Photosynthesis: Hill Reaction

Lab Report 4

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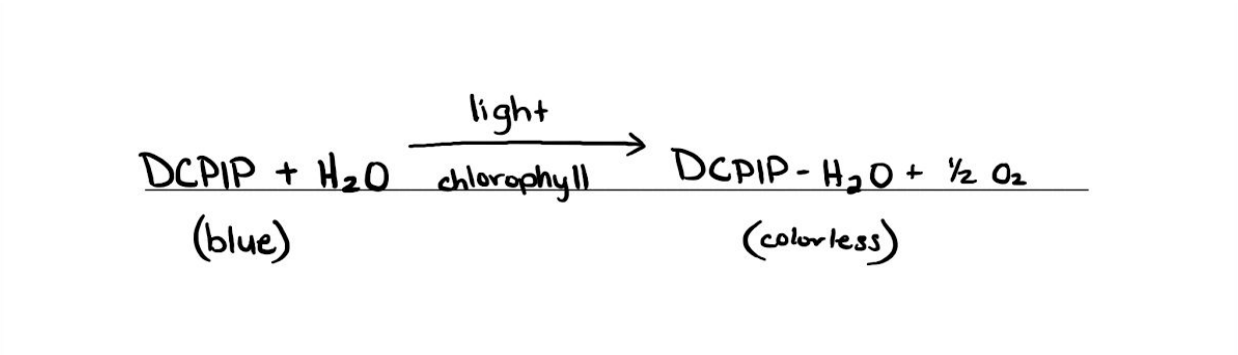
Background:

Robin Hill and his associates made this discovery in the 1930's and it has since been used to further understand the mechanism of photosynthesis. In the lab, chloroplasts are capable of releasing oxygen in the presence of light. When oxygen is released, compounds present in the leaf accept electrons. If 2,6-dichlorophenol-indophenol (DCPIP) is used, however, it can replace electron acceptors and serve as an indication of oxygen release by chloroplasts. DCPIP is blue when oxidized and become colorless when reduced:



Introduction:

In this experiment students will learn how solar energy is converted into chemical energy and the mechanisms involved in this reaction. Students will also learn the fundamentals of the Hill Reaction as well. Plant tissues contain a combination of various chemicals and pigments. These are necessary for the plants to continue living. The pigments students will learn about today will be chlorophyll. This organelle is essential photoreceptor that initiates the light reactions of photosynthesis. It enables the conversion of light energy to electron excitation energy which is transported through various systems to enable phosphorylation of ADP to ATP and reduction of NADP to NADPH. The Hill Reaction explains the mechanism by which light splits a water molecule releasing oxygen, electrons, and hydrogen protons.

Methods:

Chloroplast Isolation:

1. Obtain a chilled bag of spinach and cut 8 grams of leaf tissue into small pieces. Be sure to keep the cut spinach cold to prevent degradation
2. Place cut pieces of spinach into a 150mL beaker with 40 mL of cold .50 M sucrose

3. Blend the spinach/sucrose mixture for 15 seconds at high speed, wait for about 10 seconds and then blend again for 10 seconds
4. Filter the solution through 4 layers of cheesecloth and squeeze the cheesecloth to ensure you get all the solution filtered into a 150mL beaker .
5. Pour the green filtrate into a 30 mL centrifuge tube and place on ice.
6. Centrifuge the filtrate at 200 g for 3minutes to pellet nuclei, cell wall debris and other material not needed in the sample
7. Decenat the supernatant into another 30 mL centrifuge tube and spin at 2000 g for 10 minutes to pellet the chloroplasts.
8. Discard the supernatant and resuspend the chloroplasts in 10 mL of cold .5 M sucrose or other grinding solution
9. After resuspension, centrifuge again at 300g for 3 minutes to pellet additional contaminants
10. Resuspend supernatant and spin at 2000 g for 10 minutes to pellet relatively pure chloroplast
11. Resuspend the pellet in 10mL of cold .10 M phosphate buffer at pH of 6.5. Keep on ice
12. Examine the chloroplast suspension microscopically and record your observation

Treatment for detecting Hill Reaction

1. Prepare solutions in test tubes as shown in table 1.
2. Place test tubes the appropriate distance from the light source
3. Record your observation

