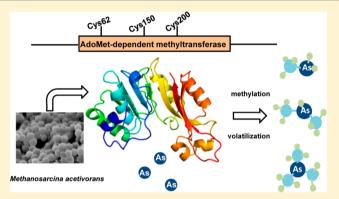# Identification and Characterization of Arsenite Methyltransferase from an Archaeon, *Methanosarcina acetivorans* C2A

Pei-Pei Wang,[†,‡] Guo-Xin Sun,*,[†] and Yong-Guan Zhu*,[†,§]

[†]State Key Lab of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, People's Republic of China

[‡]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[§]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, People's Republic of China

Ⓢ *Supporting Information*

ABSTRACT: Arsenic is a ubiquitous toxic contaminant in the environment. The methylation of arsenic can affect its toxicity and is primarily mediated by biological processes. Few studies have focused on the mechanism of arsenic methylation in archaea although archaea are widespread in the environment. Here, an arsenite [As(III)] methyltransferase (ArsM) was identified and characterized from an archaeon *Methanosarcina acetivorans* C2A. Heterologous expression of *MaarsM* was shown to confer As(III) resistance to an arsenic-sensitive strain of *E. coli* through arsenic methylation and subsequent volatilization. Purified MaArsM protein was further identified the function in catalyzing the formation of various methylated products from As(III) in vitro. Methylation of As(III) by MaArsM is highly dependent on the characteristics of the thiol cofactors used, with some of them (coenzyme M, homocysteine, and dithiothreitol) more efficient than GSH. Site-directed mutagenesis demonstrated that three conserved cysteine (Cys) residues (Cys62, Cys150, and Cys200) in MaArsM were necessary for As(III) methylation, of which only Cys150 and Cys200 were required for the methylation of monomethylarsenic. These results present a molecular pathway for arsenic methylation in archaea and provide some insight into the role of archaea in As biogeochemistry.

■ INTRODUCTION

Arsenic (As) has received considerable attention worldwide, since As pollution in soil or water is widespread and often leads to a multitude of toxic effects. Arsenic methylation is a ubiquitous phenomenon in the environment and is primarily mediated by biological actions. Arsenic methylation is acknowledged to be a significant mechanism involving As detoxification since the pentavalent methylated As are less toxic than the inorganic As. Moreover, volatilized As as the final product of methylation has been suggested to contribute to global As cycling.[1,2] One methylcobalamin-dependent methylation of As, which does not require an enzyme, has been reported in methanoarchaea.[3,4] Whether there exists an enzymatic pathway for As methylation in archaea is still unclear. Arsenic methyltransferase which is responsible for As methylation, has been identified and characterized in members of several kingdoms, from bacteria to human,[5−8] but it has yet to be conclusively identified in the archaea.

The archaea domain represents a third line of evolutionary descent, separate from bacteria and eukarya.[9] These prokaryotes not only predominate in separate niches characterized by extreme temperatures, pH, salinity, or anaerobiosis,[10,11] including hot springs, hydrothermal vents, salt lakes, soda lakes, sewage digesters, and the rumen, but also exhibit a wide distribution in common habitats,[12,13] such as ocean or lake waters, soil etc. Despite the abundance and ubiquity in the environment, the archaea have not been studied as much as bacteria, especially on the As transformation in the environment.

Archaea can thrive in various anaerobic environments. Anaerobic environments, e.g., sediments and wetlands, provide an excellent setting to study microbe-arsenic interactions,[14,15] since they often act as the sinks or secondary sources of As due to natural and anthropogenic activities.[16,17] Generally, arsenic methylation is preferred under anaerobic conditions.[1,18] For example, arsenic volatilization has been observed in many paddy soils or wetlands.[19−23] Methylation and volatilization of metal(loid)s have been regarded as an inherent feature of

methanoarchaea,[24,25] though the enzymatic mechanism of As methylation by archaea remains unknown.

In this study, we focused on the molecular mechanism of As methylation by a methanoarchaea. *Methanosarcina acetivorans* C2A was chosen as a model organism because of its available genomic information and the physiological, metabolic and environmental diversity.[26] Our main objectives were (1) to identify As methylation and the involved enzyme in *M. acetivorans* C2A, (2) to provide insight into the molecular mechanism of As methylation in archaea, and (3) to elucidate some characteristics of As(III) methyltransferase from archaea.

