Hill Reaction and Photosynthesis

Prior to lab you should understand:

- How differential sedimentation separates chloroplasts from other organelles in the plant cells.
- The movement of electrons during the light reaction of photosynthesis.
- How an alternative electron acceptor like DCIP interacts with the chloroplast.

I. <u>Objective</u>:

- **Isolate chloroplast by differential sedimentation**
- **Measure the light dependent movement of electrons using the Hill reaction**

II. <u>Background</u>:

Photosynthesis is the energy-storing process where light energy is captured and converted into chemical energy in the form of organic molecules such as glucose. The chemical reactions of photosynthesis can be grouped into the <u>light reactions</u>, which harness light energy from the sun, and the <u>Calvin cycle</u>, which use this energy to fix carbon dioxide and synthesize organic compounds (Figure 1).

<u>PHOTOSYNTHESIS:</u>

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

In the light reaction, photons are absorbed at two photosystems (I and II). At each photosystem, the light energy excites electrons at the reaction center. These electrons transfer to the primary electron acceptor and then travel through an electron transport chain. Eventually these electrons reduce $NADP^+$ to form NADPH (Figure 2). (The electrons at photosystem II are replenished from water during photolysis.) The movement of electrons down the electron transport chain powers the pumping of protons into the thylakoid lumen. This proton gradient then powers the production of ATP through chemiosmosis.

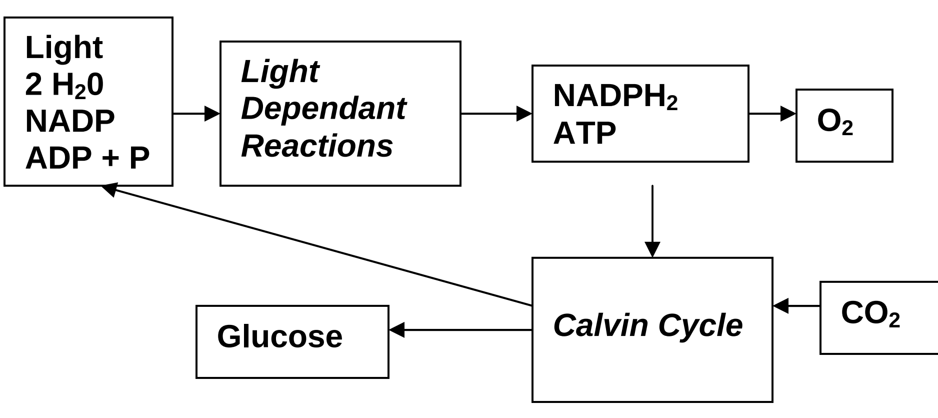

Figure 1: The light dependent reactions produce Reduced NADP and ATP which are required for the fixing of CO_2 and generation of glucose in the Calvin Cycle.