Table 1: Description of treatments for studying the Hill Reaction

Tube	Treatment	Chloroplast Suspension	Phosphate Buffer	H_2O	DCPIP
1	DCPIP control	none	1.0 mL	4.0 mL	0.5 mL
2	Darkness (wrap in foil)	2.0 mL	1.0 mL	2.0 mL	0.5 mL
3	Light treatment 1 (12 inches from light)	2.0 mL	1.0 mL	2.0 mL	0.5 mL
4	Light treatment 2 (24 inches from light)	2.0 mL	1.0 mL	2.0 mL	0.5 mL
5	Light treatment 3 (36 inches from light)	2.0 mL	1.0 mL	2.0 mL	0.5 mL
6	Light treatment 4 (48 inches from light)	2.0 mL	1.0 mL	2.0 mL	0.5 mL

Materials:

- Varian Cary WinUV Spectrophotometer
- Tissue homogenizer and vortex
- Centrifuge
- Analytical balance
- 1 1000 uL pipetman
- High intensity light source
- Knife, scissors, or razor blade
- Aluminum foil
- Cheese cloth
- 4 150 mL beakers
- 2 30 mL centrifuge tubes
- 6 10 mL test tubes
- 250 mL 0.50 M sucrose or other grinding solution
- 250 mL 0.10 M phosphate buffer (pH = 6.5)
- 50 mL 0.20 mM DCPIP solution
- 8 grams of spinach leaves, preferably chilled

Results and Discussion:

As seen in Figure 8 of this lab report, our results concluded that we successfully suspended chloroplasts and that the intensity of light affected the amount of light reactions happening in our chloroplast suspensions. The solution in test tube number 2 labeled “Darkness” remained the darkest green/blue because there were little to no light reactions happening in the suspension. The test tube number 3 labeled “Light Treatment 12 inches” received the most intense light and therefore completely turned green because there were lots of light reactions happening. Expectedly the test tubes number 4 and 5 received intermediate amounts of light and turned similar colors that were intermediates of the two extreme test tubes 2 and 3.

Conclusion:

In conclusion, collectively as a group, we learned a great deal about the pigment chlorophyll. Not only is it a vital part if of the plants biology, it’s important for us as consumers of oxygen that plants have chlorophyll. In this lab we learned how plants are able to convert solar power into chemical energy all via inductive resonance transfer. Inductive resonance transfer is the idea that chlorophyll converts solar energy into excitable electron energy that able to fuel the photosynthetic system of plants.

You might ask yourself where does all this endless supply of oxygen come from. Well, by using the Robin Hill reaction, we are capable of explaining the end results in plants supplying us with high quality O_2. in Hill reaction he demonstrates that in an event called photolysis, water is spilt by light to give us H+ and O_2 as a side product.

We also have to mention that Dr. Bidlack single handedly saved our experiment from falling flat on its face when he recognize that Marcus (intentionally) tried to sabotage our experiment by not having the right pH level for our buffer. Buffer should be blue not that brownish -purple.

Article Summary:

“Evolution of the Z-scheme of photosynthesis: a perspective” discusses the evolutionary history of photosynthesis’ conceptual understanding since its birth in 1956. Welcome to the world of human knowledge, photosynthesis! Unknown intermediates labeled with letters, became identified as the famous friends we now know and love, photosystem II and I, as well as the cytochrome team. By playing with the oxygen in the plant, it was shown that the two-light effect took place in the light reaction phase of photosynthesis, called the Hill reaction, rather than in respiration. Soon after, the Z-scheme was discovered by Hill and Bendal; this brought clarity to all the parts and pieces of photosynthesis previously acknowledged! Modern equipment has allowed researchers to now see atomic level crystal structures of BOTH PSII and PSI.

That...is...incredible! The paper is concluded with a reminder of how far knowledge can evolve in a few decades.

Something new I learned is that there is more than one type of chlorophyll a, and they each have different functionality based on the proteins to which they are attached. I gained an appreciation for how many great minds and how much time and devotion goes into studying each little part to everything there is to be studied within photosynthesis. I also learned that there is something called a picosecond! Rationalizing that the probability of picosecond being a taco topping, or perhaps a time reference to a type of Mexican food was unlikely, I looked it up to find that a picosecond is a trillionth of a second; this is equal to what one second would be compared to 31,710 years! It is mind blowing how FAST just one part the teeny little superhero, photosynthesis, is!