■ MATERIALS AND METHODS

Organisms and Cultivations. *M. acetivorans* C2A was purchased from American Type Culture Collection (ATCC) and anaerobically grown in ATCC Medium 1355 at 37 °C in the dark. Media were boiled and cooled to room temperature under an atmosphere of N_2 gas (purity ≥99.999%). The media were dispensed into acid-cleaned serum bottles, sealed with butyl rubber stoppers and covered with aluminum foil, autoclaved at 121 °C for 20 min. The headspace of serum bottles were exchanged by the filtered gas mixture (80% H_2-20% CO_2) before inoculation.

E. coli were grown at 37 °C in LB media. Strain DH5α (Promega) was used for plasmid replication. Strain AW3110-(DE3) [ΔarsRBC; ArsR-repressor; ArsB-As(III) efflux pump; ArsC-As(V) reductase] was used for the functional verification of *arsM* gene. Strain BL21(DE3) was used for protein purification.

Chemicals. Arsenicals were bought from J&K Chemical Ltd. The thiol compounds in Supporting Information (SI) Table S1 and *S*-adenosyl-L-methionine (SAM) were bought from Sigma. All reagents are analytical grade or better.

Arsenic Methylation by *M. acetivorans* C2A. With 2% (v/v) inoculum, *M. acetivorans* C2A was inoculated into the medium supplemented by sodium arsenite [As(III)] or sodium arsenate [As(V)] to a concentration of 5 or 10 μM. After a growth of 20 days, the cultures were centrifuged at 8000 r.p.m. The supernatants were filtrated by 0.45 μm disposable filter, and oxidized by 10% (v/v) H_2O_2 overnight, then kept at 4 °C until analysis.

Cloning of the *arsM* Gene from *M. acetivorans* C2A and Gene Expression in *E. coli*. The putative *arsM* gene in *M. acetivorans* C2A and other *arsMs* from different species were searched in NCBI database. The *arsM* gene alignments were carried out on DNAMAN software (Lynnan Corp, Pointe-Claire, QC, Canada). Genomic DNA was isolated from *M. acetivorans* C2A by using Genomic-Tip 20/G and Buffer set (Qiagen). A 763 bp fragment containing ATG start codon and excluding TGA stop codon was PCR amplified from the genome of *M. acetivorans* C2A by using the forward primer: 5′-CGGAATTCATGGATGCCGCTGAAAAAAA-3′ (*Eco*RI restriction endonuclease site underlined) and the reverse primer: 5′-CCCTCGAGTACCCAGGCCTTTAATTTGA-3′ (XhoI restriction endonuclease site underlined). PCR reaction (94 °C for 5 min, 94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, 30 cycles) was performed with LA Taq DNA polymerase (Takara Bio). The PCR product was cloned into PMD19T simple vector (Takara Bio) to create the plasmid PMD19T-*arsM*. Plasmids were extracted with a QIAPrep Spin Miniprep kit (Qiagen) and sequenced to obtain a positive cloning. After digested with *Eco*RI and XhoI, *arsM* fragment was again inserted into pET28a+ vector to create plasmid pET28a+*arsM*.

The His-tagged ArsM established was composed of 293 amino acid residues, with a predicted molecular mass of 31.56 kDa.

Plasmid pET28a+ or pET28a+*arsM* was transformed into *E. coli* AW3110(DE3). Transformed cells were grown in LB medium containing 50 μg mL⁻¹ kanamycin overnight, then diluted 50-fold into fresh medium containing 50 μg mL⁻¹ kanamycin, 0.3 mM IPTG, and a series of concentrations of As(III) (10, 20, and 50 μM). After a growth of 20 h, the culture was centrifuged at 8000 r.p.m. The supernatant was filtrated by 0.45 μm disposable filters, oxidized by 10% (v/v) H_2O_2 overnight and analyzed As species.

E. coli AW3110 expressing pET28a+ or pET28a+*arsM* were inoculated into LB medium containing 50 μg mL⁻¹ kanamycin and cultured overnight. Late exponential phase cells were diluted 100-fold into fresh medium containing 50 μg mL⁻¹ kanamycin, 0.3 mM IPTG and a series of concentrations of As(III) (0, 50, and 100 μM). Cellular growths were monitored as absorbance at 600 nm via UV−vis spectrophotometer (New Century, Model T6) until the cultures reached stationary phase.

