Enzyme Kinetics of Serine Hydroxymethyl Transferase 8 Variants

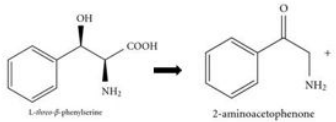
Jamie Hammond, Luckio Owuocha, and Lesa J. Beamer
Department of Biochemistry, University of Missouri, Columbia, MO

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 is 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 than for '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 a UV plate reader.



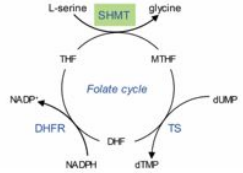
L-*threo*-β-phenylserine 2-aminoacetophenone

The mechanism in a Phenylserine kinetic assay 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

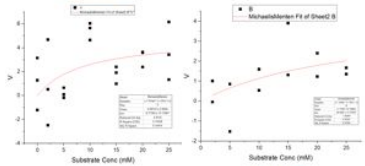
Mutants	Reported Km	Reported Vmax
Forrest	1.12 +/- 2.68	3.02 +/- 1.02
S44F	12.93 +/- 32.06	4.37 +/- 4.91
G106S	6.66 +/- 8.46	19.82 +/- 8.49
A109T	26.68 +/- 71.67	4.16 +/- 6.76

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

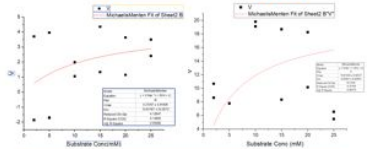
Enzymatic Activity

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

Nonaka, H., Nakanishi, Y., Kuno, S., Ota, T., Mochidome, K., Saito, Y., Sugihara, F., Takakusagi, Y., Aoki, I., Nagatoishi, S., Tsumoto, K., & Sando, S. (2019). Design strategy for serine hydroxymethyltransferase probes based on retro-aldol-type reaction. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-08833-7

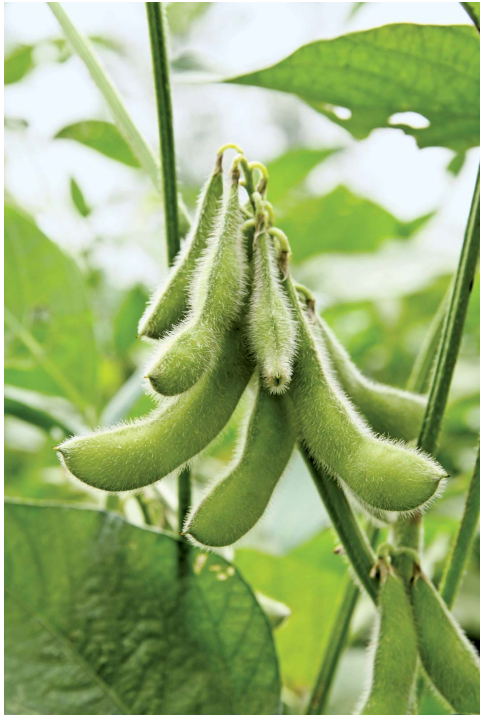
Korasick, D. A., Kandoth, P. K., Tanner, J. J., Mitchum, M. G., & Beamer, L. J. (2020). Impaired folate binding of serine hydroxymethyltransferase 8 from soybean underlies resistance to the soybean cyst nematode. *Journal of Biological Chemistry*, *295*(11), 3708–3718. https://doi.org/10.1074/jbc.ra119.012256

Background

- Soybean cultivation is a billion dollar industry in the united states
- 46.1 billion dollars in the US in 2020

Every year, over 1 billion is lost to the soybean cyst nematode

- Type of parasitic roundworm
- The nematode cannot produce folate of its own, so it attacks the roots of the soybean plant
- Extremely difficult to get rid of the infestation



