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Permalink
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Journal
ACS omega, 3(3)

ISSN
2470-1343

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Publication Date
2018-03-14

DOI
10.1021/acsomega.8b00197

Peer reviewed
Reorientation of the Methyl Group in MAs(III) is the Rate-Limiting Step in the ArsM As(III) S-Adenosylmethionine Methyltransferase Reaction

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Supporting Information

ABSTRACT: The most common biotransformation of trivalent inorganic arsenic (As(III)) is methylation to mono-, di-, and trimethylated species. Methylation is catalyzed by As(III) S-adenosylmethionine (SAM) methyltransferase (termed ArsM in microbes and AS3MT in animals). Methylarsenite (MAs(III)) is both the product of the first methylation step and the substrate of the second methylation step. When the rate of the overall methylation reaction was determined with As(III) as the substrate, the first methylation step was rapid, whereas the second methylation step was slow. In contrast, when MAs(III) was used as the substrate, the rate of methylation was as fast as the first methylation step when As(III) was used as the substrate. These results indicate that there is a slow conformational change between the first and second methylation steps. The structure of CmArsM from the thermophilic alga Cyanidioschyzon merolae sp. SS08 was determined with bound MAs(III) at 2.27 Å resolution. The methyl group is facing the solvent, as would be expected when MAs(III) is bound as the substrate rather than facing the SAM-binding site, as would be expected for MAs(III) as a product. We propose that the rate-limiting step in arsenic methylation is slow reorientation of the methyl group from the SAM-binding site to the solvent, which is linked to the conformation of the side chain of a conserved residue Tyr70.

INTRODUCTION

Arsenic is the most ubiquitous environmental toxic substance that enters our food and water supply from both geochemical and anthropogenic sources.1 It poses a serious threat to human health and, consequently, ranks first on the 2017 Environmental Protection Agency’s comprehensive environmental response, compensation, and liability act list of hazardous substances (https://www.atsdr.cdc.gov/spl/). This group 1 carcinogen is associated with a number human diseases including lung, bladder, and skin cancers, as well as neuropathy, cardiovascular disease, and diabetes.2,3 It causes developmental delay in infants and children if the mother is chronically exposed to arsenic during pregnancy or if infants are fed rice baby food containing arsenic (https://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm367263.htm).4 Arsenic is acted on biologically, creating an arsenic biogeocycle.5 Members of every kingdom, from bacteria to humans, biomethylate arsenite, producing the trivalent species methylarsenite (MAs(III)), dimethylarsenite (DMAs(III)) and, to a limited degree, volatile trimethylarsine (TMAs(III)).5,6 The reaction is catalyzed by the enzyme As(III) SAM methyltransferase (EC 2.1.1.137) (termed ArsM in microbes and AS3MT in animals). In microbes, arsenic methyltransferase is a detoxification process, but in humans, the production of MAs(III) and DMAs(III) has been proposed to increase arsenic toxicity and potentially carcinogenicity.7,8

CmArsM from the acidothermoacidophilic eukaryotic red alga Cyanidioschyzon merolae sp. SS08 from Yellowstone National Park is a 400-residue thermostable enzyme (44 980 Da, accession number ACN39191) that methylates As(III) to a

Received: February 1, 2018
Accepted: March 2, 2018
Published: March 14, 2018

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DOI: 10.1021/acsomega.8b00197
ACS Omega 2018, 3, 3104−3112

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final product of volatile \( \text{TMAs}(\text{III}) \), conferring arsenic resistance.\(^\text{1,13} \) Nearly all ArsM orthologs have four conserved cysteine residues, which are Cys44, Cys72, Cys174, and Cys224 in \( \text{CmArsM} \) (Supporting Information Figure S1).\(^\text{15,17} \) Substitution of any of the four eliminates the first methylation step (As(III) to MAs(III)), but either the C44A or C72A derivative is still able to carry out the second step, methylation of MAs(III). Cys44 and Cys72 form a disulfide bond that we hypothesize reduces a transient enzyme-bound pentavalent intermediate, whereas Cys174 and Cys224 form the binding site for trivalent arsenicals. We previously solved the structure of \( \text{CmArsM} \) to 1.6 Å without ligands and with As(III) or SAM. From a combination of structural and biochemical results, we proposed a catalytic cycle involving a disulfide bond cascade.\(^\text{1,13} \)

