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Mechanistic Studies of the tRNA-Modifying Enzyme QueA: A Chemical Imperative for the Use of AdoMet as a "Ribosyl" Donor

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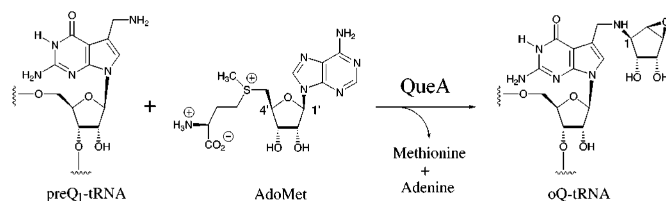
ABSTRACT



The enzyme *S*-adenosylmethionine:tRNA ribosyltransferase-isomerase (QueA) catalyzes the penultimate step in the biosynthesis of the tRNA nucleoside queuosine, a unique ribosyl transfer from the cofactor *S*-adenosylmethionine (AdoMet) to a modified-tRNA precursor. The use of AdoMet in this way is fundamentally new to the chemistry of this important biological cofactor. We report here the first mechanistic studies of this remarkable enzyme, and we propose a chemical mechanism for the reaction consistent with our experimental observations.

Posttranscriptional processing of transfer RNA (tRNA) produces a rich variety of structurally modified nucleosides that typically make up ∼10% of the nucleosides in a particular tRNA, and in some cases constitute over 25% of the nucleosides.[1] The nature of nucleoside modification varies from simple methylation of the base or ribose ring to extensive "hypermodification" of the canonical bases, the latter of which is characterized by radical structural changes and can involve multiple enzymatic steps to complete. Arguably the most remarkable modified nucleoside known to occur in tRNA is queuosine (Q, Figure 1), a 7-deazaguanosine containing an aminomethyl cyclopentendiol side chain. Queuosine and its derivatives occur exclusively at position 34 (the wobble position) in the anticodons of bacterial and eukaryotic tRNAs coding for the amino acids asparagine, aspartic acid, histidine, and tyrosine.[2] The enzyme *S*-adenosylmethionine:tRNA ribosyltransferase-isomerase (QueA) catalyzes the penultimate step in the biosynthesis of queuosine (Figure 1), an unprecedented transfer and isomerization of the ribose moiety from *S*-adenosylmethion-

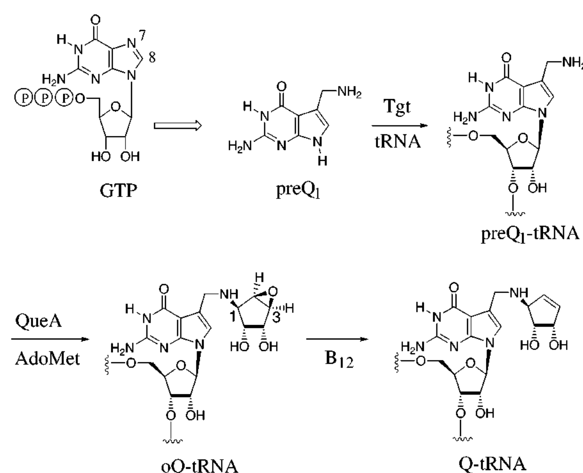


Figure 1. The biosynthesis of queuosine in bacteria.

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ine (AdoMet) to the appropriate 7-aminomethyl-7-deazaguanosine ($preQ_1$) modified tRNAs, a process that includes the elimination of both methionine and adenine from AdoMet and the rearrangement of the ribosyl moiety to form an epoxy-carbocycle.[3,4]

A definitive picture of the biochemical function or functions of queuosine has yet to emerge, but it has been correlated with eukaryotic cell development and proliferation,[5] neoplastic transformation,[5b,6] tyrosine biosynthesis in animals,[7] translational frameshifts essential to retroviral protein biosynthesis,[8] and the ability of pathogenic bacteria to invade and proliferate in human tissue.[9] Underlying most, if not all, of these phenomena is a role in modulating translational fidelity, consistent with queuosines location in the anticodon. However, evidence is also accumulating which implicates queuosine (or the free base queuine) as a modulator of signal transduction,[10] potentially by tRNA-independent mechanisms.

