Review

Thiolated arsenicals in arsenic metabolism: Occurrence, formation, and biological implications

Yuzhen Sun[1], Guangliang Liu[1], Yong Cai[1,2,*]

1. Institute of Environment and Health, Jianghan University, Wuhan 430056, China
2. Department of Chemistry and Biochemistry&Southeast Environmental Research Center, Florida International University, Miami, FL 33199, USA

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ABSTRACT

Arsenic (As) is a notoriously toxic pollutant of health concern worldwide with potential risk of cancer induction, but meanwhile it is used as medicines for the treatment of different conditions including hematological cancers. Arsenic can undergo extensive metabolism in biological systems, and both toxicological and therapeutic effects of arsenic compounds are closely related to their metabolism. Recent studies have identified methylated thioarsenicals as a new class of arsenic metabolites in biological systems after exposure of inorganic and organic arsenicals, including arsenite, dimethylarsinic acid (DMA^V), dimethylarsinous glutathione (DMA^{III}GS), and arsenosugars. The increasing detection of thiolated arsenicals, including monomethylmonothioarsonic acid (MMMTA^V), dimethylmonothioarsinic acid (DMMTA^V) and its glutathione conjugate (DMMTA^{V}GS), and dimethyldithioarsinic acid (DMDTA^V) suggests that thioarsenicals may be important metabolites and play important roles in arsenic toxicity and therapeutic effects. Here we summarized the reported occurrence of thioarsenicals in biological systems, the possible formation pathways of thioarsenicals, and their toxicity, and discussed the biological implications of thioarsenicals on arsenic metabolism, toxicity, and therapeutic effects.

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Contents

* Corresponding author. E-mail: cai@fiu.edu (Yong Cai).

Introduction

Arsenic (As) is a notoriously toxic metalloid of worldwide health concern, as exposure to inorganic arsenic (iAs) has resulted in various adverse health effects (Naujokas et al., 2013; Wang et al., 2014a). Chronic exposure through drinking iAs-contaminated water increases the risk of cancers such as carcinomas of the skin, lungs, and urinary bladder (Cohen et al., in press; Hong et al., 2014; Rossman, 2003; Saint-Jacques et al., 2014; Wang et al., 2014b). Ingestion of vegetables and rice irrigated by iAs-contaminated water, medicine (Waxman and Anderson, 2001), and seafood (Raml et al., 2005) is another major pathway of human exposure to arsenic causing serious health problems (Foster and Maher, in press; Thomas and Bradham, in press). Anthropogenic activities including mining, smelting, and burning coal can cause occupational arsenic exposure, resulting in a concern of occupational health for workers in these fields (Mandal and Suzuki, 2002).

