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# Catalysis of a New Ribose Carbon-Insertion Reaction by the Molybdenum Cofactor Biosynthetic Enzyme MoaA

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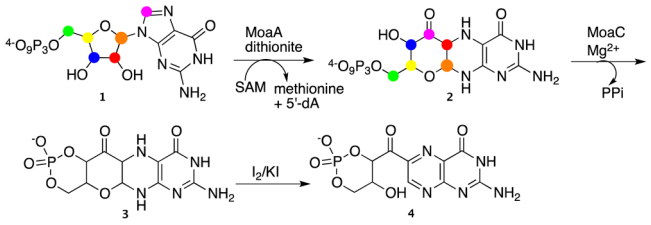
Supporting Information

**ABSTRACT:** MoaA, a radical *S*-adenosylmethionine enzyme, catalyzes the first step in molybdopterin biosynthesis. This reaction involves a complex rearrangement in which C8 of guanosine triphosphate is inserted between C2′ and C3′ of the ribose. This study identifies the site of initial hydrogen atom abstraction by the adenosyl radical and advances a mechanistic proposal for this unprecedented reaction.

Molybdenum is a required nutrient for plants, animals, and microorganisms and is involved in many redox reactions implicated in the global carbon, sulfur, and nitrogen cycles.[1,2] With the exception of nitrogenase, all molybdenum-requiring enzymes use molybdopterin as the metal binding ligand. Nitrate reductase, sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase are well-known molybdopterin-dependent enzymes. The biosynthesis of molybdopterin is similar in plants, animals, and microorganisms where the first steps utilizing guanosine 5′-triphosphate (**1**, GTP) as the precursor are catalyzed by the enzymes MoaA and MoaC (Scheme 1). MoaA is a member of the

**Scheme 1. Reactions Catalyzed by MoaA and MoaC**[a]




[a]MoaA catalyzes the rearrangement of GTP **1** to **2** in an adenosyl radical-dependent reaction involving the insertion of C8 of guanine between C2′ and C3′ of the ribose. MoaC catalyzes the formation of **3**. Also shown in the figure is the oxidation of **3** to **4** by $I_2$/KI. This compound is fluorescent and is more stable than **3**.

radical-*S*-adenosylmethionine (SAM) superfamily of proteins and harbors two [4Fe-4S] clusters. The N-terminal [4Fe-4S] cluster is likely to be responsible for the reductive cleavage of SAM to the 5′-deoxyadenosyl (5′-dA) radical and L-methionine,[3,4] and the second cluster binds to N1 of GTP.[5] Upon generation of the 5′-dA radical, GTP is transformed into **2** via a complex rearrangement reaction in which C8 of the purine is inserted between C2′ and C3′ of the ribose moiety (Scheme 1). This is an unprecedented reaction in ribose chemistry. MoaC then catalyzes the intramolecular cyclization reaction of **2** to **3**, which is oxidized to **4** prior to analysis because of the instability of **3**.[4] The goal of this study was to obtain mechanistic insights regarding the rearrangement reaction catalyzed by MoaA by identifying the initial site of hydrogen abstraction on GTP by the 5′-dA radical.

The 5′-dA (**5**) generated in the MoaA-catalyzed reaction was first isolated and characterized. The high-performance liquid chromatography (HPLC) chromatograms in Figure 1A were obtained from anaerobic reaction mixtures containing MoaA (125 mM), GTP (1 mM), SAM (1.5 mM), and sodium dithionite (excess). The blue trace corresponds to data from the assay in which all the components were present; the other traces are controls from which MoaA, SAM, or sodium dithionite was omitted.

The product 5′-dA, eluting at 14.5 min, is formed only in the reaction mixture with all components present. ESI-MS analysis (Figure 1B) and nuclear magnetic resonance (NMR) analysis (Figure S9 of the Supporting Information) confirmed the identity of the 14.5 min eluting compound.

Several (Figure 1D) site specifically deuterium-labeled GMP isotopomers were synthesized and converted to GTP in situ using guanylate kinase, nucleoside diphosphate kinase, and ATP/$Mg^{2+}$ (Figures S2−S7 of the Supporting Information). The transfer of deuterium from labeled GTP to the 5′-dA was monitored by liquid chromatography and mass spectrometry (LC−MS) (Figure S1 of the Supporting Information). Analysis of the 5′-dA generated using universally labeled GTP (i.e., deuterium on all nonexchangeable sites) reveled an increase of a single mass unit ([M + H] − 253.1 Da), demonstrating that only one of the deuterium atoms was incorporated into 5′-dA. No deuterium incorporation was observed with [8-$^2$H]GTP, demonstrating that the deuterium was derived from the ribose. No deuterium incorporation was observed with [2′-$^2$H]GTP, and a single deuterium incorporation was observed with [3′,4′,5′,5′-$^2H_4$]GTP, further localizing the site of deuterium incorporation to C3′, C4′, or C5′ of the ribose. The final