In the de novo biosynthesis of queuosine, GTP appears to be the principal precursor,[11] which in an ill-defined series of steps is converted to $preQ_1$[12] (Figure 1). Carbon-8 (and possibly N-7) of guanine are lost in this transformation,[11] which may have important parallels to the biosynthesis of the 7-deazaadenine nucleoside antibiotics (e.g., toyocamycin),[13] as well as the pterins and folic acid.[14] $preQ_1$ is subsequently inserted into the appropriate tRNA's by the enzyme Tgt[15] in a transglycosylation reaction in which the genetically encoded guanine is eliminated. Addition of the cyclopentenediol ring then occurs in an *S*-adenosylmethionine-dependent step by the enzyme QueA to give epoxyqueuosine (oQ),[3,16] which in an apparent B_{12}-dependent step is reduced to queuosine.[17]

In considering plausible mechanisms for the conversion of the ribosyl ring of AdoMet to the epoxycyclopentandiol ring of oQ, the paradigm of enzyme-catalyzed transglycosylation reactions[18] makes nucleophilic addition at C-1′ of AdoMet by the $preQ_1$ methylamine an obvious and attractive process. Indeed, a mechanism has been proposed in which $preQ_1$ addition occurs at C-1′ as the initial step.[4] Such a process establishes direct correspondence between C-1′ of AdoMet and C-1 of the epoxycyclopentandiol ring of oQ (numbering in oQ is that employed in ref 16). If one assumes, however, that the *cis*-diol present in AdoMet remains unchanged in the conversion to oQ, then the absolute stereochemistry of oQ and AdoMet renders C−N bond formation at C-1′ of AdoMet untenable (Figure 2; path A).

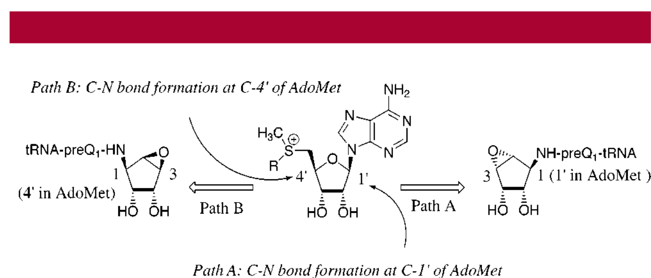

Figure 2. Regio- and stereochemical constraints on carbon−nitrogen bond formation in the QueA-catalyzed conversion of AdoMet to oQ when it is assumed that the diol remains unchanged in the conversion of AdoMet to oQ. The correct chemical structure of the epoxycarbocyclic ring of oQ is shown on the left (see also Figure 1). Path A: The relative stereochemistry of the newly formed C−N bond is shown anti to the diol since transglycosylations can occur with retention or inversion of stereochemistry,[18] and the relative stereochemistry is known to be anti. The relative stereochemistry of the epoxide is syn to the diol due to the *S* absolute stereochemistry at C-4′ of AdoMet. Note however that if the stereochemistry at C-4′ inverts, the epoxycarbocycle formed would be *enantiomeric* to the epoxycarbocycle found in oQ. Path B: Both the newly formed C−N bond and the epoxide are shown anti to the diol since bonding changes occur at the relevant carbons.

Instead, C-4′ of AdoMet is implicated as the site of C−N bond formation (Figure 2; path B). Establishing the regiochemistry of C−N bond formation is therefore fundamental to any serious exploration of the mechanism of the reaction. This issue can be addressed directly through the utilization of [^{13}C]AdoMet specifically labeled in the ribose ring in the QueA-catalyzed reaction, followed by identification of the isotopically enriched carbon in the oQ product.

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The commercial availability of [1-^{13}C]ribose led us to choose to locate the ^{13}C-label at C-1′ of AdoMet. The synthesis utilized standard carbohydrate/nucleoside chemistry[19] and was accomplished in eight steps in an overall yield of 5%. QueA was overexpressed and purified as a GST-fusion protein as previously described.[3] The RNA substrate[3] was prepared by in vitro runoff transcription[20] using a template corresponding to the complement of the anticodon stem-loop sequence of *Escherichia coli* tRNA^{Asn} (5′-GCGGACUGUUAAUCCGC-3′). Modification of the RNA by incorporation of preQ$_1$ (synthesized as described)[21] into the appropriate position of the RNA was carried out in situ by reaction with recombinant *E. coli* Tgt, which catalyzes the base exchange reaction in vivo.[15] Recombinant Tgt was obtained after amplification of the *tgt* gene from the plasmid pHH1,[22] cloning into pET-11a, and overexpression in *E. coli* BL21(DE3). Purification of the recombinant enzyme was achieved by ion-exchange chromatography. Following the Tgt/QueA reaction, the RNA was digested, the mononucleotides dephosphorylated, and the [^{13}C]oQ isolated by HPLC. Using the extinction coefficient for the 260 nm absorption of 7-deazaguanosine,[23] approximately 22 μg of oQ was isolated.

