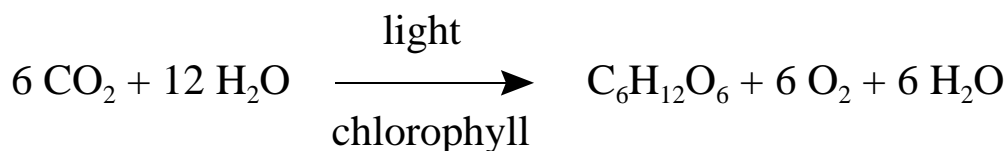


PHOTOSYNTHESIS: The HILL REACTION

Readings: Review pp. 165-176 in your text (POHS, 5th ed.).

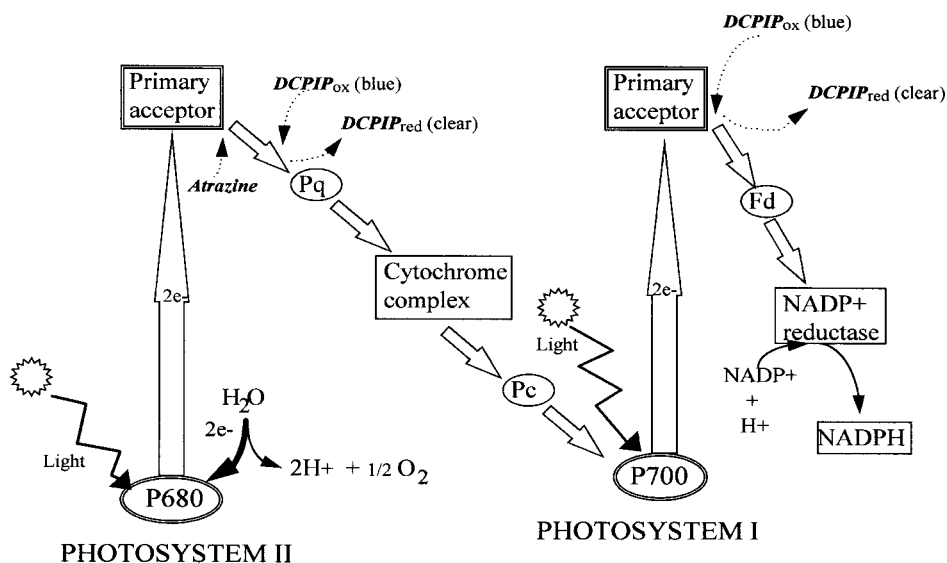
Introduction

The term photosynthesis is used to describe a remarkable and complex series of membrane-associated reactions which result in atmospheric CO₂ being *fixed* or reduced to glucose and other organic compounds. The reactions that comprise photosynthesis can be summarized as:

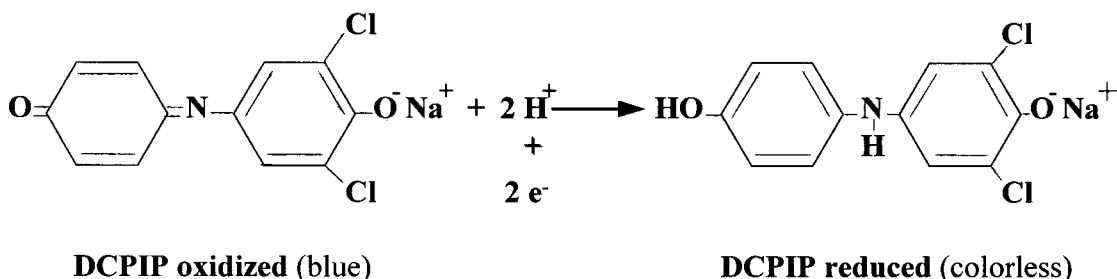


Photosynthesis occurs only in green plants, algae, and a few genera of bacteria. Molecular oxygen is a by-product of photosynthesis; thus, all organisms that require oxygen for life (including *Homo sapiens*) are ultimately dependent on photosynthesis. The energy that drives photosynthetic reactions is light, captured by photosynthetic pigments such as chlorophyll. This energy excites electrons taken from H₂O. Excited electrons are passed through a series of membrane-bound carriers and acceptors, and ultimately reduce NADP⁺ to produce NADPH + H⁺ which is then used to reduce CO₂.

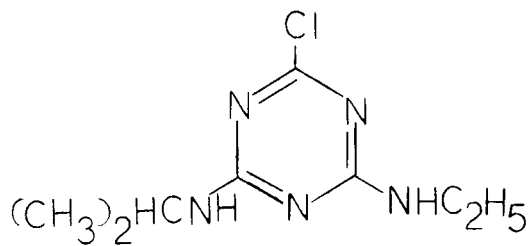
In this exercise, you will measure the rate of photosynthesis by monitoring the flow of electrons as shown schematically below:



DCPIP (2,6-dichlorophenol indophenol) is a dye able to accept electrons from a variety of donors. When DCPIP is oxidized, it is blue. Reduced DCPIP is colorless. The reduction of DCPIP by electrons derived from H₂O coupled with the production of O₂ is known as the Hill Reaction.



By following the rate of conversion of DCPIP from a blue (oxidized) state to a colorless (reduced) state, you will be able to monitor electron flow in the presence of light in spinach chloroplasts. You will also use the herbicide, Atrazine, to disrupt photosynthesis by blocking electron movement from the primary acceptor in PSII to pQ. The structure of atrazine is shown below:



LABORATORY GOALS

Determine and analyze the effects of light intensity, temperature, and herbicide concentration on the rate of photosynthesis via the Hill Reaction.

EXPERIMENTAL PROTOCOL. Work in groups of four.

