Importance of Being Thiomethylated: Formation, Fate, and Effects of Methylated Thioarsenicals

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · DECEMBER 2014

Impact Factor: 3.53 · DOI: 10.1021/tx500464t · Source: PubMed

1 AUTHOR:

Hua Naranmandura

Zhejiang University

48 PUBLICATIONS **800** CITATIONS

SEE PROFILE

Chemical Research in Toxicology

Perspective

pubs.acs.org/crt

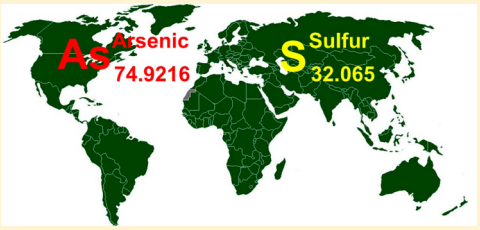
Importance of Being Thiomethylated: Formation, Fate, and Effects of Methylated Thioarsenicals

Qian Qian Wang,[†,‡] David J. Thomas,[§] and Hua Naranmandura*[,†,‡]

[†]Department of Toxicology, School of Medicine and Public Health, [‡]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

[§]Pharmacokinetics Branch, Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, Durham, North Carolina 27711, United States

ABSTRACT: Although inorganic arsenic has long been recognized as a potent toxicant and carcinogen in humans, recent evidence shows that at least some of its effects are mediated by methylated metabolites. Elucidating the conversion of inorganic arsenic to mono-, di-, and trimethylated species has provided insights into the enzymology of this pathway and identified genetic and environmental factors that influence the susceptibility of individuals to this metalloid's adverse health effects. Notably, almost all work on the formation, fate, and effects of methylated arsenicals has focused on oxoarsenicals in which arsenic is bound to one or more oxygen atoms. However, thioarsenicals are a class of arsenicals in which a sulfur atom has replaced one or more oxygens that are bound to arsenic. Thioarsenicals have been identified as urinary metabolites in humans and other animals following exposure to inorganic arsenic. Studies find that methylated thioarsenicals exhibit kinetic behavior and toxicological properties that distinguish them from methylated oxoarsenicals. This perspective considers that formation, fate, and effects of methylated thioarsenicals with an emphasis on examining the linkages between the molecular processes that underlie both methylation and thiolation reactions. Integrating this information will provide a more comprehensive view of the relationship between the metabolism of arsenic and the risk posed by chronic exposure to this environmental contaminant.



CONTENTS

1. INTRODUCTION

Depending on circumstances and intensity of exposure, inorganic arsenic (iAs) can be a threat to health or a life-saving drug. Various iAs-containing compounds have long been used in traditional Chinese and Mongolian medicine, and arsenic trioxide is currently used to treat acute promyelocytic leukemia.[1−3] Inorganic arsenic is classified as a human carcinogen;[4] chronic exposure to inorganic arsenic increases risk of skin, liver, and lung cancers.[5−7] Increased risks for a variety of other adverse health effects are also associated with chronic exposure to this metalloid making it a significant public health hazard.[8,9] Use of iAs-contaminated groundwater as a drinking water source exposes millions of people worldwide to this toxin.[10,11] Assessing risk from chronic exposure to arsenic must consider its metabolic transformation. Ingested iAs is extensively methylated and is excreted in urine as mono- and dimethylated products.[12] Research on the metabolism and effects of arsenic has focused on the formation, fate, and effects

Special Issue: Chemical Toxicology in China

Received: November 17, 2014
Published: December 22, 2014

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

of methylated oxoarsenicals in which there is one or more bonds between arsenic and oxygen atoms. However, recent studies have identified thioarsenicals as a novel class of arsenic-containing metabolites. Thioarsenicals are structural homologues of oxoarsenicals in which one or more oxygen atoms bound to an arsenic atom is replaced with a sulfur atom. Studies with thioarsenicals indicate that their distribution and fate and their toxicities are distinct from those of oxoarsenicals. Here, we consider the origin of thioarsenicals and the linkage between processes that convert iAs into methylated oxoarsenical species and processes that convert oxoarsenicals into thioarsenicals. Comparing relative production of methylated oxoarsenicals and methylated thioarsenicals is difficult as our estimates depend primarily on the quantitation of these metabolites in urine; there is little data on levels of these species in tissues. Despite the lack of quantitative information, understanding the chemical basis of thioarsenical formation and connections between pathways that produce methylated oxoarsenicals and thioarsenicals should contribute to understanding arsenic's action at the molecular, cellular, and organismic levels and assist those who must make decisions about the risk associated with its presence in water supplies or its benefit when used in the treatment of cancer.

2. METABOLISM OF ARSENIC

2.1. Formation of Methylated Oxoarsenicals. The capacity of organisms to convert iAs into methylated metabolites is a common feature in all three domains of the tree of life.[13] Pioneering work of Frederick Challenger and his colleagues[14] first described a sequence of alternating reactions in microorganisms that reduce pentavalent arsenic to trivalency and oxidatively methylate the trivalent arsenical (Figure 1).

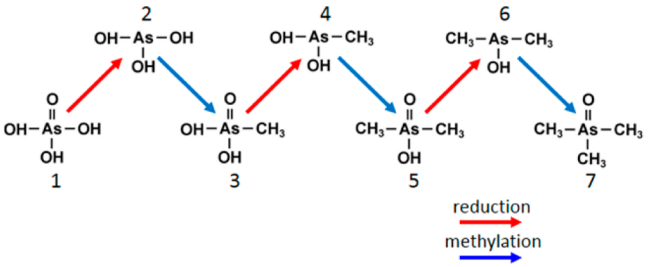


Figure 1. The Challenger scheme for methylation of oxyarsenicals. Arsenate (1) is reduced to arsenite (2), which is oxidatively methylated to monomethylarsonic acid (3). Monomethylarsonic acid is reduced to monomethylarsonous acid (4), which is oxidatively methylated to dimethylarsinic acid (5). Dimethylarsinic acid is reduced to dimethylarsinous acid (6), which is oxidatively methylated to trimethylarsine oxide (7).

Formation of methylated metabolites in the Challenger scheme has long been regarded as a process that detoxifies iAs by producing less reactive and less toxic methylated metabolites. In fact, the role of methylation of arsenic in this metalloid's toxicity is complex. Methylated arsenicals containing trivalent arsenic formed as pathway intermediates are more reactive and toxic than iAs or methylated pentavalent arsenicals.[15−19] Interindividual differences in capacity to produce methylated arsenicals are linked to differences in susceptibility to arsenic-induced diseases.[20] Notably, alternative schemes for arsenic methylation have been proposed (Figure 2).[21,22] These schemes permit the addition of a methyl group to a trivalent arsenic atom without its concurrent oxidation and posit a

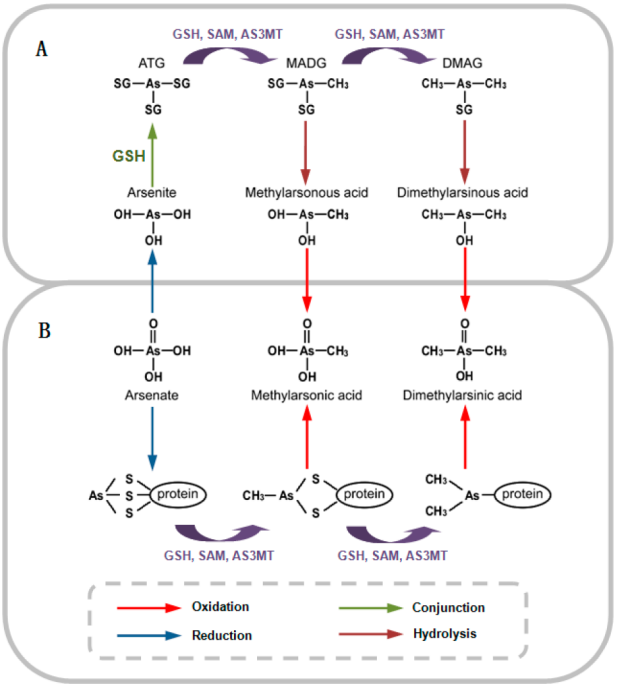


Figure 2. Alternated schemes for methylation of inorganic arsenic. (A) Hayakawa and co-workers[21] proposed a pathway in which arsenic−glutathione (GSH) complexes are substrates for *S*-adenosylmethionine (SAM)-dependent methylation catalyzed by an arsenic methyltransferase (AS3MT) that converts arsenite triglutathione ($iAs^{III}(GS)_3$) to monomethylarsonic acid diglutathione $MMA^{III}(GS)_2$ and this intermediate's conversion to dimethylarsinous acid glutathione $DMA^{III}(GS)$. (B) Naranmandura and co-workers[22] proposed a pathway in which trivalent arsenic remains bound to proteins during successive rounds of reductive methylation catalyzed by AS3MT. Here, methylated arsenicals containing pentavalent arsenic are produced by release and oxidation of protein-bound arsenicals.

