

Isolated chloroplasts bad example



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Light Reactions in Isolated Chloroplasts Racquel Currie University of Minnesota Minneapolis, Minnesota Hypothesis If isolated chloroplasts are boiled, the enzymes that occur in photosynthesis are disrupted and the DCIP will not reduce. Chloroplasts are a type of plastid found within the plant they are the basis of photosynthesis. Without photosynthesis there would be no life on earth. Photosynthesis takes the energy from sunlight and converts it into a chemical energy that can be used by the plant.

Isolating chloroplast is a way to separate plastids in order to study the details of the single cell. Isolating the chloroplast helps better understand the functions and helps determine if the isolated chloroplasts are adequate to complete photosynthesis. Biological activity will be determined by using a dye, dichlorophenolindophenol (DCIP), as a final electron acceptor. Using spectrophotometry, the reduction of DCIP will be measured to follow the dye from an oxidized (DCIP) to a reduced state (DCIPH₂) as it accepts electrons from photosystem I (1). Boiling affects the Materials and Methods

In order to isolate the chloroplasts, the process had to have been completed rapidly in subdued light. When the solutions were not being used they were stored on ice. Ten spinach leaves were rinsed and deveined. They were then placed over ice and chopped with a razor. After, the spinach was placed between eight layers of cheesecloth to squeeze all the liquid derived from the spinach. The liquid was then diluted by adding 20mL of homogenizing buffer (HB). The solution was added to a pre-cooled conical centrifuge tube, and was then centrifuged at 1000 x g for one minute.

After, the solution was transferred to another pre-cooled centrifuge tube and was centrifuged at 3000 x g for one minute. Centrifugation resulted in the

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deposition of a green pellet on the wall of the centrifuge tube near the surface of the liquid (2). This green pellet, together with the green liquid, was separated from the more dense material that had been deposited at the bottom of the tube (2). The supernatant was decanted, and the pellet was re-suspended in 10 mL of HB. The final centrifuged process occurred at 3000 x g for one minute.

After the final centrifuge the supernatant was poured off and discarded. The isolated chloroplasts were re-suspended and mixed with 10ML of suspension solution (SS). After this process to isolate the chloroplast it was stored on ice and was stable for two to four hours. After the isolation of chloroplasts the question was asked if boiled chloroplast would reduce DCIP reaction mixture, and would this affect the activity of electrons from photosystem 1. 0.1 mL of chloroplast was measured with a pipette into a tube and then suspended in boiling water for one minute.

A spectrometer was used and set to 0 (set at 600nm). There was also a control used, which was chloroplast that went through the same methods as above, although it did not get boiled (heated). The control was measured by the spectrometer and the treated (boiled) sample was measured immediately after the boiling process. The control and treatment was then exposed to bright light for 15 second intervals. After each interval the color density was measured by the spectrometer. This process continued until there was no change in the color density. Results

Figure 1 Figure 2 Discussion DCIP was used as a marker to indicate if the photosynthesis was still taking place in the isolated chloroplasts after boiling. The DCIP replaced the NAPD enzyme. The hypothesis, if isolated chloroplasts

are boiled, the enzymes that occur in photosynthesis are disrupted and the DCIP will not reduce was shown to be true based on the results of figure one and two. Where as the control's color density did decrease with the exposed light intervals. Literature Cited 1. K. Sami Nichols Light reactions in isolated chloroplasts Lab 2. C.

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