Here, we determined the rates of the first two steps of methylation, As(III) to MAs(III) and MAs(III) to DMAs (the presumed product, DMAs(III), is rapidly oxidized to DMAs(V) in air, so it will be referred to simply as DMAs in this study). When As(III) was used as the substrate, the first methylation step, As(III) \( \rightarrow \) MAs(III), was rapid, whereas the second methylation step, MAs(III) \( \rightarrow \) DMAs, was considerably slower. In contrast, when methylation was assayed using MAs(III) as the substrate, the rate was somewhat faster than the first methylation step. Thus, the rate of the second methylation step, MAs(III) \( \rightarrow \) DMAs, depends on whether the initial substrate is As(III) or MAs(III). Immediately following the methylation of As(III) to MAs(III), the orientation of the methyl group is inferred to be facing the S-adenosylhomocysteine (SAH) product in the SAM-binding site. In order to carry out the next methylation reaction, the methyl group must leave the SAM site to allow a new molecule of SAM to bind. We propose that, when MAs(III) is the product of As(III) methylation, the orientation of the methyl group is adjacent to the SAM-binding site and prevents the SAH product from leaving. This hinders the entrance of another SAM molecule, creating a kinetic block between the first and second methylation steps. To examine the structural basis for this slow step, we obtained several new crystal structures, including MAs(III)-bound \( \text{CmArsM} \). In this structure, the orientation of the methyl group is facing toward the solvent, the predicted conformation when MAs(III) is the substrate. In this conformation, an aqueous channel to the active site is occluded, preventing exchange of the product SAH for another SAM substrate. In addition, the aromatic side chain of Tyr70, a residue conserved in most bacterial and animal ArsM orthologs, appears to block the aqueous channel when the arsenic binding site is filled but not when SAM is bound. We propose that slow reorientation of the methyl group from the SAM-binding site to the solvent is rate-limiting in methylation of inorganic arsenic, with the position of the side chain of Tyr70 gating the ligand access.

**RESULTS**

**Rate-Limiting Step between the First and Second Methylation Steps.** Methylation was assayed with either As(III) or MAs(III) as the substrate (Figure 1). \( \text{CmArsM} \) is a heat-stable enzyme; hence, the initial methylation reactions were assayed by HPLC ICP-MS at 60 °C.\(^\text{18} \) When As(III) was the substrate, MAs was formed slightly faster than DMAs (Figure 1A, inset), but after 10 min, the amount of MAs decreased, and DMAs continued to increase (Figure 1A) (because the reactions were terminated by the oxidation with \( \text{H}_2\text{O}_2 \) to liberate bound products, the oxidation state of the arsenicals cannot be deduced). When MAs(III) was the substrate, methylation to DMAs was also rapid (Figure 1B). As a thermostable enzyme, \( \text{CmArsM} \) is much more active at higher temperatures, which makes it difficult to observe the early events. To slow the reaction, the temperature was decreased to 37 °C, and methylation from As(III) to MAs and then to DMAs was assayed. At 37 °C, the conversion of As(III) to MAs was clearly faster than the methylation of As(III) to DMAs (Figure 2). This indicates that the second methylation step (MAs \( \rightarrow \) DMAs) is considerably slower than the first methylation step (As \( \rightarrow \) MAs) when As(III) is the initial substrate. To directly compare the rates of methylation with either As(III) or MAs(III) as the substrate, a FRET assay was used to measure SAH production at 37 °C (Figure 3).\(^\text{15} \) In this assay, the rate of conversion of MAs \( \rightarrow \) DMAs with MAs(III) as the substrate was greater or equal to the rate of conversion of As \( \rightarrow \) MAs with As(III) as the substrate. Thus, when As(III) was the substrate, the rate of MAs \( \rightarrow \) DMAs was slower than As \( \rightarrow \) MAs, but with MAs(III) as the substrate, the rate of MAs \( \rightarrow \) DMAs was as fast or faster than As \( \rightarrow \) MAs. These results show that the overall rate of As(III) to DMAs is slower than the individual rates of the first and second methylation reactions, which are intrinsically similar. This demonstrates that there must be a rate-limiting reaction or conformational change.

