about formation on the substrate specificity of the enzyme, the physical and chemical structure of the active site, the functional groups involved in the active site and the kinetic mechanisms of the enzymatic reactions can be predicted. In this way, the effect of drugs and toxic substances can also be examined. Penicilline is an example of the enzyme inhibitors used as drugs which a commonly used antibiotic. Penicillin acts by inhibiting one or more enzymes that synthesize the bacterial wall. There are 2 types of inhibition;

1) Irreversible inhibition: Inhibitor inhibits the enzyme by covalently bonding to the active site.

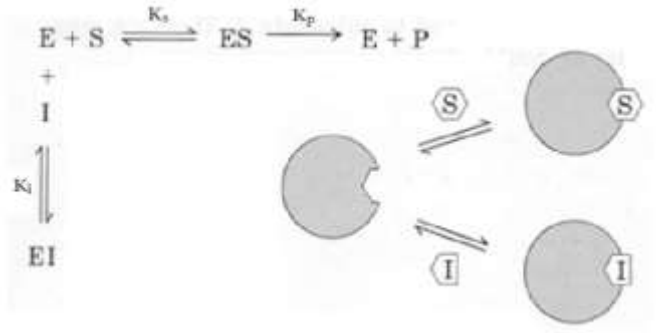
2) Reversible (reversible) inhibition:

a) Competitive inhibition

b) Semi-competitive (uncompetitive) inhibition

c) Noncompetitive inhibition

a) Competitive inhibition: The inhibitor (I) is similar to the substrate (S) in point of molecular structure. Inhibitor binds to active site of the enzyme and forms the enzyme-inhibitor (EI) complex where resultant enzyme-inhibitor (EI) complex prevents binding of the substrate. As the EI complex cannot turn into product, the activity of the enzyme is lowered. As the substrate concentration in the medium increases, the affinity of the enzyme toward inhibitor. Thereby inhibition is annihilated.



Vmax: Unchanged.

Km: Increased.

Figure 8.6. Competitive enzyme inhibition

Michaelis-Menten equation of competitive inhibition;

$$V = \frac{V_{max} \cdot [S]}{[S] + K_m (1+[I]/K_i)}$$

Such inhibition can be readily observed by experiments. At constant inhibitor concentration, inhibition decreases as substrate concentration increases. The substrate and the inhibitor compete since they will bind to the same region on the enzyme. As the substrate concentration increases, the inhibitor replaces and inhibition decreases.

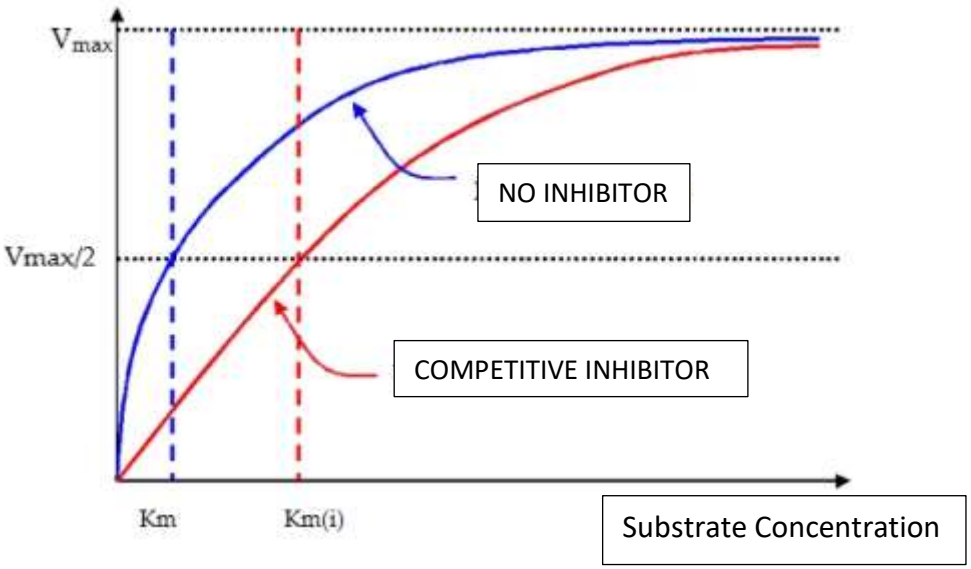
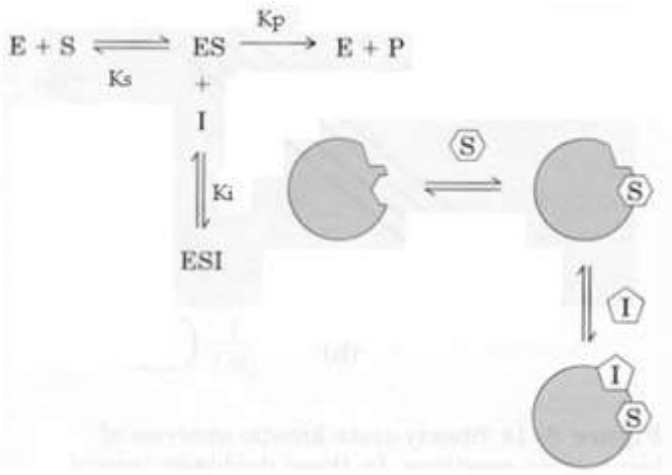


