MEASURING ENZYME CATALYSIS WITH INTEGRATION OF LIPID ADDITIVES

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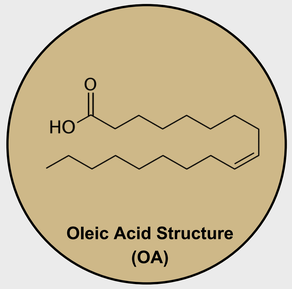
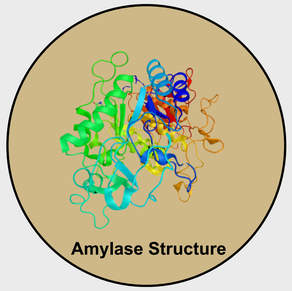
Introduction

Research Question: How does the addition of oleic acid affect the rate of amylase's catalysis of starch?

- Enzymes play a critical role in regulating processes in our bodies, such as digestion, DNA replication, and protein synthesis.
- Our enzyme, amylase, catalyzes (or breaks down) complex carbohydrates into simple starches (Dewey, 2024).
- Enzymes are commonly found in cellular environments that contain lipids, so scientists hypothesize that those lipids could have significant effects on enzyme regulation.
- Many enzymes have a hydrophobic interior and a hydrophilic exterior. It is predicted that the carboxylic acid on oleic acid could interact with the polar region of the enzyme, while the nonpolar carbon chain could interact with the hydrophobic region.
- Oleic acid is a naturally occurring, monounsaturated (saturated with hydrogens, except for one double bond) fatty acid. It is synthesized by many plants and animals, including humans (Cremerna, 2024).
- Some research suggests that oleic acid inhibits amylase; as amylase activity is inhibited, fewer polysaccharides are broken down into monosaccharides (Gaenssle et al., 2020).
- With no conclusive results, we performed our own research investigating how varying concentrations of oleic acid, with ethanol as a cosolvent, impact amylase activity.



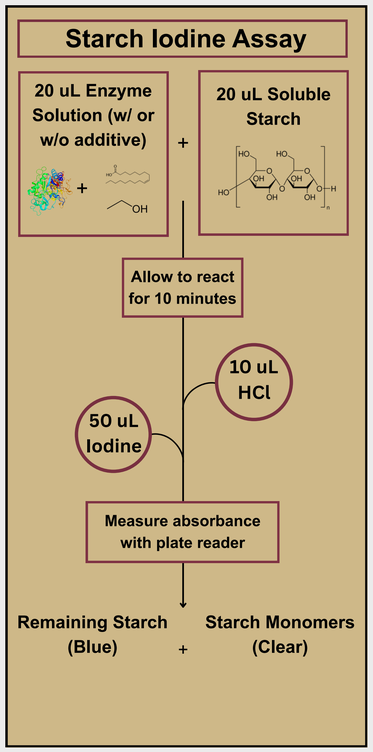
Oleic Acid Structure (OA)



Amylase Structure

Methods

- Stock additive solutions: oleic acid was dissolved in ethanol at ratios of: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. PBS was added to keep the volume constant and percent of ethanol in the total solution at 20%. The solutions were vortexed until homogeneous.
- Amylase solution: amylase was added to PBS to achieve a concentration of 30 units/mL. Equal parts of the stock additive solutions and the amylase solution were mixed and vortexed to create the final amylase-additive solutions used for the assay.
- Stock starch solution:
  - Hand mixed 220 mg of soluble starch in 20 mL of DI water.
  - 80 mL of boiling DI water was then added.
  - The solution was left on a hot plate with a stir rod until fully dissolved.
- The Starch Iodine Assay was then conducted as shown in the diagram on the right. The final solutions were 5% ethanol (by volume), 1.1 mg/mL starch, and 7.5 units/mL amylase.
- The absorbance of the remaining solution was then measured with a plate reader to quantify enzyme activity.
- Absorbance values were compared against a control without enzyme at each concentration and a control without oleic acid.



