6. PHOTOSYNTHESIS

Energy is essential for all biochemical processes that are the basis of life. The source of all energies is the sun. No living thing can directly use the light energy of the sun, but can transform it into other forms of energy. The most important energy conversion of the biosphere is realized by photosynthesis. Sunlight reaching our world is transformed into a form of energy that can be used by cells by algae, bacteria, and plants with special pigments such as chlorophyll.

Photosynthesis is defined as organic nutrient synthesis using solar energy by green plants. Based on the formation of glucose, the net equation of the photosynthesis reaction is as follows:

$$6CO_2 + 6H_2O \xrightarrow{\text{Light, chlorophyll}} C_6H_{12}O_6 + 6O_2$$

The photosynthesis is an oxidation-reduction reaction between carbon dioxide and water. Because hydrogen atoms in water are transported to carbon dioxide, carbon dioxide is chemically reduced in photosynthesis and water is oxidized. The energy required to achieve this reaction is derived from sunlight. In addition, many environmental factors have important effects for the formation of this reaction.

Reactions related to photosynthesis have been investigated since the end of the 18th century. The fact that oxygen produced in photosynthesis was discovered by Priestley in 1780. Later, a Dutch physicist, Ingenhousz, found that light is required to form oxygen. The role of water was found by Theodore de Saussure.Jean Senebier found that CO2 was spent on photosynthesis. The fact that the oxygen release during photosynthesis is not due to reduction of carbon dioxide but it is due to decomposition of water has been demonstrated by the isotope labeling technique.

Hill Reaction

In 1937, Robert Hill, using chloroplasts isolated from leaves, found that photosynthesis was carried out in a system of light-containing receptors (water) in the presence of water, and that the water was decomposed and formed O_2 by using a ferrioxalate solution.

light

$$4\ Fe^{+3} + 2H_2O \quad 4Fe^{+2} + O_2 + 4H^+$$

During photosynthesis, some reactions require light. These reactions are called light reactions. At this stage, light absorbent pigments are present on the thylakoid membranes. The reduced coenzyme and ATP obtained as a result of the reactions occurring here are used in the dark reactions of photosynthesis. However, the name 'dark reactions' does not mean that reactions occur in the dark, but that there is no need of light for these reactions.

6.1. Chloroplast

In eukaryotic cells that make photosynthesis, they are organelles where photosynthesis occurs (Figure 6.1). Chloroplasts are found in mesophyll cells near the leaf surface in plants. A plant cell has 30-40 chloroplasts. Its composition contains 50% protein, 40% lipid and 10% pigment. Chloroplasts have their own ribosomes and DNA. Thus, they can synthesize the necessary proteins and enzymes for themselves. They have double membrane. The permeability of the outer membrane is quite higher compared to the inner membrane. The inner membrane surrounds a liquid called stroma which is similar to a matrix. Enzymes are dissolved in the stroma. These enzymes are involved in the transformation of CO_2 into carbohydrates in the dark reactions of photosynthesis. Flat stacks of thylakoids called Grana (singular granum) which are arranged in a row like coins. Granas are connected with each other and with flat, membranous channels called stromal lamellae. There are enzymes and carrier molecules on the tilakoid membranes related to the light reactions of photosynthesis. The inner space of the thylakoid discs that is surrounded by thylakoid membrane is called lumen. The amount of phospholipid in the structure of the thylakoid membranes is low and the amount of glycolipid is high.

