Chemical Research in Toxicology

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Chemical Mechanism of Arsenic Biomethylation

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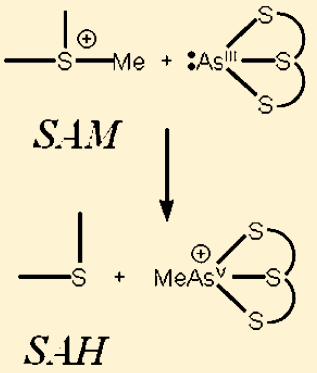
ABSTRACT: The bioconversion of inorganic arsenic to methylated metabolites affects the tissue distribution and retention of arsenic and its actions as a toxicant or carcinogen. Although enzymes that catalyze the methylation of arsenicals have been identified in all branches of the tree of life, fundamental questions persist about the chemical processes that underlie reactions that methylate this metalloid. Here, several reaction schemes for arsenic methylation are considered to encourage careful consideration of the chemical plausibility of these schemes.



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SIGNIFICANCE OF ARSENIC BIOMETHYLATION

All living organisms are exposed to arsenic in one form or another and have evolved strategies to cope with this exposure. One of the most commonly encountered is the reduction of arsenate, which is adventitiously taken up into a cell via the phosphate transport system, to arsenite that is subsequently transported out of the cell with the help of an efflux protein. Because arsenite is more toxic than arsenate, the cell interior cannot be considered to be detoxified. The same is true for the cell exterior. Even this process is not straightforward because the arsenic(III) can be further metabolized to nontoxic trimethylarsine oxide. This methylation of arsenic is another process that was once regarded as detoxification because the widely distributed, stable, and easily analyzed methylarsenic(V) species are much less toxic than their inorganic precursors. However, the finding that the less stable, and harder to analyze, methylarsenic(III) species are more toxic than inorganic arsenic species has slowly put an end to that belief.[1,2]

The oxidation state of arsenic in inorganic and methylated species is an important determinant of toxicity in a wide range of biological systems. For example, arsenicals containing As(III) are typically more cytotoxic and genotoxic than are homologues containing As(V), and in a transplacental exposure model in the mouse, methylarsonous acid (MMA(III)) is a carcinogen.[3−7] These findings and the detection of MMA(III) species in mammalian urine, especially in human populations exposed to arsenic in their drinking water, has provoked considerable interest in the mechanism of their formation.[8,9] Indeed, the linkage between the biomethylation process and formation of methylated metabolites containing either As(III) or As(V) remains a central question in the study of arsenic as a toxicant and carcinogen.

Biomethylation of arsenic to trimethylarsine was confirmed in fungi by Frederick Challenger and his co-workers in 1933.[10] Figure 1 shows the stepwise path involving oxidative addition followed by the reductive elimination he later proposed for enzymatically catalyzed methylation.[11] Challenger suggested that "active methionine," later identified as *S*-adenosylmethionine (SAM), was the methyl group donor. Notably, the Challenger pathway is analogous to the uncatalyzed oxidative

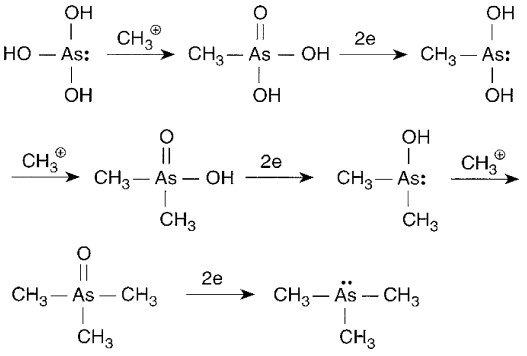

Figure 1. Challenger pathway for biological methylation of arsenic.

