Plant pigments and photosynthesis

Analysis

1. The solubility and the intermolecular bonds formed between the solute and the solvent are involved in the separation of pigments as it moves through a filter paper.

2. The Rf values would be different if a different solvent was used because the solvent would have different characteristics which affects the capillary action (because the Rf value is distance pigment migrated (mm) / distance solvent front migrated (mm), the capillary action would have a large impact on the Rf value), attraction of solvent molecules to one another, and each pigment will not be equally soluble to the original solvent.

3. The reaction center of photosynthesis contains chlorophyll a. Other chlorophyll a molecules, chlorophyll b, carotenes and xanthophylls capture light energy and transfer it to the chlorophyll a located in the reaction center. Carotenoids also protect the photosynthesis system from damaging ultraviolet rays.

Part B

Purpose

Condition of Chloroplast vs. Rate of Photosynthesis:

The purpose of this lab is to observe and measure the effect of boiled and unboiled chloroplast on the rate of photosynthesis of a chloroplast suspension made from spinach leaves.

Presence of Light vs. Rate of Photosynthesis:

The purpose of this lab is to observe and measure the effect of the presence of light on the rate of photosynthesis of a chloroplast suspension made from spinach leaves.

Variables

Condition of Chloroplast vs. Rate of Photosynthesis

· Independent Variable: Condition of chloroplast

· Dependent Variable: Rate of Photosynthesis; this will be measured by determining the percent transmittance of each chloroplast suspension.

· Controlled Variables: Amount of DPIP (mL), Temperature (°C), and Amount of Phosphate Buffer (mL)

Presence of Light vs. Rate of Photosynthesis

· Independent Variable: Presence of Light

· Dependent Variable: Rate of Photosynthesis; his will be measured by determining the percent transmittance of each chloroplast suspension.

· Controlled Variables: Amount of DPIP (mL), Temperature (°C), and Amount of Phosphate Buffer (mL)

Hypothesis

If the condition of the chloroplast in the suspension was unboiled, and there was light present, then there will be photosynthesis occurring in the cuvette. Photosynthesis the process by which the chloroplast within the leaf cells of green plants use sunlight to synthesize foods from carbon dioxide and water.

In order for photosynthesis to occur, the chloroplast needs to be functioning, and light needs to be present to excite electrons for NADP to bind with. Because unboiled chloroplast and light are both present in cuvette 3, photosynthesis occurred rapidly. But if boiled chloroplast and light were present, photosynthesis would not occur. Boiling the chloroplast would rupture and destroy the chloroplast, therefore ceasing the process of photosynthesis. If unboiled chloroplast was in the cuvette, but light was absent, photosynthesis would not occur. Light is important in the process of photosynthesis. Light striking photosystem II is the cause of the excited electrons that bind to the NADP, but in this experiment, the compound, DPIP, will be used as a substitute to determine percent transmittance. Therefore, without properly functioning chloroplast and light present, photosynthesis in the cuvette will not occur.

Procedure

First set up an incubation area that includes a light and a heat sink. Use a 100 mL beaker or flask filled with water to be placed between the light source and the cuvettes. Then, because you need to keep the chloroplast suspension cool, fill a bucket three quarters full with ice. Prepare the cuvettes by wiping all sides clean. Remember to handle them by touching the sides with the ridges. All solutions should be free of bubbles. Place cuvette position with the clear side facing the light source in the colorimeter. Label the caps of the cuvettes with numbers 1, 2, 3, 4, and 5. Then make a foil container and a cap for cuvette 2 and make sure it can be easily removed so you it can be placed into the colorimeter for percent transmittance readings. This will keep the light out of cuvette 2 because it is