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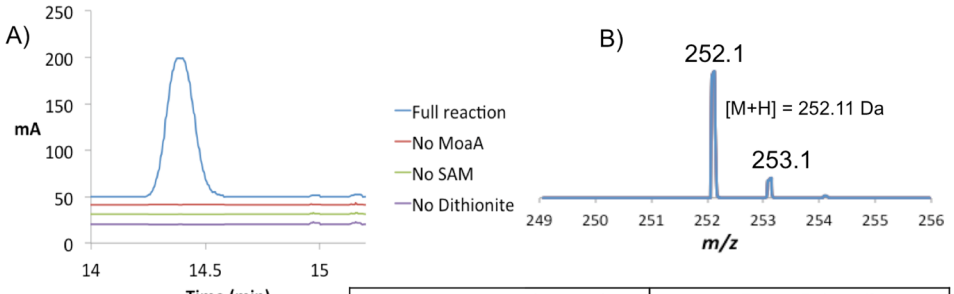


D)

| Deuterated analogs of GTP | LCMS – [M+H] for 5′-deoxyadenosine (Da) |
|---|---|
| U-$^2H_{10}$-GTP | 253.1 |
| 3′,4′,5′,5′-$^2H_4$-GTP | 253.1 |
| 2′-$^2$H -GTP | 252.1 |
| 8-$^2$H –GTP | 252.1 |
| 3′-$^2$H -GTP | 253.1 |

**Figure 1.** Determination of the regiochemistry of the MoaA-catalyzed hydrogen atom abstraction from GTP. (A) HPLC analysis of the MoaA reaction mixture. The blue trace is for the reaction mixture containing MoaA, SAM, GTP, and dithionite. The red, green, and violet traces are for controls from which MoaA, SAM, and sodium dithionite, respectively, were omitted. The peak at 14.4 min is 5′-dA. All the reactions were performed under anaerobic conditions. (B) ESI-MS analysis of the 14.4 min peak indicates an ion at $m/z$ 252.1 corresponding to [M + H] for 5′-dA. (D) MoaA assays were performed with GTP isotopomers, and the resulting 5′-dA was analyzed by liquid chromatography and mass spectrometry for deuterium incorporation. [U-$^2H_{10}$]GTP is universally deuteriated GTP. The data show that a deuterium atom at C3′ of GTP is incorporated into 5′-dA, demonstrating that MoaA catalyzes the generation of a radical at C3′ of GTP.