![Figure 1](https://example.com/fig1.png)
between the addition of the first and second methyl groups. We propose that this slow step involves reorientation of the methyl group of MAs(III) from SAH-facing to solvent-facing.

**New CmArsM Crystal Structures Provide Insights into the Rate-Limiting Step.** To gain insights into the nature of the rate-limiting step, we solved the structure of MAs(III)-bound wild-type CmArsM at 2.27 Å resolution (PDB ID 5JWN). The overall structure consists of an N-terminal domain, As(III) binding domain, and a C-terminal domain. Inset: the expanded view of the active site shows the conserved cysteine residues represented by ball-and-stick and colored green (carbon), blue (nitrogen), red (oxygen), or yellow (sulfur). The purple sphere is the arsenic atom, and the light blue sphere is a Ca$^{2+}$ ion found in the SAM-binding site. MAs(III) is bound between conserved residues Cys174 and Cys224.

In addition, we solved the structure of a PhAs(III)-bound C72A derivative with an alanine substitution for conserved cysteine residue Cys72 at 1.97 Å resolution (PDB ID 5EG5) (Supporting Information Figure S2). CmArsM is able to methylate aromatic arsenicals such as PhAs(III). Methylation of PhAs(III) is the equivalent of the second methylation step (MAs → DMAs). The structure of PhAs(III)-bound wild-type CmArsM (PDB ID 4KW7) has been previously reported. The benzene ring cannot fit into the space between SAM and the arsenic atom and, as expected, is oriented toward the solvent. The structure of MAs(III)-bound CmArsM is superimposable with PhAs(III)-bound CmArsM with an rmsd of 0.23 Å (Supporting Information Figure S6A). The methyl group of MAs(III) and the benzene ring of PhAs(III) are both oriented toward the solvent, the expected substrate-bound form. In the structure with the trivalent form of the aromatic arsenical poultry growth promoter roxarsone (4-hydroxy-3-nitrobenzenearsenite), the hydroxynitrobenzene ring is similarly oriented.
In addition to the MAs(III) ligand were identified using LigPlot+ analysis (Supporting Information Figure S5A).17 In the MAs(III)-bound structure, Tyr70 is closer to the As(III) binding site than in the As(III)-bound structure, suggesting that the hydroxyphenyl side chain of Tyr70 sterically hinders binding of arsenicals when SAM is bound. The surface representation of the MAs(III)-bound structure (light gray) shows that the Tyr70 is in the solvent channel oriented toward the As binding site and a SAM molecule modeled into the SAM-binding site in ball-and-stick. (C) Surface representation of SAM-bound wild-type CmArsM in the same orientation. In this structure, the side chain of Tyr70 is oriented away from the SAM-binding site, allowing SAM and SAH access to the active site.

Thus, in the structure of CmArsM with three different bound organoarsenicals, the molecule is in the substrate-bound form, and the methylation reaction is the equivalent of the second round of methylation.

