Original Research Article

Chelating Agents Effects on Cofactors of Enzymes

Abstract

This study aimed to investigate the effect of chelating agents on catecholase enzyme activity and identify the role of cofactors in the catechol to benzoquinone reaction. Catecholase is an essential enzyme in plants' defense mechanisms against injury and pathogens. We hypothesized that the enzyme activity would be negatively impacted by the presence of citric acid and phenyl thiourea (PTU), which bind to copper. To test our hypothesis, we conducted an experiment using four tubes, each treated with different chelating agents or serving as a control. Enzyme activity was assessed by measuring absorbance changes and the resulting color of the reaction products. Our results demonstrated a decrease in catecholase activity in the presence of PTU and citric acid, supporting our hypothesis and suggesting copper as a crucial cofactor in the catecholase enzyme reaction. Furthermore, the lower pH induced by citric acid appeared to have a negative impact on enzyme activity, aligning with previous research indicating that catecholase functions optimally at a pH of 8. These findings contribute to our understanding of the role of cofactors in catecholase enzyme function and the factors that affect its activity, providing valuable insights for future research and potential applications in agriculture and plant protection.

Keywords Catecholase, Chelating agents, Cofactors, Enzyme activity, Plant defense mechanisms

I. Introduction

Enzymes are proteins that play a crucial role in regulating the cell processes of converting one substance to another through chemical reactions. These reactions are essential for maintaining the balance, regulation, and overall functionality of life in organisms. Enzymes act as catalysts inside our cells, accelerating the rate of reactions by reducing the activation energy without altering the chemical structure, making them reusable for subsequent reactions (Alberts et al., 2014). This feature is critical for maintaining homeostasis in living organisms, as slow reaction speeds could result in imbalances and dysfunctions.

An enzyme-based reaction consists of the enzyme and the substrate(s). Enzymes possess specific structures that directly fit the shape of particular molecules, known as substrates. These substrates are the molecules that undergo reactions, either being broken down or combined to create new molecules called products (Berg et al., 2012). The specificity of enzymes for their substrates is crucial for ensuring that reactions occur only when necessary and at the appropriate rate.

Enzymes sometimes require cofactors, which are inorganic substances that help substrates fit the shape in the active site, the location where substrates meet. Cofactors can be either metal ions or small organic molecules known as coenzymes. Examples of metal ion cofactors include copper, magnesium, and iron, while examples of coenzymes include NAD+ and FAD (Berg et al., 2012).

In our study, we investigated the enzyme catecholase, which plays a vital role in the plant's response to injury, such as bruising or cuts. When injured, vesicles of catecholase are released, and the substrates catechol and oxygen, from the outside, come together in a reaction. The enzyme facilitates the reaction of catechol to benzoquinone, which acts as a protector against pathogens, sealing off injuries from the outside and preventing infection (Mayer & Harel, 1979). The reaction can be represented as follows:

Catechol + ½ O2 catecholase → benzoquinone + H2O

We used chelating agents to determine the cofactor of the catechol-benzoquinone reaction. Chelating agents are compounds that bind to metallic ions, inhibiting enzyme activity when they bind to the necessary cofactor. In this experiment, we employed ethylenediamine tetraacetic acid (EDTA) to bind to calcium and magnesium ions, while using citric acid and phenyl thiourea (PTU) to bind to copper (Yildirim et al., 2013).

EDTA is a material commonly used to preserve foods, as it prevents vital enzymes of bacteria from functioning. Citric acid is an organic compound found in citrus fruits that lowers the pH of solutions, and PTU is an organic substance with a bitter taste, used in genetic testing.

We hypothesized a loss in catecholase enzyme activity due to PTU and citric acid, given that catecholase functions most effectively at a pH level of 8 and citric acid lowers the pH of solutions (Olukunle et al., 2021). To test our hypothesis, we used a spectrometer to monitor

enzyme activity and color changes, which served as a dependent variable. In contrast, we varied the chelating agents as independent variables to determine the cofactors of catecholase.