Pictures:

Figure 1: In this photo you will see Hannah cutting the spinach into small pieces. We do this until we have measured out 8 grams of spinach. These small pieces are there to allow the grinding of the spinach to help isolate chloroplasts.

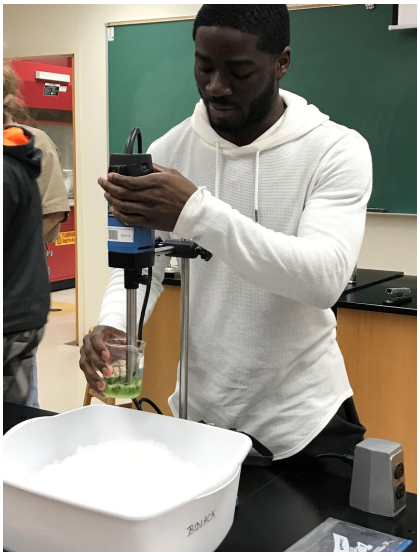


Figure 2: In this picture you will see Fon grinding and separating the spinach and buffer into a solution. He is using the tissue homogenizer to help separate organelles. He is doing this in bursts of 15 seconds for homogenizing and 10 seconds rest in ice bath.

Figure 3: In this picture you can see Fon separating solid from liquid. We only need the liquid when using the centrifuge.

Figure 4: In this picture you can see the FAMOUS Dr. Bidlack explaining the centrifuge and how to use it.

Figure 5: In this picture you can see the HANDSOME Dr. Bidlack WITH HIS NEW HAIRCUT measuring all the supernatant until all 4 groups were within a gram of each other so they are all balanced while in the centrifuge.

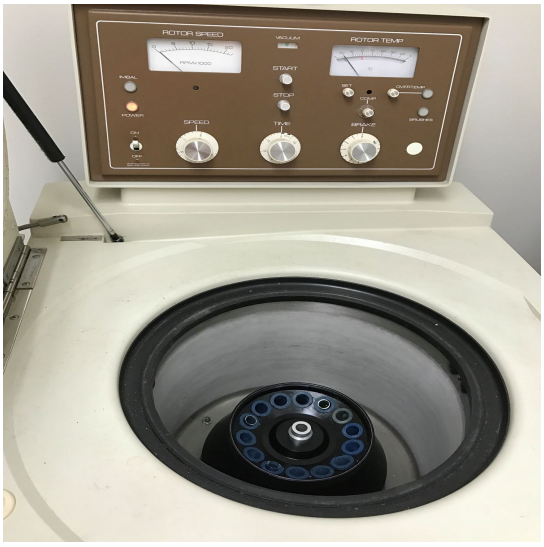


Figure 6: In this picture you can see the inside of the centrifuge.

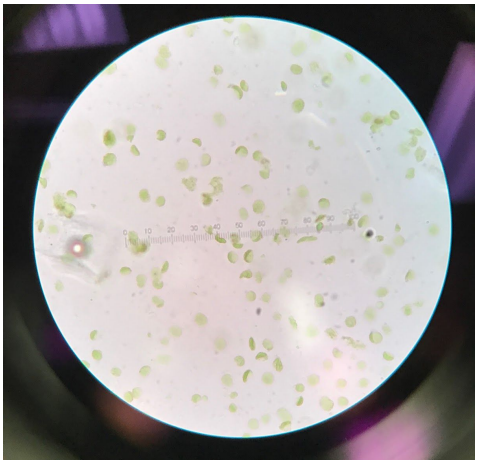


Figure 7: In this picture students can see what the chloroplasts that group 1 isolated looks like.

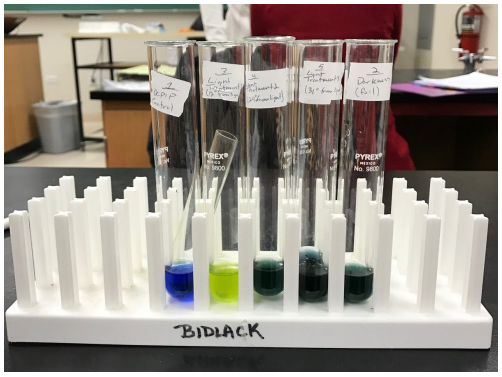


Figure 8: This is what the results of the light reactions look like.

References

Govindjee, Shevela, Dmitriy, Bjorn, Lars Olof. “Evolution of the Z-scheme of photosynthesis: a perspective.” Hisotry and Biography. 2016