critical role for thiol−arsenic interactions in methylation reactions. The chemical plausibility of different methylation schemes has been recently evaluated and questioned.[23] Studies of reaction mechanisms and kinetic behaviors of bacterial and human arsenic methyltransferases are supportive of at least some aspects of the alternative methylation schemes.[24−26]

2.2. Enzymology of Oxoarsenical Methylation. Genomes of diverse organisms contain genes that encode proteins that catalyze arsenic methylation. These arsenic methyltransferases include products of the *ArsM* gene in bacteria and archaea and the *AS3MT* gene in higher organisms.[27] These enzymes require a thiol-containing cofactor to catalyze *S*-adenosylmethionine-dependent methylation of trivalent arsenicals.[28,29] Methylated oxoarsenicals containing either trivalent or pentavalent arsenic are products of these reactions.[23] Enzymatically catalyzed methylation of arsenic is a prime determinant of the formation and fate of methylated metabolites involved in the toxic actions and clearance of arsenic. In *AS3MT* knockout mice, reduced capacity to methylate iAs results in prolonged and elevated tissue retention of iAs which exacerbates tissue damage in a target tissue, the uroepithelium.[30−33]

2.3. Formation of Thioarsenicals. *2.3.1. Definition and Occurrence in the Environment.* A thioarsenical contains an arsenic−sulfur bond analogous to an arsenic−oxygen bond in its oxoarsenical analogue.[34] Thus, thioarsenate ($(OH)_3$-As(=S)) and arsenate ($(OH)_3$-As(=O)) are thioarsenical-oxoarsen-

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

ical analogues. Trivalent arsenic in thioarsenicals readily oxidizes to pentavalency.[35] In groundwater, iAs occurs as the oxyanions arsenite or arsenate. In contrast, arsenic in soils and rocks is usually present in combination with hydroxides, sulfides, iron, and manganese.[36] Redox conditions and pH control formation of inorganic thioarsenicals in water. Under sulfidic conditions, the inorganic thioarsenicals ($AsO_3S_3^-$, $AsO_2S_2^{3-}$, $AsOS_3^{3-}$, and AsS_4^{3-}) are formed.[37] Bacteria can reduce sulfates and organic sulfur compounds in groundwater to produce sulfide that converts oxoarsenicals to thioarsenicals. Thermophilic algae from hot springs convert iAs into both methylated oxo- and methylated thio-arsenicals.[38]

2.3.2. Stability and Analysis. An overarching concern in the study of the metabolism of oxo- and thio-arsenicals is accurate, sensitive, and reproducible determination of these compounds in biological samples. Optimal conditions for collection, processing, and storage of samples preserves arsenicals in their native state until analysis. For example, the oxidation state of arsenic in biological samples can change during storage. In urine stored for 24 h at −20 °C, dimethylarsinous acid (DMA^{III}) oxidized to dimethylarsinic acid (DMA^V).[39,40] Both abiotic conditions and high pH promote oxidation of arsenite.[41] Acidification, filtration, and low temperature storage of natural water samples retard microbial activity that affects arsenic speciation.[41,42] These approaches might be used to preserve biological samples for arsenic analysis. Preservation of thioarsenicals in biological samples poses special problems. In oxygen-rich cellular environments, trivalent thioarsenicals (thioarsenites) are probably quickly converted to pentavalent thioarsenicals (thioarsenates). Oxygen and reactive iron reduce the stability of thioarsenate in aqueous samples, and acidification of sulfide-rich water may precipitate arsenic−sulfur complexes.[37,43] Both storage temperature and time affect the stability of thioarsenicals in human urine.[44,45] These findings suggest that standard protocols are needed for collection, processing, and storage of samples for thioarsenical analysis.

Detection of a thioarsenical by methods dependent on hydride generation (HG) may be affected by the extent to which it is reduced to a volatile arsine. Hydrogen peroxide converts thioarsenicals to oxoarsenical analogues so that their unique identities as thioarsenicals are not discerned,[41] for example, both DMA^{III} and a $DMMTA^V$ generate arsines at neutral pH.[46] Thus, analytical methods that use pH-specific HG for speciation of urinary arsenicals cannot easily discriminate between these arsenicals. Formation of dimethylarsine from $DMMTA^V$ at neutral pH suggests that replacement of an oxygen atom by a sulfur atoms produces a more reactive electrophile that readily forms a volatile product.

Liquid chromatography (LC) coupled with ICP-MS has proven to be a successful approach for arsenic speciation in biological samples.[40,47] Progress on detection of thioarsenicals in biological samples has been linked to studies designed to determine the oxidation state of arsenic in methylated arsenicals. Despite analytical difficulties, several studies have identified thioarsenicals including monomethylmonothioarsonic acid ($MMMTA^V$), $DMMTA^V$, and dimethyldithioarsinic acid ($DMDTA^V$) in urine and experimental species following exposure to iAs or DMA^V.[30,48,50] Urine from sheep that graze on arsenic-rich seaweed on beaches in the Orkney Islands contain several methylated oxy-arsenicals and a novel thioarsenical metabolite, 2-dimethylarsinothioyl acetic acid.[51,52] In humans, ingestion of an arsenosugar or chronic use of iAs-contaminated well water results in urinary excretion of methylated thioarsenicals.[44,46] These results suggest that formation of thioarsenicals must be considered part of the metabolic process that occurs after exposure to iAs.

2.3.3. Hydrogen Sulfide Production and Thioarsenical Formation. Hydrogen sulfide (H_2S) has long been known to convert alkylarsine oxides to thiolated products.[53] For example, H_2S converts the oxoarsenical, DMA^V, to $DMMTA^V$ and $DMDTA^V$. Computational analysis of H_2S-dependent formation of thioarsenicals from their oxoarsenical analogues indicates that formation of the As−S bond precedes rearrangement of OH and SH moieties and elimination of water.[54]

H_2S is produced in higher organisms by cellular metabolism and by the gastrointestinal microbiota. The gastrointestinal microbiota, the community of microorganisms that reside in the gastrointestinal tract, produces H_2S by scavenging hydrogen formed by fermentation and by dissimilation of reduced organic molecules. H_2S is the most abundant S-containing gas formed in the rat cecum where bioavailability of organic sulfate in diet affects H_2S production.[55] In mice, altered gastrointestinal microbiota affects H_2S levels in the gut lumen and in plasma and tissues.[56] Rapid H_2S oxidation probably prevents lethal concentrations of this gas in the gastrointestinal tract.[57,58]

H_2S is also synthesized in mammalian cells where it functions as a signaling molecule in various pathways.[59−62] As shown in Figure 3, mammalian cells produce H_2S from homocysteine (Hcy) through the reverse transsulfuration pathway and through the cysteine (Cys) oxidation pathway with cystathionine (Cyst) as a key intermediate in both processes.[63] Reactions in the reverse transsulfuration pathway are catalyzed by cystathionine β-synthase (CBS) and cystathionine-γ-lyase (CSE). Reactions in the Cys oxidation pathway that are catalyzed by cysteine aminotransferase and mercaptopyruvate sulfurtransferase generate 3-mercaptopyruvate (3MP) and

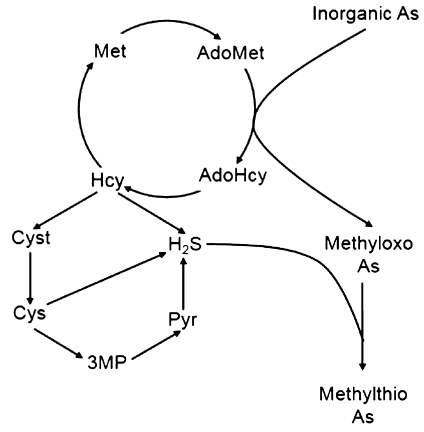


Figure 3. Origin of hydrogen sulfide production and linkage to arsenic methylation in mammalian cells. Homocysteine (Hcy) is formed by the degradation of *S*-adenosylhomocysteine (AdoHcy). Through the reverse transsulfuration pathway, Hcy is converted to hydrogen sulfide (H_2S) or converted to cystathionine (Cyst). Cyst is converted to cysteine (Cys) and through the Cys degradation pathway is converted to H_2S or converted to 3-mercaptopyruvate (3MP) and pyruvate (Pyr) as intermediates in H_2S production. Hcy is also recycled to produce methionine (Met), which is converted to *S*-adenosylmethionine (AdoMet), the methyl group donor for enzymatically catalyzed arsenic methylation which produces methylated oxoarsenicals (methyloxo As). H_2S converts methylated oxoarsenicals into methylated thioarsenicals (methylthio As). Transfer of a methyl group from AdoMet produces AdoHcy.

