Characterization and Analysis of T352V Mutation in the Enzyme β-Glucosidase B

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Introduction

A current goal of biochemistry is to develop software which predicts the function of proteins based on their primary structure. This goal is important as it could assist in understanding the mechanisms of diseases or discovering drug targets (Kulmanov & Hoehndorf). The research project Design to Data (D2D) collects protein data for β-Glucosidase B (BglB), an enzyme which catalyzes the hydrolysis of certain sugars, from labs across the country. This creates a dataset that aims to predict protein function (D2D website). We contributed to the D2D dataset by first expressing and purifying a T352V mutant of BglB. We then characterized the mutant enzyme by using the protein modeling software FoldIt, and by gathering data on the enzymatic activity of the mutant enzyme.

We hypothesize that T352V mutant β-Glucosidase B will demonstrate decreased catalytic efficiency compared to the wild type because of the change in local interactions due to the mutation, including the new presence of hydrophobic interactions and lack of hydrophilic interactions near the catalytic site (Ribeiro, et. al). Additionally, the FoldIt score of the T352V mutant indicates that that mutant has decreased protein stability and activity in comparison to the wild type, which also indicates that the mutant has decreased catalytic efficiency.

Methods

FoldIt Modeling
Phylogenetic Preprocessing, Sequencing, and Quantification
Transformation
Protein Expression and Purification
SDS-PAGE, Kinetic and Thermal Assays

Discussion

• The data collected suggests that our T352V mutant enzyme has decreased enzymatic activity, which supports our hypothesis.
• The SDS-PAGE indicates that the protein was expressed and purified, but at a low yield.
• The results of the kinetic and thermal assays of the T352V mutant suggest that there is negligible to no enzymatic activity, due to the spread and overlap of the data points.
• The yield of the mutant enzyme was low at 0.0695 mg/mL; however, the concentration is not low enough to conclude that the decreased enzymatic activity was due to insufficient expression of the enzyme.

Thus, our data suggests that the T352V mutant of β-Glucosidase B has decreased enzymatic activity compared to the wild type.

• The next steps would be to continue testing T352V and even other point mutations of BglB and uploading the data to D2D. This would contribute to the dataset that continues to improve protein function modeling software.

References


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