BIOLOGY 163 LABORATORY

LIGHT REACTIONS OF PHOTOSYNTHESIS

(Revised Fall 2011)

Photosynthesis is the process by which plants convert light energy into chemical energy in the form of carbohydrates. Energy stored in the bonds of these carbohydrates can then be utilized to produce ATP through cellular respiration.

Photosynthesis is comprised of two major reactions, both occurring in the chloroplasts. The *light dependent reactions* occur in association with the thylakoid membranes; these utilize light energy to power an electron transport chain that produces O_2, ATP, and NADPH (Figure 1). The O_2 formed during the light reactions is released into the air, replenishing the supply of molecular oxygen present in the atmosphere. The ATP and NADPH provide chemical energy to power the *light independent reactions* of the Calvin cycle. The Calvin cycle takes place in the stroma of the chloroplasts; it is responsible for converting atmospheric CO_2 into carbohydrates.

Both sets of reactions may be demonstrated using isolated chloroplasts. In this exercise, you will focus on the light dependent reactions by investigating factors that influence the rate of the reactions. These factors may involve varying light conditions (e.g., intensity, duration, or wavelength), or other environmental conditions unrelated to light exposure (e.g., temperature, pH, or the concentration of chloroplasts or substrates).

In order to quantify the light dependent reactions, you will use the dye 2,6-dichlorophenolindophenol (DCPIP) as an artificial electron acceptor in the place of the natural acceptor $NADPH^+$. In its oxidized form, DCPIP is blue and absorbs light at 600nm. However, in its reduced form, it is colorless and does *not* absorb light at this wavelength. Therefore, a *decrease* in absorbance at 600nm (easily quantifiable with a spectrophotometer) is a measure of DCPIP reduction to $DCPIPH_2$. Since $DCPIPH_2$ is the end product of our reaction *in vitro*, it is therefore a reliable measure of how much of the entire process is taking place.

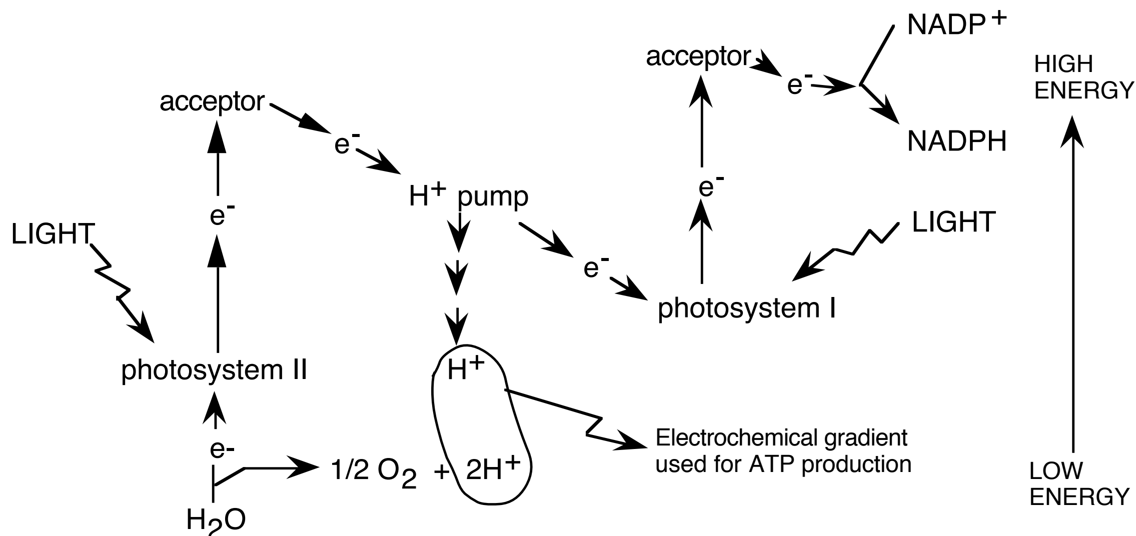

FIGURE 1: Schematic representation of electron flow in the light dependent reactions.

EXPERIMENTAL DESIGN

Standard procedures for isolating chloroplasts and quantifying DCPIP reduction are provided in Appendix A. You are responsible for designing, conducting, and presenting a study to thoroughly investigate at least one factor that may influence the reducing power of isolated chloroplasts. Remember that scientific inquiry is a *process*; as you work through it, carefully consider the following guidelines:

- Start with a clear purpose. Be sure to state your experimental question clearly and develop a hypothesis before you begin. (Appendix B provides a list of equipment and materials available in the lab.)

- Determine differing experimental conditions to which your sample will be exposed. Try to examine your chosen variable across a broad spectrum, while being careful not to manipulate multiple variables at the same time. (Appendix C provides a data table to facilitate this--the first line has been filled out as a suggested baseline from which to design additional trials.)