Arsenic Volatilization by *E. coli* AW3110 Expressing *arsM*. Volatile As produced by *E. coli* expressing *arsM* was chem-trapped as described.[21] *E. coli* AW3110 bearing pET28a+*arsM* or pET28a+ were cultured in triangular flasks [250 mL volume; 100 mL LB medium with a series of concentrations of As(III)]. Every flask was capped with a T-junction stopper having a gas inlet and a gas outlet. The inlet was linked to a super silent adjustable air pump (ACO-9601; 2-W power), to pump filtered air at a gas flow rate of 40 mL min⁻¹. The outlet of T-junction stopper was connected to a trapping tube filled with $AgNO_3$-impregnated silica gel beads. After a 48 h growth, the total As absorbed by the trapping tube was eluted by 1% (v/v) HNO_3 (5 mL) in a microwave digestion system (Mars II, CEM, U.S.A.). The eluted suspension was centrifuged at 3000 r.p.m. The supernatant was filtrated by 0.45 μm disposable filter and analyzed the quantity and species of As by HPLC-ICP-MS.

Purification of ArsM Enzyme and As(III) Methylation by Purified Enzyme in Vitro. *E. coli* BL21(DE3) bearing pET28a+*MaarsM* was grown in LB medium (200 mL) to an A600 of 0.4−0.6 when 0.3 mM IPTG was supplemented to induce expression of MaArsM. After a 8 h growth at 19 °C, *E. coli* was harvested by centrifugation (8000 r.p.m.) at 4 °C for 20 min. The wet cells were suspended in 30 mL of buffer A (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) and lysed in ultrasonic cell disruption system (JY92−2D) at a program: working for 5 s followed by a rest of 5 s, 20 min at 4 °C. The lysate was centrifugated at 8000 r.p.m. for 30 min. The supernatant was filtrated by 0.45 μm disposable sterile filter, and then loaded onto Ni(II)-NTA column (Qiagen; 5 mL) pre-equilibrated with buffer A. The column was washed with 200 mL of buffer B (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0) and finally eluted with 5 mL of buffer C (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0). The eluate bearing purified MaArsM protein was removed to a 10-kDa ultrafiltration centrifuge tube (Sartorius) and concentrated by centrifugation at 8000 r.p.m. for 1 h. Finally, the buffer C bearing MaArsM protein was exchanged into MOPS buffer (50 mM MOPS, 150 mM KCl, pH 7.4) using Micro Bio-Spin Chromatography Column (Bio-Rad). MaArsM protein was identified by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and determined its concentration by the method of Bradford (Bio-Rad Protein Assay),[27] using BSA (Sigma) as a standard.

Arsenic methylation in vitro was performed in MOPS buffer containing MaArsM protein, glutathione (GSH), SAM, and As(III). The As species during the reaction process was monitored at the different time (0.5, 1.5, 3, 4.5, 6, 20 h). In some cases, a series of concentrations of MaArsM protein, GSH, SAM, or As(III) were used in corresponding assays. Different kinds of thiol compounds in SI Table S1 were used and analyzed their effects on the enzymatic methylation of As(III). The reactions were terminated by boiling for 5 min, oxidized by 10% (v/v) H_2O_2 overnight, finally filtered by 0.45 μm filters and analyzed As species by HPLC-ICP-MS.

Preparation of MaArsM Mutants. A certain cysteine (Cys) of MaArsM was substituted for serine, through overlapping extension PCR with plasmid pET28a+*arsM* as DNA template. The primers used for site-directed mutagenesis are listed in SI Table S2. PCR products were double-digested by *Eco*RI and *Xho*I, and cloned into the *Eco*RI/*Xho*I-digested pET28a+ vector. The plasmids containing *MaarsM* mutants were sequenced to ensure that no errors had been introduced during amplification. The plasmid vectors carrying different *MaarsM* mutants were transformed into *E. coli* BL21(DE3), respectively. Protein purification and concentration determination were performed as described above. Seven mutants of MaArsM (C24/25S, C30S, C31S, C62S, C150S, C194S, and C200S) were established.