Received: November 27, 2013
Published: February 11, 2014

   dx.doi.org/10.1021/tx400441h | *Chem. Res. Toxicol.* 2014, 27, 457−461

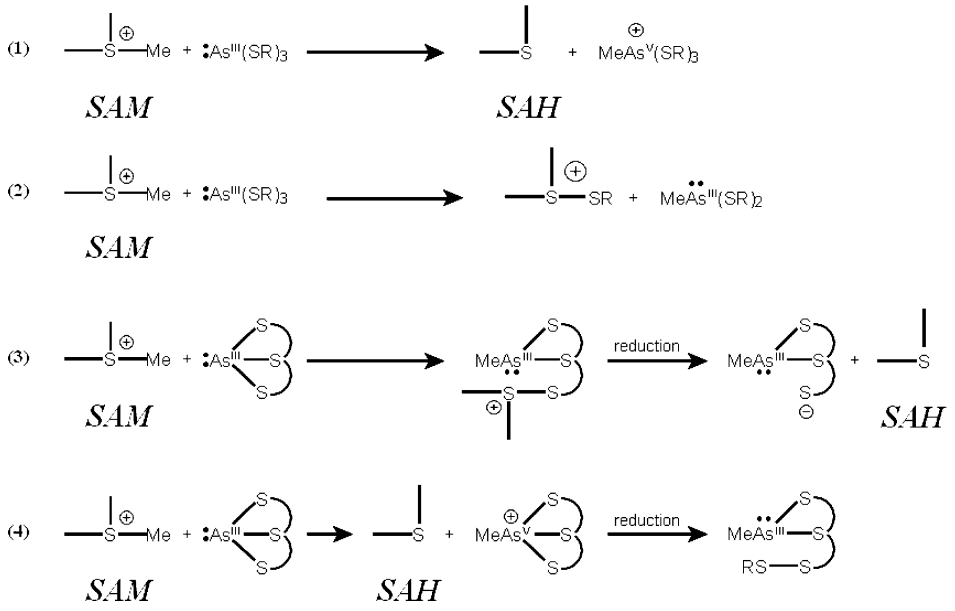

Figure 2. Equations describing the biological methylation of arsenic. Equation 1: methylation by the Challenger pathway with the As(III) species shown ligated to three RS moieties. Abbreviations: *S*-adenosylmethione, SAM; *S*-adenosylhomocysteine, SAH. Equation 2: methylation by the pathway proposed by Hayakawa and coworkers.[22] Equation 3: methylation by the pathway proposed by Wang and co-workers based on studies with arsenic (+3 oxidation state) methyltransferase (As3mt).[31] Equation 4: a modified methylation scheme consistent with the Challenger pathway and based on studies with arsenite methyltransferase (ArsM).

addition reaction known as the Meyer reaction that is used to prepare MMA(V) from arsenite and methyl halide.[12] Indeed, the Challenger pathway can be fully modeled by using the trimethylsulfonium ion as methyl donor and sulfur dioxide as the reducing agent.[13]

It should be noted that Challenger's proposed pathway was largely based on the finding that inorganic arsenic(III) and (V) and mono- and dimethylarsenic(V) species were converted to trimethylarsine (TMA) by fungi such as *Scopulariopsis brevicaulis*. Challenger was not able to identify any of the postulated intermediate species of the pathway in the culture medium due to the lack of analytical tools with the required sensitivity. Hence, he viewed the transformations as occurring in a fungal black box. Some years later, Cullen and co-workers identified methylated intermediates in culture media and used deuterium labeling to provide further evidence supporting the Challenger pathway in fungal and algal cultures.[14−19] Initially, researchers in this field assumed that Challenger's description should be taken literally with each redox step well delineated and associated with the release of specific intermediates or final products. However, it became clear that more than one redox step could take place before the "release" of an intermediate or final product and that these depended on the organism, the culture medium, and the composition and concentration of the arsenical substrate.[14,15,18,19] For example, arsenate (1 mM) is rapidly (2 days) reduced to arsenite by the fungus *Apotrichum humicola* (formerly *Candida humicola*).[19] This metabolite is then slowly (30 days) converted to trimethylarsine oxide (TMAO) that is released into media along with small amounts of other methylarsenic(V) species: monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)). In contrast, the unicellular alga *Polyphysa peniculus* rapidly metabolizes arsenate to arsenite and DMA, but MMA is absent, and trimethylarsenic species are not found in either the media or the cells. Notably, neither DMA nor MMA are metabolized by this alga.[18] Once again, Challenger was not able to demonstrate the methylation of arsenic by bacteria due to the lack of the analytical approaches with the sensitivity required for detection of the methylated products. However, in modern times such methylation has been frequently reported; for example, Michalke and coworkers[20] confirmed that anaerobic bacteria, typically those found in sewage digesters, are capable of methylating arsenic, and they report, for example, that trimethylarsine is the main product from the methogenic archaea *Methanobacterium formicicum*, *Methanosarcina barkeri*, and *Methanobacterium thermoautotrophicum.*

■ CHEMICAL PATHWAYS FOR ARSENIC METHYLATION

Questions about the validity of the Challenger pathway have prompted speculation about alternative pathways for arsenic methylation. Any proposed pathway must be chemically plausible; that is, each proposed step in the reaction scheme must conform to our knowledge of chemical kinetics and thermodynamics. In the following paragraphs, I consider the Challenger pathway and several alternatives in these terms.