Figure 8.7. Michaelis - Menten graph of competitive inhibition

b) Uncompetitive inhibition: In this type of inhibition, the inhibitor binds directly to the ES complex and not to the enzyme. Thus, product formation stops since the structure of the complex will be degraded. Inhibitor slows down the reaction either by binding the free enzyme or ES complex.



Vmax: Decreases

Km: Decreases

Figure 8.8. Uncompetitive enzyme inhibition

Michaelis-Menten equation of this type of inhibition;

$$V = \frac{Vmax\,.\,[S]}{Km + [S]\,(1 + [\dot{I}]\,/\,Ki)}$$

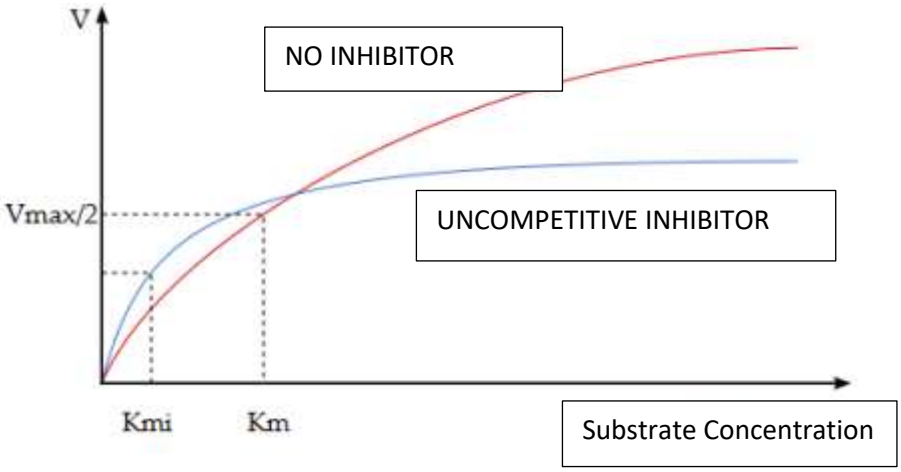
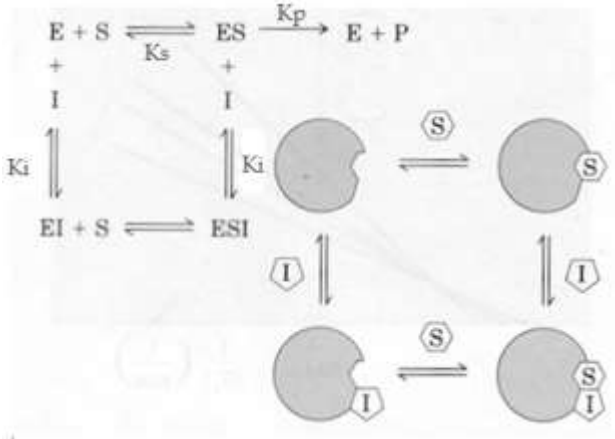