The MAs(III)-bound structure includes an N-terminal domain (the SAM-binding domain), a middle As(III)-binding domain, and a C-terminal domain of unknown function. In the PhAs(III)-bound structure of wild-type CmArsM, a disulfide bond is observed between Cys44 and Cys72, which has been proposed to be an obligatory intermediate in the first methylation step. In that structure, the N-terminal domain contains two small $\beta$-sheets and is followed by a long mobile loop, which moves from the SAM-binding site toward the As(III) binding site during the catalytic cycle. However, the loop containing the conserved residue Cys44 is not resolved in the CmArsM MAs(III)-bound structure, perhaps because it is disordered when the disulfide bond is not present. The electron densities of the bound MAs(III) and conserved cysteine residues Cys174 and Cys224 are shown in Supporting Information Figure S4A. MAs(III) occupancy was partial during several rounds of the structural refinement, with a high temperature factor for MAs(III) (71.61 Å$^2$). The CmArsM-bound MAs(III) structure adopts a pyramidal geometry in which the As atom of MAs(III) is coordinated with the S atoms of Cys174 and Cys224 (Figure 7A). The two S atoms and the C atom of the methyl group are each at an average distance of 3.7 Å from each other. Potential hydrogen bonds and hydrophobic interactions of the protein with the MAs(III) ligand were identified using LigPlot+ analysis (Supporting Information Figure S5A).17 In addition to the sulfur atoms of Cys174 and Cys224, only Glu223 interacts with the bound ligand.

To further examine the structural basis for the second methylation step, the structure of the PhAs(III)-bound C72A derivative was compared with the MAs(III)-, PhAs(III)-, and SAM-bound structures of wild-type CmArsM. In the C72A structure, PhAs(III) is bound between conserved cysteines residues Cys174 and Cys224 (Supporting Information Figure S3A). The distances of the S-methyl group of SAM to the sulfur atom of conserved cysteine residues and to the arsenic atom of MAs(III) are indicated. (B) The PhAs(III)-bound C72A structure was superimposed with the SAM-bound wild-type CmArsM structure, with an rmsd of 1.25 Å. The distances of the S-methyl group of SAM to the sulfur atom of conserved residues Cys174 and Cys224 and to the arsenic atom of PhAs(III) are indicated. The atoms representation and the coloring are the same as that described in Figure 4.
Amino Acid Residues Near the Binding Site

Table 1. Ligands MAs(III) and PhAs(III) Interaction with Amino Acid Residues Near the Binding Site

<table>
<thead>
<tr>
<th>ligand/atom</th>
<th>amino acid atom</th>
<th>distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAs(III)-Bound CmArsM</td>
<td>(O)→C174</td>
<td>3.9</td>
</tr>
<tr>
<td>MAs(III)-Bound CmArsM</td>
<td>(O)→C174</td>
<td>3.5</td>
</tr>
<tr>
<td>MAs(III)-Bound CmArsM</td>
<td>S→C174</td>
<td>3.3</td>
</tr>
<tr>
<td>MAs(III)-Bound CmArsM</td>
<td>O→E223</td>
<td>3.6</td>
</tr>
<tr>
<td>MAs(III)-Bound CmArsM</td>
<td>Cβ→C224</td>
<td>3.2</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>O→Y70</td>
<td>3.8</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>Cβ→C174</td>
<td>3.2</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>C→C174</td>
<td>3.4</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>S→C174</td>
<td>3.4</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>O→G222</td>
<td>3.8</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>C→E223</td>
<td>3.8</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>Cβ→C224</td>
<td>3.6</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>S→C224</td>
<td>3.4</td>
</tr>
<tr>
<td>PhAs(III)-Bound C72A</td>
<td>S→C224</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Moderate hydrogen bonds. Non-bonded interactions between atoms of MAs(III) or PhAs(III) with amino acid atoms.

This is not unexpected because neither Cys72 nor a Cys44—Cys72 disulfide bond is required for the methylation of either MAs(III) or PhAs(III).

