Photosynthesis

Objectives

1. Describe the roles played by light and pigments in photosynthesis.
2. Identify the pigments involved in photosynthesis by paper chromatography.
3. Determine the wavelengths most useful for photosynthesis by determining the absorption spectrum of a pigment extract.
4. Demonstrate the effect of changing light levels on electron transport in photosynthesis as measured by oxygen production.
5. Describe an experiment that demonstrates that carbon dioxide is utilized during photosynthesis.

Introduction

The equation for photosynthesis is:

$CO_2 + H_2O \rightarrow (CH_2O)_n + O_2$

In this equation, (CH_2O) represents the general carbohydrate. Usually, this equation is multiplied by six so that glucose ($C_6H_{12}O_6$) is the end product of photosynthesis. The equation is then written as follows:

$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$

Photosynthesis takes place in the chloroplasts. In the last 40 years, the details of chloroplast ultrastructure have been determined and this has helped explain the process. The arrangement of thylakoid membranes in the grana is critical to energy harvesting. The matrix or stroma has an important role in producing storage molecules.

The overall process of photosynthesis can be understood as two closely linked sets of reactions. The light-dependent reactions occur in the thylakoid membranes, where electron transport produces NADPH and creates a hydrogen ion gradient across the thylakoid membrane that drives ATP synthesis by chemiosmosis. ATP and NADPH are then used by the light-independent reactions. These occur in the stroma regions, where carbon fixation is carried out by the enzymes of the Calvin cycle to convert CO_2 to carbohydrates such as glucose.

The driving force for photosynthesis is light absorption by the photosynthetic pigments of photosystems I and II. Only the wavelengths of light absorbed by the photosystems can be used to drive photosynthesis. Light energy drives the flow of electrons from water molecules, which are split to form oxygen and hydrogen by a complex of proteins and metal ions associated with photosystem II, through photosystem II, along a chain of electron carriers, through photosystem I, and eventually to $NADP^+$. The flow of electrons through the chain of electron carriers also creates a gradient of H^+ that is used for the synthesis of ATP by chemiosmosis. The entire process by which light energy is converted into chemical energy in the form of ATP and NADPH is called noncyclic photophosphorylation. The process is so named because the flow of electrons is noncyclic from the source (water) to the destination (NADPH). The energy to drive the electron flow "uphill" comes from light (photo-). ADP undergoes phosphorylation (addition of a phosphate group), carried out by the ATP synthetases in the thylakoid membrane using the energy provided by the proton (H^+) gradient. Sometimes ATP alone is the product of some light-dependent reactions. This is due to a process called cyclic photophophorylation, in which the electrons pass through the electron transport carries but do not get passed to $NADP^+$. Instead the electrons pass from photosystem I to the electron transport chain and back to photosystem I. While passing through the electron transport chain they contribute to the size of the hydrogen ion gradient which increases the output of ATP. Oxygen and NADPH are not products of this process.

In the light-independent reactions of the Calvin cycle, the chemical energy stored in ATP and NADPH is used to reduce CO_2 to carbohydrate, an "uphill" or endergonic, process. Carbon fixation begins with carbon dioxide uptake and incorporation into an organic molecule, a reaction catalyzed by the enzyme ribulose bisphosphate carboxylase/oxygenase. The energy stored in the ATP and NADPH is then used to convert

the low energy CO_2 to high-energy carbohydrates. While these reactions are typically referred to as the light-independent reactions, in intact plants they are dependent on light as they are driven by ATP and NADPH, which are the products of the light-dependent reactions. As long as noncyclic photophosphorylation continues, sufficient NADPH and ATP will be available for the Calvin cycle reactions, CO_2 will be fixed, and carbohydrate will be synthesized. If light is removed, or electron flow chemically blocked, ATP and NADPH are quickly used up and the Calvin cycle stops working. (This is the mechanism of action of a number of popular herbicides.) In intact photosynthesis systems, then, light is necessary for the Calvin cycle as it is tightly linked to the synthesis of ATP and NADPH.

Recently, it has been recognized that water is both a reactant and product of photosynthesis. This realization has come about as biologist better understood the formation of oxygen as a product of photosynthesis. It is now known that a total of 12 water molecules are required as reactants for photosynthesis. In the space below, write out a balanced equation for the synthesis of one glucose molecule from 6 carbon dioxide molecules and 12 water molecules. Referring back to the equations at the beginning of the introduction should provide you with a clue for how this might work.