Challenger's pathway (Figure 1) makes clear predictions about the reaction in which a methyl group is transferred to an arsenic atom, about the charge on the methyl group, and about the oxidation state of the arsenic atom during and after the transfer. The pathway is usually written in terms of oxy-species, but we can be reasonably sure that As−S bonding plays a major role because of the kinetic stability of the As−S bond to hydrolysis (one of the sources of the well-known affinity of As for S). Electrons for reduction of the methylarsenic(V) species to methylarsenic(III) probably come from oxidation of two thiols to a disulfide as in the real or notional reductive elimination reaction suggested for model systems: $R_3As(SR')_2 \rightarrow R_3As: + R'S\text{-}SR'$.[16] In enzymatically catalyzed reactions, physiological dithiols such as thioredoxin or glutaredoxin which are reversibly oxidized likely provide these electrons.[21]

dx.doi.org/10.1021/tx400441h | *Chem. Res. Toxicol.* 2014, 27, 457−461

Figure 2 summarizes postulated steps in the methylation of arsenic by the Challenger and alternative pathways. Equation 1 in Figure 2 shows a variant form of Challenger's pathway in which the As(III) reactant is written as a tris-thiol derivative such as arsenic tris-glutathione. Here, transfer of the electrophile CH_3^+ from SAM to an As(III) atom yields *S*-adenosylhomocysteine (SAH), a neutral species, and a methylated arsenical containing an As(V) atom. This is a chemically plausible reaction scheme because there is no possibility of an unfavorable electrostatic interaction between the positive leaving group and the uncharged SAH.

Hayakawa and co-workers[22] have postulated that arsenic(III) species persist during methylation reactions and that oxidation to arsenic(V) species occurs (somehow) after methylation. In their proposed scheme (Figure 2, eq 2), methylation initially requires the unfavorable release of a negatively charged methyl group (CH_3^-) from positively charged SAM, which then displaces an RS-group from As(III). To minimize but not eliminate unfavorable electrostatic interactions, the authors suggest that the doubly charged sulfur species formed in this reaction by loss of CH_3^- from SAM interacts with the thiol displaced from arsenic to revert to a singly charged sulfonium species. Hayakawa and co-workers suggest that the methylated product of reactions shown in eq 2 is either released as a methylated As(III) species or oxidized during release to produce a methylated As(V) species. However, isolation of TMAO from cultures of *A. humicola* described above is an unambiguous example of the direct release of an As(V) metabolite. In this instance, the postulated precursor As(III) metabolite, the gas trimethylarsine, is actually produced by the fungus only at much higher concentrations (>1 ppm) of arsenical substrates.[23,24] Therefore, release of TMAO into the media of fungal cultures containing lower concentrations of arsenical is not the result of atmospheric oxidation of trimethylarsine (half-life in air at 20 °C: two days[25]), which is not consistent with the pathway of eq 2. The putative methylarsenic(III) precursors are not very stable and would not have been detected in the media in the early studies because hydride generation under acid conditions was used for the analysis: both MMA(III) and MMA(V) would be detected as methylarsine and both DMA(III) and DMA(V) as dimethylarsine.

"Reductive methylation", is the heart of an alternative pathway proposed by Naranmandura and co-workers.[26] They suggest that the oxidative methylation and reductive elimination reactions of the Challenger pathway occur simultaneously so that the "real" reaction product is an arsenic(III) species. In our opinion, this reaction scheme would significantly reduce the nucleophilicity of the lone electron pair on arsenic and inhibit the reaction.

