Photosynthesis

1. Pre-Lab Reading

Chapter 8 "Photosynthesis" Biology 2e, OpenStax

2. Objectives

The purpose of this lab is to explore various facets of photosynthesis.

Upon completion of this activity, you should be able to:

1. Explain the general process of photosynthesis.
2. Separate and identify plant pigments involved in photosynthesis using paper chromatography.
3. Determine the absorption spectrum for leaf pigments.
4. Explain the function of chlorophyll and accessory pigments in photosynthesis.

3. Safety Guidelines

1. Wear safety goggles when working with chemical solutions.
2. Handle all glassware with care.
3. Upon completion of all exercises, clean and straighten you lab space.
4. Wash hands after leaving the lab.

4. Background Information

Most life on our planet is either directly or indirectly dependent on the radiant energy of sunlight. An important step in the evolution of life was development of the ability to covert this energy into a useful form of chemical energy. This process is known as **photosynthesis** and involves numerous chemical reactions that are still not completely understood.

Photosynthesis occurs in most plants and algae as well as in some prokaryotes. Organisms that have the ability to manufacture their own food are called **autotrophs**. Autotrophs that utilize photosynthesis are termed **photoauotrophs**. Some prokaryotes have the ability to produce organic compounds through the oxidation of inorganic substances such as sulfur or ammonia. These organisms are referred to as **chemoautotrophs**. Organisms that lack the capacity to produce their own food are called **heterotrophs**. All heterotrophs are ultimately dependent on autotrophs as sources of organic molecules essential for their survival.

Photosynthesis in plants occurs in membrane-bound structures called **chloroplasts**. The complicated series of reactions that comprise the process of photosynthesis can be summarized by the following equation:

$$6CO_2 + 12\,H_2O \rightarrow C_6\,H_{12}\,O_6 + 6\,O_2 + 6H_2O$$

Light, chlorophyll and numerous enzymes are also essential components of the process. Photosynthesis is divided into two sets of reactions: light-dependent reactions (light reactions) and light-independent reactions (Calvin cycle or dark reactions).

The light-dependent reactions convert light energy into chemical energy in the form of ATP and the reduced electron carrier NADPH. Light is a form of electromagnetic energy that behaves both as waves and as particles called photons. The energy of a photon is inversely proportional to its **wavelength**. The wavelength of light correlates with the color that that light appears to the human eye. When a photon meets a molecule it can be:

- *Scattered* or reflected
- *Transmitted* or pass through the molecule
- *Absorbed*—the molecule acquires the energy of the photon. The molecule goes from **ground state** to **excited state**.

When a molecule absorbs light energy, electrons in that molecule are boosted to a higher energy orbital. Usually this means that the exited electrons orbit the molecule further from atomic nuclei. In order for a molecule to absorb light of a given wavelength or color the energy of the photons must match the energy difference in the molecule's ground state and exited state orbitals. Because different molecules have different molecular structures they will interact differently with different wavelengths of light or other electromagnetic energy.

Molecules that absorb energy in the visible portion of the electromagnetic spectrum are called pigments. Almost all photosynthetic organisms produce one or more types of chlorophyll, a molecule which appears green in color to the human eye. Plants have two chlorophylls, chlorophyll a, which is a blue-green color and chlorophyll b, which is more yellow-green. Most organisms also produce accessory pigments such as the carotenoids which appear orange (carotene) or yellow (xanthophylls) in color. These pigments absorb light of different wavelengths than those absorbed by chlorophyll. They then pass this energy to chlorophyll. Accessory pigments also function to protect plants from excessive light absorption if they are in bright light.

Additional details of these reactions are provided in your text. In this lab, you will investigate various aspects of photosynthesis including the extraction and isolation of photosynthetic pigments, the observation of the fluorescence of chlorophyll and determination of the absorption spectrum of photosynthetic pigments.

5. Leaf Pigment Extract Preparation

Procedure

1. Wearing disposable gloves, tear several spinach (*Spinacia oleracea)* leaves apart into small pieces and place in a mortar. **Do this once per two lab tables.**

2. Add 25 mL 95% ethanol and a very small amount of sand.

3. Grind the mixture with a pestle until the leaves are well macerated and the solution is a deep green.

4. Using cheesecloth, filter the solution into a 50 mL Erlenmeyer flask, being careful not to include any solids. Transfer an aliquot of the solution to another small container so that each table has some.