Identifying Plant Pigments by Paper Chromatography

The primary photosynthetic pigment in most plants is chlorophyll *a*. At the center of each photosystem are is pair of chlorophyll *a* molecules that are responsible for generating high energy electrons using light energy; these are known as the reaction center chlorophylls.. Surrounding the reaction center chlorophylls are more chlorophyll *a* molecules, together with chlorophyll *b* molecules, carotenes and xanthophylls. These pigments are known as secondary, or ancillary, pigments. They also absorb light energy but funnel it towards the reaction center chlorophylls for use in making high energy electrons.

In this exercise you are going to use a technique called paper chromatography to separate out these four types of pigments. Chromatography is a technique that separates molecules from each other on the basis of their solubility in particular solvents. The more soluble the pigment is in the solvent, the faster and farther it moves up the chromatography paper. The size of the molecule also affects the rate it moves up the paper with smaller molecules progressing further up the paper than large molecules.

Procedure:

1. Obtain a strip of chromatography paper, taking care to hold it only by the top and/or sides. While the strips have been cut to the correct length, you need to cut one end (the bottom) to form a point.
2. Draw a faint horizontal line in pencil only about 2cm from the tip and place a small “X” in the center of the line.
3. Use a capillary tube to apply a small amount of extracted spinach pigments to the “X”. Do this by barely touching the end of the capillary tube to the paper and then immediately withdraw it. A small green spot should appear on the paper.
4. Allow this drop to dry completely and then repeat step 3. Repeat this process at least 15 times. The resulting spot should be small and very dark. It may take 10-15 minutes to achieve this.
5. Obtain a large test tube containing a pre-measured amount of chromatography solvent and place it in a test tube rack. **CAUTION: Ether (which is a part of the chromatography solution) is toxic and extremely flammable. Do not breathe the fumes or allow others to breathe the fumes. Under no circumstance is the stopper to be removed from the test tube except to add or remove the chromatography strip. Do not place the chromatography solution near a heat source.**

6. Carry the test tube rack and your chromatography strip to one of the porches outside the building. Quickly remove the stopper, attach your chromatography strip to the hook on the stopper and replace the strip and stopper in the test tube, making certain the stopper is securely seated in the test tube. Return the test tube rack and test tube to the lab.
7. After about 20-30 min. the solvent will be close to the top of the strip and you must remove the strip immediately. Carry the rack outside again, remove the stopper, remove the strip from the hook and replace the stopper securely in the test tube.
8. Immediately mark the location of the solvent front (edge of the part wet by the solvent) with a pencil. You will be unable to analyze your chromatogram if you fail to do this before the rapidly drying solvent dries.
9. Allow the strip to dry for a few minutes before you return to the classroom.
10. After returning to the classroom, mark the upper edge of each pigment spot with a pencil as the colors will fade over time.

Analysis of the pigments:
Measure the distance, in millimeters, from your original line with the pigment spot and each of the other pencil marks you have made on the chromatography strip. You can use these measurements to calculate what is known as the R_f of each pigment (the ratio of the distance each pigment ran versus the distance the solvent ran). Depending on the quality of the pigment extract, there should be four pigment spots on the chromatogram:

Chlorophyll *a* – a blue-green pigment
Chlorophyll *b* – a yellow-green pigment
Carotenes – yellow-orange accessory pigment
Xanthophylls – yellow accessory pigment

The last two pigments (carotenes and xanthophylls) may contain more than one spot.

Complete table 1 with your measurements and R_f calculations and identify each spot using the descriptions above in the appropriate box.

Table 1. Data from Chromatography

Spot	mm moved	R_f (mm/mm solvent)	Color	Identification
Solvent front		–	–	–
Fastest spot				
Slowest spot				

Absorption Spectrum of Photosynthetic Pigments

Only the light absorbed by chloroplasts can be used in photosynthesis. The pigments of a green plant look green to the eye because they permit green light to pass through, but absorb the red and blue light. The particular wavelengths of light absorbed by a substance form a pattern called its absorption spectrum. The spectrum is determined by illuminating a solution of a substance with each wavelength of light in turn and measuring the absorption in each case. Using visible light, we can determine the absorption spectrum of a mixture of the pigments extracted from chloroplasts.

Procedure:

1. Fill a spectrophotometer cuvette with a dilute solution of chloroplast pigments. Fill a second cuvette with 80% acetone (the solvent used to extract the pigments from the chloroplasts). The second tube will be your control for measuring the absorption of the pigments at each wavelength.
2. Your instructor will help you obtain a spectrum using the spectrophotometer. Do not attempt to use this complex piece of equipment without your instructor as it is a delicate piece of equipment and easily damaged.
3. Attach a copy of the absorption spectrum in your lab report below and answer the following questions.

Absorption spectrum

1. How many peaks are there in this absorption spectrum?

2. What wavelength(s) (approximately) does/do the peak(s) occur?

3. What portions of the visible spectrum, based on the absorption spectrum, do you think contribute the most energy to photosynthesis?