The PhAs(III)-bound C72A structure superimposes less well with the wild-type SAM-bound structure (rmsd of 1.25 Å), again because of a difference in the position of the N-terminal loop. The sulfur atoms of Cys174 and Cys224 in the C72A PhAs(III)-bound structure are 7.8, 5.5, and 5.7 Å, respectively, from the S-methyl group of SAM in the superimposed structures (Figure 6B). The distance of the S-methyl group of SAM to the As atom of PhAs(III) is 4.1 Å, indicating that it is poised for transfer (Figure 6B). The S-methyl group of SAM is 7.8 Å distant from the Cβ atom of PhAs(III)-bound C72A. In these structures, the SAM entry is predicted to be restricted in part because the R group of either MAs(III) or PhAs(III) interacts with residues Tyr70, Gly222, and Glu223 (Supporting Information Figure S5) neighboring the As(III) binding site.

Tyr70 Gates Ligand Binding. We propose that the orientation of Tyr70 also provides a gating mechanism for substrate access to the active site. In both MAs(III)-bound CmArsM (Figure S5A,B) and PhAs(III)-bound C72A (Supporting Information Figure S3A,B), the hydroxophenyl ring of Tyr70 is oriented toward the SAM site, but the arsenic binding site is open. In the SAM-bound structure, the Tyr70 side chain is flipped away from the SAM-binding site (Figure S5A,C). In this conformation, the SAM site is open but the arsenic binding site is occluded. This is reflected in a 4.1 Å movement of the Cα of Tyr70 in the SAM-bound structure compared with the MAs(III)-bound wild type enzyme (Figure 5A). In the PhAs(III)-bound C72A structure, this difference is 3.5 Å (Supporting Information Figure 3A). In the SAM-bound structure, the loop that includes Try70 toward the As(III) binding site by 6.3 Å is relative to either the MAs(III)- or PhAs(III)-bound structure. With wild-type CmArsM, this movement brings Cys72 and Cys174 close enough to each other to be able to form the disulfide bond observed in the SAM-bound structure. These results indicate that the Tyr70 hydroxophenyl ring occludes the aqueous channel to bulk solvent when either MAs(III) or PhAs(III) is bound, preventing the SAM molecule from leaving the active site. In the SAM-bound conformation, the Tyr70 ring is oriented away from the aqueous channel, allowing the exchange of the SAH product for another SAM substrate but forming a Cys72—Cys174 disulfide that prevents binding of arsenicals. We conclude that Tyr70 gates the substrate access to the active site.

DISCUSSION

Arsenic methylation is a widespread biotransformation process that is catalyzed by the enzyme As(III) S-adenosylmethionine methyltransferase. The enzyme methylates trivalent As(III) up to three times, producing mono-, di-, and trimethylated species. In microbes, ArsM clearly catalyzes the detoxification of this toxic metalloid. In humans, arsenic methylation by the orthologous AS3MT has been proposed to transform inorganic arsenic into the more toxic and potentially more carcinogenic species MAs(III) and DMAs(III). When MAs(III) and DMAs(III) are excreted in urine, oxidation by air converts them to the pentavalent species MAs(V) and DMAs(V). A high ratio of urinary MAs/DMAs has been associated with various arsenic-related diseases, while the reverse appears to be protective. We previously showed that MAs(III), the product of this first methylation step (As(III)→MAs(III)), dissociates very slowly from both human AS3MT and CmArsM. This increases the retention time of MAs(III) in the cytosol of human cells, which may be a critical factor in arsenic toxicity and carcinogenicity. At longer times the second methylation step (MAs(III)→DMAs(III)) predominates, and the major product is the dimethylated species, which rapidly leaves the cells. Thus, the faster the MAs(III) is converted to