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

pyruvate (Pyr), which is converted to sulfane sulfur (S^0); reduction of S^0 yields H_2S. Notably, H_2S production is also linked to production and use of *S*-adenosylmethionine (AdoMet), the primary methyl group donor for enzymatically catalyzed reactions including the methylation of arsenicals.[29] H_2S can originate from food-derived organic polysulfides, although this pathway is not well characterized.[64] Availability of substrates and cofactors and kinetic characteristics of enzymes determine relative contributions of various pathways to H_2S production.[58,65] Estimates of H_2S levels in mammalian tissues vary widely, reflecting both intertissue variation in production and degradation and use of different analytical methods. Recent measurements find low nanomolar concentrations of H_2S in mammalian tissues.[66,67] In perfused mouse tissues, H_2S production averages about 0.45pmol/min/mg tissue.[68] In cytosol, H_2S is metabolized to methanethiol and dimethylsulfate or oxidized to thiosulfate and sulfate.[69−71] Oxidation of H_2S in mitochondria is linked to ATP production.[72]

2.3.4. Preabsorptive Metabolism and Formation of Methylated Thioarsenicals. Preabsorptive metabolism of ingested arsenicals is a molecular transformation mediated by the gastrointestinal microbiota that occurs before transport across the gastrointestinal barrier. Early work focused on the conversion of inorganic arsenicals into methylated species. In in vitro systems, arsenite and arsenate are converted to monomethylated species by anaerobic microorganisms from mouse or rat cecum.[73,74] Analytical methods used in these studies could not determine whether metabolites were methylated oxoarsenicals or methylated thioarsenicals. In an anaerobic reactor system, human fecal microbiota convert inorganic arsenate to thioarsenicals.[75] Anaerobic microorganisms from the mouse cecum which produce H_2S rapidly convert an arsenosugar from an oxoarsenical to thioarsenical species.[76] Both oxo- and thio-arsenosugars are accumulated in the Caco-2 cell intestinal barrier model, albeit at lower rates than seen for arsenite.[77,78]

DMA^V is converted to trimethylarsine sulfide by the anaerobic microbiota of the mouse cecum, demonstrating that these organisms not only methylate arsenicals but also convert metabolites from oxo- to thio-species.[79,80] The order of reactions involved in the conversion of substrate to product is not clear from these studies. Studies[34] with S-labeled compounds suggest that thiolated species could be substrates for methylation reactions; however, the evidence is not definitive. Additional evidence on reaction order is provided by a comprehensive study of the metabolism of inorganic arsenate by the anaerobic microbiota of mouse cecum.[81] This study shows that both methylation and thiolation reactions occur in this system and yield a range of mono-, di-, and trimethylated oxo- and thio-arsenical products. The integration of these pathways is shown in Figure 4 in which processes producing methylated oxoarsenicals and converting these species to their thiolated analogues are linked.

2.3.5. Postabsorptive Metabolism and Formation of Methylated Thioarsenicals. H_2S formed in tissues can convert oxoarsenicals to thioarsenicals. In *in vitro* reaction systems, DMA^{III} added to rat liver supernate is converted to $DMMTA^V$ or $DMDTA^V$.[82] The pathway involved in the formation of thiolated arsenicals from DMA^{III} is not known. In dialyzed rat liver supernate, DMA^{III} binds to proteins, suggesting that loss of sulfides during dialysis disrupts conversion to a thiolated species. *In vitro*, human red blood cells take up DMA^{III} and rapidly convert it to $DMMTA^V$, which quickly effluxes from the cell.[83] In the medium of these cells, $DMMTA^V$ is the most abundant arsenical species. The pathway for the conversion of DMA^{III} to $DMMTA^V$ in red cells could proceed by the formation of dimethylmonothioarsinous acid ($DMMTA^{III}$) that oxidizes to $DMMTA^V$ or by simultaneous thiolation and oxidiation of DMA^{III} by sulfane sulfur (S^o). Notably, in an *in vitro* reaction system containing lamb liver cytosolic supernate, an arsenosugar is quantitatively converted from an oxo species to its thio analogue.[84]

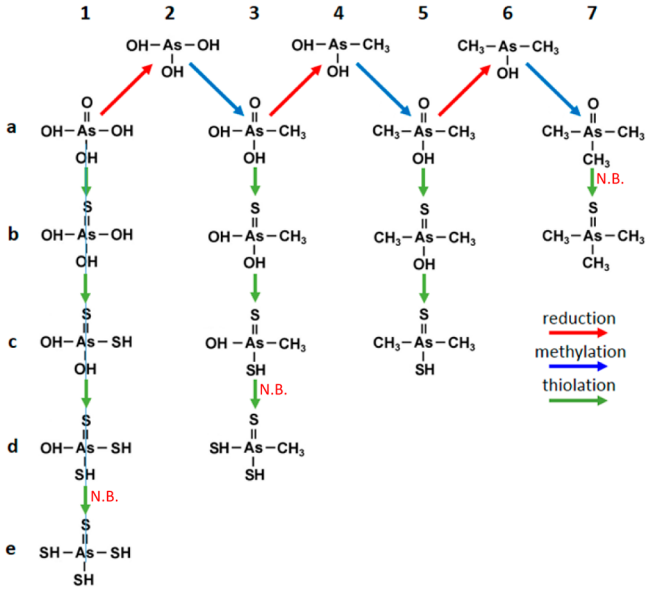


Figure 4. Linked pathways for the formation of oxo- and thio-arsenicals. Arsenate (1a) is converted to monothioarsenate (1b), dithioarsenate (1c), trithioarsenate (1d), and tetrathioarsenate (1e) or reduced to arsenite (2). Arsenite is oxidatively methylated to monomethylarsonic acid (3a) that is converted to monomethylmonothio (3b)-, monomethyldithio (3c)-, and monomethyltrithio (3d)-arsonic acid species. Monomethylarsonic acid is reduced to monomethylarsonous acid (4) that is oxidatively methylated to dimethylarsinic acid (5a), which is converted to dimethylmonothio (5b)- and dimethyldithio-arsinic acid species. Dimethylarsinic acid is reduced to dimethylarsinous acid (7) that is oxidatively methylated to trimethylarsine oxide (7a), which is converted to trimethylarsine sulfide (7b). N.B. indicates not observed.