Because the ^{13}C NMR spectrum of oQ has not been reported, the location of the isotopically enriched carbon was determined through the effect of ^{1}H−^{13}C coupling on the ^{1}H NMR spectrum; specifically through a difference spectrum of the ^{1}H−^{13}C decoupled and coupled 1-D ^{1}H NMR spectra. NMR analysis was carried out in D_2O on a Bruker AMX 400 instrument operating with water suppression, with acetone added as a chemical shift reference (2.22 ppm).[24] Clearly visible in the difference spectrum (Figure 3B) is the resonance of the hydrogen directly bonded to the ^{13}C-enriched site, which shows a positive peak at 3.77 ppm arising from the decoupled spectrum and flanking negative peaks at 3.53 and 4.01 ppm (J_{C-H} = 193 Hz) arising from the coupled spectrum.

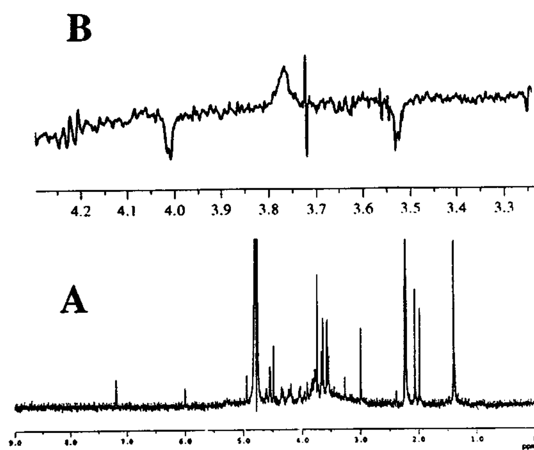
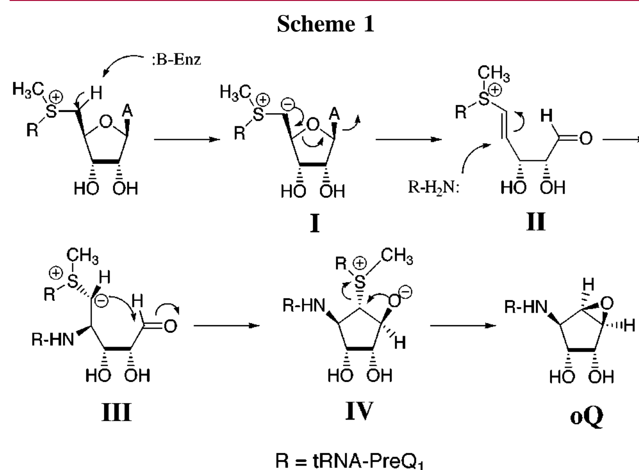


Figure 3. 1-D ^{1}H NMR spectra of [^{13}C]oQ generated from [1′-^{13}C]AdoMet. Spectra were acquired at 400.14 MHz with a spectral width of 5618 Hz using a Bruker AMX 400 MHz spectrometer. Carbons were decoupled with a GARP sequence centered at 100.61 Hz. Coupled and decoupled spectra were acquired simultaneously, with 2048 scans collected for each. (A) The carbon−hydrogen coupled ^{1}H NMR spectrum. The carbon−hydrogen decoupled spectrum (not shown) was qualitatively identical to the coupled spectrum. (B) A portion of the ^{1}H NMR difference spectrum (decoupled minus coupled) showing the resonance of the hydrogen directly bonded to the ^{13}C-enriched carbon.