Figure 8.9. Michaelis - Menten graph of uncompetitive inhibition.

c) Noncompetitive inhibition: In this type of inhibition, the inhibitor is binds to either the enzyme or ES complex at a point other than the active center to cause inhibition. Such inhibitors usually cause changes in the three-dimensional structure of the enzyme. Therefore, the formation and decomposition of ES does not occur with normal rates.

Vmax / 2 Km →[S]



Vmax: Decreases.

Km: Unchanged.

Figure 8.10. Non-competitive enzyme inhibition.

Michaelis-Menten equation of non-competitive inhibition;

$$V = \frac{Vmax\,.\,[S]}{Km\,(1+[\dot{I}]/Ki\,) + [S]\,(1+[\dot{I}]/Ki\,)}$$

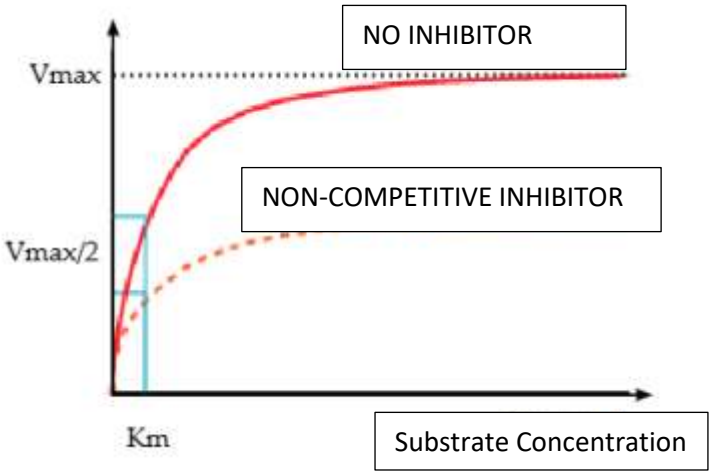


Figure 8.11. Michaelis is Menten graph of non-competitive inhibition.

8.4. Enzyme Activity Units.

Enzymes are found in a small amount in the biological environment and are very difficult to quantify. However, they can be analysed by measuring the reaction rates catalyzed under optimum conditions. It is often difficult to measure decrement in the amount of substrate in the environment because there is enough substrate to saturate the enzyme. Instead, it is preferable to measure the rate at which the product is formed. If the product is colored or is absorbing light in the UV region, the speed can be measured spectrophotometrically. Under optimum conditions, the amount of enzyme that converts the substrate into the product is called **enzyme activity.** Units used to demonstrate enzyme activity are as follows;

Enzyme unit (U): It is the amount of enzyme that converts 1 µm substrate to product at 25 ˚C, under optimum conditions, in 1 minute.

Specific activity: Specific activity is 1 mg protein per enzyme unit and calculated as U / mg protein which indicates the purity of the enzyme.

Molar activity (number of turnovers): The number of substrate molecules converted to the product in unit time by a single enzyme molecule.

Catal: It is the amount of enzyme that converts 1 mole of substrate to the product in 1 second under optimum conditions.

8.5. Classification of Enzyme

Enzymes are divided into 6 groups according to the type of reaction they catalyzed.