Photosynthesis in *Elodea*

In an intact plant, both electron transport and carbon fixation will continue as long as the plant is illuminated, and it is possible to study the effect of light intensity and the concentration of CO_2 on the rate of photosynthesis. A simple way to measure the rate of photosynthesis is to observe the rate at which oxygen bubbles are produce as a result of water splitting. An actively photosynthesizing aquatic plant will produce an abundance of oxygen bubbles.

Procedure:

1. Obtain a healthy green sprig of *Elodea*, and measure back about 10cm from the tip.
2. Wipe a sharp razor blade with alcohol, let it dry, and make a diagonal cut across the stem of the plant as gently as you can. Save the tip.

3. Put the tip into a test tube upside down co that the cut end of the stem is about 3cm from the top of the test tube.
4. Fill the test tube with tap water so that the cut end is completely covered.
5. Obtain a lamp and place it on the bench. Fill a large glass beaker with water and place it in front of the lamp to act as a heat filter. Lay a meter stick along side your set up so that the zero mark is level with the face of the lamp and the meter stick extending out from the lamp.
6. Place the test tube containing the *Elodea* sprig at a distance of 50cm from the lamp in a test tube rack. Wait a few minutes for the plant to adjust to the light level. If there are hardly any bubbles coming from the cut end after 15 minutes, ask your instructor for help.
7. Once there are two or more bubbles per minute, begin counting, and record the number of bubbles produced in a 5 minute interval in table 2a.
8. Repeat the count for another 5 minute interval and calculate the average bubble count per minute for the light intensity at 50 cm.
9. Move the tube to 25cm, allow the plant to adjust. Repeat your measurements and record the results.
10. Now move the plant to 75cm. Allow the plant to adjust and repeat the measurements.

Did you see a different rate of oxygen evolution when the light intensity was increased or decreased?

11. Replace the water in the tube with 0.5% $NaHCO_3$ (sodium bicarbonate, which will function as a carbon source for the *Elodea*).
12. Place the plant back at 25cm and measure the rate of bubble release for two 5 min intervals. Record the results in table 2b.

Table 2. Oxygen Evolution by *Elodea*

A. Tap water

Distance	Bubbles/5min	Average bubbles/min
25cm (1)		
25cm (2)		
50cm (1)		
50cm (2)		
75cm(1)		
75cm (2)		

B. $NaCO_3$

Distance	Bubbles/5 min	Average bubbles/min
25cm (1)		
25cm (2)		

What effect did decreasing the light (increasing distance) have on the rate of photosynthesis?

What effect did increasing CO_2 ($NaHCO_3$) have on the rate of photosynthesis?

Is oxygen produced as a result of carbon fixation or of electron transport? (Hint: compare the rate of bubble production at 25cm from the light in the presence and absence of $NaHCO_3$)

The Uptake of Carbon Dioxide During Photosynthesis

(Note: This procedure will be done as a demonstration by your instructor. You will need to record the results in table 3 and answer the questions relating to this experiment.)

During the second stage of photosynthesis, the plant takes up carbon dioxide (CO_2) and reduces it to carbohydrate. Therefore, the carbon dioxide in the solution surrounding aquatic plants should disappear as photosynthesis takes place. We have already used sodium bicarbonate as a carbon source but in this procedure we will use the carbon dioxide in expired breathe as a carbon dioxide source.

Procedure:

1. Fill two test tubes with phenol red solution. (Phenol red is a pH indicator; it is yellow in an acid and red in a neutral solution.)
2. Using a straw, breathe bubbles into both the test tubes until the phenol red turns from red to yellow. When carbon dioxide dissolves in water, it forms the acid carbonic acid (H_2CO_3).
3. Place a sprig of *Elodea* in one test tube and place both test tubes side by side in a test tube rack in front of a lamp. Protect the *Elodea* from the heat of the lamp with a large beaker filled with water.
4. Check the color of the solutions periodically until you detect a change in color from yellow to red. (The time required for the color to change varies but generally takes from ½ - 1 hour.

Table 3. Carbon dioxide uptake

Test tube	Color before light exposure	Color after light exposure
+ *Elodea*		
- *Elodea*		

Answer the following questions.

1. What does the gradual change to red indicate in terms of the pH of the solution?

2. What does the change in pH indicate about the carbon dioxide in the solution?

3. What happened to the carbon dioxide?

4. Would you expect the pH of the water in a shallow pond with a lot of submerged plants and algae to change over a 24 hour period? If so, how would it change?

5. Why did we include the tube without *Elodea* in the experiment?

6. You may notice small bubbles in the solution with the *Elodea*. What would you expect these bubbles to be?