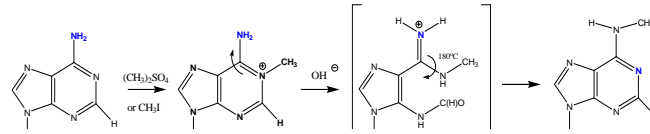


ABSTRACT

The objective of this study was to determine the mechanism of adenosine N6-dimethylallylation by *E. coli* MiaA. Bacterial MiaA (35kDa) does not contain the conserved motif found in the isopentenyltransferases, adenosine-N1 or adenosine-N6 methyltransferases, and its structure has not been solved. Our strategy was to examine the anticodon loop of the *E. coli* tRNA^{Phe} containing N6-¹⁵N labeled adenosines and the product of its reaction with MiaA. We have excluded the Dimroth rearrangement as the mechanism reaction catalyzed by MiaA and postulate direct attack of the DMAPP on the N6 group of A37-ACSL^{Phe}.

METHODS

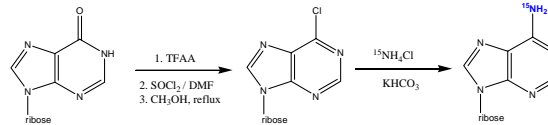
Our work focused on the mechanism of adenosine N6-dimethylallylation by *E. coli* MiaA. Studies on the chemical methylation of adenosine have shown that its reaction proceeds through the Dimroth rearrangement presented on the Scheme 3.



Scheme 3

However, the enzymatic N6-methylation of adenine catalyzed by DNA methyltransferase likely involves the direct alkylation of its amino group.

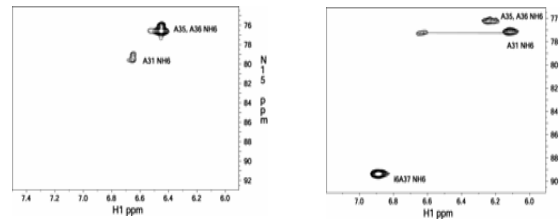
We compare structures of the anticodon loop of *E. coli* tRNA^{Phe} containing N6-¹⁵N labeled adenosines and the product of its reaction with MiaA. The synthesis of N6-¹⁵N-labeled adenosine is shown on Scheme 4.



Scheme 4

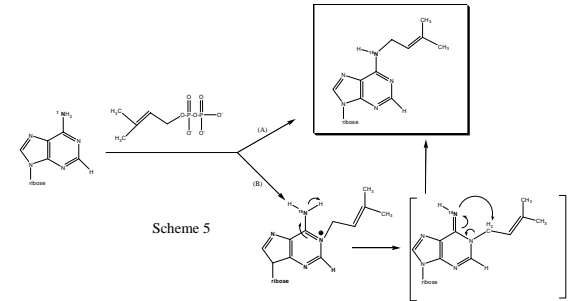
RESULTS

If dimethylallylation proceeded *via* initial attack on the N1 of A37, the Dimroth rearrangement would give N1-¹⁵N-labeled i6A37. However, 2D ¹⁵N-¹H HSQC experiments showed that the i6A37-ACSL^{Phe} contains N6-¹⁵N group instead of N1-¹⁵N.

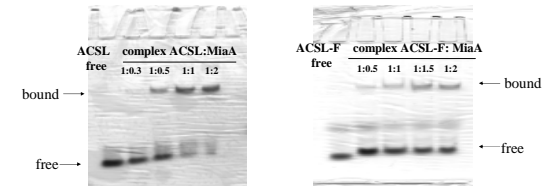
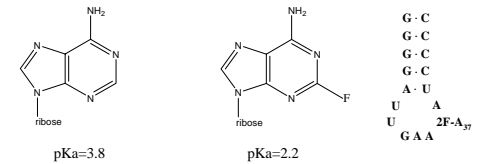


¹⁵N-¹H HSQC for the amino region of N6-¹⁵N-labeled ACSL (left) and the i6A37-ACSL (right) in the presence of Co(NH₃)₆³⁺ (1:2 molar ratio).

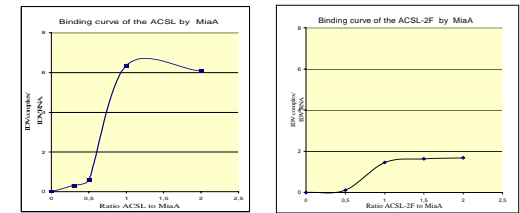
We postulate that MiaA catalyzes the direct transfer of the dimethylallyl group from DMAPP to the A37 amino group (route A on Scheme 5). Alternatively, this reaction could proceed through the initial alkylation to N1 of adenosine (route B on Scheme 5) followed by migration of dimethylallyl group to N6 to give i6A37.



We used 2-fluoroadenosine with shifted N1 pKa value to assess the importance of A37 N1 group in the recognition and binding of ACSL by MiaA. We found that 2-fluoroadenosine modified ACSL is recognized as a substrate by the enzyme.



Non-denaturing PAGE stained with Stains-All for the ACSL and its complex with MiaA (left); and ACSL-2F and its complex with MiaA (right).



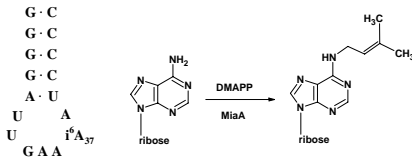
SUMMARY

We excluded the Dimroth rearrangement as the mechanism reaction catalyzed by MiaA and postulate direct attack of the DMAPP on the N6 group of A37-ACSL^{Phe}. Kinetics studies showing binding of the modified ACSL (variants with acidic N1 pKa and alkaline N1 pKa) by MiaA are progress.

INTRODUCTION

The N6-dimethylallyl modified adenosine (i6A) occurs in position 37 of the anticodon loop in prokaryotic and eukaryotic tRNAs. i6A37 and its derivatives are required for the efficient interaction of tRNA with ribosomes and they prevent misreading of the genetic code.

The DMAPP: tRNA dimethylallyltransferase (MiaA, 35kDa) is responsible for the A37 modification and utilizes dimethylallyl pyrophosphate (DMAPP) as the isopentenyl group donor (Scheme 1).



Scheme 1

Kinetic analysis of the MiaA catalyzed reaction has been carried out using variants of full length tRNA^{Phe} 1

G · C	G · C	G · C	G · C
G · C	G · C	G · C	G · C
G · C	G · C	G · C	G · C
G · C	G · C	G · C	G · C
A · U	U · A	C · G	A · U
U A	U A	U A	U A
U A ₃₇	U A ₃₇	U A ₃₇	U A ₃₇
G A A	G A A	G A A	G A A
Wild Type ^{Phe}	U ₁₇ -A ₃₈	C ₁₇ -G ₃₈	G ₃₇

k_{cat} (s ⁻¹)	0.114±0.008	0.0234±0.017	0.029±0.002
K_M (nM)	11.0±1.7	100±8	149±19
K_{cat}/K_M	1.09±0.11	0.0236±0.001	0.0198±0.002

G/C · C/G
G/A · C/U
G · C
A/u · U/a
C/U · A
U · ms ^{2,6} A ₃₇
N N A ₃₆