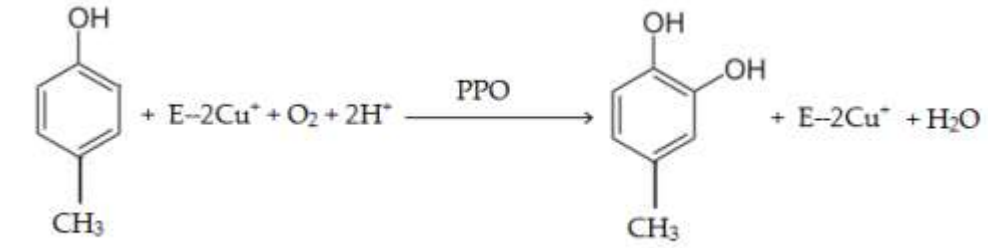
1.Oxidoreductases

Enzymes that catalyze oxidation-reduction reactions. Examples of this enzyme group are lactate dehydrogenase, catalase, polyphenol oxidase enzymes.

Catalase is an enzyme found in protein structure abundantly. This enzyme is commonly found in plants, animals and microorganisms. It is particularly available in the peroxisome organelles of animal cells. It also exists in the blood, mucous membranes, erythrocytes, liver, kidney, bone marrow and various tissues of the organism.The main function of the catalase is to break down the hydrogen peroxide in especially liver and tissues to O_2 and H_2O.This protects the cell from oxidative damage of free radicals and other reactive molecules.

Polyphenol oxidase (PPO; monophenol dihydroxyphenyl alanine) is an oligomeric enzyme containing copper in its structure and consisting of more than one subunit.Besides being found in many fruits and vegetables, it is also found in crustaceans such as lobster, shrimp, crab, and some microorganisms.The amount and distribution of the PPO enzyme from different sources depends on the type, age and maturity of the plant.PPO is an enzyme that oxidizes phenolic compounds in presence of molecular O_2. PPO catalyzes two kinds of reactions;

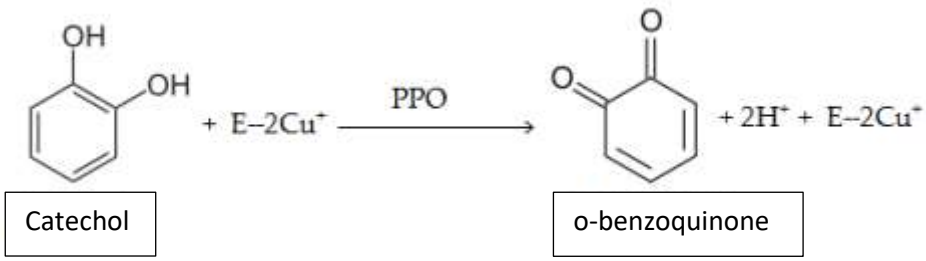
1) Hydroxylation reaction; oxidation of o-dihydroxyphenols by hydroxylation of mono phenols.



p-cresol

4-methyl catechol

2) Oxidation reaction; oxidation of o-dihydroxyphenols to o-quinones.



Catechol | o-benzoquinone

The enzymatic blackening of fruits and vegetables catalyzed by PPO enzyme occurs because of being scratched out during the storage and industrial processing of these plants and also because of influence of O_2 in air when the frozen fruit is thawed during the storage and industrial processing. This situation is not desirable because it reduces the nutritional value of the product and causes bad taste and appearance. Such blackening reactions are attempted to be prevented by inactivating the enzyme.

2. Transferases

It allows an atom or group of atoms to be transferred from one molecule to another. To illustrate; glutamic pyruvic transaminase, phosphorylase, etc.

Glutamic Pyruvic Transaminase (GPT): Alanine amino transferase (ALT, ALAT) is another name of this enzyme which is found in many tissues. GPT transfers the amino group of alanine to α-keto glutarate, resulting in pyruvate and glutamate.