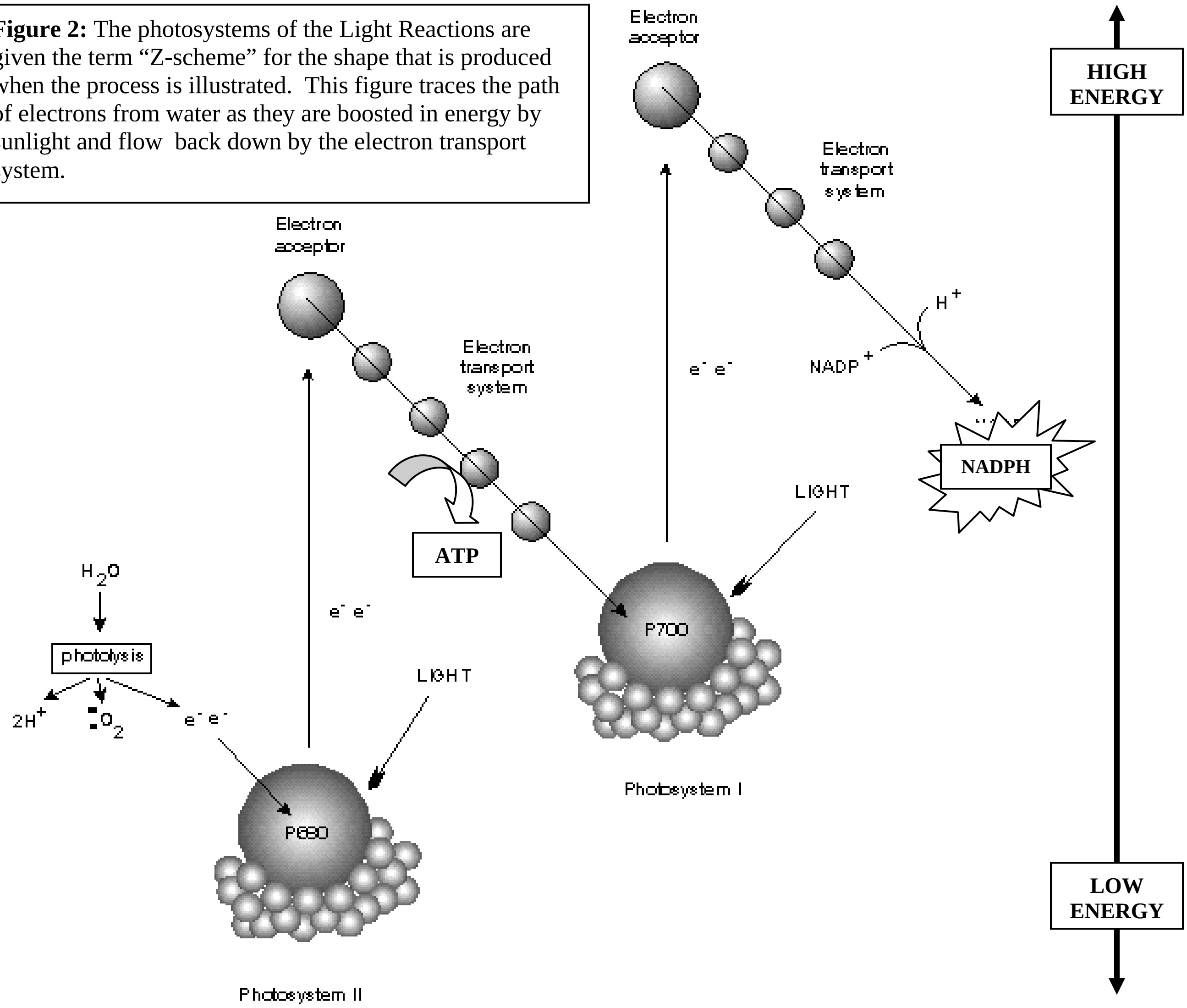

Figure 2: The photosystems of the Light Reactions are given the term "Z-scheme" for the shape that is produced when the process is illustrated. This figure traces the path of electrons from water as they are boosted in energy by sunlight and flow back down by the electron transport system.

The movement of electrons in the figure above can be summarized as below:

H_2O → PS II → electron transport chain→ PS I → electron transport chain → NADP

It can be difficult to directly observe the movement of electrons during photosynthesis. After all, electrons are very small. Some of the first evidence for this electron movement was developed in 1937 by Robert Hill. He studied the movement of electrons by adding an artificial electron acceptor to the chloroplast. An artificial electron acceptor is a molecule that can draw off electrons as they move through the electron transport chain. Some artificial electron acceptors change color as they are reduced by picking up these electrons. For example the artificial electron acceptor 2,6-dichlorophenolindolphenol (DCIP) is blue in its oxidized state, but is colorless when it is reduced. This change in color is an indirect way to monitor the movement of electrons. The movement of electrons in the Hill reaction can be summarized as follows:

$2H_2O$ → PSII→ electron transport chain → DCIP

As the electrons move to DCIP it will change from blue to colorless.

In this experiment, you will isolate *chloroplasts* from the cells of spinach leaves using differential sedimentation. You will then test whether the Hill reaction as summarized above is dependent on light.

III. <u>Materials</u>:

Each Station should have:
Ice Bucket
1 g spinach leaves
Scissors
Sand
Cold Tris-NaCl Buffer
Chilled Small Mortar and Pestle
Spatula
Small Square of Cheese cloth
2ml microfuge tubes
small funnel
microfuge
small waste beaker
spectrophotometer
cuvettes
2 15ml test tubes
150ml Beaker and aluminum foil
Small Graduated Cylinder
DCIP reagent
Microscope light source
Water bottles

IV. <u>Procedure:</u>

A. Isolation of chloroplasts

1. Line small funnel with a square of cheese cloth and place the funnel in a microfuge tube on ice.
2. Cut the spinach leaf into small pieces with the scissors and place in chilled mortar.
3. Add 4 ml of cold Tris-NaCl buffer and a pinch of sand and grind spinach for 2 minutes with pestle.
4. Use the plastic spatula to scrape the ground spinach into the funnel. Wring out the cheese cloth to fill the microfuge tube with spinach extract. (Keep chloroplast cold for remainder of experiment.)
5. Centrifuge the extract for 200g for 2 min in microfuge tube.(At this speed, nuclei and cell wall fragments should sediment into pellet)
6. Pour supernatant into new tube. Store on ice.
7. Spin this supernatant a second time at 1200g for 5 min. (At this speed the chloroplasts and the mitochondria should sediment into the pellet.)
8. Pour off supernatant into waste beaker.
9. Add 2 ml of ice-cold Tris-NaCl buffer to the tube with the pellet, mix by gentle inversion. Store on ice.

B. Hill Reaction

1. Turn on spectrophotometer and adjust wavelength to 600nm.

2. Add some ice to the 150ml beaker and fill to ~100ml mark with water.

3. Cover the top of the beaker with aluminum foil. Using the scissors, pierce the top of the foil so that it will hold up two test tubes.

4. Cover one test tube with aluminum foil. Place both test tubes into foil covered beaker.

5. Add 0.5 ml of chloroplasts to each test tube. Using the graduate cylinder, add 9.5 ml of DCIP reagent. Cover the end of the tube with parafilm and mix by inversion. Place back in ice water filled beaker.

6. Working efficiently remove 1 ml from each tube and place it in a separate cuvette. Use the spectrophotometer to measure the absorbance at 600nm. Record your data in the data collection table. (Zero minutes)

7. Turn on the microscope lamp and aim it at the tubes.

8. Every two minutes remove 1 ml from each tube, place it into a cuvette and measure its absorbance at 600nm. Repeat for 10 minutes. Rinse your cuvettes with water between measurements.

V. Short Report

Name: ___________________________
Lab Section: ______________________

Data Collection Sheet

Absorbance at 600nm

	0 min	2 min	4 min	6 min	8 min	10min
With Light						
Without Light						

1. On a line graph plot the change in absorbance of both treatments (with and without light.)

2. Discuss whether your data support the hypothesis that the movement of electrons in the electron transport chain of the chloroplast is light dependent.

3. Consider how the Hill Reaction might be used to investigate how the wavelength of light influences the rate electrons move in the electron transport chain of the chloroplast.

   A. Design an experiment to compare the effects of blue light and green light on the Hill Reaction. (Assume that you have filters that you can attach to your light source to control the wavelength of light.)

   B. Based on your knowledge of photosynthesis, how would you expect the color of the light to effect the movement of electrons.

   C. Draw a line graph of your expected results.