Soybean Crop

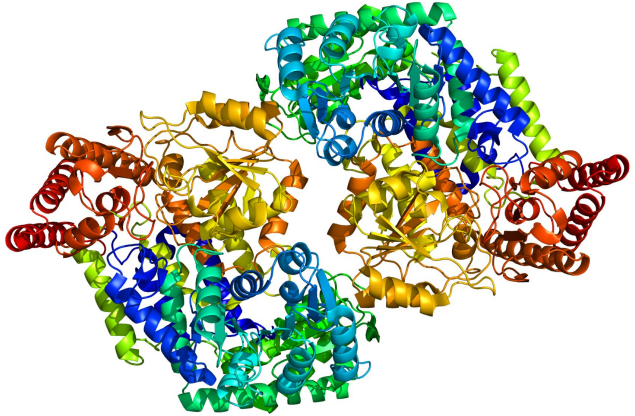
SHMT

Protein that concerts L-serine to Glycine

- Plays a huge role in folate metabolism
- Found in many organisms including soybeans

Targeting the enzymatic activity of this protein can lead to changes in folate metabolism

Doing this can be done in Soybean SHMT could create a path for solving a billion dollar problem

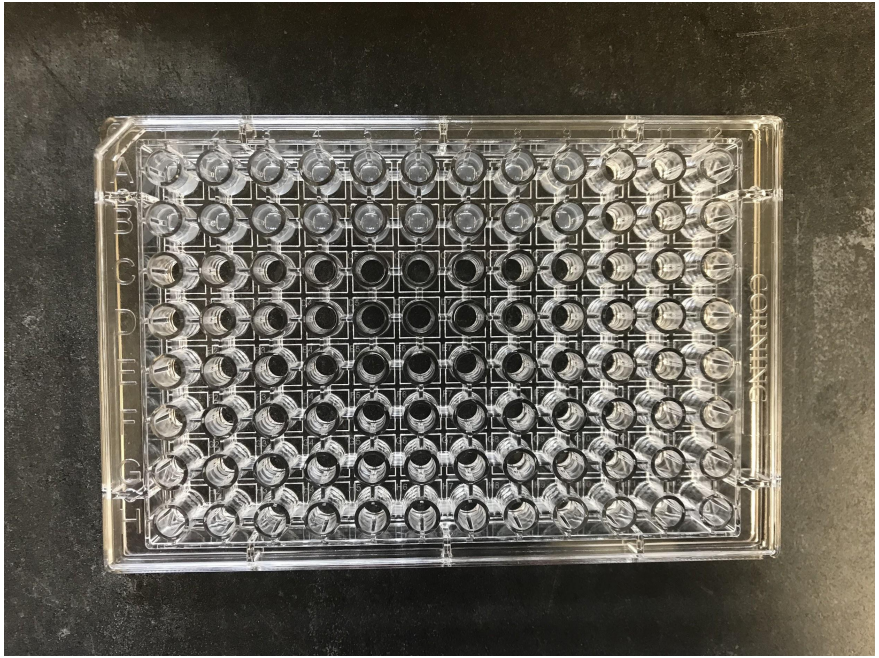


(SHMT) Serine Hydroxymethyl Transferase

Phenylserine Assay

1. Spot the well plate with a small amount of the protein (2.5ul)

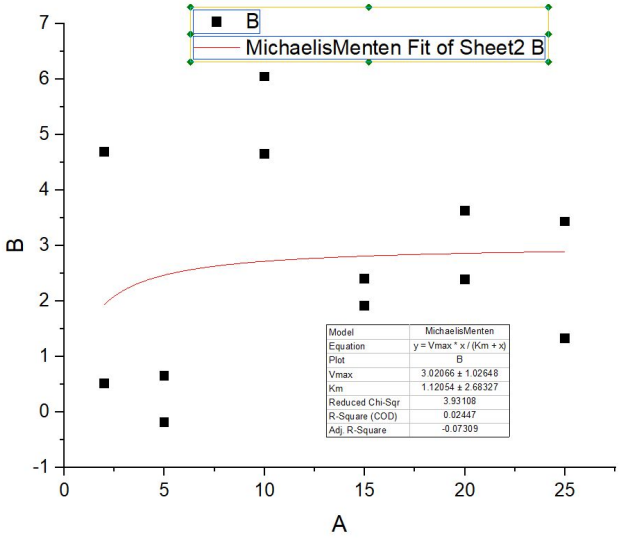
2. Pipet various substrate solutions of different concentrations into the well (100ul)



