**An Investigation of Enzyme Purification**

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**Introduction**

This report explores the process of enzyme purification. Enzyme purification can be done using a number of processes. There is no single process or string of processes that can be used to purify every enzyme, every enzyme requires processes calibrated to most effectively purify them. Enzyme purification is necessary to study various enzymes like insulin and human growth hormone (Krane, 2019).

In this experiment we attempted to purify phosphate-monoester phosphohydrolase through the use of MnCl2 precipitation, (NH4)2SO4 precipitation and centrifugation (Krane, 2019). The hypothesis is after every step, even if total protein goes down, the concentration should increase as a greater number of contaminating molecules are removed after every step than protein.

**Methods**

We labeled 5 different microcentrifuge tubes 0, S1, S2, S3, S5 respectively then added 990μl of water into each of them. We then placed all of them into a small bucket of ice. We then poured the supernatant (S1) from the wheat germ that was already centrifuged for us into a 15ml tube to measure the amount of supernatant present to record in table 6.1. We then transferred 10μl of S1 into the microcentrifuge tube labeled S1 and placed it back into the ice. We then discarded the 15ml tube with the pellet in it. We poured the remaining S1 into a beaker that was in the bucket of ice. We slowly added 20μl of MnCl2 for every ml of S1 in the beaker to the beaker. We then transferred the contents of the beaker back into the 15ml tube, washed the beaker and returned the beaker to ice. The 15ml tube was then given to the graduate teaching assistant (GTA) to centrifuge at 6000xg for 5 minutes. We then transferred the new supernatant (S2) into a clean 15ml tube, recorded its value into table 6.1 and washed the tube with the pellet to reuse it. We then transferred 10μl of S2 into the microcentrifuge tube labeled S2 and returned it to the ice. We transferred the remaining S2 into the beaker, slowly added 0.5ml of (NH4)SO2 for every 1ml of S2 and cleaned the 15ml tube. We then transferred the contents of the beaker into a cleaned 15ml tube to be centrifuged at 6000xg for 5 minutes by the GTA. We then transferred the new supernatant (S3) into a cleaned 15ml tube, recorded its value into table 6.1 and washed the tube with the pellet to reuse it. We then transferred 10μl of S3 into the microcentrifuge tube labeled S3 and returned it to the ice. We transferred the remaining S3 into the beaker, slowly added 0.8ml of (NH4)SO2 for every 1ml of S3 and cleaned the 15ml tube. We then transferred the contents of the beaker into a flask to place it into the 60˚C water for 2 minutes. After the 2 minutes, we placed the flask into ice and transferred its contents into a cleaned 15ml tube for the GTA to centrifuge at 6000xg for 5 minutes. We then transferred the new supernatant (S4) into a cleaned 15ml tube and discarded the S4 to wash the tube. We then resuspended the pellet with a third of the volume of S3 of cold distilled water and handed the 15ml tube with the resuspended pellet to the GTA to centrifuge at 6000xg for 5 minutes. We then transferred the new supernatant (S5) into a cleaned 15ml tube, recorded its value into table 6.1 and discarded the tube with the pellet. We then transferred 10μl of S5 into the microcentrifuge tube labeled S5 and returned it to the ice. We then obtained 5 cuvettes and labeled them 0, S1, S2, S3, S5 respectively. We then transferred 100μl of solution from the microfuges to their corresponding cuvettes. We then quickly transferred 1000μl of the Bradford reagent into each cuvette and allowed them to sit at room temperature for 5 minutes. We then wiped down the cuvette labeled 0 with a Kimwipe and placed it into the spectrophotometer at a wavelength of 595nm to blank the spectrophotometer. We then measured the absorbance of every other cuvette after wiping them down with a Kimwipe and recorded their absorbance into table 6.1.

**Results**

Figure 6.1. The figure shows how the equation used to calculate concentration from absorbance was obtained using data given to us by the GTA.

Table 6.1. Change in protein concentration and total protein with their calculations included.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Supernatant** | **Volume** | **Bradford absorbance** | **Protein concentration** | **Equations used for protein concentration calculation** | **Total protein** | **Equations used for total protein calculation** |
| 0 | 0 | 0 | -0.0903 | =0.5301\*B2-0.0903 | 0 | =C2\*A2 |
| S1 | 3.5 | 0.569 | 0.2113269 | =0.5301\*B3-0.0903 | 0.7396 | =C3\*A3 |
| S2 | 2 | 1.124 | 0.5055324 | =0.5301\*B4-0.0904 | 1.0111 | =C4\*A4 |
| S3 | 3.25 | 0.692 | 0.2765292 | =0.5301\*B5-0.0904 | 0.8987 | =C5\*A5 |
| S5 | 2 | 0.51 | 0.180051 | =0.5301\*B6-0.0904 | 0.3601 | =C6\*A6 |

**Conclusion**

The results show that some steps did increase the protein concentration while some decreased the protein concentration and that the total protein content is largely unrelated to the protein concentration. Our results had the total protein increase from the original for the first two supernatants and end with 48.7% of the initial amount of protein. The results do not support the hypothesis as protein concentration fluctuated throughout the steps. Literature says that precipitation is an effective way of concentrating enzymes, however, in our experiment concentration did not consistently go up with each precipitation step (Chaplin, 2014). Literature also says that enzyme purification can be achieved with centrifugation, but in our experiment, centrifugation greatly reduced our protein concentration (Tymoczko, 2002). At the end of the experiment, we ended up with a protein concentration that was only 85.2% as concentrated compared to the initial concentration. MnCl2 precipitation was the most effective step during our experiment, but that is likely because it is data acquired from another group. Excluding MnCl2 precipitation, 0.5ml of (NH4)2SO4 per ml of supernatant was the most effective step. However, simply repeating these steps would not increase the purity of because the step would have already removed most of the impurities it could.

In this experiment we were limited by our ability to separate the supernatant and the pellet properly, in many cases the pellet was not completely solid and may have transferred some of the pellet when we were attempting to transfer the supernatant. We had also forgotten to take a sample of S2 so we had to acquire the data from a different group who may have had results that greatly differed from our results. An improvement that could have been made to the experiment would be to increase the time spent in the centrifuge in order to better compact the pellets to minimize any pellet mixing with the supernatant when we transferred the supernatant. Under the assumption that the enzyme can be purified, it is reasonable to assume that a blood test for prostate cancer can be created by purifying a sample of blood and testing the enzyme activity with and without tartrate ions as tartrate ions inhibit the activity of only the prostate gland enzyme (Krane, 2019).

**References**

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