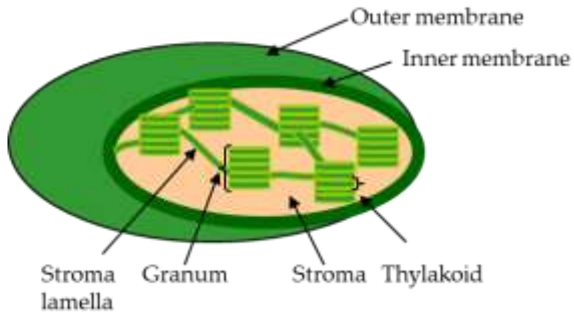


Figure 6.1. Chloroplasts and their parts

6.2. Chlorophyll

Plants have various pigments for the absorption of rays in the UV region and visible coming from the sun. Most important pigment is chlorophyll. The structure of chlorophyll molecule composed of four pyrrole rings located around a Mg atom in the center. This tetra pyrrole structure is called 'Mg-porphyrin'. The phytol, which is a long and straight chain alcohol, is attached to 7th carbon of one of the pyrrole rings via ester bond. Chlorophylls are divided into two groups as 'chlorophyll-a' and 'chlorophyll-b'. The difference between the chlorophyll a and b molecules is due to the groups bound to the 3rd carbon atom of the 2nd pyrrole ring. The methyl (CH_3) group of chlorophyll a is attached to the 3rd carbon of chlorophyll a, while the aldehyde group (CHO) is attached to the 3rd carbon of chlorophyll b (see Fig.6.2). Depending on this difference, molecules are separated from each other in terms of solubility and light absorption. Chlorophyll a is dissolved in petroleum ether, chlorophyll b is dissolved in methyl alcohol. Chlorophyll a absorbs wavelengths of 429 nm and 662 nm, and chlorophyll absorbs 453 nm and 654 nm (see Fig. 6.3). The formula of chlorophyll a is $C_{55}H_{72}O_5N_4Mg$ and chlorophyll b is $C_{55}H_{72}O_5N_4Mg$.

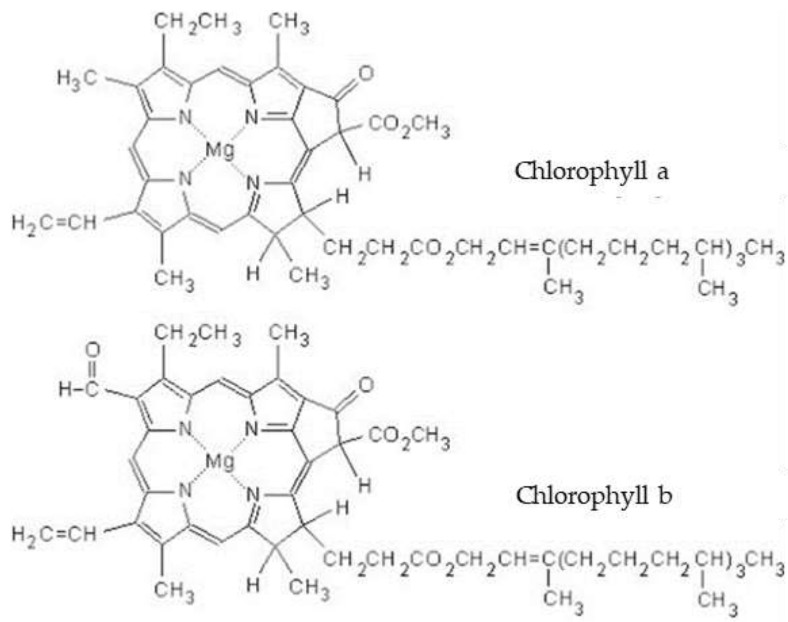


Figure 6.2 Molecular structure of chlorophyll a and b

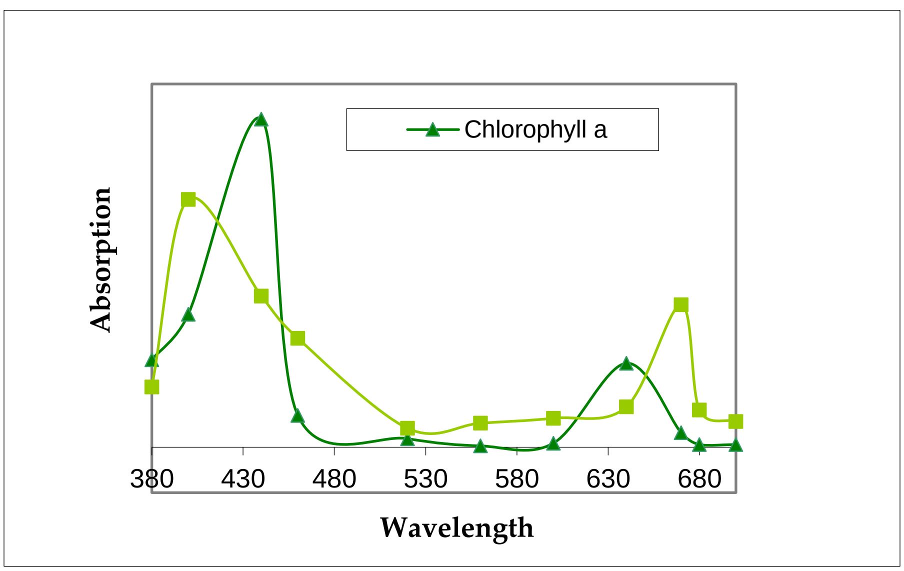
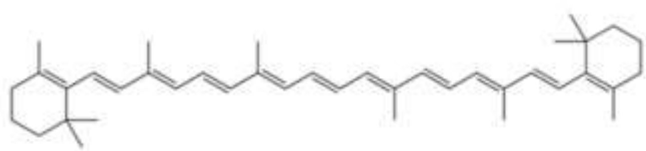


