Determining the Catalytic Efficiency of β-Glucosidase B (BglB) H328N Mutant

Emma Katubig, Marian Castro, Emma Feeney PhD.
Department of Biology — Loyola University of Chicago

Preparing people to lead extraordinary lives

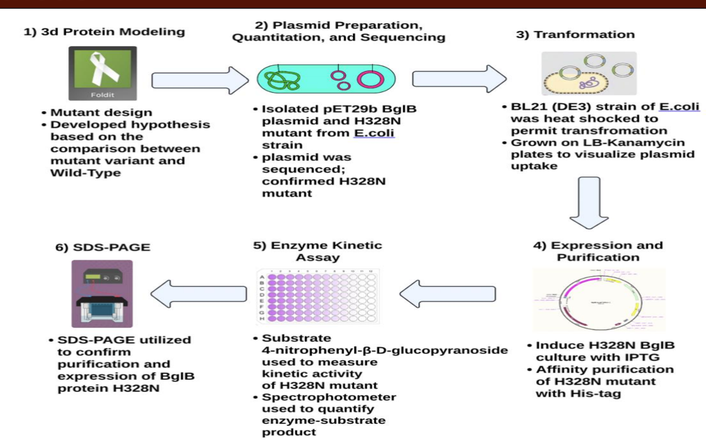
Abstract

This presentation will describe the experience of generating a mutant enzyme, characterizing its function, and making protein function predictions. This project began by designing the H328N mutation for our selected protein, β–glucosidase B (BglB), using the molecular modeling tool, Foldit. Following preparation of plasmid DNA containing H328N, DNA sequencing was performed preliminary to expression and purification of the mutant. An enzyme kinetic assay was conducted to facilitate characterization of the mutant's enzymatic activity. Additionally, the purity of H328N was analyzed using SDS-PAGE. In this study, we utilized our calculated system energy values obtained from the Foldit models in conjunction with examination of intermolecular interactions to predict the outcome of our experiment.

Introduction

Knowledge of a protein's amino acid sequence is useful in determining structural outcomes including tertiary or quaternary protein structure. However, prediction of protein function from a molecular level presents a challenge, especially when considering that proteins can have multiple functions [1]. Design to Data (D2D) is a national network database developed by Dr. Justin Siegel's Lab at UC Davis with the central goal of addressing the predictive limitations of current computational design algorithms which lack large quantitative datasets [2]. For this reason, D2D was devised to tabulate data from various single point mutations of BglB and provide accurate predictions to assist researchers in designing novel proteins or evaluating structural stability. BglB is a monomeric enzyme encoded by the Paenibacillus polymyxa bacterium that catalyzes the hydrolysis of cellulose to produce glucose and other sugars at a β-glycosidic linkage [3]. We hypothesize that the BglB mutant H328N will be similar in catalytic efficiency (K_{cat}/K_M) 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 slightly increased local interactions as both histidine and asparagine are hydrophilic. This small increase in score is attributable to deterred substrate binding due to the amino acid residue changing from positive to neutral. This small change in charge impacts the affinity and specificity of the enzyme for the substrate, producing the change in local scores. Furthermore, previously published data on a similar mutation, H328K bolsters this hypothesis.

Methods



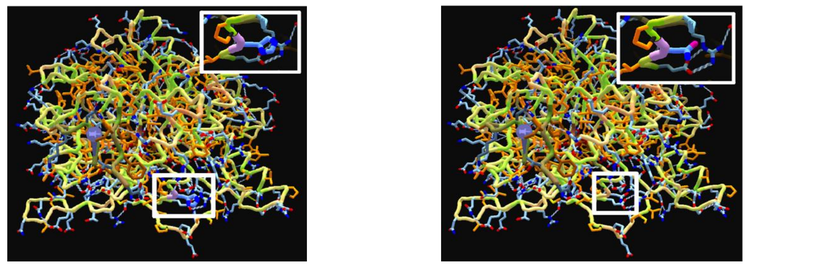


Figure 1. Fold-It generated 3D-models of Wild-Type BglB (left) and the mutant variant H328N (right). The image on the left shows a featured Histidine residue, while the image on the right shows the H328N mutation. The Foldit score changes from -1089.089 to -1089.697 after the mutation. The biomolecular protein complex features polar residues in cyan, hydrophobic residues in orange and hydrogen bonds in blue and white.

