Kinetic Efficiency Determination of Mutant Enzyme Bgl B (Y118F)

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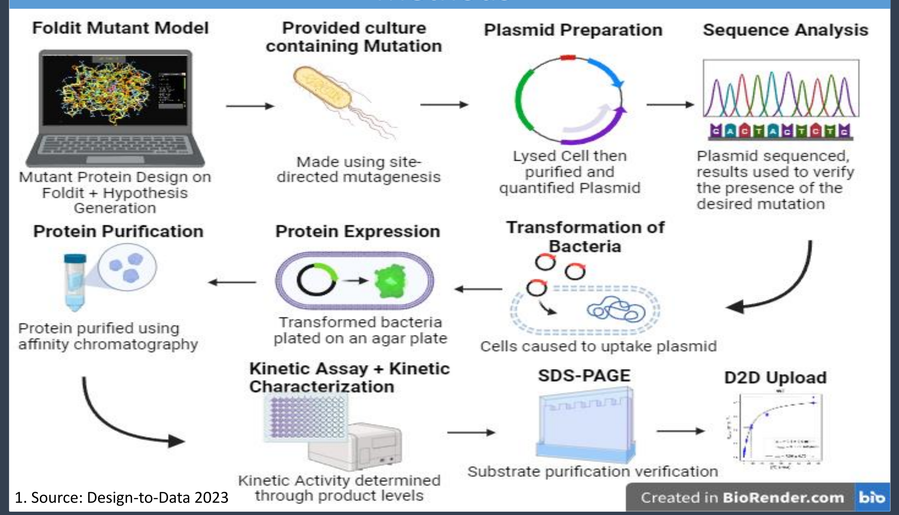
Abstract

This experiment's purpose was to characterize Bgl B mutant (Y118F) by its enzyme kinetics. The wild type Bgl B and the Y118F mutant were compared in Foldit, a chemical structure modeling software, and it was determined that our mutation would see a decrease in catalytic efficiency, substrate binding ability, and overall effectiveness. The experiment was completed with the goal of gathering data to submit to Design2Data a protein modeling algorithm. The experimentation entailed preparing the plasmid, expressing and purifying the mutant protein, analyzing the mutant using a kinetics assay and SDS-PAGE, and then interpreting and visualizing the results. The results of the SDS-PAGE did not provide much use due to little visible banding present, indicating that there are low concentrations of the mutant present. The kinetic assay results support the Kcat decreasing for the mutant but results were vague and inconclusive for the Km.

Introduction

Design to Data (D2D) is an ever-changing website filled with mutant protein data run by the Siegel Lab at the University of California-Davis. The project is generating data to improve predictions of protein modeling algorithms based on single-nucleotide changes and their effects on protein activity (Design-to-Data 2023). Running experimentation on different mutations is expensive and time-consuming. Using software to predict mutant protein behavior and function could save scientists time and money, additionally, it allows protein research to be more publicly available and studies may be able to generate data and solutions utilizing publicly generated data (Khatib et al. 2011). Generating data to improve protein modeling software can also allow theoretical enzymes to be modeled and have their activity determined more accurately. This could have potential applications in designing enzymes for use in therapeutic medical treatments (Wolf et al. 2015). The project contains data on experimentation done on different mutations of β-Glucosidase (Bgl B) (Design-to-Data 2023). Bgl B is an enzyme used primarily in the hydrolysis of glucose from cellulose; this is needed for bacteria and fungi to function (Labonte 2022). Our experimentation was done to gather more data on the Bgl B (Y118F) mutant and add the data to the D2D website. If Bgl B undergoes the mutation (Y118F) then the enzyme is expected to have an overall decrease in kinetic enzymatic activity and effectiveness. This is because the Y118 position is between the active site and the exterior so a change in structure here may have a large impact on protein activity. The change from tyrosine to phenylalanine causes a hydrogen bond to be destroyed which will decrease protein stability. The change from a hydrophilic to a hydrophobic amino acid will cause a shift in structure due to changes in the hydrophobic interactions further destabilizing the protein. The modeled Rosetta score decreases by approx. 3 which indicates expression is likely, however, activity may be decreased. These changes in structure as well as the location of the changes will most likely decrease substrate binding effectiveness as well as kinetic enzymatic activity and effectiveness. A model of the WT and mutant protein can be seen in Figure 1.

