Synthesis of S-Adenosylmethionine Using *Escherichia coli* SAM Synthetase

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Background

S-adenosylmethionine (SAM) is an important molecule that participates in many biological processes, such as antibiotic biosynthesis in bacteria.[1] The commercially available SAM usually contains 20-30% impurity. This is because SAM isomerizes from (R,S)-configuration to (S,S)-configuration, only the former of which is biologically active. In this study, *Escherichia coli* BL21(DE3) was used to overexpress SAM synthetase, which was then used to synthesize SAM *in vitro*.[2] SAM synthetase acts as a biocatalyst, facilitating the chemical reaction between methionine and ATP. I adapted and optimized the protocol of SAM synthesis and analysis. My research offers insights into enhancing the efficiency of SAM production, thereby fostering the development of novel antibiotics in the future.

Enzymatic SAM Synthesis

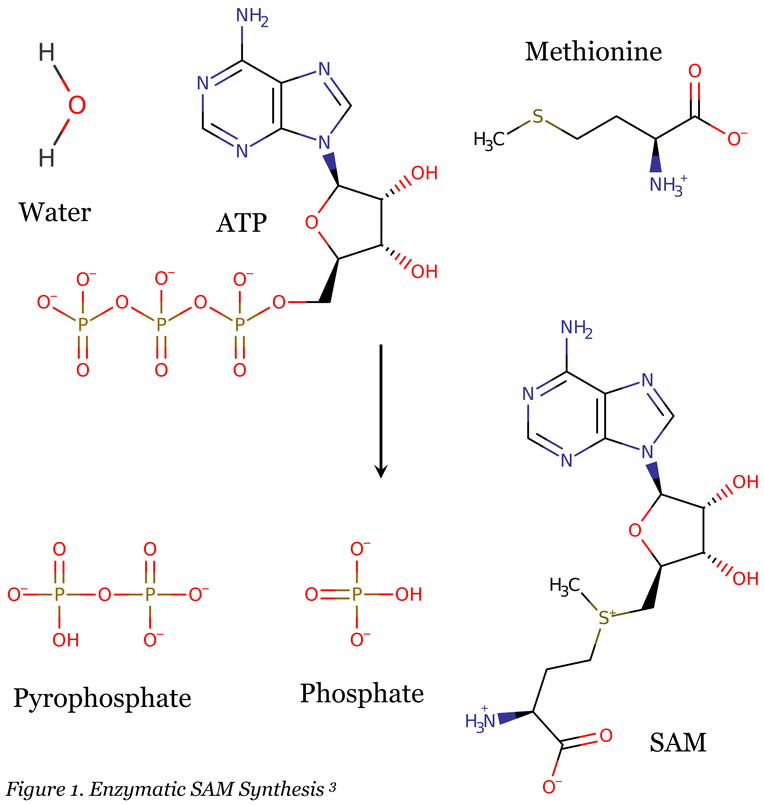
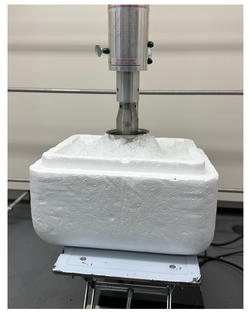


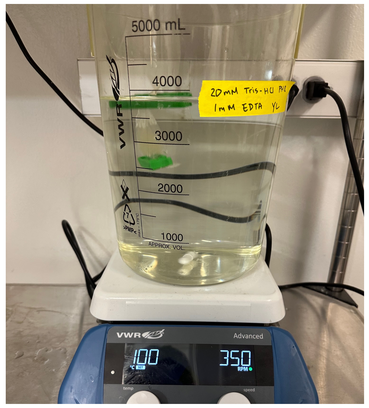
Figure 1. Enzymatic SAM Synthesis [3]