Arsenic Speciation Analysis. Arsenic speciation was analyzed by HPLC-ICP-MS (Agilent 1200 series and Agilent 7500 series, Agilent Technologies, U.S.A.) as described.[28] An anion-exchange column (PRP-X100, Hamilton Company, U.S.A.) was used and consisted of a precolumn (11.2 mm; 12−20 μm) and a PRP-X100 10-μm anion-exchange column (250 × 4.1 mm^2). The mobile phase consisted of 10 mM diammonium hydrogen phosphate $[(NH_4)_2HPO_4]$ and 10 mM ammonium nitrate $[NH_4NO_3]$, adjusted to pH 6.2 with nitric acid. The mobile phase was pumped through the column at a flow rate of 1.0 mL min^{-1}.

■ RESULTS

Arsenic Methylation by Methanoarchaea. Under anaerobic conditions, *M. acetivora* C2A had the capacity of methylating As, as monomethylarsenic (MMAs) was detected in the medium containing As(III) (Figure 1). About 10% of the total As was transformed into MMAs in the medium containing 10 μM As(III), and 13% in that with 5 μM As(III) (SI Figure S1). In addition, MMAs was detected in the medium added with As(V), but less than that in the medium with As(III). There was no methylated As in the control (no bacterial inoculation).

Cloning of the *arsM* Gene. PCR cloning using the total DNA extracts of *M. acetivora* C2A yielded a putative *arsM* gene (750 bp), designated as *MaarsM* (NCBI Reference Sequence: NP_618654.1). *MaarsM* gene encoded the MaArsM protein of 249 residues, with a predicted molecular mass of 26.67 kDa. Sequence homology analysis showed that MaArsM had high similarity with the ArsM of *Rhodopseudomonas palustris* (43% identity and 75% similarity).[29] With the ArsMs from a series of cyanobacterial species,[7] MaArsM shared lower similarity (average 23% identity and 51% similarity). In addition, MaArsM also exhibited significant, but low similarity to mammalian AS3MT methyltransferase (21% identity and 50% similarity),[5] and to the ArsM of eukaryotic alga *Cyanidioschyzon* sp. isolate 5508 (21% identity and 45% similarity).[6]
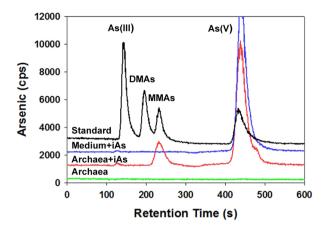

Figure 1. Arsenic transformation by *M. acetivorans* C2A. Archaea C2A were cultured in the presence of 5 μM As(III) for 20 days. The medium without inoculation or As addition was controls. The As species in the medium were analyzed as described in "Materials and Methods".

MaArsM Enzyme Detoxified As(III) in Vivo. To elucidate the function of MaArsM *in vivo*, *MaarsM* gene was expressed in As-sensitive *E. coli* AW3110 (Δ*arsRBC*), which had no orthologous *arsM* gene. Expression of *MaarsM* clearly conferred the As(III) resistance to *E. coli*. Cells expressing *MaarsM* gene grew much better than that bearing vector plasmid pET28a+ when exposed to 50 μM As(III) (Figure 2A). Furthermore, expression of *MaarsM* allowed the cellular growth at As(III) concentration as high as 100 μM while that of plasmid pET28a+ failed to do that (Figure 2A).

As(III) Methylation and Volatilization by *E. coli* AW3110 Expressing *MaarsM*. In the presence of As(III), dimethylarsenic (DMAs) were detected in the medium inoculated with *E. coli* AW3110 expressing *MaarsM* gene (Figure 2B), while no methylated As was detectable in the medium of *E. coli* with vector plasmid pET28a+. This result indicated that the MaArsM enzyme encoded by *MaarsM* gene indeed catalyzed As(III) methylation. At 10 μM As(III) concentration, transgenic *E. coli* methylated 94% of the total As in the medium into DMAs.