By using this experimental approach, we aimed to provide a comprehensive understanding of the catecholase enzyme, its cofactors, and the factors affecting its activity. This information can be beneficial for a better understanding of plant defense mechanisms and can potentially lead to the development of novel strategies to enhance plant protection against pathogens.

II. Materials and Methods

Initially, the spectrometer was set on for 15 minutes at the wavelength setting of 540 nm. The spectrometer was set to warm up for 5 minutes to ensure accurate measurement. Because colors observed by the eye are all wavelengths reflected by an object, the spectrometer was set to read 540 nm, since this wavelength is the reading of green light, where benzoquinone molecules are maximally absorbed. Green wavelengths are absorbed and not reflected; this rate was analyzed. To analyze the hypothesis, a catecholase enzyme solution was created with white potatoes. The potatoes were chilled to retain the potato's structure and reduce enzyme activity, since enzymes are most active in warmer conditions. Peeling the potato, to remove unnecessary structures that do not have enzyme activity, we sliced the potato for easier blending, reducing the amount of heat needed to blend the solution, and placed the potato into a chilled blender with 500 milliliters of water to dilute the solution. The solution is hypotonic, helping the cells burst, releasing the enzymes for analysis. Blending in 3 short 10 second bursts, to prevent the potato from heating, separating

the enzymes from the starch of the potato. Keeping the enzymes cold is essential to retaining and reducing enzyme activity until the experiment, given the exposure the potato has to oxygen after peeling. Using a three-layer cheesecloth to filter out any chunks of potato, a funnel was placed to direct the flow, where vials were stored in a cooler.

The spectrophotometer had been heated up for accurate measurement, and given the enzyme converts catechol into a product, the concentration of benzoquinone slowly increased, which turned our solution orange. Because the green light is absorbed by benzoquinone, the spectrophotometer is able to measure the absorption rate, where green light absorbed should have increased as catecholase catalyzed. The spectrophotometer is used to measure an accurate representation of absorption rates, since it could not see this with the naked eye. The measurement of 540 nm was chosen for absorbency of green wavelengths rather than orange, because with an orange absorption rate there would be little activity due to a lack of absorbance. We calibrated the spectrophotometer with a blank test tube containing a liquid solution to set our minimum value for absorbency, 0. The maximum absorbency rate possible to observe is 2. The absorbency rate initially is set to 0 to ensure detection of the changes in absorbance. The relationship between color intensity and the amount of the reaction is proportional to another. The rate of absorption is calculated by subtracting the rate of absorbance at 10 minutes to the absorbency at 0 minutes. In this equation the total amount of benzoquinone produced is observed to evaluate enzyme activity. It was calculated to determine the total changes in rates of absorption to determine which cofactor helped catalyze the reaction.

The test tubes were set up, labeled 1 through 4, while tube 5, the calibration tube, contained water only. Using a pipette to ensure accuracy of the solutions without cross contaminating substances, each component of the solution was assembled. 1mL enzyme,

2mL EDTA and 2mL of catechol was put in Tube 1, which tested for cofactors calcium and magnesium. In Tube 2, 1mL of enzyme, 2mL of PTU and 2mL of catechol, which tested for copper. Tube 3 had 1mL of enzyme, 2mL citric acid and 2mL catechol, used to again test for copper. Tube 4 had 1mL enzyme, 2mL of distilled water, and 2mL catechol, which would act as the control. Tube five was the calibration tube, which was 5 mL of distilled water. Each tube had 5 mL of solution for accurate comparison. Distilled water is used to prevent unwanted ions from entering the experiment. Before testing each tube, the spectrophotometer was calibrated with Tube 5, 5 mL of distilled water, and inverted with parafilm each tube, and tested 1-4. The tubes were inverted and parafilm was used for each tube to ensure contents did not spill, and settling was alleviated, while Kim wipes removed potential fingerprints, solution, or any substance that could affect the rate of absorption. The control in this experiment was Tube 4 which will allow the enzyme catecholase to turn catechol and benzoquinone.