Figure 7. Detailed analysis of the As-binding site. (A) MAs(III) is located between conserved residues Cys174 and Cys224 in the MAs(III)-bound structure. In the pyramidal binding site, the distances between the As atom of MAs(III) and the sulfur thiolates of Cys174 and Cys224 are 2.5 and 2.4 Å, respectively. The thiolate of Cys72 is 7.3 Å away from the arsenic atom of MAs(III). The carbon atom of MAs(III) serves as a third arsenic ligand. Each of the liganding atoms are at an average distance of 3.7 Å from each other. (B) PhAs(III) in the C72A structure is located between Cys174 and Cys224. The distances between As atoms of PhAs(III) and the sulfur thiolates of Cys174 and Cys224 are 2.5 and 2.3 Å, respectively. In this pyramidal distances between As atom of PhAs(III) and the sulfur thiolates of the C72A structure is located between Cys174 and Cys224. The are at an average distance of 3.7 Å from each other. (B) PhAs(III) in the C72A structure is located between Cys174 and Cys224. The distances between As atoms of PhAs(III) and the sulfur thiolates of Cys174 and Cys224 are 2.5 and 2.3 Å, respectively. In this pyramidal
DMAs(III), the faster the arsenic can be cleared from the body and the less time it resides inside of cells. It is therefore crucial to understand what governs the ratio of MAs to DMAs, which we predict is related to the catalytic mechanism of AS3MT.

Here, we examined the first two methylation steps in more detail with the AS3MT ortholog CmArsM. With As(III) as the substrate, MAs(III) is formed rapidly, and DMAs(III) more slowly, especially at 37 °C, where the reaction rate is slower. With MAs(III) as the substrate, MAs(III) → DMAs(III) is as rapid as As(III) → MAs(III) when As(III) is the substrate. These results demonstrate that the two steps have intrinsically similar rates, indicating that the slower rate of the second methylation step when As(III) is the substrate is due to a kinetic block that prevents MAs(III) from dissociating from the enzyme. Retaining a substrate in the active site might be expected to accelerate the catalysis because it would eliminate a diffusion-limited step, but that is not the case in this situation. Thus, the MAs(III) product of the first step is not equivalent to the MAs(III) substrate, even though there is a single active site. We propose that the position of the methyl group in MAs(III) differs in two stages (Figure 8). When As(III) and SAM are both bound, the S-methyl group of SAM must face the arsenic atom (Figure 8A). When MAs(III) is the product of As(III) methylation, the methyl group is expected to be located between the sulfur of SAH and the arsenic atom (Figure 8B). For the second methylation to occur, the SAH product must exchange with another molecule of SAM. However, the As-methyl group of the MAs(III) product could sterically clash with the S-methyl group of SAM unless it is reoriented away from the SAM-binding site (Figure 8C).

Reorientation of the As-methyl group is rate-limiting for catalysis. (A) As(III) is positioned between its binding site composed of Cys174 and Cys224 and the SAM S-methyl group. (B) The methyl group is transferred from SAM to As(III), forming a MAs(III)-bound intermediate in which the As-methyl group is oriented toward SAH. (C) Slow reorientation of the As-methyl group toward bulk solvent opens the SAM-binding site, allowing exchange of SAH for SAM. In this conformation, MAs(III) becomes the substrate for the second round of methylation.

**MATERIALS AND METHODS**

**Reagents.** All chemicals were obtained from Sigma-Aldrich (St Louis, Missouri, USA) unless otherwise mentioned. **Purification of CmArsM Enzymes.** CmArsM lacking 31 residues from the N-terminus and 28 residues from the C-terminus with a C-terminal histidine tag (termed simply wild-type CmArsM) was expressed and purified as described previously. The C72A derivative was constructed by site-directed mutagenesis using a QuickChange mutagenesis kit (Stratagene, La Jolla, California, USA). The forward and reverse oligonucleotide primers used for cysteine to alanine mutagenesis (changes underlined) were 5′-GTCCCTGGAAAAAGTTTCTACGTGCGGTCTACGC-3′ and 5′-GGGTTAGACCCGGCACCATTCCAAGGAC-3′. The mutation was confirmed by sequencing the entire gene. When expressed in Escherichia coli, C72A was produced in a soluble form in amounts comparable to the parental CmArsM. Purified enzymes were stored at −80 °C until use. Protein concentrations were estimated from the absorbance at 280 nm. Before crystallization, purified proteins were exchanged into a buffer containing 50 mM MOPS, pH 7.0, containing 0.5 M NaCl and 5 mM dithiothreitol (DTT).