Figure 6.3. Visible region spectra of chlorophyll a and b

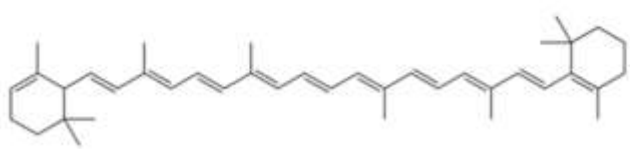
Chlorophyll contains 50% protein, 7-8% inorganic clay and lipids. The chlorophyll molecule shows polar properties, the hydrophilic portion of the porphyrins in the molecule structure is hydrophilic and the tail part of the phytol group is lipophilic.

6.3. Carotene and Xanthophyll

Carotene is another photosynthetic pigment important for photosynthesis. It contributes to photosynthesis by transferring absorbed light to chlorophyll. It gives an orange color to vegetables and fruits. Carotene having the closed formula $C_{40}H_{56}$ is a terpene and biochemically synthesized from eight isoprene units. There are mainly two isomers which are alpha-carotene (α-carotene) and beta-carotene (β-carotene). The main difference between α-carotene and β-carotene is the double bond in the ring group (Figure 6.4). β-carotene consists of two retinyl groups and is destroyed by beta-carotene dioxygenase in the small intestinal mucosa and transformed into retinol (a type of vitamin A). Retinol is a provitamin which can be stored in the liver and converted into vitamin A when necessary.



β-carotene



α-carotene

Figure 6.4. the molecular formula of β-carotene and α-carotene

Yellow xanthophiles with closed formula $C_{40}H_{56}O_2$ are oxidized derivatives of carotene. It acts as a accessory pigment in photosynthesis. Because of the hydrophilic groups it contains, they are more polar than carotene. In autumn, when the weather is cooled, chlorophyll is denatured and xanthophyll's typical yellow color emerges.

6.4. Environmental and internal factors affecting photosynthesis

CO_2 concentration, intensity of light, temperature, water and mineral salts are environmental factors that directly affect the rate of photosynthesis.

(a) CO_2 concentration: Since the main raw material used in photosynthesis is CO_2, an increase in the amount in the environment (0.03%) 15-20 times increases the rate of photosynthesis (figure 6.5). When this value is exceeded, the rate of photosynthesis remains constant. In extreme increases, the rate of photosynthesis slows down.

Figure 6.5. Effect of CO_2 concentration on photosynthesis rate

(b) Light intensity: Intensity, wavelength and duration of light used in photosynthesis are important (Figure 6.6).

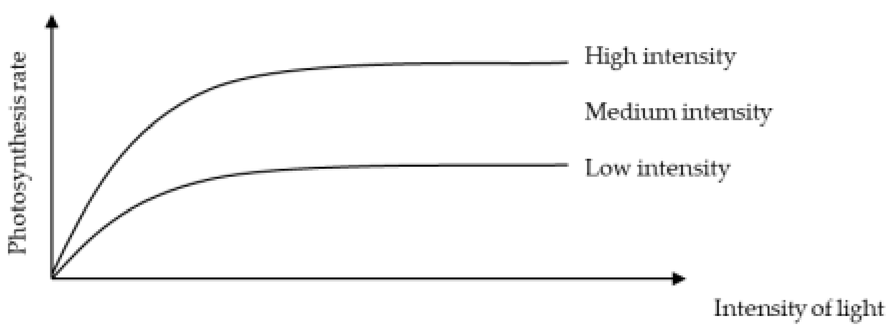


Figure 6.6. Effect of light intensity on photosynthesis rate

Light leads the opening of pores in the leaves, and hence;

(1) allows more CO_2 to enter the leaf

(2) heats the leaf and increases the sweating and thus effects of photosynthesis.

(c) Wavelength of light: Photosynthesis is fastest in presence of purple and red light. Since green light is reflected, it is the least used light in photosynthesis.

(d) Temperature: Since dark reactions of photosynthesis occur with enzymes, the rate of photosynthesis changes with temperature. High temperature will disrupt the structure of enzymes adversely affect photosynthesis (Figure 6.7).