Methods[1]



1. Source: Design-to-Data 2023

Foldit Protein Models

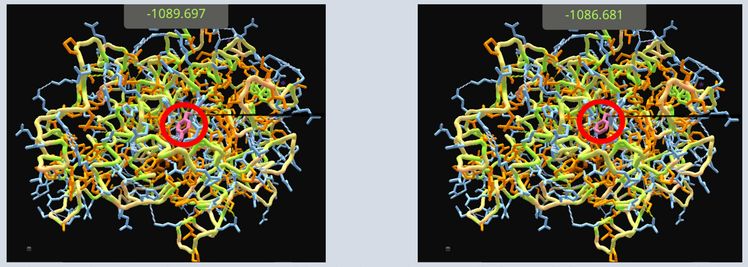


Figure 1: This figure displays the structure of both the wild type (left) and the mutant (right). The site of the mutation is pink and circled in red The Tyrosine mutates into a phenylalanine causing a conformational change in the structure. The Foldit Rosetta score (at the top in green) is also displayed which is a measure of catalytic efficiency and substrate binding ability (Khatib et al. 2011). . A decrease is shown meaning that our mutant is predicted to be less stable, less efficient, and less useful than the wild type.

Purification & SDS-PAGE

Figure 3: This shows the SDS-PAGE of the standard ladder (left) and mutant (Y118F) (right) proteins. The SDS-Page showed light banding throughout with slightly darker bands present between 50kDa-37kDa, the expected range of Bgl B. This indicates our protein may be present in the purification but in very low concentrations and likely contaminated with other material. The A280 value of 0.224 indicates a pure sample. The concentration was found to be 0.102mg/mL which is very low as expected.

Sample	A280	Concentration (mg/mL)
Y118F	0.224	0.102

Sequence Analysis

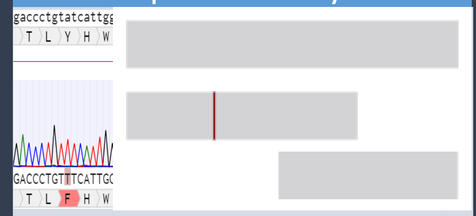


Figure 2: This figure displays both the segment of the sequence that is mutated (left) and the entire sequence (right). The section of the sequence illustrates that the change of base pairs (a to T) also changes the amino acid chain (Y to F). The entire sequence was displayed in order to illustrate that the select mutation is the only one present in the entire sequence.

Michaelis-Menten Plots

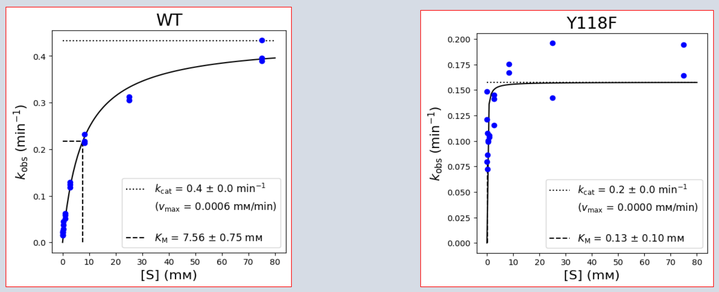


Figure 4: This shows the Michaelis-Menten plot for the wild-type (left) and the mutant (Y118F) (right). These graphs were generated using the D2D database. The graphs omitted outliers resulting in more accurate depictions of the curves. The wt displays a typical MM curve as is expected. The Km was 7.56, the Kcat was 0.4min^{-1}, and the Vmax was 0.0006. The mutant displayed an atypical curve. This is likely due to low [S] concentration levels having a similar activity to that of 0 [S]. This is likely due to a lack of activity at low levels. This likely affected the Km significantly and it is likely invalid, however, the Kcat has some statistical significance as the high [S] did display more typical activity. The Km was 0.13, the Kcat was 0.2min^{-1}, and the Vmax was lower than the detection range.

Discussion

The result generated partially support our hypothesis. The SDS-PAGE showed very little banding for our protein indicating there likely was some of the mutant protein present but in very low concentrations with possible contaminants. For our plots in figures 4 and 5 the Km for the mutant was lower which indicates it had a higher affinity of binding for the substrate (Tansey 2020), this was the opposite of what was anticipated so the hypothesis that the substrate binding effectiveness will decrease is not supported by the data. The Kcat for the mutant went down which is what was expected, this means that the kinetic catalytic activity decreased (Tansey 2020) due to the mutation which does support our hypothesis. The lower points of [S] data had similar levels of activity to the levels of 0 substrate. This means that those points have no statistical value so the generated values may not be accurate. The high [S] levels did yield activity that was noticeably above the 0 [S] levels of activity so there was not a complete loss of enzymatic activity. These irrelevant points may be responsible for the Km value being lower so it is difficult to conclude in that regard but the Kcat values are likely still valid due to being calculated based on the higher [S] values. May be best to calculate values w/o the irrelevant data points or to rerun the experiment to either verify the lack of activity or determine if there should have been activity. Overall the protein was likely expressed and displayed a decrease in kinetic activity, however, the data for substrate binding is vague so repeat testing would be beneficial.

Acknowledgements

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