Three hydrogens on the epoxycyclopentandiol ring have resonances in the immediate vicinity of 3.77 ppm: the two epoxide hydrogens at C-2 and C-3 (with chemical shifts of 3.71/3.76 ppm) and the hydrogen at C-5 (3.80 ppm).[16] Importantly, C-1, which is bonded to the nitrogen, and C-4 are both immediately excluded as possible sites for the ^{13}C-label since the chemical shifts for the corresponding hydrogens are significantly upfield (C-1, 3.37 ppm)[16] and downfield (C-4, 4.29 ppm)[16] of the observed resonance. Of the remaining carbons, C-5 can be excluded as the site of enrichment on the basis of the magnitude of the C−H coupling constant (J_{C-H} = 193 Hz), which is indicative of a C−H bond with substantial s-character, consistent with an epoxycyclopentane C−H bond but not a hydroxylcyclopentane C−H bond.[25] Although the chemical shifts of the epoxide hydrogens were not differentiated in the original structural elucidation of oQ,[16] on the basis of the substituent effects of the adjacent heteroatoms,[25] the C-2 hydrogen resonance is expected to be the upfield resonance (3.71 ppm) given its position adjacent to the C-1 alkylamine, with the resonance at 3.76 ppm due to the C-3 hydrogen as a result of the more electronegative hydroxyl at C-4. Thus, the ^{1}H NMR data supports C-3 as the site of ^{13}C-enrichment but does not rigorously rule out C-2.

While mechanistic paths to either [3-^{13}C]oQ or [2-^{13}C]oQ from [1′-^{13}C]AdoMet can be envisioned, in keeping with the maxim of Occam's razor, the route to [3-^{13}C]oQ is more reasonable as this does not require any reorganization of the diol moiety in the conversion of AdoMet to oQ (Figure 2). Proposed in Scheme 1 is a mechanism which is consistent with our data and the analysis presented in Figure 2. Initial enzyme-catalyzed deprotonation of AdoMet at C-5′ generates the sulfonium ylide **I**, which can collapse to the vinyl sulfonium **II** by opening of the ribose ring with concomitant elimination of adenine. Addition of the preQ$_1$ amine to the

Scheme 1



re-face of C-4′ (C-1 in oQ numbering) then generates the new sulfonium ylide **III**, which can subsequently attack the *re*-face of the C-1′ (C-3 in oQ numbering) aldehyde to give alkoxy-carbocycle **IV**. Intramolecular S_N2 attack of the alkoxy oxygen on the adjacent carbon with elimination of methionine then gives oQ.

The mechanism proposed in Scheme 1 provides a logical series of chemical steps to account for the observed reaction, and good chemical precedence exists for the basic features of the proposal.[26,27] Indeed, a considerable chemical literature exists on the formation of epoxides from the condensation of sulfonium ylides with carbonyls, and examples of nucleophilic addition to vinyl sulfonium species followed by intramolecular condensation of the resulting ylide with a carbonyl to generate an epoxide have been described.[26] It has also been proposed that deprotonation at C-5′ of AdoMet followed by ring opening to vinyl sulfonium **II** and loss of adenine is the chemical basis for the conversion of AdoMet to pentosylmethionines in alkaline solution.[28] Importantly, this mechanistic proposal reveals a chemical imperative for the unusual utilization of AdoMet as a ribosyl donor, namely, the increased acidity of the 5′-methylene hydrogens of AdoMet ($pK_a \approx 23$) due to the adjacent sulfonium ion.[26]

In conclusion, the use of AdoMet as a "ribosyl" donor in the QueA-catalyzed reaction is fundamentally new to the biochemistry of this important cofactor. The results of our experiments implicate C-3 of oQ as the site of isotopic enrichment arising from [1′-^{13}C]AdoMet, which is consistent with C−N bond formation at C-4′ of AdoMet, a conclusion further supported by mechanistic economy. We are currently engaged in additional experiments that address specific issues raised by our proposed mechanism and will report the results of this work in due course.

Acknowledgment. We thank Dr. Helga Kersten (Universität Erlangen) for plasmids pHH1 and pGEX-QueA and for her enthusiastic support of our work and Dr. David Peyton (Portland State University) for technical expertise with the NMR experiments. Acknowledgment is made to the NSF (MCB-9733746) and to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for generous financial support of this work.

Supporting Information Available: Detailed experimental conditions for enzyme reactions and purification of oQ (including sample HPLC and UV traces). This material is available free of charge via the Internet at http:/pubs.acs.org.

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