5. Stopper the flask with a cork and **keep the extract on ice** when not using.

6. Fluorescence

Fluorescence is emission of light from a material. It occurs when that material has absorbed energy and then releases it in the form of light. This occurs in chlorophyll when it absorbs uV radiation and then releases light in the visible wavelength range. **Note that this is not what happens in photosynthesis and the color seen here does not tell you what wavelengths of light plants use for photosynthesis.** It is simply an illustration of the process that happens when molecules absorb energy, boosting electrons to a higher energy state. In photosynthesis pigments absorb energy in the visible wavelength range and this energy is transferred to a component of an electron transport pathway rather than released.

Procedure

1. Transfer 2 mL extract to a small test tube. Add 0.5 mL dH_20 and mix gently.

2. Hold the test tube containing your extract up to the light and note its color.

3. Now, place the test tube inside the uV Viewing Chamber and turn on the long-wave light. Note the color of the extract and record your observations.

7. Separation and Identification of Leaf Pigments by Paper Chromatography

Paper chromatography is a method of separating a mixture of dissolved compounds based on differences in their solubilities in the solvent used and affinity for the paper. The leading edge of the solvent is called the **front**. As the pigments travel up the paper from their point of origin with the solvent, discrete bands for each pigment will form.

The ratio of the distance a substance moves up the paper to the distance the solvent front travels is called the **retardation factor**, R_f. The R_f value is characteristic of a substance when the same solvent system and type of chromatography paper are used.

The rate at which pigments will move in the paper chromatography experiment depends largely on their polarity. Recall the meaning of the terms polar and nonpolar. Polar covalent bonds form between atoms that differ in electronegativity and electrons in these bonds are shared unequally. Bonds between oxygen and hydrogen are among the most polar. Other polar bonds include carbon-oxygen, carbon-nitrogen and nitrogen-hydrogen bonds. Nonpolar covalent bonds form between atoms that are similar in electronegativity and electrons in these bonds are shared equally. Examples are carbon-carbon bonds and carbon-hydrogen bonds. Overall polarity of a molecule depends on additional factors such as geometry, but, in general, molecules with oxygen-containing groups (especially hydroxyl or "OH" groups) are more polar than molecules with only carbon and hydrogen. In addition, charged groups (ions) will interact only with polar solvents.

The solvent used in this experiment is 90% petroleum ether. Petroleum "ether" is a common name for a mixture of hydrocarbons (primarily pentane) and does not actually contain molecules with ether or other oxygen-containing groups. It is therefore a very nonpolar solvent.

The following information will help you interpret your results:

- Polar substances dissolve in polar molecules.
- Nonpolar substances dissolve nonpolar molecules.

- Chromatography paper is a polar substance (cellulose).
- The solvent (90 parts petroleum ether:10 parts acetone) is relatively nonpolar.
- The less polar the substance is the more quickly it will dissolve in the solvent and move up the paper.
- The most polar substances will be attracted to the chromatography paper and therefore move last.

Procedure

1. Using a pencil, place a line on your strip of chromatography paper 1.5 cm from the pointed end of the paper.

2. Dip a capillary tube into your leaf extract.

3. Cover the other end of the capillary tube with your finger and lightly touch the tube to the center of the pencil line on the chromatography paper.

4. **Allow the spot to dry** and repeat this process several more times to create a dark green spot on your paper. The darker your spot, the better your results.

5. While you are waiting for your spot to dry take a look at the pigment structures projected on the screen. Write a hypothesis regarding the following question: Which pigment molecule is least polar? Write a prediction based on your hypothesis regarding which pigment molecule will travel furthest on the paper.

6. Working in the fume hood, pour enough chromatography solvent into the test tube provided to just reach the sharpie mark below the red stripe on the side of the test tube.

7. Pin the non-pigment end of your chromatography paper to the cork stopper provided and carefully place the paper inside the test tube. The tip (but not the green spot) of the pigment end of the paper should be touching the solution.