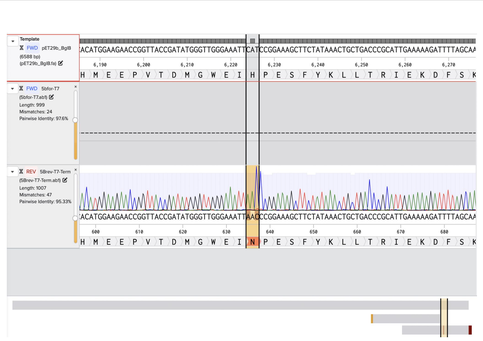
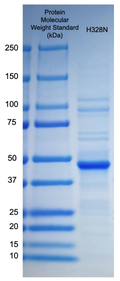


Figure 2. DNA Sequence Analysis of pET29b-Mutated Bglb Plasmid variant H328N. Wild-type amino acid sequence displayed on top while H328N variant sequence presented on the bottom.



Sample	A280	Concentration (mg/mL)
H328N	0.782	0.3560

Figure 3. SDS-PAGE analysis and Spectrophotometer reading of BglB variant H328N. Assay displays expression and purification of H328N variant.

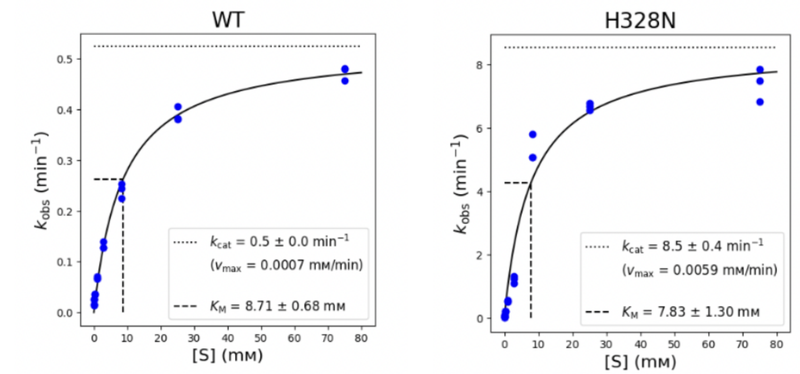


Figure 4. Comparative analysis of kinetic assay for Wild-Type (left) and H328N variant (right). A 1:10 dilution of purified protein concentration was utilized. The graphs displayed represent a Michaelis-Menten plot. The graphs display a K_{cat} value, which measures the rate in which product is formed, and a K_M values, which suggests the affinity of substrate to enzyme.

Discussion

We aim to characterize the structural and functional significance of H328N mutations in BglB using a range of experimental methodologies encompassing SDS-PAGE and kinetic assays. The outcomes of our study provided valuable insights into the catalytic efficiency and expression levels of the H328N mutant relative to the wild type BglB.

- SDS-PAGE analysis revealed a pronounced expression of the H328N mutant protein, indicative of successful expression and purification processes.
- According to the results of our kinetic assay, the obtained data demonstrated that the H328N mutant exhibited increased catalytic efficiency relative to the wild type, as corroborated by the higher K_{cat} value.
- Upon completion of our analysis, it was determined that our results do not support our initial hypothesis. Considering that the K_{cat} of our variant significantly increased when compared to the wild type, our results suggest that the variant generates and releases product at a greater rate than the wild type.
- Ultimately, our results were unexpected as we had initially anticipated that the mutant K_M and K_{cat} be similar or less than the wild type due to the small increase in local score and decrease in Foldit Energy Score.
- A potential explanation for the obtained results could be attributed to the chemical properties of asparagine. Given that asparagine is a polar and neutral amino acid, this allows for the amino group to function as a hydrogen bond donor. Correspondingly, the carbonyl group can serve as a hydrogen bond acceptor. Together, both groups of asparagine can participate in hydrogen bonding with nearby amino acid residues, resulting in increased molecular interactions.
- Our findings underscore the need for further research such as a thermostability assay that may provide further understanding of the H328N effects on the protein's overall stability.

References

1. Whisstock, J.C., and Arthur M. L. (2003). Prediction of protein function from protein sequence and structure. *Quarterly reviews of biophysics*, 36(3), 307-340, doi:10.1017/S0033583503003901. PMID: 15029827
2. Huang, X., Kim, D., Huang, P., Vater, A., and Siegel, J.B. (2020). Design to Data for mutants of β-glucosidase B from *Paenibacillus polymyxa*: L171M, H178M, M221L, E406W, N160E, F415M. b*ioRxiv,* https://doi.org/10.1101/2020.11.17.387829
3. Isorna, P., Polaina, J., Latorre-Garcia, L., Canada, F.J., Gonzalez, B., Sanz-Aparicio, J. (2007). Crystal Structures of *Paenibacillus polymyxa* β-glucosidase B complexes Reveal the Molecular Basis of Substrate Specificity and Giv eNew Insights into the Catalytic Machinery of Family I Glycosidases. *Journal of Molecular Biology*, 371(5), 1204-1218. https://doi.org/10.1016/j.jmb.2007.05.082.

Acknowledgements

We would like to thank our collaborators at the Siegel Lab at the University of California, Davis, the National Science Foundation, and Loyola University of Chicago and the Department of Biology.