On the other hand, arsenicals have been used as medicines to treat various diseases since over 2000 years ago and some of them are still in use (Bouteille et al., 2003; Chen et al., 2007; Zhou et al., 2005). A number of arsenic-containing drugs, including Flowler's solution, Donovan's solution, Asiatic pills, de Valagin's solution, sodium cacodylate, arsphenamine, neoarsphenamine, oxophenarsine hydrochloride, arsthinol, acetarsone, tryparsamide, and carbarsone, have been historically used for the treatment of respiratory diseases, head lice, and plague, among others (Dan and Tallman, 2005; Mandal and Suzuki, 2002). Arsenicals have also been investigated for the treatment of hematological cancers such as leukemia, lymphoma, and solid tumor (Gibaud and Jaouen, 2010; Stice, 2014). Arsenic trioxide has been successfully used to treat acute promyelocytic leukemia patients (APL). Some organoarsenicals, such as darinaparsin (dimethylarsinous glutathione) and 4-(N-(S-glutathionylacetyl)amino)phenylarsonous acid (GSAO) are in clinical trials for hematological cancers and refractory
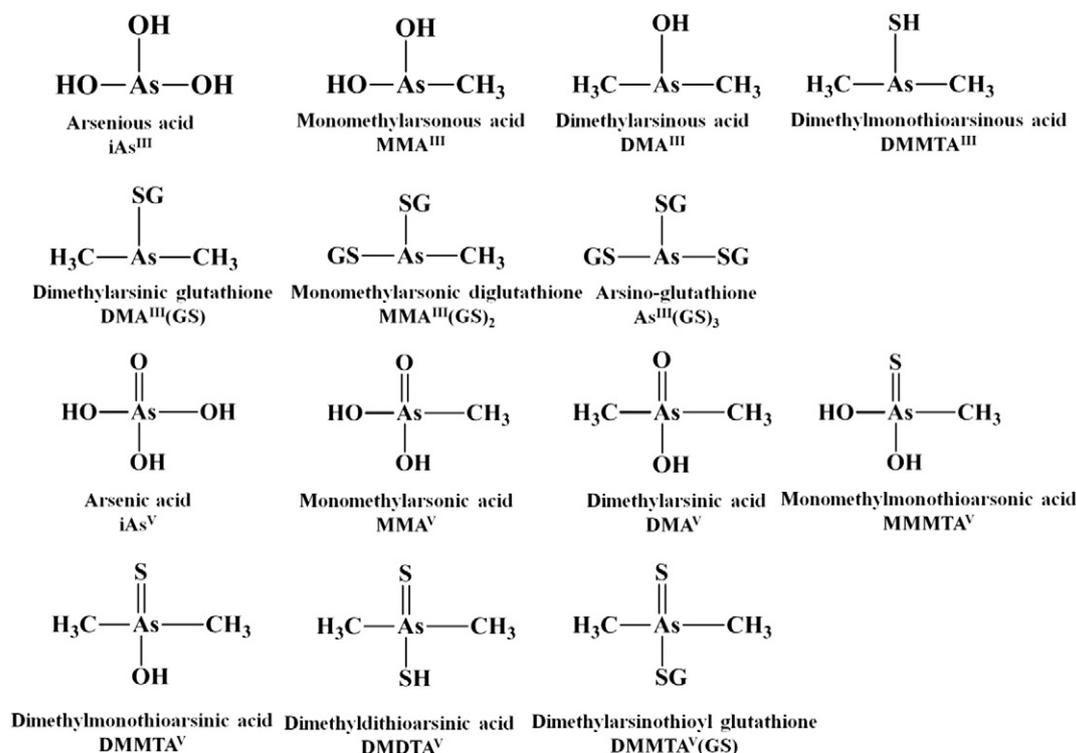
Fig. 1 – Structures, names and abbreviations of major arsenic compounds discussed in this paper.

solid tumors (Garnier et al., 2013; Tsimberidou et al., 2009; Waxman and Anderson, 2001; Wu et al., 2010).

Arsenic can undergo extensive metabolism and transformation once being taken up in biological systems, and both toxicological and therapeutic effects of arsenic compounds are closely related to their metabolism in biological bodies (Challenger, 1945; Hayakawa et al., 2005; Naranmandura et al., 2006). The metabolism of arsenic involves a variety of arsenicals with drastically different chemical properties and toxicities, with the biomethylation of iAs to form methylated arsenic species being regarded as the critical processes that have been extensively studied (Loffredo et al., 2003; Mandal et al., 2004; Mandal and Suzuki, 2002; Valenzuela et al., 2005). These methylated arsenicals include monomethylarsonic acid (MMA^V), dimethylarsinic acid (DMA^V), monomethylarsonous acid (MMA^{III}), and dimethylarsinous acid (DMA^{III}). Fig. 1 lists the names, abbreviations and chemical structures of major trivalent and pentavalent arsenicals involved in arsenic metabolism.

In addition to these methylated oxoarsenicals, recent studies have identified thiolated arsenicals, which contain As–SH and/or As=S substructures (possibly present as interchangeable tautomeric forms), as a new class of arsenic metabolites (Kala et al., 2004; Raab et al., 2007; Raml et al., 2005, 2007; Suzuki et al., 2004b, 2007). For instance, dimethylarsionthioyl glutathione complex ($DMMTA^VGS$), a pentavalent thioarsenical bound to glutathione (GSH), was detected in cabbages after exposure to DMA^V (Raab et al., 2007) and in human cell lines after darinaparsin exposure (Yehiayan et al., 2009, 2014). Dimethylmonothioarsinic acid ($DMMTA^V$), a thiolated pentavalent sulfur-containing derivative of DMA^V, has been found in urine (Mandal et al., 2008; Raml et al., 2007), liver and kidney homogenates, plasma, and red blood cells (Naranmandura and Suzuki, 2008; Raml et al., 2009). Monomethylmonothioarsonic acid ($MMMTA^V$) has been identified in the urine of hamsters and rats as arsenic metabolite (Naranmandura et al., 2007b; Suzuki et al., 2010). These thioarsenicals were sometimes mistaken as oxoarsenicals and consequently, thioarsenic species were overlooked in the metabolic pathway of iAs (Helle Rusz et al., 2004). It should be noted that the term thiolated arsenicals (or thioarsenicals) here do not include the conjugated arsenic (usually trivalent species) complexes with thiol group (e.g., from glutathione and

Table 1 – Major thiolated arsenicals that have been detected in biological systems.