8. Secure the stopper in place and put your test tube in the rack provided.

9. After about 10 minutes, remove the paper from the test tube and **immediately** mark the solvent front on the chromatography paper. Also mark the location of any pigment bands that appear on the paper.

10. Replace the stopper of your tube and leave it in the fume hood.

11. Tape the chromatogram into your lab book and/or draw a diagram of the bands. **Label** the pigments based on the colors described above.

12. Measure and record the distance traveled in cm from the starting line for each pigment observed.

13. Calculate the R_f values for each pigment using the following formula:

$$R_f = \frac{\text{distance pigment travels}}{\text{distance solvent front travels}}$$

The distance the solvent travels is measured from the center of the pigment spot to the solvent front.

14. Record your calculations and R_f values in your lab book.

8. Determining the Absorption Spectrum for Leaf Pigments

Procedure

1. Turn on the spectrophotometer and allow it to warm up at least 10 minutes before using. Set the wavelength on the machine to 360 nm.

2. Make a table in your lab book, one column for Wavelength and the other for % Absorbance. Wavelength values will range from 400 nm to 720 nm, increasing each time by 20nm increments.

3. Write a hypothesis and prediction for this experiment.

4. Fill one of your cuvettes with 3 mL 80% acetone, insert it into the sample holder and close the lid. This cuvette will serve as your blank throughout this exercise and will be used to recalibrate the machine each time you change the wavelength.

5. Fill a second cuvette with 3 mL 80% acetone and add 3 drops of your leaf extract. Mix gently but thoroughly (do not invert the tube) and then place the cuvette into the sample holder with the wavelength set at 360nm. If the reading is below 0.4% absorbance, add more extract one drop at a time until the reading is close to 0.4%.

6. Change the wavelength to 400 nm, insert your blank, and recalibrate the instrument to 0% absorbance with the blank tube. Remove the blank and insert your sample. Record your absorbance reading.

7. Continue making observations, increasing the wavelength by 20 nm increments until you reach 720 nm. Be sure to recalibrate the instrument each time you change the wavelength.

8. Make a scatter/line graph of Wavelength (x-axis) vs. % Absorbance. Graph only the 400 thru 720 wavelengths. Label the graph Absorption Spectrum of Spinach Pigment Extract.

9. Conclusion and Summary

1. What 3-4 pigments were isolated using paper chromatography? Of these pigments, which was the most soluble in the solvent used? Which pigment would you consider

the most polar?

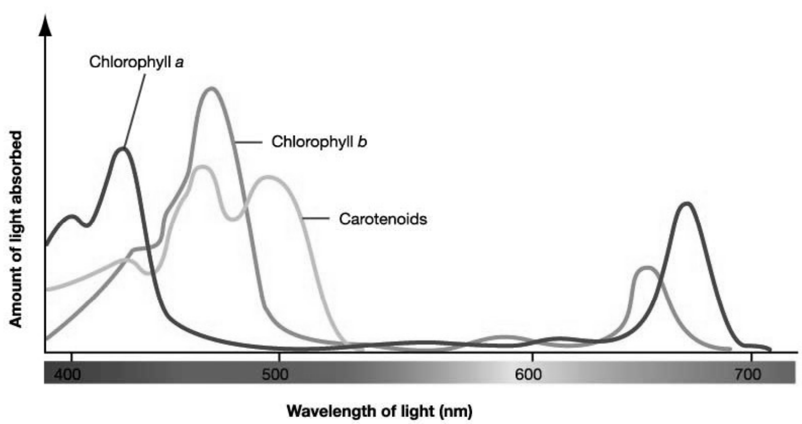
2. Write a conclusion regarding your hypothesis for the paper chromatography experiment.

3. What wavelengths (two peaks) of light had the highest absorption by leaf pigments in the chromatography experiment? What colors of light do plants use for photosynthesis?

4. Write a conclusion regarding your hypothesis for the Absorption Spectrum for Leaf Pigments experiment.

5. Describe the similarities and differences between the graph you made from data obtained in Absorption Spectrum for Leaf Pigments with the graph below. What pigments are most likely in your sample according to the absorbance data (Does the absorbance pattern correspond exactly with the absorbance spectrum of chlorophyll a or is there at least some absorbance at other wavelengths)?

6. Do the Absorption Spectrum results agree with the paper chromatography result?



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