Characterizing the Enzymatic Activity and Thermostability of Protein β-Glucosidase B (BglB) Mutant Y118F

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ABSTRACT

The purpose of this experiment was to characterize the functionality of BglB mutants and generate datasets to train artificial intelligence algorithms to predict mutant functionality. It was hypothesized that mutant Y118F would demonstrate decreased catalytic efficiency and thermostability compared to WT based on the predictive capacities of Foldit, a predictive modeling software. Foldit predicted decreased local interactions such as hydrogen bonds for mutant Y118F, suggesting decreased stability. Experimentation was performed to analyze the enzymatic activity and thermostability of mutant Y118F.

INTRODUCTION

Design to Data (D2D) is a research initiative directed by the Siegel Lab at the University of California-Davis. The "big-picture" objective of the initiative is to train artificial intelligence (AI) algorithms to predict enzyme structure and function (Design2Data). The development of accurate prediction modeling algorithms is important because they allow scientists to test hypotheses without the need of wasting time and other resources conducting laboratory experimentation. Analyzing the catalytic activity and thermostability of one mutant protein takes days or weeks of work and costs several thousand dollars. Having to run such experiments on many different mutations is tedious and costly.

These AI algorithms are trained using datasets produced by research students that perform experimentation on specific mutations of β-Glucosidase (BglB). This enzyme aids in the breakdown of cellulose and other (β-1→ 4) linked sugars (Feeney, 2022A). It completes the last step during cellulose hydrolysis to generate glucose. Experimentation was performed to characterize the catalytic efficiency and thermostability of individual BglB mutants and data was submitted to the D2D database.

The hypothesis of this research is as follows: BglB mutant Y118F will demonstrate decreased catalytic efficiency and thermostability in comparison to the wild type because its overall Foldit score suggests a high likelihood of expression, and intermolecular modeling analysis (local score) also points to decreased local interactions, such as hydrogen bonds. Furthermore, previously published data on similar mutations like W120A supports this hypothesis (Design2Data).

DISCUSSION

- The SDS-PAGE results confirm that the Y118F mutation was both expressed and purified. If impurities were present, there would be other markings present in other molecular weight lanes.
- The specificity constant of the mutant (77.73 mM^{-1} min^{-1}) is greater than that of the WT (45.26 mM^{-1} min^{-1}). This indicates that the mutant is better at producing products than the WT (Feeney 2022j). Therefore, the mutant demonstrates increased catalytic efficiency compared to the WT. Given that the WT had a hydrogen bond that was lost in the mutant, and other local interactions such as clashes increased in Foldit, it is unclear why the mutant displays increased catalytic efficiency. The data does not support the proposed hypothesis.
- The T_{50} of the mutant (36.6 °C) is slightly less than that of the WT (38.6 °C). This indicates that the mutant has decreased thermostability compared to the WT, but not by much. This deviation could be explained by the variation in results across the three wells used to generate T_{50} data, which varied by up to 0.4 in some cases. Therefore, the temperature difference between WT and Y118F is likely due to experimental error.

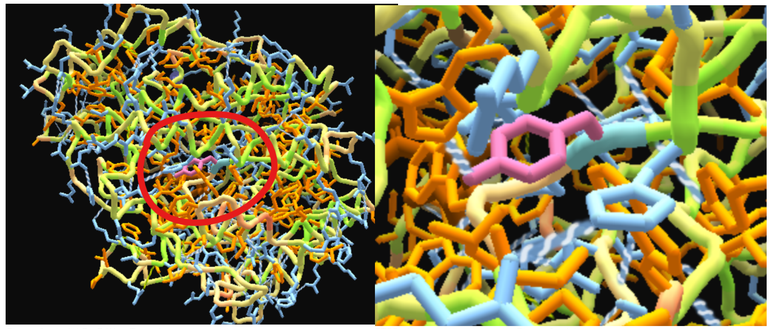


Figure 1a. (left) BglB Wild Type. Illustration of the arrangement of wild-type pET29b β-glucosidase protein. **Figure 1b. (right)** is the zoomed-in version of pET29b_BglB Wild Type. This image highlights amino acid 118 where Tyrosine (pink structure) can be seen, which has no local interactions.