In the diagnosis of liver diseases, glutamic oxalacetic transaminase (GOT) and glutamic pyruvial transaminase (GPT) are analysed. These two enzymes are very important in terms of liver function in clinical biochemistry. As a result of damage to liver cells, these enzymes are released out of the cell. GPT is found in the cytoplasm of liver cells and GOT is found in both the cytoplasm and mitochondria. In cases of acute, viral, toxic and drug-associated hepatitis, liver necrosis, severe heart failure, and chronic active hepatitis, GPT and GOT in serum may increase to 10-100 times the normal limit. Analysis of these enzymes is of great importance in monitoring the course of the disease. In serum, GPT enzyme normally ranges between 15-56 units in men and 6-44 units in women.

3. Hydrolases

It is the enzyme group that catalyzes the breakdown (hydrolysis) of water by adding water. For example; carbohydrases (enzymes that provide hydrolysis of carbohydrates such as amylase, lactase, invertase), lipase (hydrolysis of lipids), proteases (hydrolysis of proteins), etc.

Amylase is one of the most important enzymes in the hydrolase family and its molecular mass is 45,000 Daltons. It is found in saliva, saliva and pancreatic secretion of animals. This enzyme acts on the (1,4) glycoside bonds of starch (see Chapter 2) and glycogen and randomly splits the linear amylose chains. Partial hydrolysis of starch begins with its interaction with salivary amylase during chewing in the mouth. The enzymatic activity depends on the acid or base (pH) concentration of the medium. Saliva amylase works only in basic environment. Since stomach sap is very acidic, it does not in amylase acidic medium. The digestion of starch is completed in the small intestine. **Amylase is the only plasma enzyme normally found in urine**, as it has a molecular structure small enough to pass through kidney glomeruli.

Excess of this enzyme in serum indicates that there is a disorder in the pancreas. In liver diseases, the level of amylase in plasma may be low. There may be an increase in plasma amylase level in intestinal obstruction, mumps and diabetes. In pancreatic diseases, α-amylase enzyme level increases in serum.

The α-amylase enzyme is the first commercially available enzyme. In the food industry, products which are dextrin, glucose and maltotetrose released by the hydrolysis of starch with the α-amylase enzyme are widely used. Dextrin is used as a thickener filler in foods. Produced glucose is used in production of fructose syrup. Glucose is obtained first either as a syrup or as a crystalline powder and then, glucose isomerase syrup is added to the glucose syrup in order to produce fructose syrup. Maltotetrose is used as moisture retainer. In the textile industry, the yarns are treated with a starch-containing solution to ensure that the yarns are durable and smooth and do not broken during weaving. After the fabric is knitted, the α-amylase enzyme is used to remove excess starch in the fabric.

β -amylase in plants, on the other hand, creates maltose by breaking down the bonds of the amylase on the chain ends where the amylase does not have the ability to reduce the bonds of the amylase on the chain ends. Alpha and beta-amylases also affect amylopectin. However, they can disentangle the straight chains up to their branching points.

4. Liases

They are enzymes that break or form bonds through oxidation or hydrolysis. To illustrate; pyruvate decarboxylase, citrate synthase, adenylate cyclase etc.

5. Isomerases

They are enzymes that catalyze the transformation of the molecule into an optical, geometric or structural isomer of the molecule such as triisophosphate isomerase, glucose-6-phosphate isomerase.

6. Ligases (Sentetases)

Enzymes that bind two molecules by using energy such as DNA ligase etc.

8.6. Clinical Enzyme Activity Assays

Activity measurements of enzymes in body fluids or tissues are very important for the diagnosis of diseases. The quantity of enzymes in body fluids or tissue extracts is very low, so it is difficult to measure directly. To determine the amount of an enzyme, the rate of the reaction catalyzed by the enzyme in the sample is measured. The rate of the measured reaction is proportional to the enzyme concentration.

For this purpose, blood, cerebrospinal fluid (CSF), amniotic fluid, urine, seminal fluid, erythrocytes, leukocytes, tissue biopsy samples, tissue cell cultures are used as the biological samples in the measurement of enzyme activity. For most diagnostic tests, serum or plasma are used. Analysis of urine for the measurement of the enzyme activity is limited in the diagnosis since very few enzymes can be filtered from kidney glomeruli.