A. Prepare chloroplasts.

1. Choose 10 average sized spinach leaves and rinse them in cool water if they are dirty. Remove the stems and discard. Put the spinach leaves into a Waring Blendor with 100 ml of ice-cold 0.4 M sucrose in 10 mM Tris (pH 7.8; extraction buffer). Blend at top speed for approximately 30 seconds.

2. Filter the spinach extract through 4 layers of cheesecloth into a beaker. From this point on, keep the chloroplast suspension on ice until you are ready to do the experiment.
3. Place 35-40 ml of chloroplast suspension into a pre-chilled 50 ml centrifuge tube. The tubes will be centrifuged at 6000 rpm at 4 °C for 5 minutes. Pour off the supernatant and resuspend the pelleted chloroplasts in an equal volume (35-40 ml) of ice-cold extraction buffer.
4. Transfer the chloroplast suspension to a pre-chilled Erlenmeyer flask.
5. Examine a drop of chloroplast suspension microscopically to make sure the chloroplasts are intact.

B. Setting up the light source and colorimetric tube racks.

1. Measure points 15, 30, 60, and 120 cm away from the center of the light bulb. Place tube racks at these points and make sure none of the racks shade any of the others.
2. Position the “Spec 20” and timer so that colorimeter tubes can be easily and quickly passed from hand to hand, and Absorbance readings can be made efficiently. Turn on the “Spec 20” and set the wavelength to 640 nm.

C. Preparing the reactions.

1. Put two colorimetric tubes into a rack at room temperature. These tubes will be used to check the accuracy of your pipetman and are not part of the experimental series. Pipet 2 ml of chloroplast suspension into each of these tubes. Pipet 4 ml extraction buffer into each tube. Add 0.1 ml DCPIP to each of these tubes, and mix by inversion. Hold them up to the light. Are they the same color? If not, have your instructor check the pipetman and your technique.
2. Number 8 colorimetric tubes with small pieces of tape at the very top and place the labeled tubes on ice. Make a foil shield and cap for tube 6.
3. Add 2.0 ml chloroplast suspension to all tubes. Swirl your flask to resuspend the chloroplasts each time you pipet them.
4. Place tube 7 in the heat block and warm it at 65 °C for 10 minutes.
5. Add 4.0 ml ice-cold extraction buffer to tubes 1-7. Add 3.9 ml extraction buffer to tube 8.
6. Add 0.1 ml of 20% Atrazine to tube 8.

D. *The Hill Reaction.*

1. Use tube 1 to zero your “Spec 20”. Set tubes 1, 3, 6, 7, 8 in the rack 30 cm from the light source.
2. Set tube 2 in the rack 15 cm from the light source.
3. Set tube 4 in the rack 60 cm from the light source.
4. Set tube 5 in the rack 120 cm from the light source.

Use the chart below to make sure your tubes are set up properly; **DO NOT ADD DCPIP YET:**

Cuvette #	Extraction buffer (ml)	Other additions (ml)	Chloroplast suspension (ml)	Distance from light (cm)	DCPIP (ml) **see below
1	4.0	None	2.0	30	None
2	4.0	None	2.0	15	0.1
3	4.0	None	2.0	30	0.1
4	4.0	None	2.0	60	0.1
5	4.0	None	2.0	120	0.1
6	4.0	None	2.0	Dark (30)	0.1
7	4.0	2.0 warmed chloroplasts	None	30	0.1
8	3.9	0.1 Atrazine	2.0	30	0.1

**** Read before proceeding.** The reactions will be started when you add DCPIP to the tubes. Once the DCPIP has been added, you cannot stop until the entire experiment is completed. *Rehearse the following steps before you add DCPIP to any of your tubes.* Immediately after the dye is added mix thoroughly by inverting it 3 times, then hand it to your partner at the “Spec 20” who will take an Absorbance reading which will be written down by another partner; this will be the zero time data point for that tube. Hand the tube back to the first partner who will replace it in the appropriate rack and hand you another tube. The fourth partner will be the time-keeper. Practice these steps. Keep in mind the room will be dark.

5. Once you have added DCPIP and read one Absorbance for every tube, immediately start over and read the Absorbance on every tube a second time in the same sequence. Repeat until you have at least 6 readings for each tube. Make sure each tube is well mixed before reading Absorbance.

DATA ANALYSIS and WRITE UP

A. *Determine the effect of light intensity on DCPIP reduction in spinach chloroplasts.*

Plot Absorbance (vertical axis) vs. time from zero for each tube using tubes 2-6. Determine the rate of DCPIP reduction (equivalent to the rate of photosynthesis) from these graphs. Use the linear portion of the graph to determine the slope. Plot each of these rates (in Absorbance units/minute) vs. the light intensities which are equal to $1/\text{distance}^2$. Use absolute values of the rates you have determined when you are plotting the second graph. Use your data to answer the following questions:

1. Explain how the rate of photosynthesis is related to light intensity. Include observations from both the highest and the lowest light intensities used.
2. Determine the intensity of light that limits the rate of photosynthesis, and at what point increased intensity no longer increases the photosynthetic rate.
3. Suggest explanations for your observations in question 2 in terms of pigments and photosystem function.

B. *Determine the effects of temperature and Atrazine on the rate of photosynthesis.*

Plot Absorbance (vertical axis) vs. time from zero for tube 7 (altered temperature) and tube 8 (Atrazine present). Determine the rate of photosynthesis in each of these tubes, as above. Compare these rates with the rate you determined for tube 3.

In your write up, comment on:

1. The effects of temperature and Atrazine on photosynthetic rate.
2. The effects of temperature and Atrazine on the structure and function of relevant proteins.

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