HOW DO YOU LIKE YOUR EGGS? PURIFICATION AND CHARACTERIZATION OF LYSOZYME

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Introduction

The goal of this research was to modify a method to extract and purify lysozyme from hen egg whites. In order to know if we had extracted the enzyme, and to test the activity of the extraction, we performed an assay measuring enzyme activity with purified lysozyme to compare to assays ran with our extraction throughout the purification process.

Background Research

Lysozyme targets bacterial cell walls which are imperative for cell stability and survival in the host. Cell walls are composed of peptidoglycan monomers made up of the N-acetylglucosamine (NAG)-N-acetylmuramic acid (NAM) disaccharide. Polymers of peptidoglycan are formed by β-1,4 glycosidic linkages between NAG and NAM. Lysozyme kills bacteria by targeting and hydrolyzing the β-1,4 glycosidic linkages between NAG and NAM, which increases cell wall permeability and causes the cell to burst from osmotic pressure. Figure 1 shows the peptidoglycan being broken into fragments through hydrolysis.

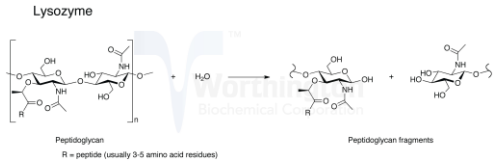
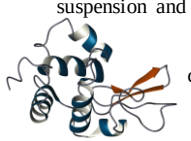


Figure 1. Enzymatic reaction of lysozyme

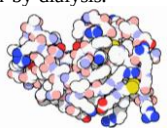
Research has also shown there is a second mechanism in which positively charged lysozymes insert themselves into the negatively charged peptidoglycan of cell walls, causing pores and again killing the bacteria through osmotic pressure (Ragland et al. 2017).

Hypothesis

We hypothesized that we would be able to extract lysozyme from the egg whites of hen eggs. We also hypothesized that we would be able to successfully purify the enzyme with a bentonite clay suspension and pyridine wash followed by dialysis.

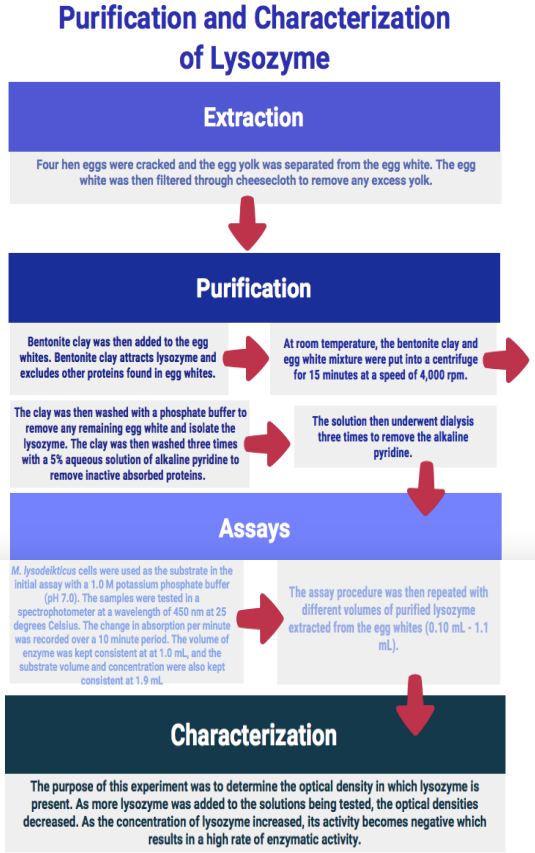


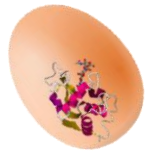
Mature lysozyme of chickens is composed of 128 amino acids.

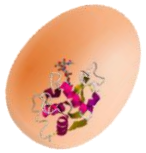


The active site of lysozyme consists of a deep crevice, which divides the protein into two domains linked by an alpha helix. One domain (residues 40 to 85) consists almost entirely of beta-sheet structure, while the second domain (residues 89-99) is more helical (Strynadka and James 1991).

Procedure







Results

Through a slope analysis in excel we were able to determine that as the purification process went on activity became less negative leading to a slower rate of the reaction (Figure 1).

The optical densities of lysozyme decreased as more lysozyme was added because the *Micrococcus lysodeikticus* cells are being broken down by the presence of lysozyme. As concentration of lysozyme increases the activity becomes more negative resulting in a high rate of enzymatic activity (Figure 2).

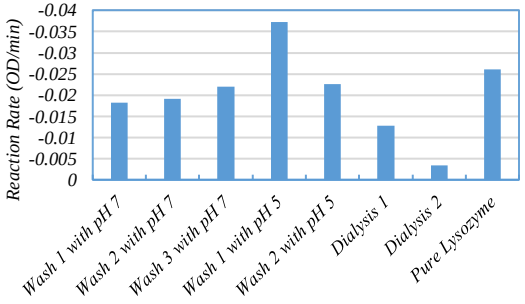


Figure 1. How Activity is Effected through Purification Process
As the purification process of lysozyme progresses the reaction rate increased with the pH 7 decreased with pH 5 washes and decreased through dialysis.

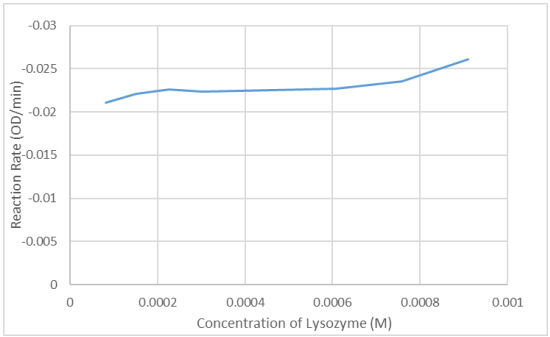


Figure 2. Comparison of Optical Density and Lysozyme Concentration. As the concentration of pure lysozyme increases the rate of the reaction increases as well.

Discussion

With the time we had we were not able to run a test to determine the purity of the lysozyme that we extracted. However, we could see from the assay with our different washes throughout the purification process that we successfully extracted lysozyme because *Micrococcus lysodeikticus* cells were being broken down. We can also see that we lost some activity throughout the process, as the enzymatic activity decreased. However, we are unsure exactly how much lysozyme was present in the final purification steps when comparing it to the given purifies lysozyme. The pure lysozyme from the assay was an original concentration of 9.09091×10^{-6}. This is important because we were able to see at which points lysozyme activity was optimal. At wash 5, before dialysis, we believe we were at optimal activity. Our results also showed that the pure lysozyme successfully broke down the *Micrococcus lysodeikticus* cells as it was supposed to, to act as a standard for comparison.

Study Limitations

- Time was limited
- Data was given to us by our professor because we could not be present in the lab to get our results.

Future Improvements

In the future it would be ideal to have more trial and error. We essentially ran out of time, and we were not able to complete all the experiments like we had hoped to. We only performed this experiment once, so there was not a lot of time for adaptation. It would have been helpful to repeat the experiment a second or third time with adjustments to better our results and smooth out any glitches. If we were unable to see results after a few more trial and errors of our experiment, we also would have wanted to try another type of purification such as one from Roy et al. (2003), which dealt with cation exchanger.

References

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