2.3.6. Possible Cross-Talk in the Formation of Thioarsenicals. Preabsorptive and postabsorptive metabolism may play coordinated roles in the formation of thioarsenicals. In wild-type rats receiving an oral dose of arsenite, biliary excretion of monomethylarsonous acid (MMA^{III}) and its diglutathione conjugate, monomethylarsenodiglutathione ($MMA^{III}(GS)_2$, precedes the appearance of MMMTA in urine.[85] In Eisai hyperbilirubinuric rats that are deficient in multidrug resistance protein (Mrp2/Abcc2), biliary transport of $MMA(GS)_2$ is minimal, and MMMTA levels in urine are much reduced.[85] Biliary transport of arsenotriglutathione ($iAs^{III}(GS)_3$) and $MMA^{III}(GS)_2$ depends on Mrp2/Abcc2 that is an essential component of enterohepatic circulation of arsenicals.[86−88] Because glutathione complexes of methylated oxoarsenicals are preferred substrates for a variety of transporters,[89,90] formation of these complexes may stimulate their biliary excretion. In the gut lumen, these complexes are converted into methylated thioarsenicals that are reabsorbed into the systemic circulation (Figure 5). Notably, $DMMTA^V$-GS, a complex of a dimethylated thioarsenical and glutathione, has been detected

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

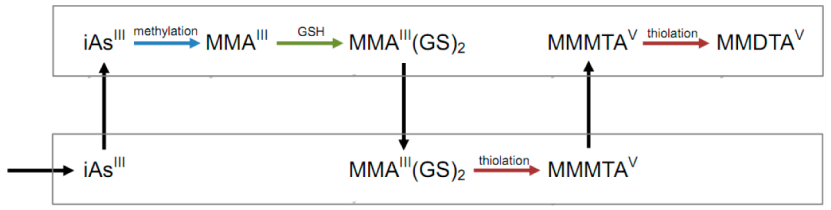


Figure 5. Cross-talk between preabsorptive and postabsorptive metabolism in the formation of methylated thioarsenicals. Ingested inorganic arsenic (iAs^{III}) is absorbed from the intestinal lumen and is enzymatically converted to monomethylated arsenical species (MMA^{III}). This compound is converted to a diglutathione complex, $MMA(GS)_2$, which is secreted in bile. In the intestinal lumen, the $MMA(GS)_2$ is converted to monomethylmonothioarsenic ($MMMTA^V$) by the microbiota. $MMMTA^V$ is absorbed across the intestinal barrier, distributed systemically, and converted to other thiolated metabolites (e.g., monomethyldithioarsenic, $MMDTA^V$).

in plants and animals.[91,92] Complexation of methylated thioarsenicals by thiols could play important roles in the distribution and metabolism of these metabolites.

2.3.7. Systemic Distribution of Thioarsenicals. Studies in experimental animals provide insights into the formation, distribution, and clearance of thioarsenicals. Oral administration of arsenite results in urinary excretion of $DMMTA^V$ and $DMDTA^V$ in hamsters and $DMMTA^V$ in rats.[34] In both species, monomethylmonothioarsonic acid ($MMMTA^V$) is found in urine. Chronic treatment of rats with an arsenite-containing diet results in urinary excretion of $MMMTA^V$ and $DMMTA^V$.[49] In rats, intravenously administered $DMMTA^V$ produces a pattern of arsenic distribution and retention that resembles that seen after treatment with DMA^{III}; in contrast, intravenously administered $DMDTA^V$ produces a pattern of arsenic distribution and retention that resembles that seen after treatment with DMA^V.[93] Intravenously administered $MMMTA^V$ produces a pattern of arsenic distribution and retention in rats that differs from that seen after treatment with MMA^V.[94] In hamsters, intravenously administered $DMMTA^V$ produces a pattern of arsenic distribution among tissues that resembles DMA^{III}, and intravenously administered $DMDTA^V$ produces a pattern of arsenic distribution and retention that resembles that seen after treatment with DMA^V.[95]

2.3.8. Toxic Effects of Thioarsenicals. Cytotoxic potency is a metric to compare the toxic properties of thioarsenicals and oxoarsenicals. In cultured A431 human epidermoid carcinoma cells, $DMMTA^V$ is more cytotoxic than DMA^V (LC_{50} 10.7 μM and 843 μM, respectively) and resembles iAs^{III} (LC_{50} 5.5 μM) or DMA^{III} (LC_{50} 2.2 μM) in potency.[96] In HepG2 human hepatocarcinoma cells, $DMMTA^V$ (LC_{50} 26 μM) is more cytotoxic than $DMDTA^V$ (LC_{50} 3660 μM) or DMA^V (LC_{50} 343 μM).[97] In EJ-1 human urinary bladder carcinoma cells, DMA^{III} and $DMMTA^V$ are equally cytotoxic (LC_{50} 13 μM and 17 μM, respectively); in contrast, both monomethylated oxo- and thio-arsenicals are relatively weak cytotoxins in these cells.[98,99] The cytotoxicities of $DMMTA^V$ and $DMA^{III}(GS)$ are similar (IC_{70} 21 μM and 6 μM, respectively) in A549 human lung adenocarcinoma epithelial cells.[100] Taken together, these results show $DMMTA^V$, a dimethylated thioarsenical containing pentavalent arsenic, to be nearly as cytotoxic as an inorganic oxoarsenical (iAs^{III}) or a dimethylated oxoarsenical that contains trivalent arsenic (DMA^{III} and $DMA^{III}(GS)$). Comparable cytotoxic potencies of $DMMTA^V$ and DMA^{III} reflect similar kinetics for cellular accumulation of these compounds. Although these compounds readily enter cells, DMA^V, $DMDTA^V$, monomethylated oxoarsenicals, and monomethylated thioarsenicals are not accumulated in cells.[98] Thus, cytotoxic potency can be rationalized as a function of the kinetic behavior of different oxo- and thio-arsenicals.

Besides cytotoxicity, $DMMTA^V$ has other adverse effects in cultured cells. In EJ-1 cells, both DMA^{III} and $DMMTA^V$ induce prooxidant states and cause DNA damage.[98,99] In Syrian hamster embryo cells, $DMMTA^V$ and $DMDTA^V$ produce a range of chromosomal abnormalities.[97] In UROtsa human urinary bladder epithelial cells, $DMMTA^V$ has larger effects on cell cycle progression, induction of apoptosis, and response to oxidative stress than do equimolar concentrations of arsenite.[101] Differences in potencies of these arsenicals for these effects reflect higher cellular accumulation of arsenic after exposure to $DMMTA^V$ than after exposure to arsenite.

2.3.9. Potential Role of Thioarsenicals in Arsenic Toxicity. Complementary pathways for the methylation of arsenicals and for conversion of oxoarsenicals into thioarsenicals complicate our understanding of the role of metabolism in the toxicity of arsenic. An example of this metabolic complexity comes from studies of carcinogenic outcomes associated with exposure to DMA^V. In rats, DMA^V exposure increases tumor incidence in urinary bladder transitional epithelium.[102,103] However, neither the proximate carcinogen nor the mechanism of action underlying tumor development has been identified. The array of metabolites found in urine from rats exposed to DMA^V includes both methylated oxoarsenicals and methylated thioarsenicals that are potential proximate carcinogens (Figure 6). Exposure of rats to DMA^V results in the appearance of methylated oxoarsenicals, dimethylarsinous acid (DMA^{III}) and trimethylarsine oxide (TMAO), in urine.[48,104] Appearance of these compounds is consistent with metabolic schemes in which arsenic in the trivalent oxidation state is enzymatically converted to methylated products. DMA^{III} is a potent cytotoxicant that could induce regenerative proliferation that underlies urinary bladder tumor formation.[104−106] TMAO exposure induces liver adenomas in rats.[107] Long-term exposure of rats to DMA^V in drinking water is associated with the appearance of sulfur-containing arsenicals in urine that are likely to be methylated thioarsenicals.[108] Both $DMMTA^V$ and trimethylarsine sulfide are found in the urine of rats that receive DMA^V in drinking water.[104] Exposure of rats to the methylated oxoarsenicals, monomethylarsonic acid (MMA^V) or DMA^V, leads to the accumulation dimethylthioarsenicals in the liver.[102,109] Although the carcinogenicity of methylated thioarsenicals has not been evaluated, $DMMTA^V$ has a cytotoxic potency that resembles DMA^{III} and could induce regenerative proliferation that underlies tumor formation.

