Expression and Purification of a Radical S-adenosyl-L-Methionine Enzyme

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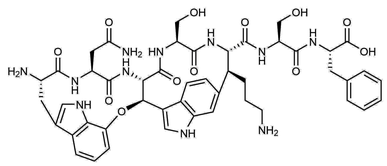
Abstract

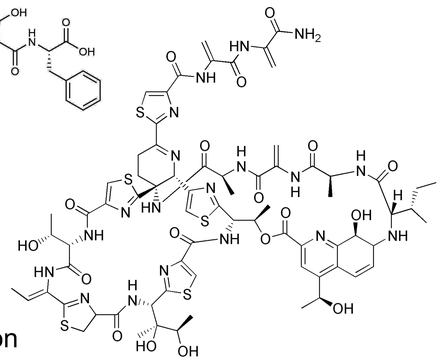
With antibiotic resistance on the rise, new antibiotics are in high demand. Many naturally occurring antibiotics are biologically synthesized by radical S-adenosyl-L-methionine (rSAM) enzymes. Our goal is to gain a better understanding of how rSAM enzymes synthesize naturally occurring antibiotics to uncover new routes for creating synthetic mimics that have antimicrobial activity. In this study, we expressed and purified a rSAM enzyme using *Escherichia coli*. Successful enzyme production lays a foundation for further studies on the biosynthesis of antibiotics.

Background

With antibiotic resistance being on the rise, there is a growing need for new antibiotics; this is especially true for antibiotics that target Gram-negative bacteria (Payne et al. 2007). New antibiotics have been discovered by screening microorganisms that share a need for antibiotics similar to our own (Imai et al. 2019). In nature, rSAM enzymes are involved in many biosynthetic pathways of antibiotics in nature, such as darobactin (Nguyen et al. 2022) and thiostrepton (Knox et al. 2022). Understanding how exactly rSAM enzymes work can help uncover new routes for producing antibiotics that are useful for humans. In this study, we used *Escherichia coli* as a host to express a rSAM enzyme and purified the enzyme anaerobically. We characterized the purified enzyme using UV-vis spectroscopy. We also performed the ferrozine assay and the Bradford assay to quantify the iron content in the protein sample.

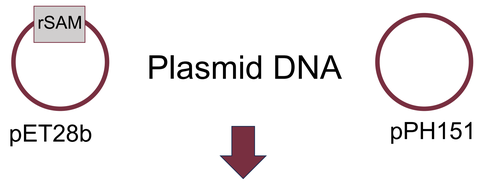


Darobactin



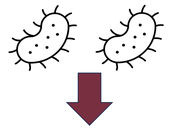
Thiostrepton

Methods



DNA Transformation

Two plasmids were co-transformed into *E. coli* BL21 (DE3) for protein expression



Protein Expression

E. coli was cultured at 37 °C and cells were harvested by centrifugation.

Protein Purification

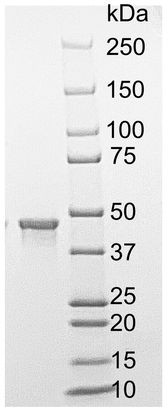
The enzyme is oxygen-sensitive because it contains iron-sulfur clusters. To maintain the integrity of these clusters, enzyme purification was performed in an anaerobic chamber.

Affinity Chromatography

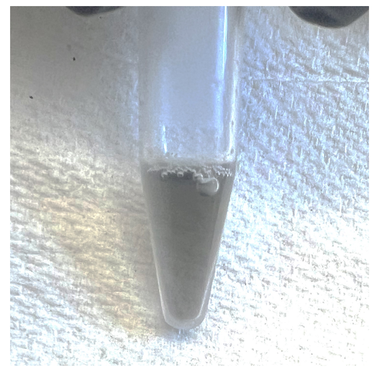
Chemical Reconstitution

Size-Exclusion Chromatography

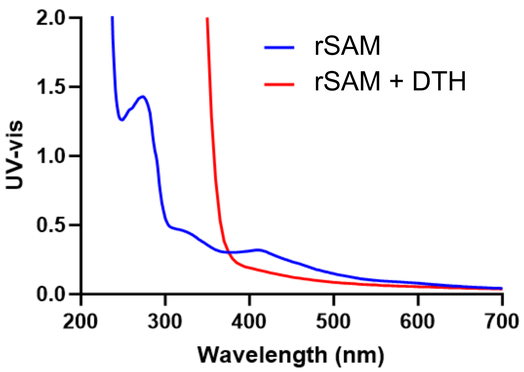
Results



Sodium dodecyl-sulfate polyacrylamide gel electrophoresis



Purified rSAM enzyme is brown because iron-sulfur clusters are colored. Iron content of rSAM = 12 ± 2 iron/protein



UV-vis spectra of rSAM enzyme and the sodium dithionite (DTH) reduced protein. The reduction of iron-sulfur clusters by DTH is shown by the missing peak at 415 nm

References

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