3. Place immediately in a plate reader to collect the data

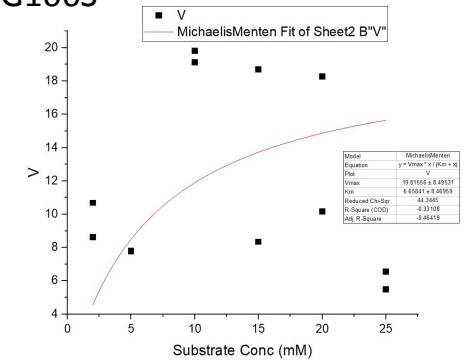
4. Analyze the data using Origin application

Enzyme Kinetics

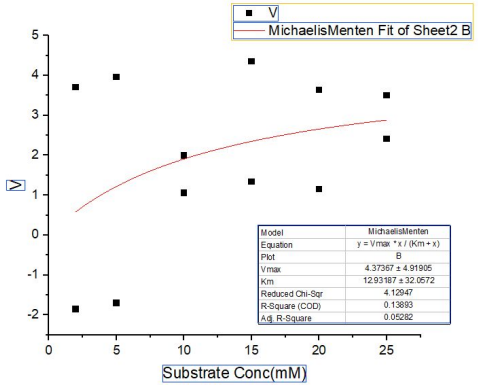


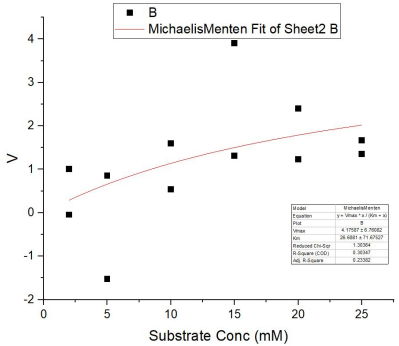
Forrest
(Original strain)

G106S



S44F





A149T

Conclusion

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

Mutants show an increased Km and Vmax

These values suggest that there is a decreased affinity for the substrate in these mutated strains

Reasoning for this conclusion:

- Mutations near the binding site for the substrate or a coenzyme (PLP) can case the protein to lose function
- In this case, loss of function for folate metabolism could be helpful

Discussion and Limitations

Difficulties with Enzymatic Assays

- Replication essential for accurate data
- High margin of error
- Several possible unknown variables
- Difficulties with substrate insolubility

Future work:

I hope to continue my work with Enzyme Kinetics and will continue to work on other mutations of SHMT8, as well as perfecting enzymatic assay skills

References

Soybean Cyst Nematode. CropWatch. (2019, July 22). https://cropwatch.unl.edu/plantdisease/soybean/soybean-cyst-nematode.

Syngenta. (n.d.). *How Costly is Soybean Cyst Nematode?* How Costly is Soybean Cyst Nematode? | Syngenta Thrive. https://www.syngenta-us.com/thrive/production/scn-yield-loss.html#:~:text=By%20some%20estimates%2C%20soybean%20cyst,than%20128%20million%20bushels%20annually.

Yokoyama, D. M. (2018, August 23). *What is an Enzyme Cofactor?* News. https://www.news-medical.net/life-sciences/What-is-an-Enzyme-Cofactor.aspx.

Ducker, G. S., Ghergurovich, J. M., Mainolfi, N., Suri, V., Jeong, S. K., Li, S. H.-J., Friedman, A., Manfredi, M. G., Gitai, Z., Kim, H., & Rabinowitz, J. D. (2017, October 24). *Human SHMT inhibitors reveal defective glycine import as a targetable metabolic vulnerability of diffuse large B-cell lymphoma*. PNAS. https://www.pnas.org/content/114/43/11404.

Nonaka, H., Nakanishi, Y., Kuno, S., Ota, T., Mochidome, K., Saito, Y., Sugihara, F., Takakusagi, Y., Aoki, I., Nagatoishi, S., Tsumoto, K., & Sando, S. (2019). Design strategy for serine hydroxymethyltransferase probes based on retro-aldol-type reaction. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-08833-7

Korasick, D. A., Kandoth, P. K., Tanner, J. J., Mitchum, M. G., & Beamer, L. J. (2020). Impaired folate binding of serine hydroxymethyltransferase 8 from soybean underlies resistance to the soybean cyst nematode. *Journal of Biological Chemistry*, *295*(11), 3708–3718. https://doi.org/10.1074/jbc.ra119.012256