3. SUMMARY AND LOOKING FORWARD

A variety of studies show that the formation of thioarsenicals is one aspect of the web of metabolic reactions that occur in organisms exposed to iAs. Methylated thioarsenicals that

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

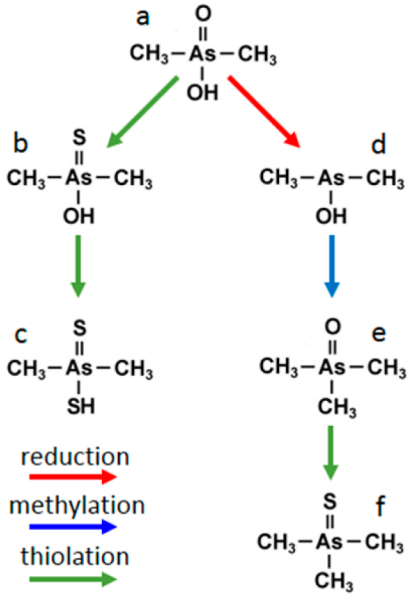


Figure 6. Parallel pathways for the formation of metabolites of dimethylarsinic acid. In animal models, exposure to dimethylarsinic acid (a) results in the production of thiolated metabolites dimethylmonothioarsinic acid (b) and dimethyldithioarsinic acid (c). Reduction of a produces dimethylarsinous acid (d) that is methylated to form trimethylarsine oxide (e), which can be converted to its thio-analogue, dimethylarsine sulfide (f).

contain pentavalent arsenic can be differentiated from methylated oxoarsenicals that contain pentavalent arsenic on the basis of their kinetic behavior and by their higher potency as cytotoxicants and genotoxicants. These findings suggest that additional work is needed to elucidate steps in pathways that convert oxoarsenicals to thioarsenicals, particularly the sequence of methylation and thiolation reactions in these pathways. Unraveling connections between these pathways will require the development of improved methods for collection and processing of samples that preserve oxo- and thio-arsenicals in their native state and analytical methods to quantify these species in biological samples. Studies of the kinetics of thioarsenicals in *in vitro* and *in vivo* systems are needed to provide a dosimetric basis for additional studies of the biological effects of thioarsenicals. Ultimately, these studies should provide insight into the modes of toxic actions of thioarsenicals which can be integrated into a broader understanding of the role of these metabolites in toxic outcomes associated with chronic exposure to iAs. In sum, this research will reduce the uncertainty in risk assessment for this metalloid.

AUTHOR INFORMATION

Corresponding Author

*Phone/Fax: (86) 571-8820-6736. E-mail: narenman@zju.edu.cn.

Funding

We acknowledge the National Natural Science Foundation of China (Nos. 81274138 and 81473289), Zhejiang Provincial Natural Science Foundation of China (No. R2110231), Zhejiang University K.P. Chao's High Technology Development Foundation (2013RC026), and Zhejiang Provincial Program for the Cultivation of Medical Rookies.

Notes

This manuscript has been reviewed in accordance with the policy of the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The authors declare no competing financial interest.

ABBREVIATIONS

iAs, inorganic arsenic; DMA^{III}, dimethylarsinous acid; DMA^{V}, dimethylarsinic acid; HG, hydride generation; $DMMTA^{V}$, dimethylmonothioarsinic acid; $MMMTA^{V}$, monomethylmonothioarsonic acid; $DMDTA^{V}$, dimethyldithioarsinic acid; H_2S, hydrogen sulfide; Hcy, homocysteine; Cys, cysteine; Cyst, cystathione; 3MP, 3-mercaptopyruvate; Pyr, pyruvate; S^0, sulfane sulfur; AdoMet, *S*-adenosylmethionine; TMAS, trimethylarsine sulfide; $DMMTA^{III}$, dimethylmonothioarsinous acid; MMA^{III}, monomethylarsonous acid; $MMA^{III}(GS)_2$, monomethylarsenodiglutathione; MRP2/cMOAT, multidrug resistance-associated protein; $iAs^{III}(GS)_3$, arsenotriglutathione; $DMMTA^{V}$-GS, complex of a dimethylmonothioarsinic acid and glutathione; MMA^{V}, monomethylarsonic acid; $DMA^{III}(GS)$, complex of dimethylarsinous acid and glutathione; TMAO, trimethylarsine oxide

REFERENCES

(1) Bouteille, B., Oukem, O., Bisser, S., and Dumas, M. (2003) Treatment perspectives for human African trypanosomiasis. *Fundam. Clin. Pharmacol. 17*, 171−181.

(2) Powell, B. L., Moser, B., Stock, W., Gallagher, R. E., Willman, C. L., Stone, R. M., Rowe, J. M., Coutre, S., Feusner, J. H., and Gregory, J. (2010) Arsenic trioxide improves event-free and overall survival for adults with acute promyelocytic leukemia: North American Leukemia Intergroup Study C9710. *Blood 116*, 3751−3757.

(3) Powell, B. L. (2011) Arsenic trioxide in acute promyelocytic leukemia: potion not poison. *Expert Rev. Anticancer 11*, 1317−1319.

(4) IARC (2004) Some drinking-water disinfectants and contaminants, including arsenic. *IARC Monogr. Eval. Carcinog. Risks Hum. 84*, 1.

(5) Hong, Y.-S., Song, K.-H., and Chung, J.-Y. (2014) Health effects of chronic arsenic exposure. *Am. J. Prev. Med. 47*, 245.

(6) Wang, W., Cheng, S., and Zhang, D. (2014) Association of inorganic arsenic exposure with liver cancer mortality: a meta-analysis. *Environ. Res. 135*, 120−125.

(7) Saint-Jacques, N., Parker, L., Brown, P., and Dummer, T. J. (2014) Arsenic in drinking water and urinary tract cancers: a systematic review of 30 years of epidemiological evidence. *Environ. Health 13*, 44.

(8) Moon, K., Guallar, E., and Navas-Acien, A. (2012) Arsenic exposure and cardiovascular disease: an updated systematic review. *Curr. Atheroscler. Rep. 14*, 542−555.

(9) Farzan, S. F., Karagas, M. R., and Chen, Y. (2013) In utero and early life arsenic exposure in relation to long-term health and disease. *Toxicol. Appl. Pharmacol. 272*, 384−390.

(10) Chung, J. Y., Yu, S. D., and Hong, Y. S. (2014) Environmental source of arsenic exposure. *J. Prev. Med. Public Health 47*, 253−257.

(11) Shankar, S., Shanker, U., and Shikha (2014) Arsenic contamination of groundwater: a review of sources, prevalence, health risks, and strategies for mitigation. *Sci. World J. 2014*, 304524.

(12) Watanabe, T., and Hirano, S. (2013) Metabolism of arsenic and its toxicological relevance. *Arch. Toxicol. 87*, 969−979.

(13) Zhu, Y.-G., Yoshinaga, M., Zhao, F.-J., and Rosen, B. P. (2014) Earth abides arsenic biotransformations. *Annu. Rev. Earth Planet Sci. 42*, 443−467.