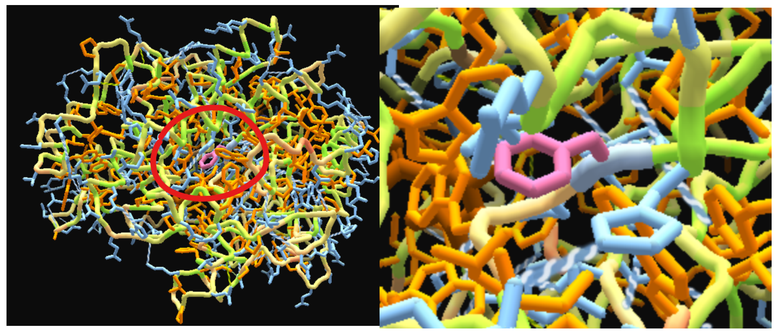


Figure 2a. (left) BglB Mutant Y118F. Illustration of the arrangement of mutant pET29b β-glucosidase protein. **Figure 2b. (right)** is the zoomed-in version of pET29b_BglB Mutant. This image highlights amino acid 118 where Phenylalanine (pink structure) can be seen which has a hydrogen bond facilitating local interactions.

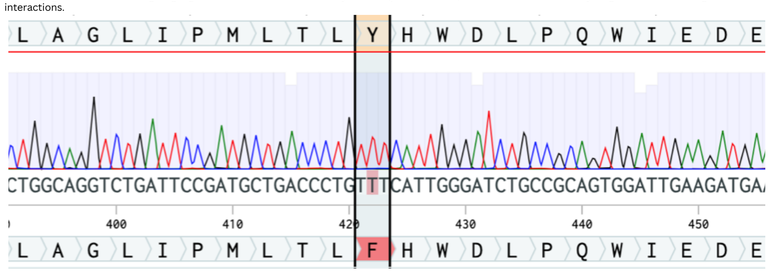
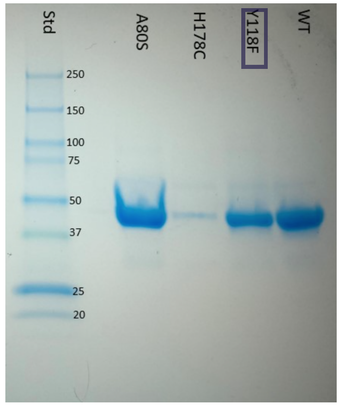


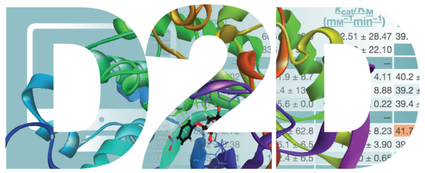
Figure 3. Transformation in pET29b-BglB and the mutation success of Y118F.

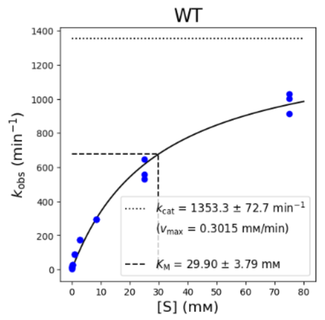


Mutation	A280	Concentration (mg/mL)
Y118F	1.465	0.6667

Table 1. Protein concentration and purification of mutant Y118F in pET29b_BglB. The absorbance was at 1.465 and the concentration was 0.6667 mg/mL, showing a high product yield.

Figure 4 (left). SDS-PAGE results of Y118F in pET29b_BglB, indicating protein expression and purification.





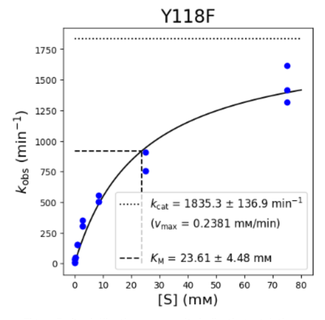
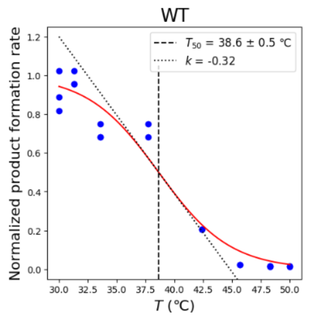


Figure 5a (top). Kinetic assay results indicating catalytic efficiency of Wild Type.

Figure 5b (bottom). Kinetic assay results indicating catalytic efficiency of mutant Y118F.