III. Results

The absorbance values were initially measured for Tubes 1, 2, 3, and 4, with readings of 0.838, 1.013, 0.410, and 0.646, respectively (table 1). Following a ten-minute resting period, during which enzyme activity occurred in the tubes that were not affected by the chelating agents, the absorbance of each tube was measured again. Notably, Tubes 1 and 4 showed significant increases in absorbance, with final readings at the ten-minute mark being 1.047, 0.396, 0.421, and 0.770 for Tubes 1, 2, 3, and 4, respectively. Tube 1 exhibited an increase of 19.96%, while Tube 4, the control, increased by 16.10% (Figure 1). In contrast, Tube 2 displayed a substantial decrease of 155.80%, and Tube 3 exhibited a smaller increment of increase, at 2.61% (table 1).

To better understand the impact of the chelating agents on enzyme activity, we calculated the change in absorbance for each of the tubes and recorded any visible coloration changes. Tube 1 had an absorbance change of 0.209, displaying a brown-orange color. Tube 2 had an absorbance change of -0.617, with no visible change in color, remaining clear. Tube 3 had a minimal absorbance change of 0.0124, also presenting no change in color and remaining clear (table 2).

Upon analyzing the data, it became evident that Tube 2 experienced the most significant change in absorbance, showing a substantial negative shift, whereas Tube 3 exhibited the smallest change in absorbance, altering by only 0.0124. Tubes 1 and 4 demonstrated positive changes in absorbance, with both values trending upwards and differing by less than 5%.

These results suggest that the presence of chelating agents had a substantial impact on the enzyme activity, as evidenced by the significant decrease in absorbance for Tube 2 and the minimal increase for Tube 3. The changes in absorbance observed in Tubes 1 and 4, which trended upwards and were within 5% of one another, further highlight the influence of the chelating agents on catecholase enzyme activity. The differences in coloration among the tubes also provided a visual representation of the effects of chelating agents on the enzyme reaction, with Tube 1 displaying a brown-orange color indicative of benzoquinone production, while Tubes 2 and 3 remained clear, suggesting minimal or no enzyme activity.

Our findings demonstrate that the chelating agents used in this experiment, specifically PTU and citric acid, had a significant impact on the catecholase enzyme's activity, as evidenced by the changes in absorbance and coloration. This supports our hypothesis that the enzyme activity would be negatively affected by the presence of chelating agents, providing further insights into the cofactors involved in the catecholase enzyme's function and the factors that affect its activity.

IV. Discussion

Based on the data collected, our hypothesis that catecholase enzyme activity would be negatively affected by PTU and citric acid was supported. Our findings suggest that copper may serve as a crucial cofactor in the catecholase enzyme reaction. The control, Tube 4, provided a baseline for the normal rate of enzyme activity without any changes in variables. Tube 1 showed a similar increase within the 5% range, indicating that EDTA binding of magnesium and calcium had no significant impact on enzyme activity. In contrast, Tubes 2 and 3, which were treated with copper-binding chelating agents, showed considerably reduced enzyme activity compared to their counterparts (Olukunle et al., 2021).

Our results demonstrated a decrease in catecholase activity in the presence of PTU and citric acid, consistent with our hypothesis. This finding highlights the importance of copper as a cofactor for the catecholase enzyme, as its activity was inhibited when copper-binding chelating agents were present. Furthermore, the lower pH induced by citric acid appeared to

have a negative impact on enzyme activity, aligning with previous research indicating that catecholase functions optimally at a pH of 8 (Olukunle et al., 2021).