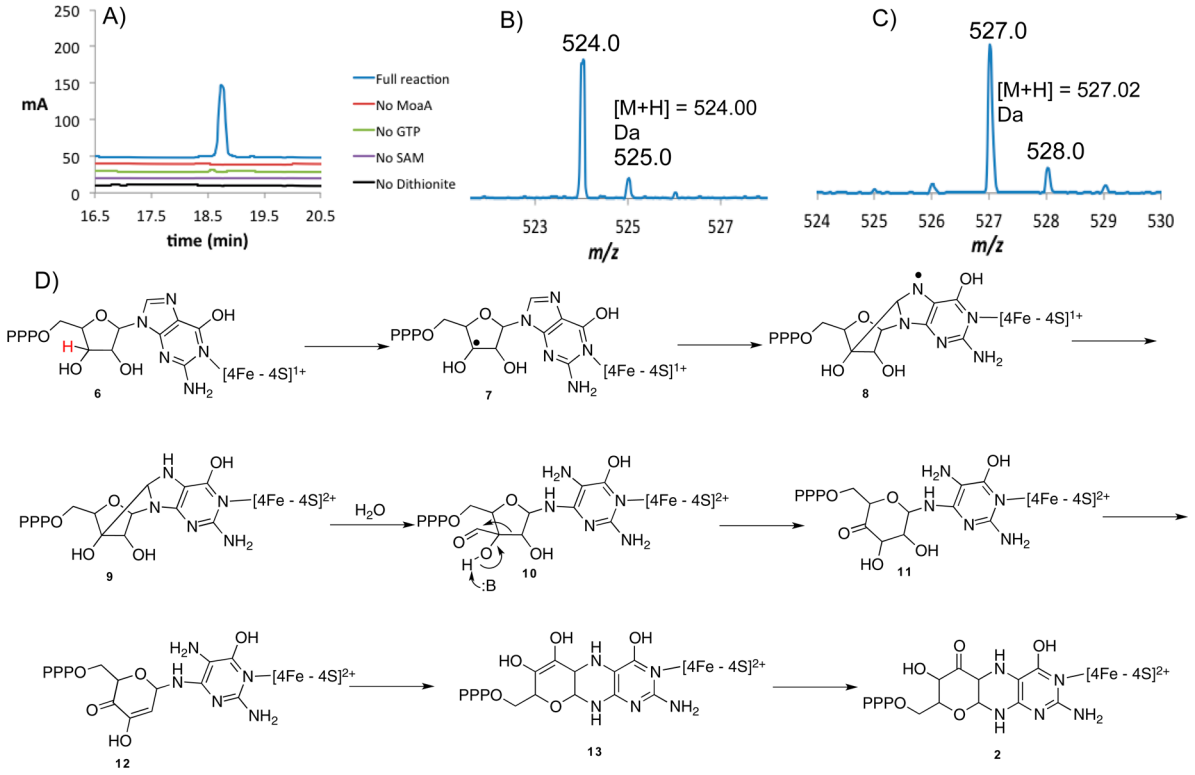


**Figure 2.** Characterization of the pterin formed in the MoaA-catalyzed reaction. (A) HPLC (320 nm) chromatogram showing the second product (blue trace, 18.6 min) formed in the MoaA-catalyzed reaction. This product was observed only in reactions in which MoaA, GTP, SAM, and sodium dithionite were present. Chromatograms from control reactions without MoaA, GTP, SAM, or dithionite are also shown (red, green, purple, or black, respectively). (B) ESI-MS of the compound eluting at 18.6 min using GTP as the substrate shows an [M + H] ion at 524.00 Da consistent with structure **2**. (C) ESI-MS of the compound eluting at 18.6 min using [3′,4′,5′,5′-$^2H_4$]GTP as the substrate shows an [M + H] ion at 527.02 Da consistent with the loss of deuterium from C3′ and retention of the three deuteria at C4′ and C5′. (D) Mechanistic proposal for the reaction catalyzed by MoaA.

localization of the hydrogen atom transferred was achieved by demonstrating the transfer of a single deuterium from [3′-$^2$H]GTP to 5′-dA.

The pterin product (**2**) of the MoaA reaction was also analyzed. This eluted after 18.6 min (Figure 2A) and was present only in the reaction mixture containing GTP, SAM, and dithionite. Because this product was oxygen sensitive, all the LC−MS buffers were degassed and the LC−MS samples were prepared in an anaerobic chamber. ESI-MS analysis revealed that


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the [M + H] ion for the signal with a retention time of 18.6 min is 524.00 Da (Figure 2B). This is consistent with structure **2**.

It was not possible to obtain an NMR spectrum of **2** because of its instability. The identity of this compound was therefore confirmed, as previously described,[6] by treatment with MoaC followed by oxidation with $I_2$/KI to give an air-stable product with structure **4** as shown by NMR analysis (Figure 1 and Figure S8 of the Supporting Information). It was not possible to analyze the pterin produced using deuteriated GTP by NMR because of the small quantities of the labeled samples available. However, when [3′,4′,5′,5′-$^2H_4$]GTP was used as a substrate, a 3 Da increase was observed to give an [M + H] ion of 527.02 Da (Figure 2C). This is consistent with abstraction of the C3′ hydrogen by the 5′-dA radical and retention of the C4′ and C5′ hydrogens in the product.

A mechanistic proposal for the unprecedented carbon-insertion reaction catalyzed by MoaA that is consistent with the labeling studies described here and the previously reported electron-nuclear double resonance and structural studies is shown in Figure 2D.[3−5] The 5′-dA radical abstracts the 3′-hydrogen atom from GTP bound to the second [4Fe-4S] cluster (**6**), resulting in radical **7** that then adds to C8 of the purine to give **8**. Reduction of this radical by the liganded cluster would give **9**. Hydrolysis of this strained system would give **10**. A benzylic-like rearrangement would give **11** completing the insertion of the purine carbon into the ribose.

Dehydration of **11** to **12** followed by conjugate addition gives **13**. A final tautomerization completes the formation of **2**. This mechanistic proposal has several testable features and is currently under investigation.

## ■ ASSOCIATED CONTENT

### Supporting Information

Detailed procedures for the synthesis of labeled GMP and GTP, HPLC methods, NMR data for **4**, and procedures for the enzymatic reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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A.P.M. and J.W.H. contributed equally to this work.


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### Notes

The authors declare no competing financial interest.

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