E. coli AW3110 expressing *MaarsM* gene effectively volatilized As when exposed to different concentrations of As(III). The gaseous products were mainly trimethylarsine (TMAs) which was oxidized and identified as trimethylarsine oxide (TMAO), together with small amount of dimethylarsine (DMAsH) detectable as oxidized dimethylarsenate [DMA(V)] (Figure 2C). At 5 and 20 μM As(III), only 0.05% and 0.02% of the total As (about 25 and 44 ng) was volatilized from the medium grown with *E. coli* bearing *MaarsM* (SI Figure S2). At higher As(III) concentrations (50, 100 μM), *E. coli* with *MaarsM* volatilized about 0.2% and 0.4% of the total As (about 0.73 and 3.135 μg) from the medium, respectively (SI Figure S2). However, when As(III) concentration up to 200 μM, volatilization was much weaker and only 0.001% of total As (about 18 ng) was released, perhaps attributed to the As toxicity and poor growth of cells.

Methylation of As(III) by Purified ArsM Enzymes in Vitro. To gain a better insight into the mechanism of enzyme, a series of in vitro methylations were assayed with purified MaArsM protein (Figure 3). MMAs and TMAO were formed after 0.5 h, with small amount of DMAs appearing at 1.5 h (Figure 3B). The concentrations of DMAs and MMAs
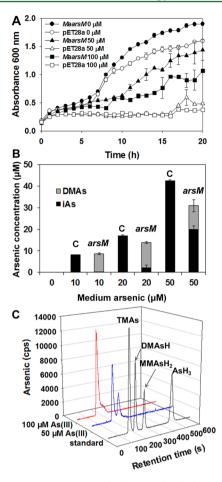
Figure 2. Arsenic resistance, methylation and volatilization by *E. coli* AW3110 (Δ*arsRBC*) expressing pET28a+*MaarsM*. (A) The growth curves of *E. coli* bearing *MaarsM* or vector plasmid pET28a+. *E. coli* was cultured in LB medium containing the indicated concentrations of As(III), 50 μg mL^{-1} kanamycin and 0.3 mM IPTG at 37 °C. Cellular growths were monitored as absorbance at 600 nm at the indicated time points. The error bars indicate the standard errors of three triplicates. (B) As(III) methylation by *E. coli* expressing *MaarsM*. *E. coli* was incubated in LB medium with the indicated concentrations of As(III) (0, 10, 20, and 50 μM), 50 μg mL^{-1} kanamycin and 0.3 mM IPTG at 37 °C for 20 h. The As species in the supernatants of cultures were analyzed by HPLC-ICP-MS as described in "Materials and Methods". C, *E. coli* bearing vector plasmid pET28a+ as control; *arsM*, *E. coli* bearing *MaarsM*. The error bars indicate the standard errors of three triplicates. (C) The species of volatile As released by *E. coli* expressing *MaarsM*. *E. coli* was grown in LB medium containing 50/100 μM As(III), 50 μg mL^{-1} kanamycin and 0.3 mM IPTG at 37 °C for 48 h. Volatile As from medium was chemo-trapped as described in the Materials and Methods section, the species of which were analyzed by HPLC-ICP-MS.

increased with the reaction time, concomitant with the decrease of inorganic As (Figure 3B). The quantity of TMAO decreased with time. At 20 h, the amount of DMAs and MMAs exceeded that of inorganic As but TMAO disappeared (Figure 3B). As(III) methylation level by a series of protein concentrations was demonstrated (Figure 3C). Result showed that the production of methylated As was promoted by increasing MaArsM concentrations in the reaction solution. The account of MMAs decreased when MaArsM concentration exceeded 1 μM. Overall, the total amount of methylated As kept on increasing, concomitant with the increase of MaArsM concentration within 10 μM.

The amount of methylated As increased with the rising As(III) concentrations in the reaction solution (Figure 3D), but the percentage of that indeed decreased. Almost 95% of the total As was transformed into methylated As when treated with 5 μM As(III), while the percentage dropped to about 75% when with 20 μM As(III).

Effects of SAM and GSH Concentrations on the Enzymatic Methylation of As(III). As(III) methylation did not occur in the absence of SAM or GSH (SI Figure S3). SAM served as a methyl donor while GSH as a thiol cofactor according to Hayakawa's pathway for As methylation.[30] We carried out As(III) methylation assays in the presence of a series of concentrations of SAM or GSH (SI Figure S3A). Results showed that the effect of SAM or GSH on the efficiency of methylation is a function of the concentration added. The level of methylated As went up with increasing GSH concentration, although MMAs declined when GSH concentration exceeding 10 mM (SI Figure S3A). Therefore, As(III) methylation was promoted by increasing GSH concentration (within 20 mM). On the contrary, excessive SAM inhibited the enzymatic reaction. Maximal level of As(III) methylation occurred at 1 mM SAM, over which the sum of methylated As began to decrease (SI Figure S3B).