a control. Remember to replace the foil between readings. Label the provided pipettes “ B” for boiled chloroplast and “ U” for unboiled chloroplast. Obtain the boiled and unboiled chloroplasts. Fill the bulb of each pipette to about one-third its total size. Invert the pipettes and place them in your ice bath. Be sure to keep both chloroplasts on ice at all times. When you are dispensing the chloroplasts into the cuvette, gently shake the pipette to resuspend the chloroplasts. To cuvette 1 add 1 mL of phosphate buffer, 2. 5 mL of distilled water, and 3 drops of unboiled chloroplasts; cuvette 2 add 1 mL of phosphate buffer, 1. 5 mL of distilled water, 1 mL of DPIP, and 3 drops of unboiled chloroplasts; cuvette 3 add 1 mL of phosphate buffer, 1. 5 mL of distilled water, 1 mL of DPIP, and 3 drops of unboiled chloroplasts; cuvette 4 add 1 mL of phosphate buffer, 1. 5 mL of distilled water, 1 mL of DPIP, and 3 drops of boiled chloroplasts; cuvette 5 add 1 mL of phosphate buffer, 1. 5 mL + 3 drops of distilled water, and 1 mL of DPIP. Link the computer to the colorimeter, and prepare Logger Pro. Add three drops of unboiled chloroplasts to the water and phosphate buffer as indicated in the table. Cap the cuvette, place it into the colorimeter and use it to calibrate the colorimeter. Finally, add three drops of unboiled chloroplasts to cuvette 2, immediately start your stopwatch, and record the time and transmittance in the data table. Return the cuvette to its foil container and place it behind the heat sink. Add three drops of unboiled chloroplasts to cuvette 3, immediately record the transmittance and time. Add three drops of boiled chloroplast to cuvette 4, and record the time and transmittance. Check and record the transmittance of cuvette 5, which is the control. Record time and transmittance. Remember to check the

transmittance of each cuvette at five-minute intervals from when the chloroplasts were added up to 15 minutes.

Conclusion

Functioning chloroplasts and the availability of light are two important factors for the rate of photosynthesis. Light is needed to excite the electrons from the water molecule. Then the excited electron binds with NADP, or in this case, DPIP. When the DPIP accepts the electron, the compound begins to degrade. A greater concentration of DPIP is easily seen inside a cuvette because of the dark blue dye associated with the DPIP. As DPIP degrades, the color of the chloroplast solution begins to get lighter. A solution without DPIP would be clear. Percent transmittance would be greater if more light passes through the solution in the colorimeter. If a cuvette had functioning chloroplasts and was exposed to light, the DPIP would be breaking down at a faster rate in the controlled time, which would mean there are less DPIP compounds in the cuvette, resulting in a lighter colored suspension; the amount of DPIP is directly related to the shade of the solution. The percent transmittance is determined from the shade of the suspension; the lighter the solution, the smaller the amount of DPIP remaining. Therefore, the decrease of DPIP in the given time would indicate that photosynthesis is indeed occurring in the cuvette.

Our hypothesis is supported by the data because we hypothesized that fully functioning chloroplast with the presence of light would result in the occurrence of photosynthesis. In this experiment, we tested and observed the effect of the condition of chloroplast and the presence of light on the rate of photosynthesis. In cuvette 2, we added unboiled chloroplast and did not

allow light to penetrate the cuvette. So we used aluminum foil to block out the light from the light source behind the heat sink. At 0 minutes, the light transmittance was at 17. 5%. But 10 minutes later the percent transmittance was at 19. 9%. The data indicates that where was a small amount of DPIP reduced. When we removed the cuvette's foil shell to measure transmittance, light was introduced to the suspension. This indicates that the very little photosynthesis that occurred was the result of the light that excited electrons for DPIP to accept during the seconds between the removing and the replacing of the aluminum cuvette castings. In cuvette 3, there was unboiled chloroplast and light introduced to the mixture of distilled water, phosphate buffer, and DPIP. As you can see from the provided data table and graph, at 0 minutes, the transmittance was 18. 09%. But 10 minutes later, the percent transmittance rose to 96. 26%. Light struck the functioning chloroplast, excited electrons, and caused DPIP to break down as it accepted the electrons. This is evidence of photosynthesis occurring at a very fast rate inside cuvette 3. But 15 minutes later, the transmittance of cuvette 3 was to 96. 83%. This shows that the rate of photosynthesis slows down, but this was the cause of the scarce amount of DPIP. The rate of photosynthesis was so fast that it used up almost all of the available DPIP in 10-15 minutes. In cuvette 4, there was boiled chloroplast in the suspension, and light was present. At the initial time, 0 minutes, percent transmittance was at 24. 32%. 10 minutes later, the solution had a 28. 47% transmittance. There is a slight increase in transmittance, but exposure to light can cause DPIP to break down. If photosynthesis had occurred, it would have occurred at a much faster rate. The data would be similar to cuvette 3's data, but because of the slight increase of transmittance, photosynthesis did not