8.7. Experimens

Experiment 8.7.1. Activity Determination of Polyphenol Oxidase Enzyme

Principle of the experiment: This experiment is a colorimetric method which based on the catalysis of catechol which is diphenol oxidized by the enzyme PPO.

Procedure:

Spectrophotometric analysis will be performed by using the catechol solutions prepared in phosphate buffer (pH 7.2). The following analysis will be carried out:

(1) Determination of activity of the polyphenoloxidase enzyme

(2) Effect of substrate concentration on activity (reaction rate) will be analyzed by plotting the V vs. [S] graph (Michaelis-Menten graph)

(3) Calculation of Vmax and Km values of the enzyme from Michaelis-Menten graph

(4) The catechol (pH 7.2) substrate and enzyme prepared at different concentrations will be taken in the amounts that are provided in the table and mixed. Absorbance spectra of them will be recorded in every 15 seconds at 420 nm for 2 minutes. The reaction velocity will be determined by drawing the absorbance time graph.

Catechol concentration	**Std.1 (0.5 mM)**	**Std.2 (1 mM)**	**Std.3 (2 mM)**	**Std.4 (3 mM)**	**Std.5 (4 mM)**
Catechol (mL)	2.8	2.8	2.8	2.8	2.8
PPO (mL)	0.2	0.2	0.2	0.2	0.2
Record the increase in absorbance spectra in every 15 seconds at 420 nm for 2 minutes via spectrophotometer.					

(5) The initial velocity (enzyme activity) is found from the slope of absorbance - time graph for each substrate concentration.

(6) If the initial velocity values are plotted against the substrate concentration, the Michaelis-Menten graph is obtained. Discuss the effect of substrate concentration on velocity according to this graph.

(7) Km and Vmax of the enzyme can be determined from the Michaelis-Menten graph.

Solutions:

1) 0.5, 1, 2, 3 and 4 mM catechol solutions prepared in phosphate buffer (pH 7.2)

2) Polyphenoloxidase enzyme: 1 mg PPO/ 20 mL phosphate buffer

Experiment 8.8.2. PPO Inhibition

Principle of the experiment: L-cysteine (pH 7.2) is used as an inhibitor of catechol (pH 7.2) and polyphenoloxidase enzyme. This inhibition of enzyme will be monitored by the decrease in speed.

Procedure:

The test will be carried out as indicated in the following table.

Blank	2.8 mL catechol
Sample	Blank + 0.2 mL PPO
Record the increase in absorbance spectra in every 15 seconds at 420 nm for 2 minutes via spectrophotometer.	
Plot the Absorbance-time graph and find the velocity from this graph.	

Blank	1.4 mL Catechol + 0.2 mL L-Cystein
Sample	Blank+ 0.2 mL PPO
Record the increase in absorbance spectra in every 15 seconds at 420 nm for 2 minutes via spectrophotometer.	
Plot the Absorbance-time graph and find the velocity from this graph.	
Change in the enzyme activity after addition of inhibitor will be discussed by comparing their velocities.	

Solutions:

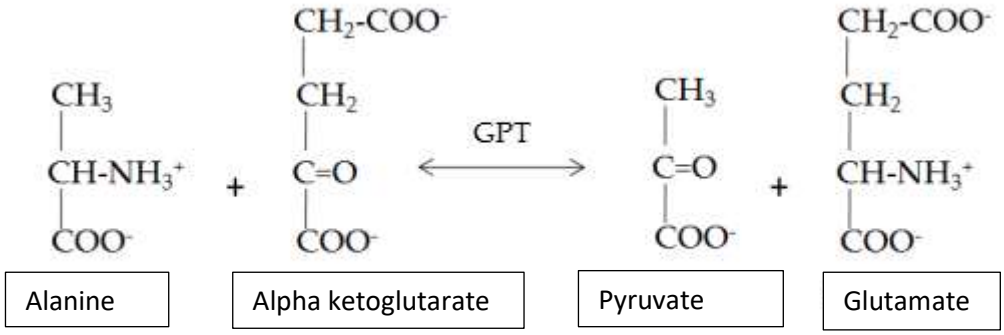
1) 2 mM L-Cysteine (pH 7.2 in phosphate buffer)