Effect of Different Thiols on the Enzymatic Methylation of As(III). GSH is not produced by archaea, although it is a major thiol in many bacteria and nearly all eukaryotes.[31] Coenzyme M (CoM; 2-mercaptoethanesulfonic acid) as an analog of GSH,[32] is almost only produced by methanoarchaea and plays roles in some thiol-associated processes, such as methanogenesis. For the As(III) methylation by MaArsM, CoM can act as a cofactor and is much more efficient than GSH (SI Figure S4). To further examine the effect of different thiol types on the enzymatic methylation, several other thiol compounds were demonstrated as the cofactors of MaArsM, respectively, and proved to be unequal in supporting As(III) methylation (Figure 4). Homocysteine was a relatively ideal cofactor. DMAs were the main methylated product, except in the cases of cysteine, thioglycolic acid, and 3-mercaptopropionic acid, in which MMAs was dominant product. In addition, some thiol compounds such as penicillamine, 2,3-dimercaptopropanol (DMP) and 2,3-dimercaptosuccinic acid (DMSA), did not work for As(III) methylation by MaArsM at all (Figure 4).

Conserved Cysteine Residues Cys62, Cys150, and Cys200 of MaArsM. Cysteines in As(III) methyltransferases are especially important in catalyzing As methylation process, attributed to their thiol groups.[33,34] The amino acid sequence of MaArsM contained eight cysteines, of which the individual function was assessed by site-directed mutagenesis. Cysteine pair or a single cysteine was substituted for serine(s), creating mutants C24/25S C30S, C31S, C62S, C150S, C194S, and C200S (SI Figure S5). Compared with normal MaArsM, C150S, and C200S completely lost their activities of As(III) methylation, while the activity of C62S severely decreased (Figure 5A). Furthermore, MMAs was transformed into DMAs by C62S, as in the case of normal MaArsM, but not by C150S or C200S (Figure 5B). Two kinds of controls (without enzyme or arsenicals) were performed together with the experimental groups. No methylated As species appeared in controls. Results showed that three cysteines (Cys62, Cys150, and Cys200) played roles in first methylation step [from As(III) to MMAs], while two of which (Cys150 and Cys200) were required for second methylation step (form MMAs to DMAs) in
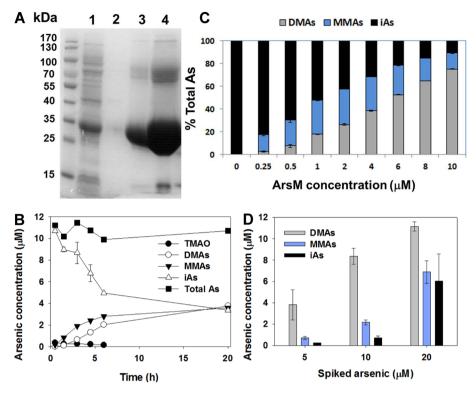
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Figure 3. Purification and functional verification of MaArsM. (A) SDS-PAGE showing the purity of the purified MaArsM. Lane 1, cell lysis solution; Lane 2, collections of the washing buffer outflowing from Ni(II)-NTA column; Lane 3, the eluate outflowing from Ni(II)-NTA column; Lane 4, concentrated MaArsM solution. (B) Time dynamic of As(III) methylation by purified MaArsM enzyme. The reaction mixture contained 10 μM As(III), 1 mM SAM, 8 mM GSH, and 5 μM pudified MaArsM in MOPS buffer at 37 °C. Arsenic species in reaction solution were analyzed at the indicated time points by HPLC-ICP-MS as described in the Materials and Methods section. (C) Methylation levels by different concentrations of MaArsM enzyme were tested in MOPS buffer containing 10 μM As(III), 1 mM SAM and 8 mM GSH at 37 °C for 20 h. (D) Methylation levels in different As(III) concentrations. The reaction mixture contained a certain concentration of As(III) (5, 10, and 20 μM), 1 mM SAM, 8 mM GSH, and 10 μM pudified MaArsM in MOPS buffer, at 37 °C for 20 h. All error bars indicate the standard errors of three triplicates.
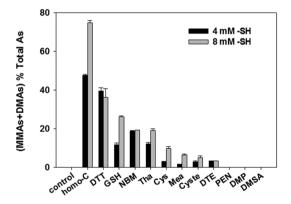