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

(14) Challenger, F. (1945) Biological methylation. *Chem. Rev. 36*, 315−361.
(15) Petrick, J. S., Ayala-Fierro, F., Cullen, W. R., Carter, D. E., and Aposhian, H. V. (2000) Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. *Toxicol. Appl. Pharmacol. 163*, 203−207.
(16) Styblo, M., Del Razo, L. M., Vega, L., Germolec, D. R., LeCluyse, E. L., Hamilton, G. A., Reed, W., Wang, C., Cullen, W. R., and Thomas, D. J. (2000) Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Arch. Toxicol. 74*, 289−299.
(17) Naranmandura, H., Xu, S., Koike, S., Pan, L. Q., Chen, B., Wang, Y. W., Rehman, K., Wu, B., Chen, Z., and Suzuki, N. (2012) The endoplasmic reticulum is a target organelle for trivalent dimethylarsinic acid (DMA^{III})-induced cytotoxicity. *Toxicol. Appl. Pharmacol. 260*, 241−249.
(18) Zhang, Y. F., Zhang, M., Huang, X. L., Fu, Y. J., Jiang, Y. H., Bao, L. L., Maimaitiyiming, Y., Zhang, J. G., Wang, Q. Q., and Naranmandura, H. (2014) Combination of arsenic and cryptotanshinone induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in breast cancer cells. *Metallomics 7*, 165−173.
(19) Rehman, K., Fu, Y. J., Zhang, Y. F., Wang, Q. Q., Wu, B., Wu, Y., Zhou, X. Y., Sun, W. H., Sun, T. F., and Naranmandura, H. (2014) Trivalent methylated arsenic metabolites induce apoptosis in human myeloid leukemic HL-60 cells through generation of reactive oxygen species. *Metallomics 6*, 1502−1512.
(20) Antonelli, R., Shao, K., Thomas, D. J., Sams, R., and Cowden, J. (2014) AS3MT, GSTO, and PNP polymorphisms: impact on arsenic methylation and implications for disease susceptibility. *Environ. Res. 132*, 156−167.
(21) Hayakawa, T., Kobayashi, Y., Cui, X., and Hirano, S. (2005) A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. *Arch. Toxicol. 79*, 183−191.
(22) Naranmandura, H., Suzuki, N., and Suzuki, K. T. (2006) Trivalent arsenicals are bound to proteins during reductive methylation. *Chem. Res. Toxicol. 19*, 1010−1018.
(23) Cullen, W. R. (2014) Chemical mechanism of arsenic biomethylation. *Chem. Res. Toxicol. 27*, 457−461.
(24) Ajees, A. A., Marapakala, K., Packianathan, C., Sankaran, B., and Rosen, B. P. (2012) Structure of an As(III) S-adenosylmethionine methyltransferase: insights into the mechanism of arsenic biotransformation. *Biochemistry 51*, 5476−5485.
(25) Dheeman, D. S., Packianathan, C., Pillai, J. K., and Rosen, B. P. (2014) Pathway of human AS3MT arsenic methylation. *Chem. Res. Toxicol. 27*, 1979−1989.
(26) Naranmandura, H. (2014) Letter to the Editor regarding the article by Dheeman et al., 2014. *Chem. Res. Toxicol. 27*, 2011.
(27) Thomas, D. J., and Rosen, B. P. (2013) Arsenic Methyltransferases, in *Encyclopedia of Metalloproteins* pp 138−143, Springer, New York.
(28) Ding, L., Saunders, R. J., Drobna, Z., Walton, F. S., Xun, P., Thomas, D. J., and Styblo, M. (2012) Methylation of arsenic by recombinant human wild-type arsenic (+3 oxidation state) methyltransferase and its methionine 287 threonine (M287T) polymorph: Role of glutathione. *Toxicol. Appl. Pharmacol. 264*, 121−130.
(29) Thomas, D. J., Li, J., Waters, S. B., Xing, W., Adair, B. M., Drobna, Z., Devesa, V., and Styblo, M. (2007) Arsenic (+3 oxidation state) methyltransferase and the methylation of arsenicals. *Exp. Biol. Med. 232*, 3−13.
(30) Drobna, Z., Naranmandura, H., Kubachka, K. M., Edwards, B. C., Herbin-Davis, K., Styblo, M., Le, X. C., Creed, J. T., Maeda, N., Hughes, M. F., and Thomas, D. J. (2009) Disruption of the arsenic (+3 oxidation state) methyltransferase gene in the mouse alters the phenotype for methylation of arsenic and affects distribution and retention of orally administered arsenate. *Chem. Res. Toxicol. 22*, 1713−1720.
(31) Hughes, M. F., Edwards, B. C., Herbin-Davis, K. M., Saunders, J., Styblo, M., and Thomas, D. J. (2010) Arsenic (+3 oxidation state) methyltransferase genotype affects steady-state distribution and clearance of arsenic in arsenate-treated mice. *Toxicol. Appl. Pharmacol. 249*, 217−223.
(32) Chen, B., Arnold, L. L., Cohen, S. M., Thomas, D. J., and Le, X. C. (2011) Mouse arsenic (+ 3 oxidation state) methyltransferase genotype affects metabolism and tissue dosimetry of arsenicals after arsenite administration in drinking water. *Toxicol. Sci. 124*, 320−326.
(33) Yokohira, M., Arnold, L. L., Pennington, K. L., Suzuki, S., Kakiuchi-Kiyota, S., Herbin-Davis, K., Thomas, D. J., and Cohen, S. M. (2011) Effect of sodium arsenite dose administered in the drinking water on the urinary bladder epithelium of female arsenic (+3 oxidation state) methyltransferase knockout mice. *Toxicol. Sci. 121*, 257−266.
(34) Naranmandura, H., Suzuki, N., Iwata, K., Hirano, S., and Suzuki, K. T. (2007) Arsenic metabolism and thioarsenicals in hamsters and rats. *Chem. Res. Toxicol. 20*, 616−624.
(35) Planer-Friedrich, B., Suess, E., Scheinost, A. C., and Wallschlager, D. (2010) Arsenic speciation in sulfidic waters: reconciling contradictory spectroscopic and chromatographic evidence. *Anal. Chem. 82*, 10228−10235.
(36) Mandal, B. K., and Suzuki, K. T. (2002) Arsenic round the world: a review. *Talanta 58*, 201−235.
(37) Wallschlager, D., and Stadey, C. J. (2007) Determination of (Oxy)thioarsenates in sulfidic waters. *Anal. Chem. 79*, 3873−3880.
(38) Qin, J., Lehr, C. R., Yuan, C. G., Le, X. C., McDermott, T. R., and Rosen, B. P. (2009) Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga. *Proc. Natl. Acad. Sci. U.S.A. 106*, 5213−5217.
(39) Gong, Z., Lu, X., Cullen, W. R., and Le, X. C. (2001) Unstable trivalent arsenic metabolites, monomethylarsonous acid and dimethylarsinous acid. *J. Anal. At. Spectrom. 16*, 1409−1413.
(40) Gong, Z., Lu, X., Ma, M., Watt, C., and Le, X. C. (2002) Arsenic speciation analysis. *Talanta 58*, 77−96.
(41) McCleskey, R. B., Nordstrom, D. K., and Maest, A. S. (2004) Preservation of water samples for arsenic (III/V) determinations: an evaluation of the literature and new analytical results. *Appl. Geochem. 19*, 995−1009.
(42) Cheam, V., and Agemian, H. (1980) Preservation of inorganic arsenic species at microgram levels in water samples. *Analyst 105*, 737−743.
(43) Planer-Friedrich, B., and Wallschlager, D. (2009) A critical investigation of hydride generation-based arsenic speciation in sulfidic waters. *Environ. Sci. Technol. 43*, 5007−5013.
(44) Raml, R., Rumpler, A., Goessler, W., Vahter, M., Li, L., Ochi, T., and Francesconi, K. A. (2007) Thio-dimethylarsinate is a common metabolite in urine samples from arsenic-exposed women in Bangladesh. *Toxicol. Appl. Pharmacol. 222*, 374−380.
(45) Conklin, S. D., Fricke, M. W., Creed, P. A., and Creed, J. T. (2008) Investigation of the pH effects on the formation of methylated thio-arsenicals, and the effects of pH and temperature on their stability. *J. Anal. At. Spectrom. 23*, 711−716.
(46) Raml, R., Goessler, W., Traar, P., Ochi, T., and Francesconi, K. A. (2005) Novel thioarsenic metabolites in human urine after ingestion of an arsenosugar, 2′,3′-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-riboside. *Chem. Res. Toxicol. 18*, 1444−1450.
(47) B'hymer, C., and Caruso, J. A. (2004) Arsenic and its speciation analysis using high-performance liquid chromatography and inductively coupled plasma mass spectrometry. *J. Chromatogr., A 1045*, 1−13.
(48) Adair, B. M., Moore, T., Conklin, S. D., Creed, J. T., Wolf, D. C., and Thomas, D. J. (2007) Tissue distribution and urinary excretion of dimethylated arsenic and its metabolites in dimethylarsinic acid- or arsenate-treated rats. *Toxicol. Appl. Pharmacol. 222*, 235−242.
(49) Suzuki, S., Arnold, L. L., Pennington, K. L., Chen, B., Naranmandura, H., Le, X. C., and Cohen, S. M. (2010) Dietary administration of sodium arsenite to rats: relations between dose and urinary concentrations of methylated and thio-metabolites and effects