Results

Figure 1

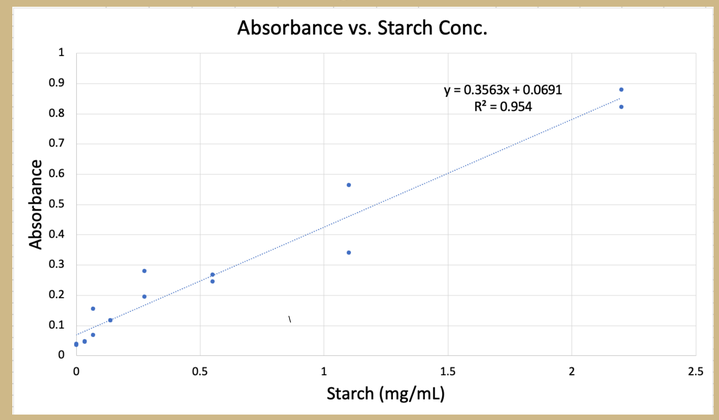


Figure 2

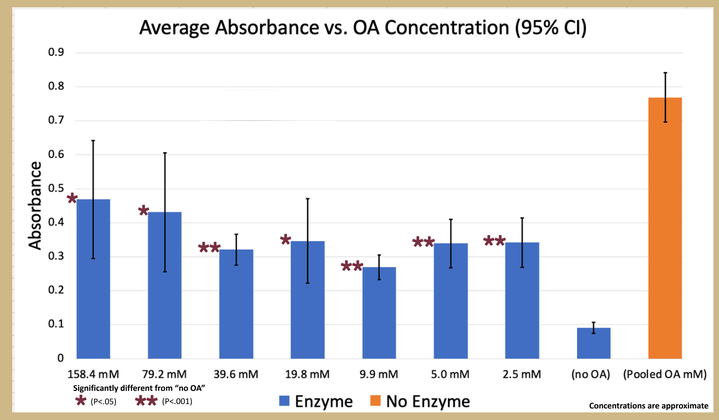
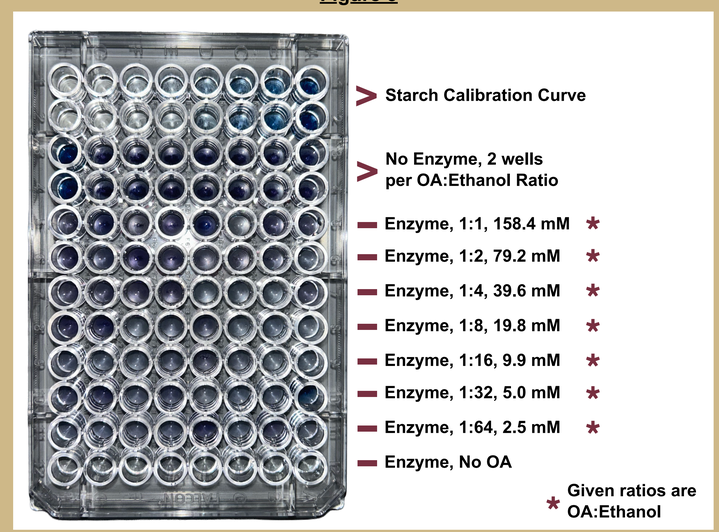


Figure 3



Discussion

- Figure 1 depicts the absorbance of a control solution with PBS substituted for amylase. This allows for the concentration of uncut starch to be determined in the experimental trial by defining absorbances at known starch concentrations for comparison.
- The absorbances in the control solutions displayed in Figure 2 did not follow a clear trend and were not experimentally significant. This suggests that the oleic acid and alcohol solution does not have a large effect on the measured absorbance. As a result, the difference between treatment groups was a result of varying enzyme activity, not solution opacity.
- Statistics:
  - Each experimental group (with OA) was compared against the control (without OA) using a Welch's T-test at a 95% confidence level.
  - All tests suggested the difference in absorbance between the control and experimental groups was significant (P<.05).
  - All groups had at eight replicates.
- **Conclusion: At the concentrations studied, our results indicate that oleic acid may inhibit enzyme function.**
- Strengths of our study:
  - Aligns with past literature suggesting oleic acid can inhibit amylase activity.
  - The methodology used for this project may provide grounds for future research involving lipid-mediated enzyme function.
- Limitations of our study:
  - Few replicates limited the strength of the statistical analysis.
  - Error due to experimental methods: inconsistent micro-pipetting, measuring absorbance of opaque solutions, and immiscibility between the oleic acid and the PBS buffer.

Future Research

- OA could be tested at lower concentrations to determine if there is a value where amylase activity is enhanced rather than inhibited.
  - This would involve repeating the 5.0 mM and 2.5 mM trials and systematically reducing OA concentration by factors of ten.
- Other additives, such as anionic and nonionic surfactants, could be tested to see if they also have an inhibitory effect on amylase.

Sources & Contributions

Conceptualization: VT, AJ, SL
Investigation: EL, SE, JMc, JMo, TA
Methodology: VT, EL, SE, JMc, JMo
Project Administration: VT, SL
Statistical Testing: EL
Supervision: VT, SL
Visualization: EL, SE, JMc, JMo
Writing: EL, SE, JMc, JMo