2) 2 mM catechol (pH 7.2 in phosphate buffer)

3) Polyphenoloxidase enzyme (1 mg / 20 mL phosphate buffer)

Experiment 8.8.3. Determination of Glutamic Pyruvic Transaminase (GPT) Activity

Principle of the experiment: In the transamination reaction catalyzed by the glutamic pyruvic transaminase enzyme, pyruvic acid and glutamic acid are produced from alanine and α-keto glutaric acid. The resulting pyruvic acid reacts with 2,4-dinitrophenyl hydrazine to form hydrazone. The color intensity of the brown compound in the basic medium is directly proportional to the GPT activity.



Procedure: Processes indicated in the following table are applied respectively.

Stage I: Case study

Substances/ mL	**Blank**	**Sample**
Substrate	0.5	0.5
Wait the tube at 37°C for 3 minutes.		
Distilled Water	0.1	-
Serum	-	0.1
Wait the tube at 37°C for 30 minutes.		
2,4-dinitrophenyl hydrazine	0.5	0.5
Wait at room temperature 20 minutes.		
0.4 M NaOH	5	5
Wait at room temperature 10 minutes.		

Absorbance spectra of blank and standards are recorded. Plot the standard calibration curve with absorbances recorded and calculate serum GPT enzyme unit by using this standard graph.

Stage II: Sketching the standard calibration graph

Substances / mL	**Blank**	**Std 1**	**Std 2**	**Std 3**
Unit Values	**-**	**28**	**57**	**97**
Substrate	1	0.9	0.8	0.7
Distilled Water	0.2	0.2	0.2	0.2
Sodium pyruvate	-	0.1	0.2	0.3
2,4-dinitrophenol hydrazine	1	1	1	1
Wait at room temperature 20 minutes.				
0.4 M NaOH	10	10	10	10
Wait at room temperature 10 minutes.				

Absorbance values of blank and standards are recorded at 510 nm. Plot the standard calibration curve with absorbances recorded and calculate serum GPT enzyme unit by using this standard graph.

Solutions

Phosphate buffer: 13.97 g of K_2HPO_4 and 2.69 g of KH_2PO_4 are dissolved in some distilled water and diluted to 1 liter(pH 7.4) and stored at + 4 ° C.

Substrate: 29.2 mg of α-keto glutaric acid and 1.78 g of dl-alanine are dissolved in 1 M NaOH. The pH is adjusted to 7.4 and then diluted to 100 mL with phosphate buffer.

2,4-Dinitrophenyl hydrazine: 19.8 mg of 2,4-dinitrophenyl hydrazine is dissolved in 10 mL of concentrated HCl and diluted to 100 mL with distilled water. It is stored in a dark bottle at room temperature.

Sodium pyruvate: 22 mg sodium pyruvate is dissolved in 100 mL phosphate buffer. It is freezed and stored.

Experiment 8.7.4. Effect of Temperature and Enzyme Amount on Enzyme Activity

Experiment principle: In the experiment, the rate of the reaction of catalase in the liver with H_2O_2 will be monitored with respect to change in the temperature and the amount of enzyme.

$$H_2O_2 \rightarrow H_2O + O_2$$

a) Effect of Temperature

Procedure: After the liver is cut into small pieces, it is divided into 2 parts. Some are boiled in a bowl full of water. The boiled liver pieces are taken up in a beaker and 3 mL of H_2O_2 is added onto it. To another beaker, pieces of liver at room temperature are taken and 3 mL of H_2O_2 is added onto it. The effect of temperature on the reaction rate is observed.

b) Effect of Amount of Enzyme

Procedure: Equal amounts of liver pieces are divided into 2 parts. One of the pieces is pounded in the grinder and crushed. Grinded liver is added to the one of the test tubes containing equal amount (3 mL) of H_2O_2 and liver pieces are added to the other tube and the reaction rates are observed.