Figure 4. Methylation of As(III) as a function of different thiol cofactors. Methylation of As was tested in MOPS buffer containing 10 μM As(III), 4 or 8 mM thiols, 1 mM SAM and 5 μM ArsM enzyme. The thiols used in the experiment are homo-C, L-homocysteine; DTT, Dithiothreitol; GSH, Glutathione; NBM, 3-Nitrobenzylmercaptan; Tha, Thioglycolic acid; Cys, Cysteine; Mea, 3-Mercaptopropionic acid; Cyste, Cysteamine; DTE, Dithioerythritol; PEN, Penicillamine; DMP, 2,3-Dimercaptopropanol; and DMSA, 2,3-Dimercaptosuccinic acid. The error bars indicate the standard errors of three triplicates.

Challenger's pathway.[35] For the mutants C24/25S, C30S, C31S, or C194S, no significant difference was observed in enzymatic activity, compared with normal MaArsM (Figure 5C, 5D). We concluded that Cys24, Cys25, Cys30, Cys31, and Cys194 were not involved in As methylation.

■ DISCUSSION

To our knowledge, MaArsM enzyme is the first identified and characterized As(III) methyltransferase from an archaeon. MaArsM enzyme provides some insights into the mechanism of As resistance in archaea. The expression of *MaarsM* gene conferred As resistance to As-hypersensitive *E. coli*, even at As(III) concentration as high as 100 μM. The *E. coli* bearing *MaarsM* gene could transform inorganic As into various methylated species. Given enough time and optimal conditions, almost all of the As(III) in medium can be methylated by *E. coli*, with DMAs as the dominant product. *E. coli* with *MaarsM* produced gaseous TMAs and DMAsH, and the quantity can reach 3 μg (0.4% of the total As). MaArsM enzyme may metabolize As(III) to a lower level permitting cellular growth in vivo, in other words, it may relieve As toxicity in cells, consistent with the idea that As methyltransferase has an important physiological role in detoxifying As.

Cysteine residues are especially important for As methylation and the most conserved as functional sites.[36] For MaArsM enzyme, Cys62, Cys150, and Cys200 are shown to determine its function in As methylation. Sequence analysis showed that these cysteines are conserved in almost all the As(III) methyltransferases identified in members of every kingdom, from bacteria to humans. For the CmArsM from eukaryotic alga *Cyanidioschyzon* sp. isolate 5508, it is proposed that Cys174 and Cys224 of the enzyme play a central role in binding As(III) or MMA(III), while Cys72 is the last residue to participate in As(III) binding but is not involved in the binding
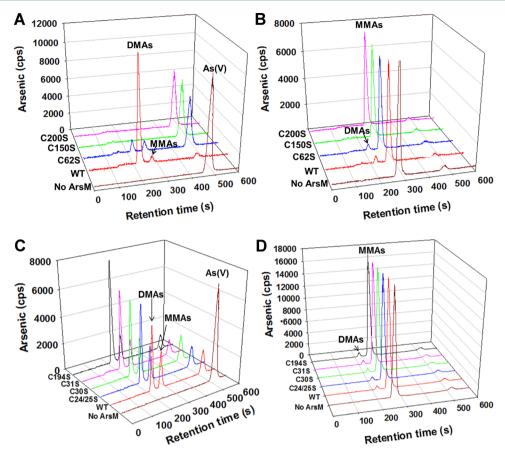
Figure 5. Methylation of As(III) or MMAs by normal or mutant MaArsM enzymes. Methylation of As was tested in MOPS buffer containing 10 μM As(III) (A and C) or 10 μM MMAs (B and D), 1 mM SAM, 8 mM GSH, and 10 μM ArsM enzyme. The reaction solutions were kept at 37 °C for 20 h and analyzed As species by HPLC-ICP-MS.

of MMA(III) or DMA(III).[34] For the mouse AS3MT, Cys157 and Cys207 are speculated to form an intramolecular disulfide bond during As methylation reaction.[37] In addition, Cys72, Cys156, and Cys206 of the human AS3MT are essential in maintaining hAS3MT structure.[33] The As methylating gene may be horizontally transferred from prokaryote to eukaryote for an environmental adaptation.[38,39] The characterization of ArsM from archaea, which are likely the oldest lives on the Earth, could be advantageous for studying the evolution of ArsMs.