Methods

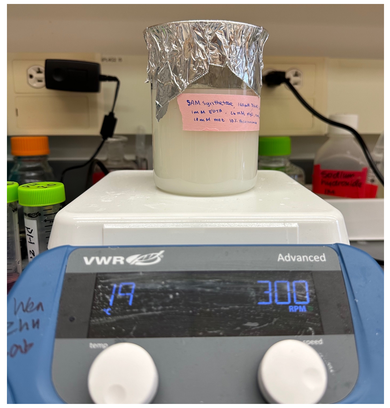
Overexpressing SAM synthetase in *E. coli* BL21(DE3)



Sonicating cell paste for dialysis

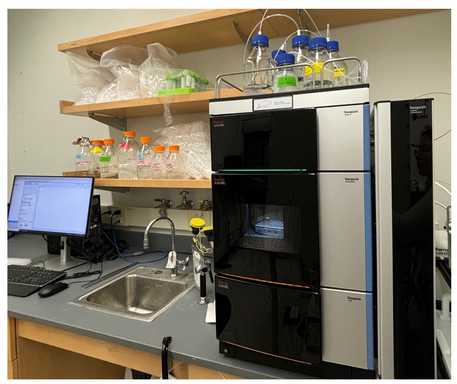


Dialysis to remove small molecule contaminates



SAM synthesis reaction

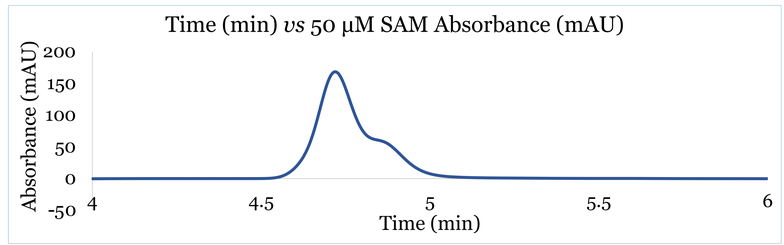
Purification of SAM using ion exchange chromatography

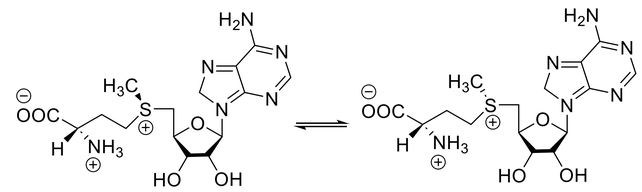


Analysis of SAM sample using high-performance liquid chromatography (HPLC)

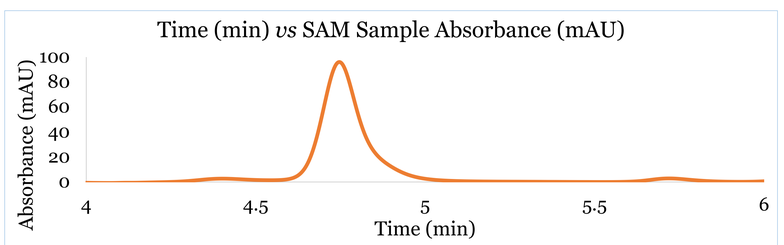
Results

HPLC was used to analyze SAM in the purified sample. The commercially available SAM (70% purity) was used as an external standard to verify the production of SAM. The major peak in the SAM standard represents the (R,S)-configuration and the shoulder peak represents the (S,S)-configuration. Notably, our HPLC results revealed a peak appearing at 4.7 min in retention time, matching the peak observed in the commercially available SAM standard. The shoulder peak in the standard, which represents the 30% impurity, was not observed in my sample. This suggests that we have successfully synthesized SAM, and our sample preparation process can provide a single diastereoisomer of SAM.





(R,S)-configuration (S,S)-configuration



References

1. Imai, Y., et al. (2019). A new antibiotic selectively kills Gram-negative pathogens. *Nature*, *576*, 459–464.
2. Young, A. P., et al. (2018). TYW1: A Radical SAM Enzyme Involved in the Biosynthesis of Wybutosine Bases. *Methods in Enzymology*, *606*, 119–153.
3. Ribeiro A. J. M., et al. (2017). Mechanism and Catalytic Site Atlas (M-CSA): a database of enzyme reaction mechanisms and active sites. *Nucleic Acids Research*, 46, D618-D623.