Testing Chelating Agents to Find the Cofactor of Catecholase

Lab Report

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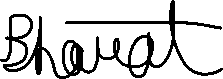
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**I pledge that no unauthorized assistance has been given or received in the completion of this work. Experiments described were performed by me and/or my lab group and this write-up is entirely my own creative work.**

**Signature:**



1. Introduction

Enzymes are proteins that act as catalysts to biological reactions. They increase the rate of reactions in organisms by lowering the activation energy required for a particular reaction to occur. Without enzymes, reactions in cells would not go at a speed necessary to maintain life. When the reaction is complete, the enzyme remains unchanged, ready to catalyze another reaction. Each enzyme has a particular shape which allows it to bind to particular substrates. The substrates in question are the molecules that are acted upon by the enzyme, and upon going through a reaction, they result in molecules known as the products.

The reaction that was studied involves the enzyme catecholase. Catecholase is an important part of plants’ responses to injury. There is a small amount of catechol that lies beneath the surface of several plants; when the inside of a plant is exposed to the air, oxygen causes this catechol to be oxidized. This produces a molecule of water as well as the compound benzoquinone, which acts as an antiseptic. Catecholase catalyzes this reaction. Without it, there would not be enough benzoquinone produced to prevent pathogens from entering the damaged tissue. An example of this reaction can be seen when apples are cut open and their insides are exposed to the air. The browning of the apple, said to occur when it “oxidizes”, represents the formation of benzoquinone.

Some enzymes require an additional substance to facilitate their activity. This substance is called a cofactor. In this case, catecholase cannot function without the presence of a particular cofactor. In this experiment, I tested whether the required cofactor was copper or a combination of magnesium and calcium. This was done by manipulating whether the enzyme had access to these ions by using chelating agents, which are substances that bind to the ions and inhibit them from reaching the enzyme. The three chelating agents tested were ethylenediamine tetraacetic acid (EDTA), phenyl thiourea (PTU), and citric acid. EDTA binds to calcium ions (Ca2+) and magnesium ions (Mg2+), while PTU and citric acid both bind to copper ions (Cu2+). As they bind, the ions become unavailable to the enzyme. We hypothesized that the necessary cofactor for catecholase was copper, as an apple slice is mostly free from browning if a lemon is squeezed on it, and citric acid binds to copper. If a solution containing catecholase was exposed to citric acid or PTU, then less, if any, benzoquinone would be produced, and the color of the solution would change very little, if at all. The independent variable in this experiment was the cofactor available to the solution (by use of the different chelating agents), and the dependent variable was the formation of benzoquinone, measured by the change in absorbance of green light.

1. Materials and Methods

Firstly, we received a catecholase enzyme solution that was created using white potatoes. The potatoes were chilled and had the peels removed, and then they were cut up into small pieces to activate the enzyme. Then, 500 milliliters of chilled, distilled water were added to a chilled blender along with the potatoes. The reason for the chilling of the blender, the water, and the potatoes was to slow the reaction when the solution was being created. Moreover, the water was distilled to remove any impurities. The blending was done in three ten-second bursts that allowed the enzyme to escape the starch matrix of the potatoes. It was done in bursts to prevent the solution from receiving enough heat for the reaction to speed up and to release more enzymes as they are released from the starch matrix. The enzyme solution was then strained through a cheesecloth in order to filter out any impurities, and then it was funneled into a beaker, poured into vials, sealed, and kept on ice to further prevent any premature reactions. When the solution was poured into the vials, it overflowed the vials to ensure that there was no oxygen within the vials that would cause benzoquinone production to begin.

Next, we warmed up a spectrophotometer for fifteen minutes, and then set the wavelength of light to be measured at 540 nm. For this reaction, we observed that exposed surfaces of potatoes would develop a brownish-orange tint, caused by an orange wavelength that is reflected due to benzoquinone. Therefore, the colors absorbed would be green, purple, and blue. we decided to choose to observe a wavelength of light corresponding to green, which was most absorbed by benzoquinone molecules. The wavelength of light corresponding to green was 540 nm. Catechol was relatively colorless, and therefore did not influence the reading of the spectrophotometer. We used a spectrophotometer for this experiment because we would be unable to see absorbed light with our eyes. If we set the spectrophotometer to observe a wavelength corresponding to orange, nothing would happen, as orange would be reflected by the solution. Hence, a more intense orange color of the solution would indicate a greater amount of reaction. A more intense orange color would indicate more absorbance of 540 nm wavelength, so a greater change in absorbance would also indicate a greater amount of reaction.