These findings contribute to our understanding of the cofactors involved in the catecholase enzyme's function and the factors that affect its activity. Our study offers valuable insights into the role of catecholase in plant defense mechanisms and underscores the significance of cofactors and optimal pH conditions for the efficient functioning of this enzyme.

Further research could explore the effects of other chelating agents or metal ions on catecholase activity to determine if additional cofactors play a role in the enzyme's function. Understanding the precise mechanisms of action for these cofactors and how they contribute to enzyme activity may lead to the development of novel strategies for enhancing plant protection against pathogens.

In practical applications, our findings can benefit various industries, including agriculture, catering, and food-related businesses. Since fruits and other plants like potatoes are prone to browning due to catecholase reactions, chelating agents such as citric acid or PTU could be used to bind to the cofactor copper, preventing browning. This approach may help delay browning in fruits like apples, preserving their appearance for customers.

Future research should focus on determining the effectiveness and safety of using citric acid and PTU in everyday scenarios to preserve foods for business activities involving the serving

of cut fruit. Investigating the potential long-term effects of these chelating agents on food quality and nutritional value is also important, as this information could inform best practices for food preservation and presentation.

In conclusion, our study has demonstrated the importance of cofactors, such as copper, and optimal pH conditions for catecholase enzyme activity. These findings contribute to our understanding of the role of catecholase in plant defense mechanisms, providing a foundation for future research and potential applications in agriculture and plant protection.

I. References

Stegenga, Barbara. “Photosynthesis.” Laboratory Exercises for Biology 101. Fall 2022-Spring 2023: Hayden McNeil, 21-32.

Alberts,B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2014). Molecular Biology of the Cell. Garland Science.

Berg, J. M., Tymoczko, J. L., & Gatto, G. J. (2012). Biochemistry. W. H. Freeman and Company.

Mayer, A. M., & Harel, E. (1979). Polyphenol oxidases in plants. Phytochemistry, 18(2), 193-215.

Olukunle, O. J., Akinpelu, B. A., Odeja, O., & Aiyegoro, O. A. (2021). Catechol Oxidase Activity and Total Phenolic Content of Four Solanum melongena L. (Eggplant) Cultivars. Journal of Advances in Microbiology, 22(2), 12-21.

Yildirim, A., Erşan, S., & Özkan, M. (2013). Inhibition of polyphenol oxidase (PPO) activity by various di- and tri-peptides in cherimoya (Annona cherimola Mill.) and avocado (Persea americana Mill.) fruit models. Food Chemistry, 141(1), 356-361.

Table 1, Absorbency Readings

Tube	Absorbency at Initial Reading	Absorbency at 10 Minutes	Percentage Change	Color Change
1	0.838	1.047	19.96%	Brown-Orange
2	1.013	0.396	-155.80%	Clear
3	0.410	0.21	2.61%	Clear
4	0.646	0.770	16.10%	Yellow-Orange