The thiols play an important role in detoxification of toxic metals, including As methylation process, in which a thiol cofactor was required. Some intracellular or extracellular thiols have been shown different abilities to support As methylation. CoM, an important coenzyme produced at millimolar concentration in methanogens,[40] is more ideal for As methylation by MaArsM in comparison with GSH. Homocysteine, existing in archaea, bacteria, and eukarya and mediating As methylation by the one-carbon metabolism mechanism in mammals,[41,42] also exhibited relatively high capability in As methylation by MaArsM. According to the Hayakawa's pathway for As methylation, the glutathionylated arsenicals are preferred substrates for the methylating enzymes.[30] The effect of thiols on As methylation may critically depend on the characteristics of the As-thiol complex (such as geometry, stability, etc.). For example, dithiothreitol (DTT) usually forms an unstable complex with As(III), while DMSA and DMP usually form stable chelate structures with As(III).[43] The complex of As-DTT is flexible enough to bind the

methylating enzyme, however, that of As-DMSA or As-DMP fails to that. DTE forms a bicyclic dithiolate with As(III), while its stereoisomer DTT, as well as GSH, cysteine, form respective trithiolates with As(III).[44,45] The trithiolate structure is preferable as substrate than the dithiolate in As(III) methylation. These may partly explain the different efficiency of thiols in methylation reaction. The thiols are ubiquitous in anoxic environments,[46−50] and may be originated from the decomposition of residues or from in situ production by microorganisms, or both. The ambient low-molecular-weight thiols can mediate metal biochemistry, such as the uptake and methylation of mercury by anaerobic bacteria.[51] The levels of As methylation and volatilization were enhanced by adding organic matter to soils, such as manure, cow dung, and dried distillers grain.[21,52,53] One possible explanation is that the concentrations of the thiols in soils were increased through the decomposition of organic matters[54] and the uptake of thiols by soil microbes (such as archaea) improved As biomethylation.

Environmental Implications. Archaea are widespread in every environment that has been explored.[55] In As-rich extreme environments, archaea including methanoarchaea, represent a considerable fraction of the prokaryotic world.[56−58] It has been suggested that archaea play a considerable role in the biogeochemistry of metal(loid)s in the environment.[59] Our results, showing the molecular mechanism of As methylation by *M. acetivorans* C2A could contribute to the understanding of archaeal role in As biogeochemistry in the environment. The potential applications of either archaeal biomass or archaeal enzymes in bioremediation is alsp possible. For example, the

ability of methanoarchaea to thrive in anaerobic or other extreme environments suggests that they are ideally suited for use in the bioremediation of anoxic sludge or marine coastal sediment, etc. Archaeal enzymes mass-produced in well-characterized *E. coli* provide an outlook for developing genetic engineering bacteria aimed to remediate As-contaminated soil or water.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Figure S1: The percentage of As species formed in the medium of *M. acetivorans* C2A. Figure S2: Arsenic volatilization by *E. coli* AW3110 ($\Delta arsRBC$) expressing *MaarsM*. Figure S3: Methylation of As(III) as a function of substrate (GSH or SAM) concentrations. Figure S4: The percentage of As species formed in the enzymatic methylation of As(III) with CoM or GSH as cofactor. Figure S5: The graph of SDS-PAGE for purified normal and mutant MaArsM proteins. Table S1: The abbreviations and structures of thiol compounds investigated in MaArsM assays. Table S2: Primers used for the site-directed mutagenesis of enzyme MaArsM. This material is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Authors

*Phone: +86-10-62-62936940; fax: +86-10-62936940; e-mail: ygzhu@rcees.ac.cn.
*Phone: +86-10-62-62936940; fax: +86-10-62936940; e-mail: gxsun@rcees.ac.cn.

Notes

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