Experiment 8.7.5. Hydrolysis of Starch with α-amylase

Principle of the experiment: When starch (see Chapter 2) is hydrolyzed with α-amylase in urine or saliva, the α-1,4 bonds in the starch molecule are broken down and form glucose, maltose and dextrins. The decomposition products of starch resulting from hydrolysis are divided according to the reactions with iodine and the degree of size of the molecule in parallel: The product that gives blue color with iodine is called 'amylodextrin'. With more hydrolysis, erythrodextrin is obtained, which is dyed red with iodine. With a further hydrolysis, a substance called 'acrodextrin' is obtained. This substance no longer gives color with iodine, its reducing feature is high. Reducing ability of acrodextrin is lower than that of the last hydrolysis product, maltose and glucose.

Hydrolysis Stage	Iodine Color
Starch	Blue
Amylo dextrin	Purple violet
Erythro dextrin	Red onion peel
Acro dextrin	Light yellow
Maltose / Glucose	Colorless

Procedure: The following study will be conducted for enzymatic hydrolysis of starch (0.1%) with α-amylase in urine.

Solutions / mL	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
α-Amylase (in urine)	-	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Distilled water	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3
0.1% Starch	2	2	2	2	2	2	2	2

After mixing the tubes, they are kept at 37^{o}C in water bath for 30 minutes. The reaction is stopped by adding 5 mL of cold water to each tube. Colors formed are monitored by adding 1 drop of iodine solution (1.5% KI and 0.3% I_2) to each tube.

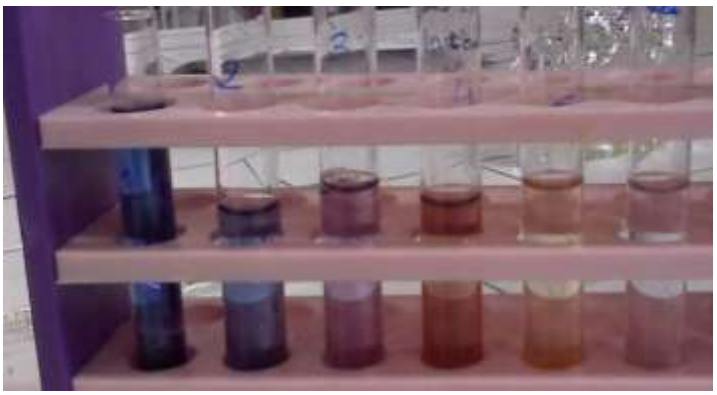


Figure 8.12. Hydrolysis of starch with alpha amylase

Solutions:

I_2 / KI solution: Prepared by dissolving 0.3 g I_2 and 1.5 g KI in 100 mL distilled water.

0.1% starch solution: Take 0.1 g of starch, dissolve it by heating with some distilled water and dilute it to 100 mL with distilled water.

REFERENCES

(1) http://www.eng.umd.edu/~nsw/ench485/lab5.htm

(2) http://themedicalbiochemistrypage.org/enzyme-kinetics.html.

(3) Berthelot M.: Report Chem.Aplique 1, 284 (1859).

(4) Bergmayer H. U., Verlag Chemie GMBH Wein Heim/Bergster Academic Press, New York and London. Pp. 779-787 & 837-853 (1956).

(5) www.mustafaaltinisik.org.uk/09-tbl102-0809Enzimler.ppt

(6) www.ctf.edu.tr/anabilimdallari/pdf/160/Enzimler.pdf