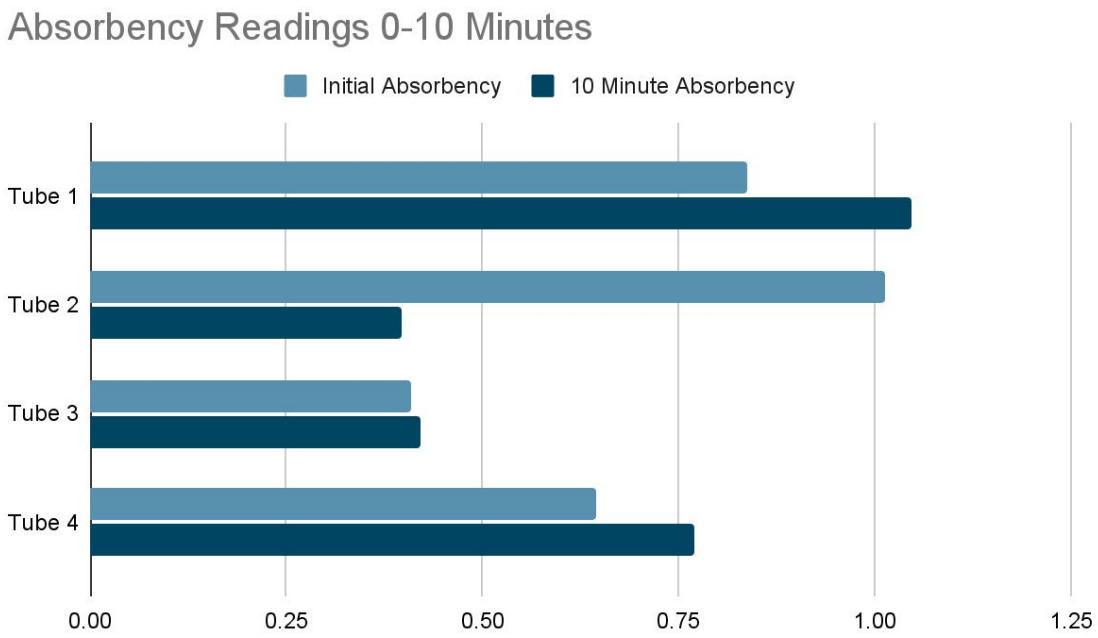

Figure 1. Absorbency Comparisons

Table 2. Change in Absorbency Calculation

Tube	Change in Absorbency (A_F-A_I)	Color Change
1	0.209	Brown-Orange
2	-0.617	Clear
3	0.011	Clear
4	0.124	Yellow-Orange

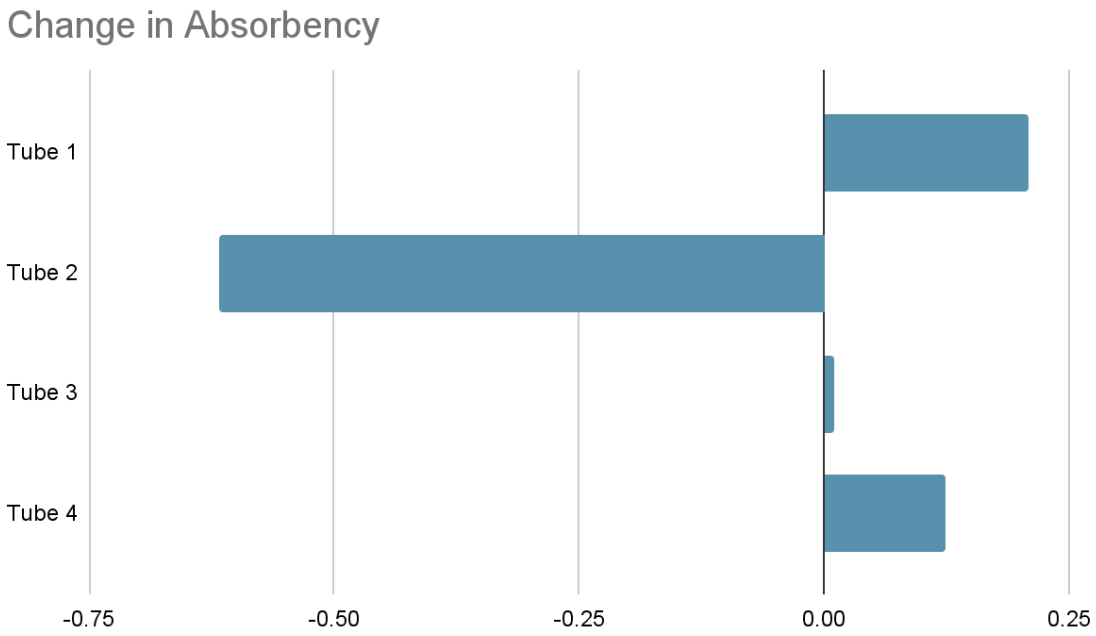

Figure 2. Change in Absorbency Analysis