**Cocrystallization of CmArsM and Derivatives with Organoaarsenicals.** Crystallization was performed by the hanging drop vapor diffusion method with a variety of crystal screen conditions from Hampton Research (Aliso Viejo, California, USA), Emerald Biosciences Inc. (Bainbridge Island, Washington, USA), and Jena Biosciences GmbH (Jena, Germany). For cocrystallization with MAs(III), 20 μL of wild-type CmArsM at 18 mg/mL was mixed with 20 μL of 2 mM MAs(III) and incubated on ice for 20 min before crystallization. Crystals were obtained by mixing 2.0 μL of protein solutions containing MAs(III) with equal volumes of reservoir solution consisting of 18% PEG 3350, 0.2 M calcium chloride, and 0.1 M TRIS (pH 7.5) until crystals appeared.
acetate, and 0.1 M Tris–HCl at pH 7.0. CocrySTALLization of C72A with 1 mM phenylarsenate (PhAs(III)) was performed as described previously.\(^{16}\)

### X-ray Data Collection and Structure Refinement.
Crystals were transferred to a cryoprotectant solution (25% PEG 3350, 0.2 M calcium acetate, 0.1 M Tris–HCl, pH 7.0, and 10% glycerol) and flash-frozen in liquid nitrogen for data collection. Data sets of wild-type MAs(III)-bound CmArsM and PhAs(III)-bound C72A were collected at the southwest regional collaborative access team (SER-CAT) facility at advanced photon source (APS), Argonne National Laboratory. Data were obtained from 360 image frames with a rotation angle about ϑ using a MAR-300 CCD detector under standard cryogenic conditions (100 K) at a synchrotron beam line 22-ID with a crystal to detector distance of 200 mm. Data sets were indexed, integrated, and scaled with the HKL2000 suite.\(^{28}\) The structures were determined by molecular replacement with Phaser in the CCP4 suite.\(^{29}\) The unliganded crystal of CmArsM (PDB ID 4FS8) was used as a template for the detection step and 10% glycerol) and 10% of the residues in the generously allowed region. The Ramachandran plot showed that 97.0% of the residues are in the most favored region, 2.4% of the residues in the additionally allowed regions, and 0.6% of the residues in the generously allowed region. The final data collection, refinement statistics, and protein data bank accession codes are given in Table 2. Molecular models were drawn with PYMOL.\(^{33}\) Ligplot+ was used to illustrate the hydrogen bonds and non-bonded interactions of MAs(III)-bound CmArsM and PhAs(III)-bound C72A.\(^{17}\)

### Assay of CmArsM Activity.
Methylation activity of purified CmArsM was assayed in 50 mM NaH₂PO₄ (pH 8.0) containing 0.3 M NaCl, 5 mM GSH, and 1 mM SAM, as described previously at 60 or 37 °C, as indicated.\(^{13}\) Unless otherwise noted, the reactions were terminated by adding 10% (v/v) H₂O₂, final concentration, to oxidize all arsenic species. Denatured protein was removed by centrifugation using a 3 kDa cut-off Amicon ultrafilter. The products of As(III) or MAs(III) methylation were separated by high-performance liquid chromatography (HPLC) using a Hamilton PRP-X100 C18 reverse phase column (Hamilton Co., Reno, NV) and quantified using a PerkinElmer ELAN DRC-e inductively coupled plasma mass spectrometer (ICP-MS), as described previously.\(^{14}\)