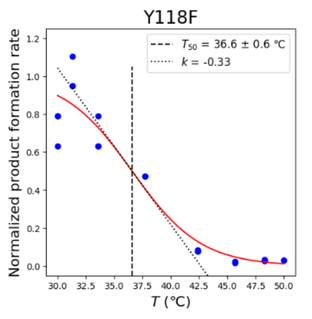


Figure 6a (top). Thermostability assay indicating T_{50} of Wild Type.

Figure 6b. (bottom). Thermostability assay indicating T_{50} mutant Y118F.

METHODS

The methods used to conduct this research include Foldit modeling, plasmid preparation, protein expression and purification, kinetic assay, thermostability assay, and characterization of purified protein. The mutation was first modeled in Foldit to determine what point mutations were expected to occur, along with the change in Rosetta energy.

Plasmid Preparation: Plasmids were first isolated from bacteria using QIAGEN Plasmid Purification Kit. The major steps included binding plasmid DNA to a column, washing the column, and eluting the plasmid DNA. Following plasmid preparation, quantitation was performed to determine the amount of plasmid that was isolated. Spectrophotometry was performed to measure DNA concentration. Next, the plasmid DNA was sequenced using the Sanger Sequencing method. The sequence was analyzed to confirm the presence of the Y118F mutation. Lastly, a chemically competent strain of E. coli (BL21(DE3)) was transformed to take up the mutant plasmid DNA. Bacterial growth replicated the plasmid and created many copies of the mutant DNA.

Protein Expression and Purification: pET29b plasmid, after transforming E. coli, was used to express mutant forms of the BglB gene. After bacterial colonies were grown overnight, the cultures were expressed by adding induction media and incubated with shaking for 24 hours. The BglB-His protein was purified by affinity chromatography using Ni2+ NTA resin, then eluted from the column. The protein was then quantified via spectrophotometry.

Kinetic Assay: The enzyme activity of BglB was analyzed by observing the release of p-nitrophenol (PNP), which produces a yellow color when deprotonated, using a spectrophotometer. As the concentration of PNP increased, so did the yellow color. The concentration of this substrate over time was used to analyze the effect of substrate concentration on the rate of reaction. The Michaelis-Menten equation was used to graph this data. The turnover rate (Kobs) was analyzed to characterize the binding affinity of the substrate to protein, which correlated to catalytic efficiency.

Thermostability Assay: The thermostability of BglB was analyzed by observing color changes in solution over time as the protein was heated to a range of different temperatures. The thermal stability (T50), the temperature at which there is 50% enzyme activity, was calculated from the data produced.

Characterization of Purified Protein: The purified BglB protein was characterized by SDS-PAGE. SDS-PAGE separated the proteins by size on a gel. Then, the separated proteins were visualized. This analysis determined which proteins were present in various samples taken throughout the experiment. Namely, this determined if the protein was purified efficiently (Feeney 2022h; Feeney 2022i).

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REFERENCES

1. Carlin DA, Caster RW, Wang X, Betzenderfer SA, Chen CX, Duong VM, Ryklansky CV, Alpekin A, Beaumont N, Kapoor H, et al. 2016. Kinetic Characterization of 100 Glycoside Hydrolase Mutants Enables the Discovery of Structural Features Correlated with Kinetic Constants. Plos One. 11(1) https://doi.org/10.1371/journal.pone.0147596
2. Design2Data [Internet]. Davis (CA): University of California, Davis; [updated 2023 Feb 26; cited 2023 Apr 13]. Available from: https://d2d.sf.ucdavis.edu/
3. Feeney E. 2022a. Designing B-Glucosidase Mutants: Foldit [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 2.
4. Feeney E. 2022b. Plasmid Preparation, Quantitation, and Sequencing [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 3.
5. Feeney E. 2022c. Transformation of Bacteria [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 4.
6. Feeney E. 2022d. Expression of a Foreign Gene in Bacteria [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 5.
7. Feeney E. 2022e. Affinity Purification of a Recombinant Protein from Bacteria [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 5.
8. Feeney E. 2022f. Background: Enzyme Kinetics [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 7.
9. Feeney E. 2022g. Background: Thermostability [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 9.
10. Feeney E. 2022h. Characterization of Affinity Purified Protein Using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 10.
11. Feeney E. 2022i. Characterization of Affinity Purified Using Western Blot Analysis [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 11.
12. Feeney E. 2022j. Understanding the Data Analysis [lab manual]. Loyola University. Department of Chemistry. BIOL 366L. Week 11.