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

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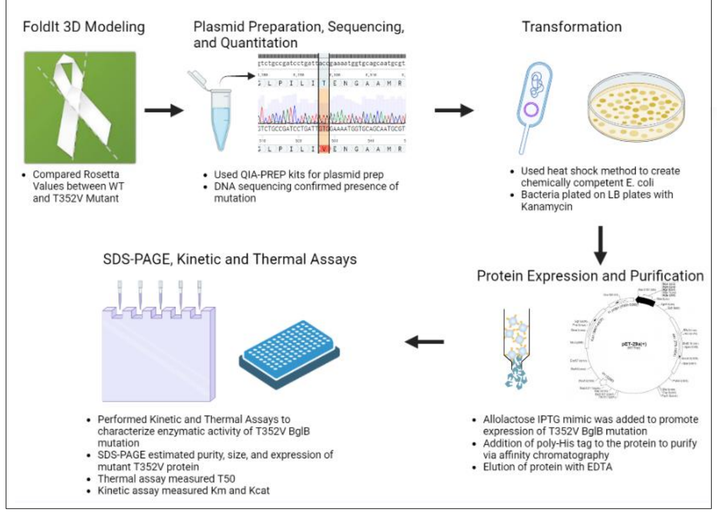
Introduction

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 T352V mutant will have decreased catalytic efficiency.

Methods



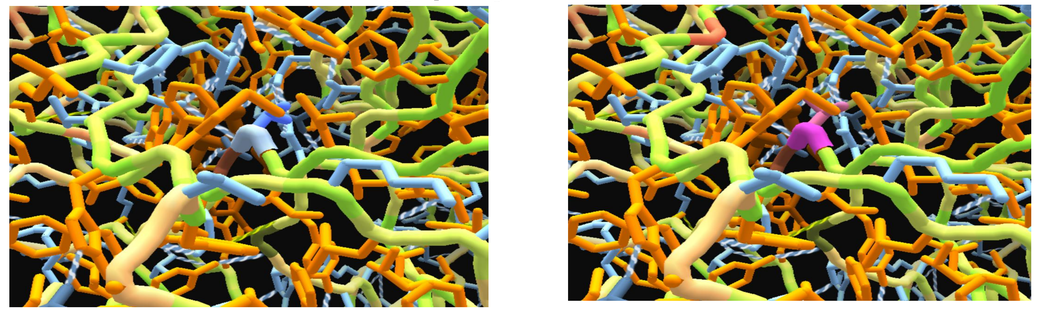


Figure 1. FoldIt 3D predictive model of β-Glucosidase WT (left) and Mutant T352V (right). The highlighted residue on the left is Threonine (T352), and the highlighted residue on the right is mutation Valine. The molecular environment consists of other residues, including hydrogen bonds (blue lines), hydrophobic residues (orange), and polar interactions (cyan). This model was used to predict catalytic efficiency of the mutant based on changes in interactions.

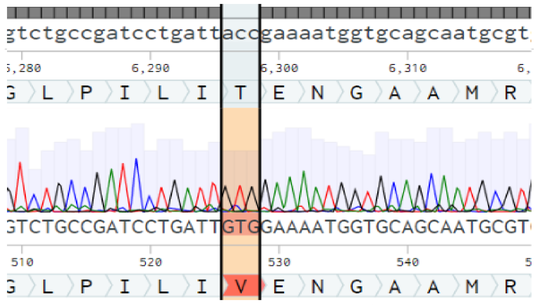


Figure 2. Sequence alignment that confirmed the presence of the point mutation for T352V variant. The WT amino acid sequence is on top, and the mutant sequence is on bottom.

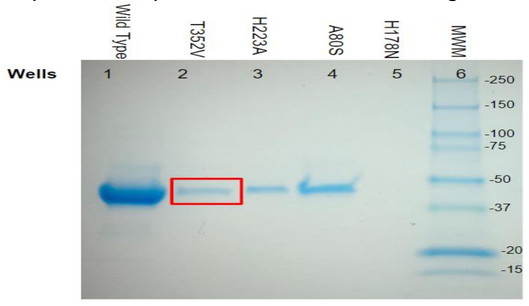


Figure 3. SDS-PAGE of β -Glucosidase Variant T352V to determine expression and purity of mutant protein. The band indicates limited purity but confirmed presence. Molecular weight was estimated by using a molecular weight ladder (lane 6).

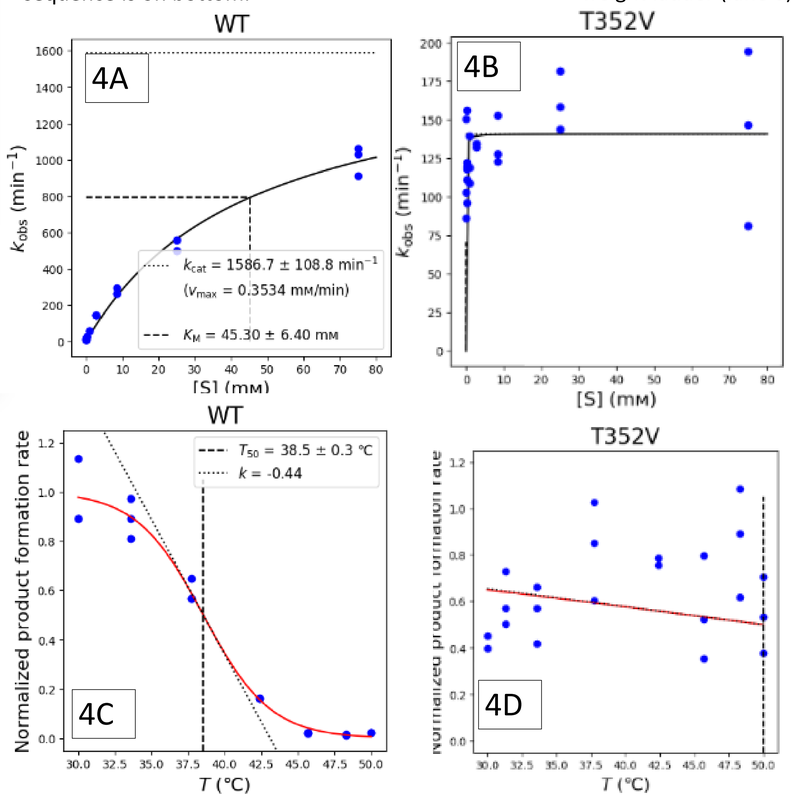


Figure 4. D2D graphical representations of Kinetic (4A, 4B) and Thermal(4C, 4D) Assays for β-Glucosidase (BglB) WT (4A, 4C) and Mutant T352V (4B, 4D). Both mutant assays demonstrate no correlation, which shows the enzymatic activity did not change as concentration nor temperature were changed. This suggests no enzymatic activity.

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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Labonte, Jason. “D2D Cure: About.” *D2D CURE | About*, https://www.d2dcure.com/about/. Accessed 14 April 2023.

Ribeiro, A. J. M., Tyzack, J. D., Borkakoti, N., Holliday, G. L., & Thornton, J. M. (2020). A global analysis of function and conservation of catalytic residues in enzymes. *Journal of Biological Chemistry*, *295*(2), 314–324. https://doi.org/10.1074/jbc.rev119.006289

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