Photosynthesis: A Simple Demonstration of the Hill Reaction

David S. Ostrovsky
Guy L. Steucek
Millersville State College
Millersville, Pennsylvania 17551

The splitting of water molecules during the light reactions is a crucial step in photosynthesis. The electrons released from the water molecules will ultimately be used by the photosystems of the light reactions to supply ATP and NADPH, key materials for the synthesis of sugar in the dark reactions.

In 1938 Robert Hill of Cambridge University demonstrated that isolated chloroplasts, in the presence of light and a suitable electron acceptor, can split water molecules. We can easily use the classic Hill Reaction to demonstrate: (1) the release of electrons from split water molecules; (2) the need for light as an energy source in this process and hence photosynthesis in general; and (3) the efficiency of different wavelengths of light in carrying out this process.

Materials and Methods

The following materials are needed:

- 500 ml beaker
- blender
- spinach leaves (enough to fill beaker)
- cheesecloth
- "miracloth" (available from Calbiochem-Behring Corp.)
- centrifuge
- four 10 ml test tubes
- foil
- cellophane, green and red

Prepare the following solutions:

1. 0.25M sucrose in tap water
2. .02% 2,6 Dichloroindophenol (the sodium salt) in tap water. This chemical is widely available and inexpensive.

Procedure for Isolating Chloroplasts

Place 200 ml of 0.25M sucrose solution in the beaker and fill it with loosely packed spinach leaves. Pour the contents of the beaker into a blender and blend at low speed for 10 seconds. Filter the solution of homogenized leaves through a filter made of two layers of cheesecloth and one layer of "miracloth." This filtration will remove the plant fibers but allow the chloroplasts to pass through. Centrifuge the chloroplast solution in an ordinary clinical centrifuge at medium speed for three to five minutes. Pour off the supernatant and resuspend the chloroplast pellet in 0.25M of sucrose. Dilute the resuspended chloroplast pellet solution to a final volume of 200 ml.

Demonstration of the Hill Reaction

Place 2 ml of the chloroplast suspension in each of the four test tubes. Cover one tube with foil leaving the top open. Cover one tube with green cellophane and one tube with red cellophane, also leaving the tops open. Allow one tube to remain uncovered. Add 0.2 ml of the .02% 2,6 Dichloroindophenol solution to each tube and mix. (We use disposable plastic syringes to measure out both the chloroplast suspension and the dye.) Expose the four tubes to light. After an exposure of one to two minutes, the solution in the foil-covered tube should be a dark blue-green, the solution in the tube covered with green cellophane should be a light blue-green, and the solutions in the tube covered with red cellophane and the uncovered tube should be very light blue-green to green.

2,6 Dichloroindophenol is a blue dye that accepts electrons given off when water is split. After accepting these electrons, the dye goes from a blue to a colorless state. This experiment, therefore, demonstrates not only the light requirement for the Hill Reaction, but also the particular effectiveness of those wavelengths of light absorbed by chlorophyll.

References

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