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

on the rat urinary bladder epithelium. *Toxicol. Appl. Pharmacol. 244*, 99−105.
(50) Naranmandura, H., Rehman, K., Le, X. C., and Thomas, D. J. (2013) Formation of methylated oxyarsenicals and thioarsenicals in wild-type and arsenic (+3 oxidation state) methyltransferase knockout mice exposed to arsenate. *Anal. Bioanal. Chem. 405*, 1885−1891.
(51) Hansen, H. R., Raab, A., Jaspars, M., Milne, B. F., and Feldmann, J. (2004) Sulfur-containing arsenical mistaken for dimethylarsinous acid [DMA(III)] and identified as a natural metabolite in urine: major implications for studies on arsenic metabolism and toxicity. *Chem. Res. Toxicol. 17*, 1086−1091.
(52) Hansen, H. R., Pickford, R., Thomas-Oates, J., Jaspars, M., and Feldmann, J. (2004) 2-Dimethylarsinothioyl acetic acid identified in a biological sample: the first occurrence of a mammalian arsinothioyl metabolite. *Angew. Chem., Int. Ed. 116*, 341−344.
(53) Fricke, M. W., Zeller, M., Sun, H., Lai, V. W., Cullen, W. R., Shoemaker, J. A., Witkowski, M. R., and Creed, J. T. (2005) Chromatographic separation and identification of products from the reaction of dimethylarsinic acid with hydrogen sulfide. *Chem. Res. Toxicol. 18*, 1821−1829.
(54) Orthaber, A., Sax, A. F., and Francesconi, K. A. (2012) Oxygen versus sulfur: structure and reactivity of substituted arsine oxides and arsine sulfides. *J. Comput. Chem. 33*, 112−117.
(55) Suarez, F., Furne, J., Springfield, J., and Levitt, M. (1998) Production and elimination of sulfur-containing gases in the rat colon. *Am. J. Physiol.: Gastrointest. Liver Physiol. 274*, G727−G733.
(56) Shen, X., Carlstrom, M., Borniquel, S., Jadert, C., Kevil, C. G., and Lundberg, J. O. (2013) Microbial regulation of host hydrogen sulfide bioavailability and metabolism. *Free Radical Biol. Med. 60*, 195−200.
(57) Martin, G. R., McKnight, G. W., Dicay, M. S., Coffin, C. S., Ferraz, J. G. P., and Wallace, J. L. (2010) Hydrogen sulphide synthesis in the rat and mouse gastrointestinal tract. *Dig. Liver Dis. 42*, 103−109.
(58) Kabil, O., and Banerjee, R. (2014) Enzymology of H_2S biogenesis, decay and signaling. *Antioxid. Redox Signaling 20*, 770−782.
(59) Gadalla, M. M., and Snyder, S. H. (2010) Hydrogen sulfide as a gasotransmitter. *J. Neurosci. 113*, 14−26.
(60) Teague, B., Asiedu, I., and Moore, P. K. (2002) The smooth muscle relaxant effect of hydrogen sulphide in vitro: evidence for a physiological role to control intestinal contractility. *Br. J. Pharmacol. 137*, 139−145.
(61) Zhao, W. M., Zhang, J., Lu, Y. J., and Wang, R. (2001) The vasorelaxant effect of H_2S as a novel endogenous gaseous K-ATP channel opener. *EMBO J. 20*, 6008−6016.
(62) Abe, K., and Kimura, H. (1996) The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci. 16*, 1066−1071.
(63) Singh, S., and Banerjee, R. (2011) PLP-dependent H_2S biogenesis. *Biochim. Biophys. Acta 1814*, 1518−1527.
(64) Benavides, G. A., Squadrito, G. L., Mills, R. W., Patel, H. D., Isbell, T. S., Patel, R. P., Darley-Usmar, V. M., Doeller, J. E., and Kraus, D. W. (2007) Hydrogen sulfide mediates the vasoactivity of garlic. *Proc. Natl. Acad. Sci. U.S.A. 104*, 17977−17982.
(65) Singh, S., Padovani, D., Leslie, R. A., Chiku, T., and Banerjee, R. (2009) Relative contributions of cystathionine β-synthase and γ-cystathionase to H_2S biogenesis via alternative trans-sulfuration reactions. *J. Biol. Chem. 284*, 22457−22466.
(66) Linden, D. R., Levitt, M. D., Farrugia, G., and Szurszewski, J. H. (2010) Endogenous production of H_2S in the gastrointestinal tract: still in search of a physiologic function. *Antioxid. Redox Signaling 12*, 1135−1146.
(67) Furne, J., Saeed, A., and Levitt, M. D. (2008) Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am. J. Physiol.: Regul., Integr. Comp. Physiol. 295*, R1479−R1485.
(68) Linden, D. R., Sha, L., Mazzone, A., Stoltz, G. J., Bernard, C. E., Furne, J. K., Levitt, M. D., Farrugia, G., and Szurszewski, J. H. (2008) Production of the gaseous signal molecule hydrogen sulfide in mouse tissues. *J. Neurosci. 106*, 1577−1585.
(69) Weisiger, R. A., Pinkus, L. M., and Jakoby, W. B. (1980) Thiol S-methyltransferase - suggested role in detoxication of intestinal hydrogen-sulfide. *Biochem. Pharmacol. 29*, 2885−2887.
(70) Furne, J., Springfield, J., Koenig, T., DeMaster, E., and Levitt, M. D. (2001) Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized function of the colonic mucosa. *Biochem. Pharmacol. 62*, 255−259.
(71) Levitt, M. D., Furne, J., Springfield, J., Suarez, F., and DeMaster, E. (1999) Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. *J. Clin. Invest. 104*, 1107−1114.
(72) Libiad, M., Yadav, P. K., Vitvitsky, V., Martinov, M., and Banerjee, R. (2014) Organization of the human mitochondrial hydrogen sulfide oxidation pathway. *J. Biol. Chem. 289*, 30901−30910.
(73) Rowland, I., and Davies, M. (1981) In vitro metabolism of inorganic arsenic by the gastro-intestinal microflora of the rat. *J. Appl. Toxicol. 1*, 278−283.
(74) Hall, L. L., George, S. E., Kohan, M. J., Styblo, M., and Thomas, D. J. (1997) In vitro methylation of inorganic arsenic in mouse intestinal cecum. *Toxicol. Appl. Pharmacol. 147*, 101−109.
(75) Van de Wiele, T., Gallawa, C. M., Kubachka, K. M., Creed, J. T., Basta, N., Dayton, E. A., Whitacre, S., Du Laing, G., and Bradham, K. (2010) Arsenic metabolism by human gut microbiota upon in vitro digestion of contaminated soils. *Environ. Health Perspect. 118*, 1004−1009.
(76) Conklin, S. D., Ackerman, A. H., Fricke, M. W., Creed, P. A., Creed, J. T., Kohan, M. C., Herbin-Davis, K., and Thomas, D. J. (2006) In vitro biotransformation of an arsenosugar by mouse anaerobic cecal microflora and cecal tissue as examined using IC-ICP-MS and LC-ESI-MS/MS. *Analyst 131*, 648−655.
(77) Leffers, L., Ebert, F., Taleshi, M. S., Francesconi, K. A., and Schwerdtle, T. (2013) In vitro toxicological characterization of two arsenosugars and their metabolites. *Mol. Nutr. Food Res. 57*, 1270−1282.
(78) Leffers, L., Wehe, C. A., Huwel, S., Bartel, M., Ebert, F., Taleshi, M. S., Galla, H. J., Karst, U., Francesconi, K. A., and Schwerdtle, T. (2013) In vitro intestinal bioavailability of arsenosugar metabolites and presystemic metabolism of thio-dimethylarsinic acid in Caco-2 cells. *Metallomics 5*, 1031−1042.
(79) Kubachka, K. M., Kohan, M. C., Conklin, S. D., Herbin-Davis, K., Creed, J. T., and Thomas, D. J. (2009) In vitro biotransformation of dimethylarsinic acid and trimethylarsine oxide by anaerobic microflora of mouse cecum analyzed by HPLC-ICP-MS and HPLC-ESI-MS. *J. Anal. At. Spectrom. 24*, 1062−1068.
(80) Kubachka, K. M., Kohan, M. C., Herbin-Davis, K., Creed, J. T., and Thomas, D. J. (2009) Exploring the in vitro formation of trimethylarsine sulfide from dimethylthioarsinic acid in anaerobic microflora of mouse cecum using HPLC-ICP-MS and HPLC-ESI-MS. *Toxicol. Appl. Pharmacol. 239*, 137−143.
(81) Pinyayev, T. S., Kohan, M. J., Herbin-Davis, K., Creed, J. T., and Thomas, D. J. (2011) Preabsorptive metabolism of sodium arsenate by anaerobic microbiota of mouse cecum forms a variety of methylated and thiolated arsenicals. *Chem. Res. Toxicol. 24*, 475−477.
(82) Suzuki, K. T., Mandal, B. K., Katagiri, A., Sakuma, Y., Kawakami, A., Ogra, Y., Yamaguchi, K., Sei, Y., Yamanaka, K., Anzai, K., Ohmichi, M., Takayama, H., and Aimi, N. (2004) Dimethylthioarsenicals as arsenic metabolites and their chemical preparations. *Chem. Res. Toxicol. 17*, 914−921.
(83) Naranmandura, H., and Suzuki, K. T. (2008) Formation of dimethylthioarsenicals in red blood cells. *Toxicol. Appl. Pharmacol. 227*, 390−399.
(84) Hansen, H. R., Jaspars, M., and Feldmann, J. (2004) Arsinothioyl-sugars produced by in vitro incubation of seaweed extract with liver cytosol analysed by HPLC coupled simultaneously to ES-MS and ICP-MS. *Analyst 129*, 1058−1064.
(85) Bu, N., Wang, H. Y., Hao, W. H., Liu, X., Xu, S., Wu, B., Anan, Y., Ogra, Y., Lou, Y. J., and Naranmandura, H. (2011) Generation of thioarsenicals is dependent on the enterohepatic circulation in rats. *Metallomics 3*, 1064−1073.