We proceeded to take test tubes and number them from 1-5. In the first 4 tubes, we pipetted 1 mL of the enzyme solution and pipetted 2 mL of EDTA, PTU, citric acid, and dH2O into tubes 1, 2, 3, and 4 respectively. Tube 4 received dH2O in order to serve as a control, as there would be no chelating agents in the tube and therefore copper, magnesium, and calcium ions would all be able to bind with catecholase and allow the reaction to proceed as usual. We put 5 mL of dH2O into tube 5. The enzyme solution was mixed before the chelating agents were pipetted in. Then, we sealed the tubes with Parafilm so that the solution would not exit the tubes, and inverted and shook them for two minutes at room temperature to mix them. After the two minutes were up, we added 2 mL of catechol to tubes 1-4, and we covered the tubes as we inverted and incubated them. Next, we calibrated the spectrophotometer using tube 5, and then measured tubes 1-4, noting down the absorbance of each at the initial reading. Then, we placed the tubes back into a test tube rack and waited for 10 minutes. We inverted the tubes to mix them and wiped them with a Kimwipe to ensure that there would be no impurities or fingerprints obscuring the reading of the spectrophotometer. We calibrated the spectrophotometer again with tube 5, and then measured the absorbance of tubes 1-4 and recorded it. Then, we subtracted the initial absorbance from the 10-minute absorbance to ascertain the change in absorbance of each tube.

1. Results

Figure 1 show a greater change in absorbance in tubes 1 and 4, which contained EDTA and dH2O (control tube) respectively. Tube 1 had an initial absorbance reading of 0.447 and a final absorbance reading 0.462, demonstrating a change in absorbance of 0.015. Tube 2, which contained PTU, had an initial absorbance reading of 0.307 and a final absorbance reading of 0.310, demonstrating a change in absorbance of 0.003. Tube 3, which contained citric acid, had an initial absorbance reading of 0.472 and a final absorbance reading of 0.475, again demonstrating a change in absorbance of 0.003. Tube 4 had an initial absorbance reading of 0.443, and had a final absorbance reading of 0.461, demonstrating a change in absorbance of 0.018. Hence, tubes 2 and 3 showed the smallest change in absorbance. This can also be observed in Figures 2 and 3, which graphically show the differences in the change in absorbance of each tube.

Figure 4 shows the color change observed in each tube after the 10 minutes had passed. Tube 1 had an original color of orange when absorbance was measured, and had a final color of pink. Tube 2 had an original milky-white color, and had the same milky-white color during the final reading. Tube 3 similarly had an initial milky-white color and preserved this color during the final reading. Lastly, tube 4, the control tube, began with a yellow-orange color and had a more pronounced orange color during the final reading.

1. Discussion

Based on the results, our hypothesis is supported. A greater change in absorbance would characterize a greater rate of formation of benzoquinone, meaning that catecholase would be functioning as usual. Conversely, a lower change in absorbance would characterize a lower rate of formation of benzoquinone, showing inhibition of catecholase. The tubes containing PTU and citric acid demonstrated the lowest change in absorbance, meaning that less benzoquinone was produced. Both PTU and citric acid bind to copper ions; therefore, when catecholase was unable to bind to copper, less benzoquinone was produced. Furthermore, the tube containing EDTA as the chelating agent had an almost identical change in absorbance as the control tube, showing that the absence of calcium and magnesium ions did not prove to be significantly influential in the production of benzoquinone. Hence, copper was the correct cofactor of catecholase, as determined by our data.

Potential errors and/or inconsistencies in our data could have stemmed from allowing the solutions to sit for too long before performing the initial reading. Another possible error could’ve been ineffective wiping of the tubes with Kimwipes prior to the reading, which would obstruct the spectrophotometer from correctly measuring absorbance. Lastly, and perhaps most crucially, cross-contamination of the pipettes and/or test tubes could’ve greatly influenced the results of this experiment.

The bigger picture of this experiment is that any chelating agent that binds to copper can be used on fruits and vegetables to prevent browning, or what is commonly known as “oxidation”. Such an effect usually causes consumers to shy away from the affected fruits and vegetables, so using chelating agents to enhance the appearance of the affected products could allow vendors to sell more product. As a whole, this experiment demonstrates that certain substances can be used to almost completely (if not completely) prevent a reaction from taking place.

1. Appendix

Figure 1

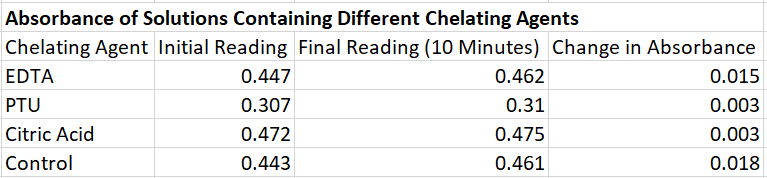
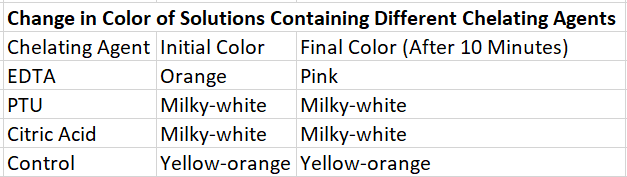


Figure 2

Figure 3

Figure 4



1. Literature Cited

Stegenga, Barbara. *Laboratory Exercises for BIOL 101, Fall 2021-Spring 2022.* Macmillan Learning, 2022. Accessed 26 March 2022.