occur. This proves out hypothesis that for photosynthesis to occur, light and functional chloroplast must be present. When the chloroplast was boiled, this destroyed the chloroplast. Therefore, without functioning chloroplast, photosynthesis will not occur. Cuvette 5 was the control; chloroplast was not added to the solution. At 0 minutes cuvette 5 had a transmittance of 25. 22%. 10 minutes later cuvette 5's transmittance was 22. 60%. This decrease is due to experimental error.

Without light, functional chloroplast would be no use. In order for photosynthesis to occur, light must be present to excite the electrons. Because of the absence of light, DPIP will not degrade due to accepting excited electrons, for instance, cuvette 2's data. Without fully functional chloroplast, there will not be any electrons in photosystem II to excite, and the DPIP will not degrade because there aren't any excited electrons to bond to, for example, cuvette 4. This data proves that for photosynthesis to occur, fully functional chloroplast and light must be present.

Analysis

The DPIP will be used to substitute the NADP electron acceptor. When light strikes the chloroplasts, the electrons are boosted to a higher energy level, which will reduce the DPIP, turning it from blue to colorless.

The DPIP replaces the NADP molecule.

Electrons used to reduce DPIP are obtained when a water molecule is split.

The colorimeter in this experiment measures the amount of light received at the sensor across from the light source in the colorimeter. If the chloroplast

suspension, which is placed in between the light sensor and the light source, is darker in color, then we can imply that the DPIP in the solution has not yet broken down, which confirms that photosynthesis is not occurring.

Darkness inhibits the reduction of DPIP; because the light waves are not exciting the electrons in the chloroplast, the DPIP is not breaking down. Therefore, the DPIP remains in great numbers in the chloroplast suspension. The more DPIP, the darker the solution.

Boiling chloroplasts does not affect the reduction of DPIP. When the chloroplast is boiled, it is nonfunctional. Because the chloroplast is nonfunctional, the photosystem II is unable to receive the light and excite the electron. Because the electrons are not excited, the DPIP is not reduced.

Chloroplasts that were incubated in the light are able to harness the energy from the light to excite electrons that is then accepted by DPIP. This causes a reduction of DPIP, which makes the originally blue chloroplast suspension to lighten in color. The lighter the solution, the greater the percent transmittance, because more light can pass through the solution in the colorimeter. Chloroplasts that were kept in the dark do not receive light and cannot excite electrons. The DPIP compounds are not broken down, which results in a darker blue suspension. When this is placed into the colorimeter to measure percent transmittance, less light will be able to travel through the suspension due to the dark-blue color of the suspension.

Cuvette 1:
was used to calibrate the colorimeter. This cuvette did not contain DPIP, which would resemble 100% transmittance because of the lack of dye in the solution.

Cuvette 2:
contained unboiled chloroplast and was kept in the dark. This was used to confirm that both functional chloroplast and light are needed for photosynthesis to occur because the combination of unboiled chloroplast and the absence of light did not produce a significance increase in percent transmittance.

Cuvette 3:
contained functional chloroplast and was placed in the light. The significant increase of percent transmittance proves that both functional chloroplast and significant light are necessary for photosynthesis to occur.

Cuvette 4:
contained boiled chloroplast and was placed in the light. The function of this cuvette was to prove that functional chloroplast and light are needed for photosynthesis to occur, but the small increase in percent transmittance may have been the result due to the light breaking down DPIP.

Cuvette 5:
did not contain any chloroplast or light. This cuvette was used as a control. It would be used as the “ baseline” when analyzing data because it can reveal any effects that is experienced by the cuvette that did not result from the presence of chloroplast or light.