INVESTIGATING THE FUNCTION OF ENZYME 2O14

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Abstract

Enzyme 2O14 is a protein of unknown function, but is hypothesized to be an esterase. We investigated the function of this enzyme using a chromogenic ester substrate, p-nitrophenyl acetate (PNPA). The product from the hydrolysis of PNPA is yellow, allowing for the measurement of absorbance as a function of activity. Enzyme 2O14 was shown to hydrolyze the ester substrate, indicated by the increase in absorbance as a result of the formation of the yellow product.

Introduction

There are almost 4,000 proteins in the Protein Data Bank that have unknown function. We investigated the function of unknown protein 2O14 using activity testing [1]. Through previous computational work, we believe that enzyme 2O14 is an esterase, a hydrolase that acts on ester bonds. P-nitrophenyl acetate (PNPA) was used as a substrate to test for esterase activity. When PNPA is hydrolyzed, p-nitrophenol is produced and a color change occurs. For this reason, PNPA is characterized as a chromogenic substrate.

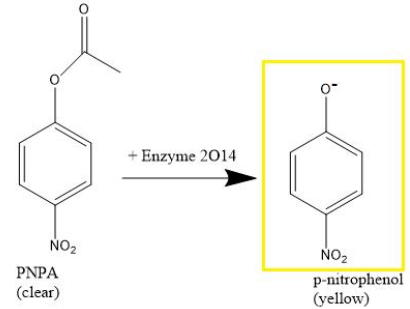

Figure 1: Hydrolysis of PNPA using enzyme 2O14 and resulting p-nitrophenyl substrate

2O14 Purification

2O14 was expressed in *E. coli* using a vector that adds 6 His-tags. These N-terminal His-tags allowed for purification using Ni-NTA chromatography. The affinity of the column for the His-tag allowed the protein of interest to bind to the column while unwanted proteins were eluted out. An elution buffer was used to remove protein 2O14 from the column, as the buffer contains imidazole that competes with the His-tag and allows protein 2O14 to be eluted. SDS-PAGE was used to determine which elution fractions contained a viable amount of enzyme and a Bradford Assay was used to determine the concentration.

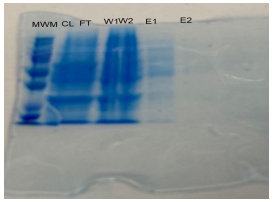

Figure 2: SDS-PAGE analysis of purification of 2O14.

Activity Testing

The use of the PNPA chromogenic substrate allows for the formation of a yellow product (p-nitrophenol). Activity tests were performed using the PNPA substrate by measuring the absorbance at 405 nm after the addition of enzyme 2O14.

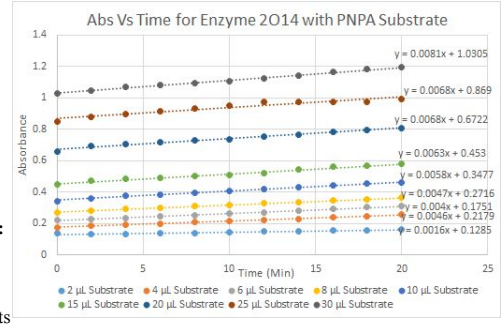

Figure 3: Plot of first activity test results

Activity Testing (cont)

A second activity test was performed in order to gain more kinetics data. In both tests, as substrate concentration increased the absorbance also increased. This indicates formation of the yellow product, meaning that enzyme 2O14 is successfully hydrolyzing the ester substrate.

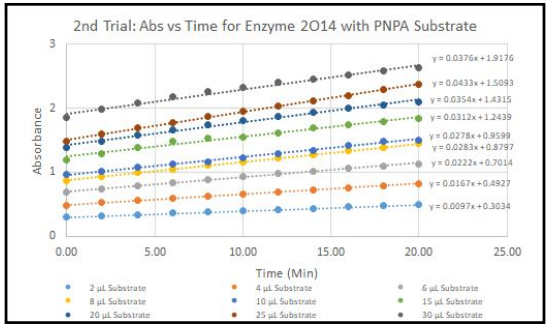

Figure 4: Plot of second activity test results

Future Work

For our future plans, we will repeat prior work to obtain further activity study results. Data will be compared to the lipase activity of 2O14 using dodecanoate substrate in DMPC vesicles to determine if the enzyme acts better as an esterase or a lipase.

References

[1]Kuzin, A.P., Chen, Y., et al.. (n.d). X-Ray structure of the hypothetical protein YXIM_BACsu from Bacillus subtilis. Kuzin, A.P., Chen, Y., et al. *To be published.*

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