Rubisco in spinach leaves

One of the many proteins found in Spinach leaves is ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco). It is an enzyme that adds carbon dioxide to ribulose 1, 5-bisphosphate during sugar production in green plants. Rubisco is the most abundant protein found on earth as well as a soluble protein (Ishimaru, et. al, 2001). The main purpose of this experiment is to isolate rubisco from spinach leaves. Three different techniques were used to isolate rubisco, first being ammonium sulfate precipitation, second ion exchange chromatography and third being SDS-Page to help isolate rubisco. The amount of rubisco in spinach is found out after SDS-page experiment is completed. Isolating rubisco is highly important in order to figure out whether spinach leaves have a high fraction or a low fraction of rubisco. Rubisco will be highly presented in the spinach leaves due to the fact it is an abundant protein in green plants.

Ammonium Sulfate Precipitation uses solubility to help separate rubisco from other proteins. In this procedure, three hundred grams of spinach was de-ribbed and blended with 200 mL of Buffer 1 (0. 01 M KPOâ,„ buffer, pH 7. 5, 0. 3 mM EDTA, 30 g/L polyvinylpolypyrrolidone) and it was blended for 1 min on medium speed. The spinach solution was filtered through a Miracloth. Ammonium sulfate was added to the filtrate to reach 37% saturation (210 g/L). The solution was centrifuged at 9, 000 x g for 15 min. Three milliliters of the resulting supernatant 1 (S1) was saved. Ammonium sulfate was added to the remaining supernatant to reach 50% saturation (85 g/L). Three point one hundred and forty-five grams of ammonium sulfate was added. The supernatant was stirred with the ammonium sulfate for 15 min. The pellet was resuspended in 4 mL of distilled water and dialyzed against distilled

water overnight. This was labeled pellet 1 (P1). The supernatant was centrifuged for 15 min at 9, 000 x g. Three milliliters of supernatant was poured into a conical tube and labeled supernatant 2 (S2). Both the pellet were resuspended in 4 mL of distilled water and dialyzed against distilled water overnight.

Ion Exchange Chromatography that uses charges to contribute in the isolation of rubisco. In order to perform ion exchange chromatography the use of DEAE Cellulose fast flow column. The columns were equilibrated by adding 30 mL of Buffer A (10 mM Tris, pH 8. 0, 3 mM EDTA). The dialyzed samples were centrifuged on a tabletop centrifuge for 3 min at 1, 000 rpm and one milliliter of P1 and P2 samples was transferred to an Eppendorf tube and labeled. P1 was diluted 1: 50 by adding 5, 000 uL of Buffer A and 100 uL of P1. Three milliliters of diluted P1 was added to one column and 3 mL of undiluted P2 was added to the other column. The elute were saved. Ten milliliters of low salt buffer (Buffer A + 50mM NaCl) was added to both columns. The elute was collected in 2 mL fractions in cuvettes and the highest one was saved for spectrum analysis. The OD at 280 nm was measured of the low salt flow through of P1 and P2. Ten milliliters of medium salt buffer (Buffer A + 200mM NaCl) was added to both columns. The flow through was collected in 1-2 mL fractions in cuvettes. The spectrophotometer was blanked with medium salt buffer. The OD of the medium salt flow through for P1 and P2 was measured. Ten milliliters of high salt buffer (Buffer A + 500 mM) was added to the columns. The high salt flow through was collected in cuvettes in fractions of 1-2 mL. The spectrophotometer was blanked with high salt buffer and the OD of the high

salt flow through for P1 and P2 was measured. The OD value of the P1 and P2 flow through was measured as well. The highest OD value for the low, medium, and high salt was recorded for both P1 and P2. The flow through with the highest OD value was saved for low, medium, and high salts for P1 and P2. The columns were washed with 10 mL of resin cleaning buffer (1 M NaOH, 3 M NaCl). The resin-cleaning buffer flowed through and before the columns were closed, Buffer A was added.

SDS-Page uses size to isolate the rubisco from spinach, the amount of rubisco in spinach is found out after SDS-Page experiment is concluded. The SDS-Page experiment required sixty microliters of each of the samples (filtrate, P1, P1 fractions, P2, P2 fractions). Thirty microliters of 3X sample buffer (bromophenol blue, glycerol, dithiothreitol, SDS) was added to each sample. All of the 9 samples plus the protein standards were heated for 4 min at 95° C. Twenty microliters of each protein samples were loaded onto the 10% acrylamide (agarose gel). Ten microliters of the MW standard was loaded. The gel was electrophoresised at 180 V for 50 min. The gel was stained in Staining/Fixing solution (Methanol, Acidic Acid, Coomassie Blue R-250) for about 30 min. The gel was destained in Destaining Solution (Methanol, Acetic Acid) and dried overnight and photographed.

Results:

P2H S F P1 P1LS P1MS P1HS P2 P2LS P2MS

Distance (cm) 75

56

45

37

Figure 1: SDS-Page

– 20 ml samples were loaded (left to right) and electrophoresis at 180V for 50 minutes. The tops of the image states, which sample were in each lane. S stands for Standard. F stands for filtrate. LS, MS, and HS stand for low salt, medium salt, and high salt (the distances migrated and molecular weights for the marker were used to make the standard curve. The standard curve was used to find the molecular weights for the proteins in the 9 samples).

The figure towards the right was obtained from the protein isolation experiment that was compared to the figure to the right. The literature compared to the Rubisco graph in many aspects. The first peak in figure right reached about the same amount of absorbance as in the literature. Even though the Experimental figure shows two peaks doesn't necessarily mean that the figures are not related; the wavelengths match up to where it shifts from one peak to another (around 250nm).

Protein concentrations in spinach leaves

Sample

Protein Concentration (ug/mL)

P1 Low Salt

7. 85

P1 Medium Salt

1. 55

P1 High Salt

5. 70

P2 Low Salt

30. 81 (high due to diluting)

P2 Medium Salt

3. 50

P2 High Salt

4. 41

Table 1: Protein concentrations in spinach leaves (OD= ƒ¥cd)

– This table shows the protein concentrations in each sample. The protein concentration was found by multiplying the OD value of the sample at 280 nm by the extinction coefficient of rubisco. The extinction coefficient of rubisco is between 14. 1 and 18. 2 ug/mL. The average of the two was taken, which was 16. 15 ug/mL, and that was used to do the calculations to find protein calculations.

Discussion: The purpose of this experiment was to be able to isolate rubisco from spinach leaves. Ammonium sulfate precipitation, ion exchange chromatography, and SDS-Page were the three different techniques used in

this lab. The molecular weight of rubisco is 55 kDa and 14 kDa. Rubisco was suppose to be found in P2 with nothing added and P2 medium salt. The molecular weight of the rubisco likely found in P2 with nothing added was 52 kDa. The extinction coefficient of rubisco is between 14. 1 ug/mL and 18. 2 ug/mL (Hudson, 1994). The average of the two numbers, which is 16. 15 ug/mL, was used to calculate the protein concentration in each sample. We multiplied the extinction coefficient of rubisco by the highest OD value at 280 nm for each sample to find the protein concentration. Pellet two with nothing added did not have a protein concentration because the OD value at 280 nm was a negative number, which is, calculated the same as zero. We found the highest protein concentration to be in P2 high salt. Errors made during this experiment may perhaps have lead to not getting accurate results. These results are important because they tell us how much rubisco is anticipated in spinach leaves.