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289

(86) Rehman, K., Chen, Z., Wang, W. W., Wang, Y. W., Sakamoto, A., Zhang, Y. F., Naranmandura, H., and Suzuki, N. (2012) Mechanisms underlying the inhibitory effects of arsenic compounds on protein tyrosine phosphatase (PTP). *Toxicol. Appl. Pharmacol. 263*, 273−280.
(87) Suzuki, K. T., Tomita, T., Ogra, Y., and Ohmichi, M. (2001) Glutathione-conjugated arsenics in the potential hepato-enteric circulation in rats. *Chem. Res. Toxicol. 14*, 1604−1611.
(88) Kala, S. V., Kala, G., Prater, C. I., Sartorelli, A. C., and Lieberman, M. W. (2004) Formation and urinary excretion of arsenic triglutathione and methylarsenic diglutathione. *Chem. Res. Toxicol. 17*, 243−249.
(89) Banerjee, M., Carew, M. W., Roggenbeck, B. A., Whitlock, B. D., Naranmandura, H., Le, X. C., and Leslie, E. M. (2014) A novel pathway for arsenic elimination: human multidrug resistance protein 4 (MRP4/ABCC4) mediates cellular export of dimethylarsinic acid (DMAV) and the diglutathione conjugate of monomethylarsonous acid (MMAIII). *Mol. Pharmacol. 86*, 168−79.
(90) Carew, M. W., Naranmandura, H., Shukalek, C. B., Le, X. C., and Leslie, E. M. (2011) Monomethylarsenic diglutathione transport by the human multidrug resistance protein 1 (MRP1/ABCC1). *Drug Metab. Dispos. 39*, 2298−2304.
(91) Raab, A., Wright, S. H., Jaspars, M., Meharg, A. A., and Feldmann, J. (2007) Pentavalent arsenic can bind to biomolecules. *Angew. Chem., Int. Ed. 46*, 2594−2597.
(92) Yehiayan, L., Stice, S., Liu, G. L., Matulis, S., Boise, L. H., and Cai, Y. (2014) Dimethylarsinothioyl glutathione as a metabolite in human multiple myeloma cell lines upon exposure to darinaparsin. *Chem. Res. Toxicol. 27*, 754−764.
(93) Suzuki, K. T., Iwata, K., Naranmandura, H., and Suzuki, N. (2007) Metabolic differences between two dimethylthioarsenicals in rats. *Toxicol. Appl. Pharmacol. 218*, 166−173.
(94) Naranmandura, H., Bu, N., Suzuki, K. T., Lou, Y., and Ogra, Y. (2010) Distribution and speciation of arsenic after intravenous administration of monomethylmonothioarsonic acid in rats. *Chemosphere 81*, 206−213.
(95) Naranmandura, H., Iwata, K., Suzuki, K. T., and Ogra, Y. (2010) Distribution and metabolism of four different dimethylated arsenicals in hamsters. *Toxicol. Appl. Pharmacol. 245*, 67−75.
(96) Naranmandura, H., Ibata, K., and Suzuki, K. T. (2007) Toxicity of dimethylmonothioarsinic acid toward human epidermoid carcinoma A431 cells. *Chem. Res. Toxicol. 20*, 1120−1125.
(97) Ochi, T., Kita, K., Suzuki, T., Rumpler, A., Goessler, W., and Francesconi, K. A. (2008) Cytotoxic, genotoxic and cell-cycle disruptive effects of thio-dimethylarsinate in cultured human cells and the role of glutathione. *Toxicol. Appl. Pharmacol. 228*, 59−67.
(98) Naranmandura, H., Carew, M. W., Xu, S., Lee, J., Leslie, E. M., Weinfeld, M., and Le, X. C. (2011) Comparative toxicity of arsenic metabolites in human bladder cancer EJ-1 cells. *Chem. Res. Toxicol. 24*, 1586−1596.
(99) Naranmandura, H., Ogra, Y., Iwata, K., Lee, J., Suzuki, K. T., Weinfeld, M., and Le, X. C. (2009) Evidence for toxicity differences between inorganic arsenite and thioarsenicals in human bladder cancer cells. *Toxicol. Appl. Pharmacol. 238*, 133−140.
(100) Leffers, L., Unterberg, M., Bartel, M., Hoppe, C., Pieper, I., Stertmann, J., Ebert, F., Humpf, H. U., and Schwerdtle, T. (2013) In vitro toxicological characterisation of the S-containing arsenic metabolites thio-dimethylarsinic acid and dimethylarsinic glutathione. *Toxicology 305*, 109−119.
(101) Ebert, F., Leffers, L., Weber, T., Berndt, S., Mangerich, A., Beneke, S., Burkle, A., and Schwerdtle, T. (2014) Toxicological properties of the thiolated inorganic arsenic and arsenosugar metabolite thio-dimethylarsinic acid in human bladder cells. *J. Trace Elem. Med. Biol. 28*, 138−146.
(102) Wei, M., Wanibuchi, H., Morimura, K., Iwai, S., Yoshida, K., Endo, G., Nakae, D., and Fukushima, S. (2002) Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors. *Carcinogenesis 23*, 1387−1397.
(103) Cohen, S. M., Ohnishi, T., Arnold, L. L., and Le, X. C. (2007) Arsenic-induced bladder cancer in an animal model. *Toxicol. Appl. Pharmacol. 222*, 258−263.
(104) Cohen, S. M., Arnold, L. L., Uzvolgyi, E., Cano, M., St John, M., Yamamoto, S., Lu, X., and Le, X. C. (2002) Possible role of dimethylarsinous acid in dimethylarsinic acid-induced urothelial toxicity and regeneration in the rat. *Chem. Res. Toxicol. 15*, 1150−1157.
(105) Cohen, S. M., Yamamoto, S., Cano, M., and Arnold, L. L. (2001) Urothelial cytotoxicity and regeneration induced by dimethylarsinic acid in rats. *Toxicol. Sci. 59*, 68−74.
(106) Cohen, S. M., Arnold, L. L., Eldan, M., Lewis, A. S., and Beck, B. D. (2006) Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. *Crit. Rev. Toxicol. 36*, 99−133.
(107) Shen, J., Wanibuchi, H., Salim, E. I., Wei, M., Kinoshita, A., Yoshida, K., Endo, G., and Fukushima, S. (2003) Liver tumorigenicity of trimethylarsine oxide in male Fischer 344 rats–association with oxidative DNA damage and enhanced cell proliferation. *Carcinogenesis 24*, 1827−1835.
(108) Yoshida, K., Kuroda, K., Zhou, X., Inoue, Y., Date, Y., Wanibuchi, H., Fukushima, S., and Endo, G. (2003) Urinary sulfur-containing metabolite produced by intestinal bacteria following oral administration of dimethylarsinic acid to rats. *Chem. Res. Toxicol. 16*, 1124−1129.
(109) Kenyon, E. M., Hughes, M. F., Adair, B. M., Highfill, J. H., Crecelius, E. A., Clewell, H. J., and Yager, J. W. (2008) Tissue distribution and urinary excretion of inorganic arsenic and its methylated metabolites in C57BL6 mice following subchronic exposure to arsenate in drinking water. *Toxicol. Appl. Pharmacol. 232*, 448−455.

DOI: 10.1021/tx500464t
Chem. Res. Toxicol. 2015, 28, 281−289