- Incorporate controls into your design. How do you know that light is required for DCPIP reduction? How do you know that chloroplasts are necessary?

- Design your experimental apparatus carefully. Putting together your physical set-up is part of the challenge. Avoid accidentally introducing extraneous variables. Also be aware of the potential for “light pollution” between groups.

- While conducting your experimental trials, don’t be afraid to adjust your design if your initial results are unexpected or unusable. What will you do, for example, if all your planned trials result in complete DCPIP reduction?

- Don't forget the importance of replication. A single trial for each treatment is not particularly convincing. Consider sharing chloroplast preparations with other groups.

PRESENTATION

At the next laboratory, your group should be prepared to orally present the study you designed and conducted. Your presentation should not exceed five minutes and length, and must include:

- Clear statement of your purpose and hypothesis.

- Summary of **experimental treatments** you applied. *DO NOT summarize standard procedures that everyone completed! (For example, isolating chloroplasts or calibrating the spectrophotometer.)*

- Visual presentation and summary of your results. *All the rules you have learned for analyzing and presenting data still apply!*

- Conclusion(s). Answer your experimental question based on your results. Provide a biologically valid explanation for your observations.

Use of PowerPoint is strongly recommended. Keep in mind that your slides should provide visual aids to augment your talk. *Avoid filling slides with text that you simply read back to your audience!*

Computer video projection will be available for your use.

APPENDIX A: STANDARD PROCEDURES

Isolation of chloroplasts from spinach leaves

The isolation of chloroplasts from spinach leaves should be carried out at 0-4°C and in the dark. Maintain the preparation at this temperature by appropriate use of ice buckets, pre-chilled solutions, and pre-chilled glassware.

1. Weight out 10 grams of spinach leaves with the central ribs removed.
2. Cut the leaves into smaller fragments and transfer to a pre-chilled mortar containing 30 ml of grinding medium (0.4M sucrose, 50 mM Tris Buffer, pH 7.5) and a pinch of sea sand. Grind with a pestle for approximately two minutes, then strain and squeeze (with hands) through two layers of cheesecloth, allowing the filtrate to flow through a funnel into a pre-chilled beaker.
3. Pour the filtrate equally into two pre-chilled centrifuge tubes, and centrifuge the homogenate at approximately 1,600 rpm for 2 minutes.
4. Pour equal amounts of the supernatant (contains chloroplasts, thylakoid membranes, and other cell components) into two clean pre-chilled centrifuge tubes, and centrifuge again at approximately 2,400 rpm for 5 minutes.
5. Pour off and discard the resulting supernatant (contains broken chloroplasts, mitochondria, various membrane fragments, and soluble components).
6. Add 1 ml of grinding medium to each tube containing the pellet (contains whole chloroplasts). Re-suspend the pellet using a clean transfer pipette. Combine the chloroplast preparations into one test tube. *Keep this suspension on ice and in the dark.*

Quantifying DCPIP reduction with the Spec-20 spectrophotometer

NOTE that in this study you are measuring a DECREASE in absorbance, as opposed to an INCREASE in absorbance (as in previous studies). Carefully read and follow all instructions for calibrating and using the spectrophotometer!

NOTE that each tube must be prepared and run individually. DO NOT add the chloroplast suspension until you are ready to begin a treatment!

1. Set the wavelength control on the spectrophotometer to 600 nm.
2. With **nothing** in the sample well, set the absorbance to **infinity** with the **left hand** control knob.
3. Add chloroplasts to an experimental tube, and mix by inverting the tube while covering the opening with a piece of Parafilm and your thumb.
4. **Immediately** place the tube in the sample well of the spectrophotometer and set the absorbance to **1.0** with the **right hand** control knob**.**
5. Remove the tube and expose it to light as per your experimental design
6. **Immediately** re-mix the sample, place the tube in the sample well, and read and record the absorbance.
7. Repeat steps 3-6 for each of your experimental tubes.

APPENDIX B: EQUIPMENT AND MATERIALS AVAILABLE IN THE LAB

In addition to the basic supplies at your table, other equipment available to you in the lab includes:

- rulers/meter sticks
- light meters
- clocks/stopwatches
- light filters of various colors
- tap water/water baths/incubators of various temperatures

Your instructor may be able to assist you in locating other equipment you may wish to use.

TABLE 1: Composition and Incubation Conditions of Experimental Tubes

Tube	Incubation Conditions	DCPIP	H2O	Chloroplast
1	30 cm from lamp, 3 minute exposure	2.0ml	2.8ml	0.1ml (100μl)
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				

Based on a laboratory in Bregman, Allyn.1990. Laboratory Investigations in Cell and Molecular Biology, 3rd. Ed., Wiley, NY. pp. 165-179.