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Functional Studies of Dessferoxiame D, an NIS Synthetase

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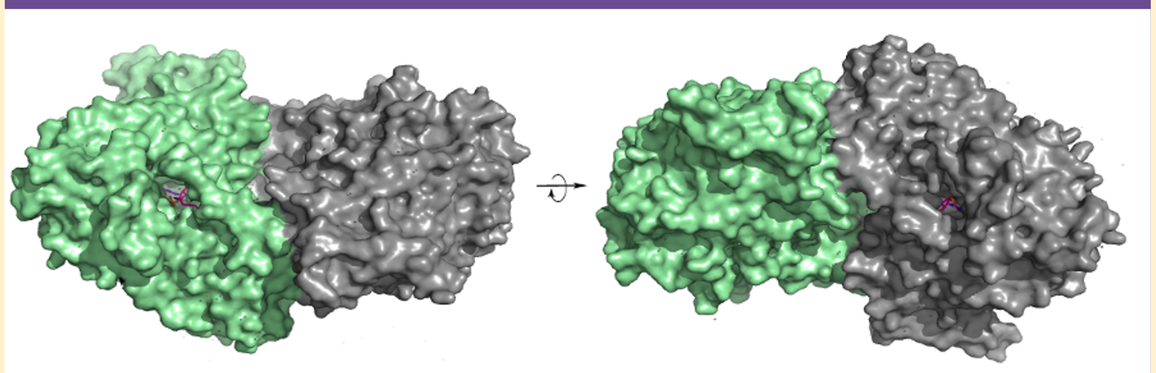
Introduction

A lack of essential iron leads to secretion of siderophores, small molecule metal chelators, by bacteria. Siderophores have high affinity for ferric iron (Fe3+) outcompeting host chelators and making them virulence factors in infection. NIS synthesized siderophores are increasingly associated with virulence in pathogenic bacteria and evade the human immune response during infection.There are three types of siderophores in the bacteria: desferrioxamine B, desferrioxamine E, and coelichelin. Desferrioxamines B and E are processed through the same NIS pathway containing four enzymes: DesA, DesB, DesC, and DesD. Of these, DesD is a hallmark NIS synthetase: a new family of enzymes unique to bacterial NIS siderophore synthesis, and a promising antibiotic target.

Kinetics is the scientific study of rates of chemical reactions. Three different assays have been used to find the kinetic constants, continuous coupled AMP turnover assay, a hydroxylamine assay and the new kinITC assay. The continuous- coupled AMP assay is successful in determining ATP to AMP reaction production but is limited by the rate of the reporter, pyrophosphate. The hydroxylamine assay is effective in reporting the carboxylate specificity of various substrates but is unable to be used on all enzymes. The new kinITC uses an ITC (Isothermal Titration Calorimetry) to obtain the kinetic constants.



Purpose of Study

The purpose of the study was to compare the kinetic constants using a variety of different techniques.

Research Question

Will the kinetic constants from varies different NIS Synthetases vary based on the technique used to obtain them?

Results

DesD is the first enzyme to use the new kinetic ITC assay and the kinetic constants obtained using this technique differed from kinetic constants obtained using a hydroxylamine assay. Table 1 shows kinetic constants from 5 different NIS Synthetases and what technique was used to obtain these kinetic constants. The general kinetic constants for NIS Synthetases range from k_{cat}= 0.28- 2,200 s^{-1}, K_M= 130- 25,000 uM, and k_{cat}/K_M= 11- 150,000 $M^{-1}s^{-1}$. The kinetic constants for Type A NIS Synthetases have a wide range from k_{cat}=0.28- 2200 s^{-1}, K_M=130-14,700 uM, and k_{cat}/K_M= 11-150,000 $M^{-1}s^{-1}$ while the kinetic constants for Type C range from k_{cat}= 0.42- 11.1 s^{-1}, K_M= 170- 5,650 uM, and k_{cat}/K_M=11- 32,200 $M^{-1}s^{-1}$. The general kinetic constants for dimers are k_{cat}= 0.89- 2200 s^{-1}, K_M= 232- 14,700 uM, and k_{cat}/K_M=158- 150,000 $M^{-1}s^{-1}$ while the kinetic constants for tetramers are k_{cat}= 0.28- 0.58 s^{-1}, K_M=130- 1,900 uM, and k_{cat}/K_M=11- 3,200 $M^{-1}s^{-1}$.

Protein	k_{cat} (s^{-1})	K_M (M)	k_{cat}/K_M ($M^{-1}s^{-1}$)	Technique
AsbB	0.89 ± 0.07	$5.65 \times 10^{-3} \pm 1.6 \times 10^{-3}$	158	Continuous-Coupled AMP Turnover
AcsD	2200	$1.47 \times 10^{-2} \pm 2.00 \times 10^{-6}$	1.5×10^5 M^{-1} s^{-1}	Continuous-Coupled AMP Turnover/ Hydroxylamine
IucA (ATP)	0.43 ± 0.02	$1.30 \times 10^{-4} \pm 3.00 \times 10^{-5}$	3200	Hydroxylamine
IucA (citrate)	0.35 ± 0.02	$1.80 \times 10^{-4} \pm 3.00 \times 10^{-5}$	1900	Hydroxylamine
IucA (hydroxylamine)	0.32 ± 0.02	$1.50 \times 10^{-2} \pm 6.00 \times 10^{-3}$	22	Continuous-Coupled AMP Turnover
IucA (N^6 -acetyllysine)	0.28 ± 0.02	$2.50 \times 10^{-2} \pm 6.00 \times 10^{-3}$	11	Continuous-Coupled AMP Turnover
IucC (ATP)	0.42 ± 0.02	$1.70 \times 10^{-4} \pm 2.00 \times 10^{-5}$	2,500	Hydroxylamine
IucC (Ciityl-ahLys)	0.50 ± 0.01	$1.32 \times 10^{-3} \pm 8.0 \times 10^{-5}$	380	Hydroxylamine
IucC (ahLys)	0.58 ± 0.02	$1.90 \times 10^{-3} \pm 2.0 \times 10^{-4}$	300	Hydroxylamine
DesD (ATP)	7.48 ± 0.6	$2.32 \times 10^{-4} \pm 3.00 \times 10^{-5}$	32,200 ± 600	kinITC
DesD (dfoG)	11.1 +/- 0.2	$3.5 \times 10^{-4} \pm 1.00 \times 10^{-5}$	32,000 ±1,000	kinITC

Conclusion

No correlation was found between the kinetics and oligomerization and or subtype, however, all ranges of kinetics were wide. Furthermore, there is a need for a standardized method to obtain kinetics and the label-free kinetics assay is the best suited kinetic assay as it does not have limitations compared to its counterparts.

For future work it is planned to test the claim the that DesD has relatively broad substrate tolerance. An ITC will a be used to test the binding affinity of different substrates and their respective analogs. An example of the kinetics curve obtained using an ITC can be seen in Figure 2. The experiments will be performed using Microcal PEAQ ITC analysis software to determine binding affinity.

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