Although these alternative pathways are consistent with evidence that methylated products containing As(III) are often bound to protein targets, they do not account for the observation that methylated products containing either As(III) or As(V) are products of enzymatically catalyzed methylation reactions.[27,28]

Other reaction schemes have been suggested based on evidence from studies that used purified arsenic methyltransferases. Two such proteins have been identified and their genes cloned. Arsenic (+3 oxidation state) methyltransferase (As3mt) catalyzes arsenic methylation in a wide range of higher organisms, and arsenic methyltransferase (ArsM) catalyzes these reactions in Archaea, some eukaryotes, and many prokaryotes.[29,30] Wang and co-workers examined methylation catalyzed by recombinant human As3mt using a physiological monothiol (glutathione, cysteine) as the sole reductant.[31] They suggested that an As(III)-containing substrate initially binds to three thiolate residues that are shown as protein-bound S atoms (curved bonds) in the first step of eq 3, Figure 2. Then, following Hayakawa and co-workers,[22] they postulate that the methylated arsenic product remains as As(III) and that a protein-bound S, displaced from the arsenic during the methylation reaction, binds to the demethylated and doubly charged SAM. This reaction is followed by a novel reduction step that does not involve an As(III) intermediate. Here, the disulfide formed between the displaced S and demethylated SAM is reduced to generate SAH. This reaction is claimed to be linked to a conformational change in As3mt that releases SAH. Unfortunately, the model also fails to address the central problem with such a reaction scheme; namely, how does CH_3^- leave a positive center?

A recent paper by Ajees and co-workers[32] offers an opportunity to examine the two pathways shown in eqs 1 and 2 (Figure 2) as they would apply to the methylation of arsenite by SAM catalyzed by ArsM. This structural study provides a model of the enzyme's active sites to which inorganic As(III) and SAM are bound. In a fully charged ArsM molecule, the methyl group of SAM is poised to be transferred to the arsenic which is initially bound to three thiolate-containing cysteinyl residues (Cys 72, Cys174, and Cys 224). These thiolates can be thought of as equivalent to the protein-bound S atoms that interact with arsenic as shown in eq 3 (Figure 2). The authors did not write out their reaction scheme but, following others,[22,31] we can use the first step of eq 3 as a framework to keep As in a trivalent oxidation state. Ajees and co-workers predict that a cysteinyl residue, Cys 72, in ArsM is the leaving group that forms the S−S bond with demethylated SAM as shown in eq 3. However, this process is not confirmed by their structural model, which shows that Cys 72 moves away from SAM during the reaction rather than binding to it.

Finally, eq 4 of Figure 2 gives our interpretation of enzymatically catalyzed methylation according to the Challenger pathway. In this scheme, there are no problems with charge, and it is easy to see that binding another thiolate to the arsonium center either from the protein, there are many cysteinyl residues in ArsM and As3mt, or from exogenous thiol reactants would neutralize the charge on arsenic and provide an opportunity for reductive elimination of a disulfide, leaving the methylarsenic(III) species bound to the protein. The final product of eq 4 is also poised to accept another methyl group from SAM to yield a DMA(V) species which would be bound to the enzyme by two As−S bonds and would be more likely to be released by hydrolysis than the precursor MMA(V) species which is bound by three As−S bonds. This difference in susceptibility to hydrolysis may account for the general preponderance of DMA(V) over MMA(V) in biological systems. The failure of rat and human As3mt to methylate MMA(V) may be because there is no available reduction path to afford the necessary enzyme bound methylarsenic(III) intermediates. [29]

CONCLUSIONS

On the grounds of chemical plausibility, the Challenger pathway with SAM as a donor of CH_3^+ remains the most rational option to describe the biological methylation of arsenic. We anticipate that future structural and functional studies with

As3mt and ArsM will further refine this model. In particular, although the reaction scheme postulated by Hayakawa and co-workers is cited (for example, see Figures 1 and 3 in ref 33), it should be regarded as very speculative, and at least, the existence of the sulfonium species with a S−S bond required in eq 2 needs to be verified experimentally. The high toxicity of the methylarsenic(III) metabolites remains a problem no matter which methylation pathway is favored.

Finally, one of the groups initially supporting Hayakawa's model now reports[34] that the "theoretical calculation shows the methyl group transfer process to be a typical in-line SN2 nucleophilic substitution reaction in many SAM-dependent methyltransferases. iAs^{3+} with lone pair can attack the CH_3."

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Funding

I am grateful to the Canadian Water Network for financial support.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

I thank David J. Thomas, US Environmental Protection Agency, and Ken Reimer, Royal Military College, Kingston, Canada, for encouragement.

ABBREVIATIONS

MMA(III), methylarsonous acid; MMA(V), methylarsonic acid; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; TMA, trimethylarsine; As3mt, arsenic (+3 oxidation state) methyltransferase; ArsM, arsenite methyltransferase

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