Bioisosteric Analogs of S-Adenosylmethionine as Potential Antibacterial SAM Riboswitch Inhibitors

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Introduction

This project was aimed to prepare stable isosteric analogs of S-adenosylmethione (SAM) whose sulfur atom is replaced by a nitrogen atom and to evaluate these analogs for the SAM riboswitch-binding activities and antibacterial activities. In bacteria, SAM binds to the SAM riboswitch, which regulates the biosynthesis of methionine and cysteine, two amino acids essential for survival. Therefore, synthetic molecules that bind to SAM riboswitches have the potential to kill bacterial cells. Three different classes of SAM riboswitches exist in bacteria (SAM I, II, and III), shown in Figure 2, and each were prepared for testing for binding to the synthesized SAM analogs.

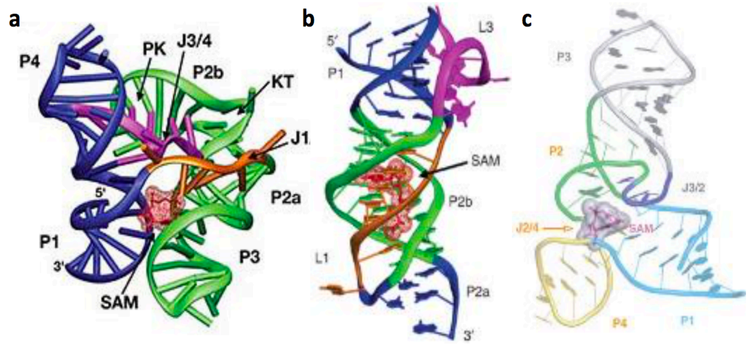

Figure 2. (a) Structures of SAM I riboswitch[3], (b) SAM II riboswitch[1], and (c) SAM III riboswitch[2]

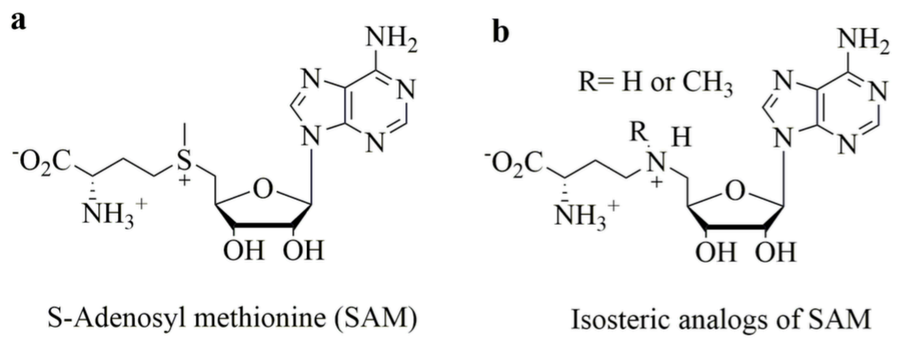

Figure 1. (a) Structures of SAM and (b) its isosteric analogs

Results

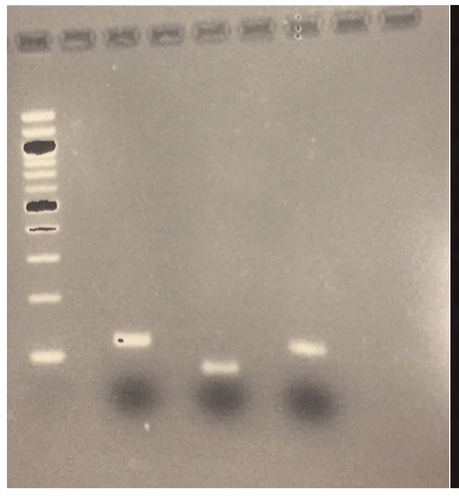

Figure 3. Gel electrophoresis of SAM riboswitch DNA molecules
Lane 1: 100 bp DNA ladder
Lane 2: SAM I riboswitch DNA (135 bp)
Lane 3: SAM II riboswitch DNA (95 bp)
Lane 4: SAM III riboswitch DNA (131 bp)

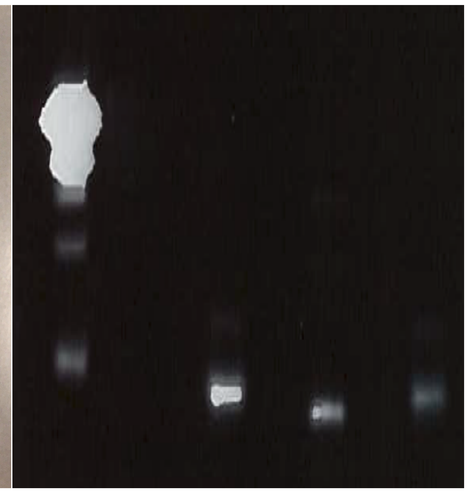

Figure 4. *In vitro* transcription of SAM riboswitch DNA molecules
Lane 1: 100 bp ladder
Lane 2: SAM I riboswitch RNA (94 bases)
Lane 3: SAM II riboswitch RNA (52 bases)
Lane 4: SAM III riboswitch RNA (89 bases)

Methods

- Each class of SAM riboswitch gene under control of T7 promoter was prepared by the overlapping extension polymerase chain reaction (PCR) of synthetic oligonucleotides
- Each SAM riboswitch gene was cloned into the pUC19 plasmid and verified by DNA sequencing
- A high concentration of each SAM riboswitch DNA was prepared by PCR
- The corresponding SAM riboswitch RNA was prepared by *in vitro* transcription using T7 RNA polymerase

Summary

Each class of SAM riboswitch was successfully prepared, cloned, and verified by DNA sequencing. High concentrations of each class of SAM riboswitch was prepared and converted to the corresponding SAM riboswitch RNA. Further studies to determine the binding of the SAM riboswitch RNA molecules to the isosteric analogs of SAM will be carried out.

1. Gilbert, S. D.; Rambo, R. P.; Tyne, D. V.; Batey, R. T. Structure of the SAM-II riboswitch bound to S-adenosylmethionine. *Nature structural and molecular biology* **2008**, *15*, 177.

2. Lu, C.; Smith, A. M.; Fuchs, R. T.; Ding, F.; Rajashankar, K.; Henkin, T. M.; Ke, A. Crystal structures of the SAM-III/Smk riboswitch reveal the SAM-dependent translation inhibition mechanism. *Nature structural and molecular biology* **2008**, *15*, 1076.

3. Montange, R. K.; Batey, R. T. Structure of the S-adenosylmethionine riboswitch regulatory mRNA element. *Nature* **2006**, *441*, 1172.

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