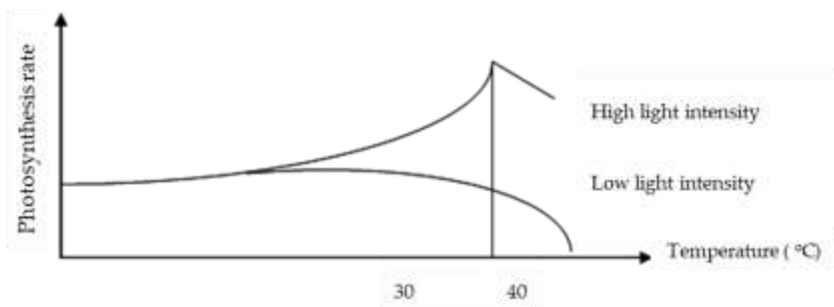


Figure 6.7. Effect of temperature on photosynthesis rate

(e) Water: causes minor changes in the rate of photosynthesis. The amount of water causes sweating.

(f) Some chemicals and mineral salts: affect directly or indirectly the photosynthesis. These substances are mainly hydroxylamine, Iodo acetate, HCN and H_2S. It has been observed that these substances have a negative effect on photosynthesis as specific enzyme inhibitors.

Internal factors affecting photosynthesis are as follows:

(a) amount of chlorophyll and chloroplast

(b) activity of enzymes

(c) the accumulation of photosynthesis products

(d) number, size and distribution of stroma

(e) the width of the leaf surface

(f) structure and distribution of the root system

6.5. Experimental

6.5.1. Half Leaf Experiment

The principle of experiment: The starch formed as a result of the reaction of photosynthesis is based on the principle that it interacts with iodine in the lugol solution to form a colored complex.

Experimental procedure:

(1) Geranium leaf is wrapped with aluminum foil and a band-shaped opening is formed on it. In this way, it is kept in a bright environment for 4 to 5 days.

(2) The leaf is removed from the aluminum foil and the leaf cell membranes are left in the beaker for 10 minutes in order to become permeable.

(3) The leaf is removed from the water, placed in a warm ethyl alcohol beaker to prevent chlorophyll from blocking the starch and is allowed to wait until the green color of chlorophyll is removed. (This step is performed by placing the beaker with ethyl alcohol in a hot water bath).

(4) The alcohol-washed leaf is washed with cold water.

(5) The leaf is immersed in a petri dish with 20 mL of lugol solution, and after 5 minutes it is taken over a watch glass and examined in light.

Solutions:

Lugol solution: 2.5 g of KI is added over 0.5 g of iodine and the resulting mixture is milled in muller, the powdered mixture is dissolved by adding distilled water to a total volume of 100 mL.

6.5.2. Spectrophotometric Measurement of the Absorption of Chlorophyll a and b in Different Wavelengths

Principle of the experiment: The optical density of the chlorophyll a and b molecules in petroleum ether and methanol solvents is measured spectrophotometrically in the wavelength range of λ= 380-700 nm. (λ= 380-400-420-440-460-520-560-600-640-660-680-700).

Procedure:

(1) Stinging nettle or spinach leaves are finely cut with the help of scissors or knives.

(2) Add the amount of acetone to cover the leaves.

(3) Wait 10-15 minutes.

(4) The dark mixture is centrifuged for 5 min, the precipitate is discarded. The solution (crude extract) is taken up in a clean tube.

(5) Samples are prepared according to the following table for measurement.

1st Tube	1st Blank tube	2nd Tube	2nd Blank tube
6.6 mL petroleum ether	6.6 mL petroleum ether	6.6 mL methanol	6.6 mL methanol
0.4 mL leaf extract	0.4 mL acetone	0.4 mL leaf extract	0.4 mL leaf extract

(6) Absorption values for both solutions are transferred to the chart against the wavelength.

6.5.3. Calculation the Amount of Chlorophyll and Carotenoids

Principle of the experiment: The amount of chlorophyll and carotenoid will be calculated according to the formula below by measuring absorbance values of the crude extract obtained from previous experiment and blank tube (acetone) at various wavelengths (λ=661,6, 644,8 and 470 nm).