CmArsM activity was also assayed with an EPIgeneous methyltransferase assay kit (Cisbio Bioassays, Bedford, MA) by measuring the conversion of SAM to SAH as described previously.\(^{15}\) The reaction has two steps: (1) the enzymatic reaction, which converts SAM to SAH, and (2) the detection step that quantifies SAH production. Both steps were carried out sequentially in the same well of a low volume 384-well microtiter plate, with a total volume of 20 μL (10 μL for the enzymatic step and 10 μL for the detection step) in a buffer consisting of 50 mM NaH₂PO₄, pH 8.0, containing 0.3 M NaCl, 2 mM GSH, 1 μM CmArsM, and 10 μM of either As(III) or MAs(III). After incubating for 1 min, 10 μM SAM, final concentration, was added to initiate the reaction. The reactions were carried out at 37 °C for the indicated times. The reactions were terminated by addition of the proprietary detection reagent, followed by SAH-d2 and anti-SAH-Lumi4-Tb reagents. Fluorescence was measured at both 665 and 620 nm with excitation at 337 nm in a Synergy H4 Hybrid Multi-Mode Microplate Reader. The homogeneous time-resolved fluorescence (HTRF) was calculated from the ratio of emission at 665 and 620 nm. The concentration of SAH was calculated

### Table 2. Data Collection, Indexing, and Refinement Statistics\(^{44}\)

<table>
<thead>
<tr>
<th>Data collection</th>
<th>MAs(III)-bound CmArsM</th>
<th>PhAs(III)-bound C72A</th>
</tr>
</thead>
<tbody>
<tr>
<td>diffraction source</td>
<td>APS 22-ID</td>
<td>APS 22-ID</td>
</tr>
<tr>
<td>wavelength (Å)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>detector</td>
<td>MARCCD 300</td>
<td>MARCCD 300</td>
</tr>
<tr>
<td>space group</td>
<td>C2</td>
<td>C2</td>
</tr>
<tr>
<td>a, b, c (Å)</td>
<td>85.56, 47.27, 100.54</td>
<td>85.26, 47.27, 100.26</td>
</tr>
<tr>
<td>α, β, γ (deg)</td>
<td>90.0, 113.9, 90.0</td>
<td>90.0, 113.6, 90.0</td>
</tr>
<tr>
<td>resolution range (Å)</td>
<td>100–2.27 (2.37–2.27)</td>
<td>50.0–1.97 (2.04–1.97)</td>
</tr>
<tr>
<td>unique reflections</td>
<td>17.063 (1624)</td>
<td>23.35 (2467)</td>
</tr>
<tr>
<td>completeness (%)</td>
<td>99.2 (95.1)</td>
<td>89.7 (96.9)</td>
</tr>
<tr>
<td>redundancy</td>
<td>7.3 (6.6)</td>
<td>7.2 (7.4)</td>
</tr>
<tr>
<td>(I/σ(I))</td>
<td>26.6 (7.3)</td>
<td>20.1 (8.6)</td>
</tr>
<tr>
<td>Rmerge</td>
<td>0.057 (0.205)</td>
<td>0.054 (0.235)</td>
</tr>
<tr>
<td>Rwilson</td>
<td>0.053 (0.223)</td>
<td>0.058 (0.253)</td>
</tr>
<tr>
<td>Wilson B-factor (Å²)</td>
<td>0.019 (0.085)</td>
<td>0.022 (0.092)</td>
</tr>
<tr>
<td>Refinement</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
from a calibration curve of the HTRF with known concentrations of SAH.

**REFERENCES**

Multiple sequence alignment of As(III) SAM methyltransferases; structure of the CmArsM C72A derivative with bound PhAs(III); superposition of the structures of C72A with bound PhAs(III) and SAM-bound wild-type CmArsM; stereo view of electron density map of (A) MAs(III) and (B) mutant C72A bound-PhAs(III) in CmArsM; residues interacting with MAs(III) or PhAs(III); and superimposition of MAs(III)- and PhAs(III)-bound structures (PDF)

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**ACKNOWLEDGMENTS**

This work was supported by NIH grants GM55425 and ES023779 and a Pilot Project award to C.P. from the Herbert Wertheim College of Medicine and supported in part by the National Institutes of Health, National Institute of General Medical Sciences, and the Howard Hughes Medical Institute. The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences of the US Department of Energy under Contract no. DE-AC02-05CH11231.

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**Notes**

The authors declare no competing financial interest.

**REFERENCES**


