Enzyme Kinetics of Serine Hydroxymethyl Transferase 8 Variants

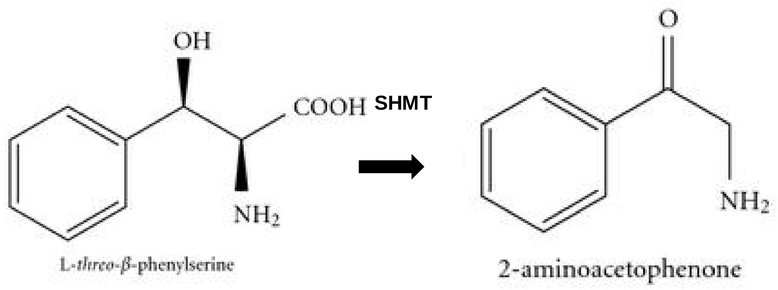
Jamie Hammond, Luckio Owuocha, and Lesa J. Beamer
Department of Biochemistry, University of Missouri, Columbia, MO

Abstract

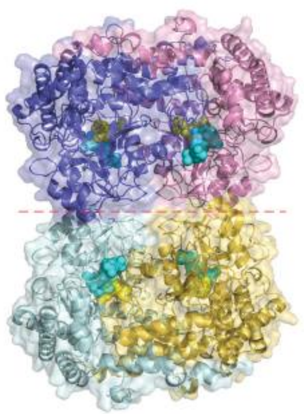
Research indicates that enzymatic activity may vary with mutations of serine hydroxymethyl transferase 8 (SHMT8). To observe the kinetic activity, a phenylserine assay was performed on a strain of soybean SHMT protein called 'Forrest', and three of its mutated variants. Production of Benzaldehyde was monitored at 279nm to observe enzymatic activity, and this was done at varying substrate concentrations of 2 mM, 5mM, 10mM, 15mM, 20mM, and 25mM. Higher Km and Vmax values were observed for the mutants of SHMT compared with 'Forrest'. This suggests that SHMT mutants have a decreased affinity for the substrate.

Phenylserine Kinetic Assay

A Phenylserine Kinetic Assay is completed to determine the Enzymatic activity of a given protein. A small concentration of enzyme is spotted on a 96 well UV plate, and substrate of varying concentrations is added to the wells and immediately read by an Epoch UV plate reader.



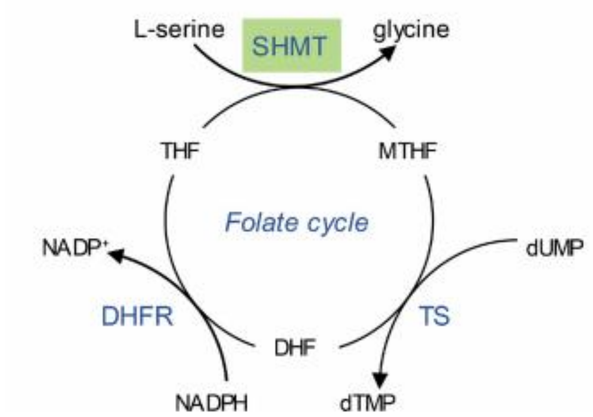
The mechanism of a Phenylserine kinetic assay is a reverse aldol, or retro aldol reaction. This mechanism works by deprotonation of an alcohol on L-b-Phenylserine, followed by loss of the carboxylic acid as an aldol. This is done by the enzyme in question, in this case SHMT. Concentration variance of either enzyme or substrate can control the rate of this reaction



Structure of SHMT8

Applications to Folate Metabolism

Observing the kinetic activity of SHMT can provide important information on its role as an enzyme. Particular interest in this topic involves SHMT metabolism of folate.



Folate metabolism is a major function of SHMT, and targeting this function can lead to decreasing in parasitic roundworms that attack soybean plants

Variant Affinity to substrate

Lower Vmax and Km of an enzyme values correlate with an increased affinity for the substrate, and higher Vmax and Km values correlate with a decreased affinity for the substrate

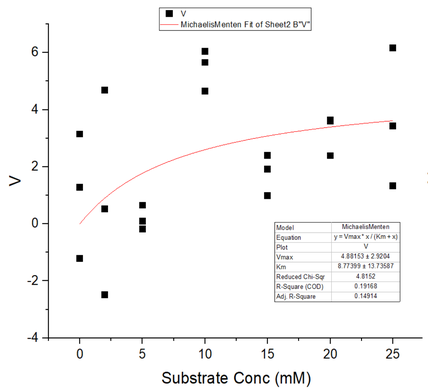
Mutants	Reported Km	Reported Vmax
Forrest	1.12 +/- 2.68	3.02 +/- 1.02
S44F	12.93 +/- 32.06	4.37 +/- 4.91
G106S	6.66 +/- 8.46	19.82 +/- 8.49
A109T	26.68 +/- 71.67	4.16 +/- 6.76

Upon completion of the Phenylserine Assay, the above values were recorded. Some mutations of Forrest are near the binding site, or cofactor binding site. Mutations near this location are likely to be less functional. Data shows a decrease in function of all three mutations, although a significant amount of error is present . Replication of data is highly important, and future work could focus on precision of enzyme assay results

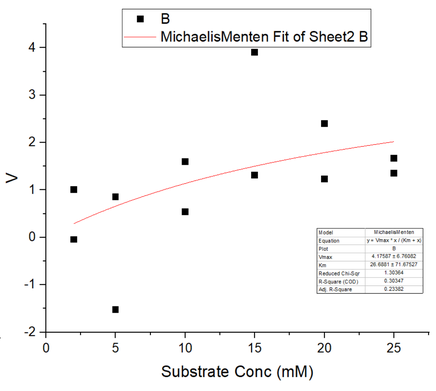
Enzymatic Activity

Enzymatic activity of Forrest may be decreased by mutations of certain amino acids. Three mutants of Forrest have been reported with lower Km and Vmax values suggesting a decreased affinity for the substrate L-b-phenylserine.

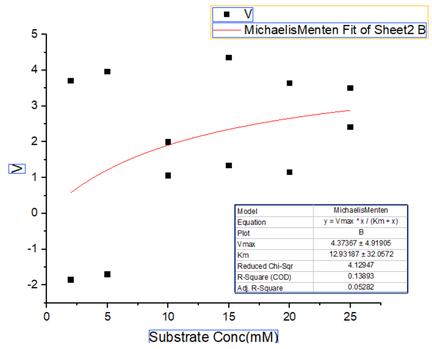
Forrest



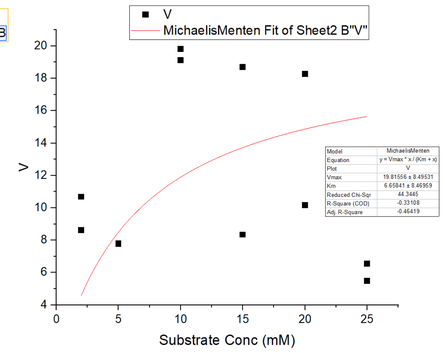
A149T



S44F



G106S



Conclusions and Future Work

Results suggest that mutations of Forrest have a decreased affinity for the substrate

Future Projects:
1. Gain proficiency in enzymatic assays for more accurate results
2. Complete assays on other mutations of SHMT8

References

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