Calculation of pigment concentrations:

$C_a = 11.24 \times A_{661.6} - 2.04 \times A_{644.8}$ C_a = Chlorophyll a concentration (µg/mL)

$C_b = 20.13 \times A_{644.8} - 4.19 \times A_{661.6}$ C_b = Chlorophyll b concentration (µg/mL)

$C_{a+b} = 7.05 \times A_{661.6} + 18.09 \times A_{644.8}$ C_{a+b} = Total Chlorophyll concentration (µg/mL)

$C_{x+c} = [1000 \times A_{470} - 1.90 \times C_a - 63.14 \times C_b] / 214$ C_{x+c} = Total carotenoid concentration (µg/mL)

6.5.4. Qualitative determination of chlorophyll and carotenoid

The principle of the experiment: It is based on the separation of the substances in the raw chlorophyll extract by taking advantage of their different solubility and absorption characteristics.

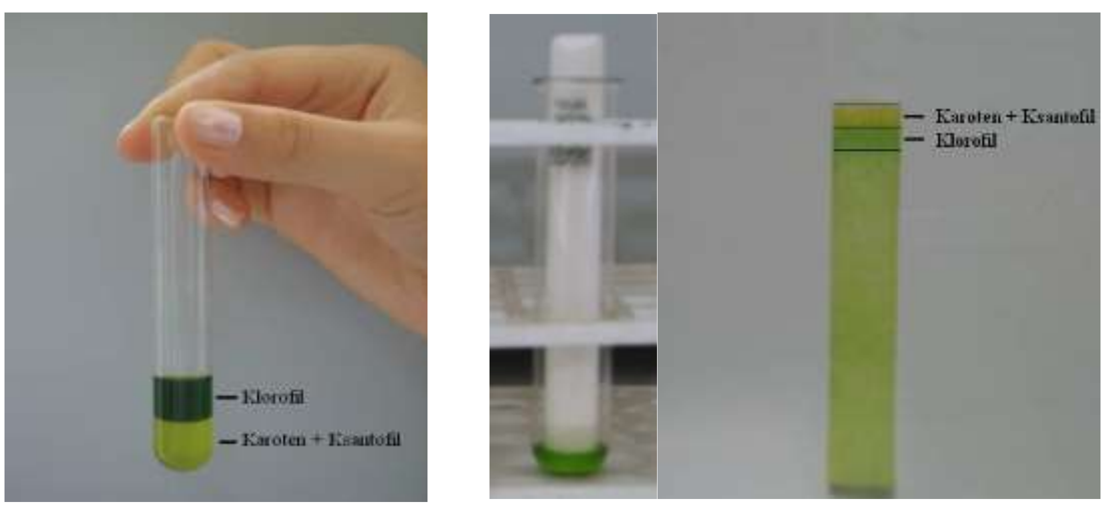
Procedure: Sting nettle is finely cut with scissors or a knife, 10 mL of ethanol is added on it and left for 10 to 15 minutes. The resulting solution is called 'crude chlorophyll extract'. To obtain a clear extract, the solution is filtered through filter paper.

(a) According to the resolution specifications;

2 mL of the filtrate is taken and transferred to a test tube. The same amount of benzene and a few drops of water are added to the tube (The purpose of adding water is to increase the density of the alcohol mixture and allow the benzene to easily rise to the top of the tube) and the solution is mixed. After a while, chlorophyll dissolves in the upper part of the tube and yellow carotene and xanthophylls remain in the lower part of the tube (Figure 6.8 a).

(b) According to absorption and molecular weight characteristics;

1 mL is taken from the filtrate and transferred to a test tube. It is placed in the form of a striped filter paper. After a while, yellow colored carotene and xanthophyll on the upper part of the paper and green colored chlorophyll at the bottom (Figure 6.8 b).



(a) **(b)**

Figure 6.8. Separation of chlorophyll, carotene and xanthophyll pigments in raw plant extract on the basis of solubility (a) and absorption (b)

Working Questions:

(1) Half of the leaf of the begonia plant is soaked in hot water, and after a few seconds it is observed that the part soaked is brown. Explain the reason for this change.

(2) After the half of the begonia plant is placed in a large tube with a KOH or NaOH solid, the mouth of the tube is tightly closed. It is seen that the leaf inside the tube turns yellow after a few days. Explain the reason for this change

(3) A few drops of HCl are added to the test tube by adding some of the previously prepared raw chlorophyll extract and the color of the chlorophyll extract is observed to turn brown. Explain the reason for this change

(4) Discs are taken from the leaves of the geranium plant, which is kept in the dark for a while, and dried in an oven at 105^{o}C. After the same plant is left in the light for a few days, discs are taken from the leaves and dried. What kind of difference would you expect between their dry weights?

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