Non-Cyclic Photophosphorylation

Summary: My group and I observed the effects of light on the rate of photosynthesis. We did this by using the synthetic dye chlorophenol indophenol (DCPIP) as a way to determine the rate photosynthesis. DCPIP serves as an artificial electron acceptor during plant energy-making reactions and changes color when it is reduced, which allows measurements to be made with spectrophotometer. Our results showed that exposing chloroplasts directly to a strong source of light has an immediate and significant effect on photosynthesis, increasing the rate considerably. Using DCPIP as a means to measure this rate has been found to be effective in other related experiments *(Cornic & Miginiac-Maslow, 1985)*. Also, additional activity due to increased light is attributable entirely to the functions of the chloroplasts, which are typically responsible for transporting the electrons that reduce NADP+ *(Al-Khatib & Paulsen, 1989)*.

Methods & Results: My group and I filled two centrifuge tubes 2/3 to the top with homogenized spinach in sucrose-phosphate buffer, which serves as a hyper osmotic buffer and causes the chloroplasts to shrivel. The two tubes were then inserted into a centrifuge where they were processed at full speed for six minutes. After pouring off the remaining liquid, a dark green pellet of concentrated plant chloroplast remained in the bottom of each tube and both were then transferred to an ice bath. We added 1mL of sucrose-phosphate buffer into each and the pellets of chloroplasts were then resuspended using a pipette. The new contents of these two centrifuge tubes were combined.

Next we obtained two Spectronic 20 tubes and pipetted 4.9mL of sodium-phosphate buffer to both while adding 0.1mL of the enriched chloroplast solution into just one. We zeroed our Spectrophotometer at 600nm with the sodium-phosphate buffer tube and took an absorption reading for the second tube containing enriched chloroplast solution, which provided a value of 0.220. We used the equation (Absorption Reading) x (33.3mL) = (Necessary Final Volume) to determine the amount of sucrose-phosphate buffer that needed to be added to the enriched chloroplast suspension for it to be 0.1mg/mL of chloroplast (this was 5.426mL of sucrose-phosphate buffer: 7.326mL – 1.9mL). The new solution was mixed thoroughly by covering the top of the test tube with parafilm and inverting it one-half dozen times.

Three new Spectrophotometer 20 test tubes were filled with the following contents (with the addition and mixing of the adjusted chloroplast solution occurring immediately before taking readings):

Test Tube A (Blank)	**Test Tube B (Dark Control)**	**Test Tube C (Light Reaction)**
3.5mL Sodium Phosphate buffer	3.0mL DCPIP	3.0mL DCPIP
1.0mL water	3.5mL Sodium Phosphate buffer	3.5mL Sodium Phosphate buffer
0.5mL adjusted chloroplast solution	0.5mL water	1.0mL water
	0.5mL adjusted chloroplast solution	0.5mL adjusted chloroplast solution

We used test tube A to zero the spectrophotometer at 600nm. After adding and mixing the adjusted chloroplast solution to test tube B, we recorded a measurement and then immediately wrapped the tube in tin foil to keep it from being exposed to light in between readings. The adjusted chloroplast solution was then added and mixed to test tube C and measurements were recorded every 15 seconds until the absorbance reached zero (this indicated the successful reduction of DCPIP, as it loses its blue color when photosynthesis occurs). In between readings, the light reaction tube was placed 15cm away from the beam of a microscope illuminating light in an attempt to increase the rate photosynthesis. After our final reading for test tube C we returned test tube B to the spectrophotometer and recorded its final value. We then consolidated all of this data into the graph found in diagram 1.

The experiment was expected to last for nearly ten minutes, but our DCPIP was reduced entirely in less than 35 seconds (even after adding six times the originally specified amount of 0.5mL in an attempt to slow down the experiment). My group and I expect that the DCPIP solution was prepared incorrectly and had a very low concentration level, which caused it to be reduced very quickly. Our limited number of readings makes it difficult to draw many conclusions, but it is clear that the presence of direct sunlight has a significant effect on increasing the rate of photosynthesis—even when it has been exposed only for a short amount of time.

Comparing the average decreasing slopes of test tubes B and C shows that the light reaction had a value of -0.0059 while the dark reaction had a value of -0.0015. While a more thorough experimental process is required, we can take this to mean that photosynthesis occurs at a much higher rate when

chloroplasts are exposed directly to light (in this particular case, nearly four times as quickly). The process of the reduction of DCPIP during photosynthesis is shown in diagram 2.

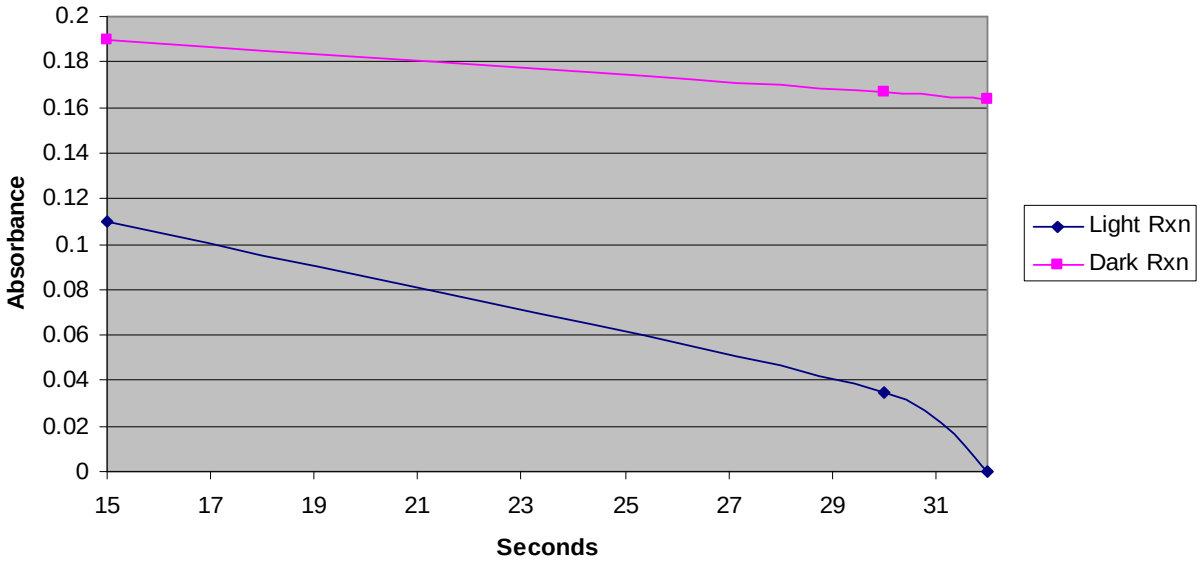

Diagram 1

Diagram 2

References:
Al-Khatib, K. & Paulsen, G., 1989. Enhancement of Thermal Injury to Photosynthesis in Wheat Plants and Thylakoids by High Light Intensity, *Plant Physiology*, 1989(90), pp. 1041-1048.
Cornic, G. & Miginiac-Maslow, M., 1985. Photoinhibition of Photosynthesis in Broken Chloroplasts as a Function of Electron Transfer Rates during Light Treatment, *Plant Physiology*, 1985(78), pp. 724-729.