Thioarsenicals detected	Animals	As species exposed	Reference
Dimethylarsinothioyl glutathione ($DMMTA^VGS$)	Human cell lines	Darinaparsin	Stice et al. (2016), Yehiayan et al. (2011, 2014)
Dimethylarsinodithioic acid ($DMDTA^V$)	Human cell lines	Darinaparsin	Stice et al. (2016)
	Mouse cecum	iAs^V	Pinyayev et al. (2011)
	Mouse cecum	^{34}S-$DMMTA^V$	Kubachka et al. (2009b)
	Mouse cecum	DMA^V	Kubachka et al. (2009a)
	Rat and human RBCs	$DMMTA^V$	Naranmandura and Suzuki (2008)
	Human urine and nails	iAs	Mandal et al. (2008)
	Hamster urine	iAs^{III}	Naranmandura et al. (2007b)
	Rat liver homogenate	DMA^{III}	Naranmandura et al. (2006)
	Rat liver	Arsenic	Suzuki et al. (2004a)
Dimethylarsinothioic acid ($DMMTA^V$)	Myeloma cells	Darinaparsin	Stice et al. (2016)
	Human urine	iAs	Raml et al. (2007)
	Human urine	Arsenosugar	Raml et al. (2005, 2006, 2009)
	Human RBCs	DMA^{III}	Naranmandura and Suzuki (2008)
	Rat plasma	iAs^{III}	Chen et al. (2013b)
	Rat urine	iAs^{III}	Bu et al. (2011), Suzuki et al. (2010)
	Mouse urine	iAs^V	(Naranmandura et al., 2013)
	Mouse cecum	DMA^V	(Kubachka et al., 2009a)
	Hamster and rat urine	iAs^{III}	(Naranmandura et al., 2007b)
	Rat liver homogenate	DMA^{III}	Naranmandura et al. (2006)
	Rat urine	DMA^V	Adair et al. (2007)
	Rat liver	Arsenic	Suzuki et al. (2004a)
	Sheep wool	Arsenosugar	Hansen et al. (2004)
Monomethylmonothioarsonic acid ($MMMTA^V$)	Mouse urine	iAs^V	Naranmandura et al. (2013)
	Rat plasma	iAs^{III}	Chen et al. (2013b)
	Rat urine	iAs^{III}	Bu et al. (2011), Suzuki et al. (2010)
	Human gut microbiota	Contaminated soil	Van de Wiele et al. (2010)
	Hamster and rat urine	iAs^{III}	Naranmandura et al. (2007b)
	Human urine	iAs	Raml et al. (2007)
Thio-dimethylarsenoethanol (thio-DMAE)	Human urine	Chinese seaweed	Wang et al. (2015a)
	Human serum	Arsenosugar	Raml et al. (2009)
	Human urine	Arsenosugar	Raml et al. (2005, 2006, 2009)
Thio-dimethylarsenoacetate (thio-DMAA)	Human serum	Arsenosugar	Raml et al. (2009)
	Human urine	Arsenosugar	Raml et al. (2005, 2006, 2009)
	Human urine	Chinese seaweed	Wang et al. (2015a)
	Sheep urine	Arsenosugar	Helle Rusz et al. (2004)

iAs: inorganic arsenic. iAs^V: arsenate. iAs^{III}: arsenite.

proteins) (Shen et al., 2013), such as glutathione arsenite $(As(GS)_3)$, dimethyl glutathione arsenious acid $(MMA(GS)_3)$, and glutathione dimethyl arsenious acid (DMA(GS)). This type of glutathione-conjugated arsenic species may be mentioned and discussed in the review as they are related to the formation and metabolism of thioarsenicals.

The increasingly detected thiolated arsenicals suggest that arsenic metabolism is more complicated and beyond the classical formation pathway of methylated oxoarsenicals and that thioarsenicals may be important metabolites playing important roles in arsenic toxicity and therapeutic effects. Here we summarized the reported occurrence of thioarsenicals in biological systems, the possible formation pathways of thioarsenicals, and their toxicity, and discussed the biological implications of thioarsenicals on arsenic metabolism, toxicity, and therapeutic effects.

1. Occurrence and detection of thiolated arsenicals

1.1. Occurrence of thiolated arsenicals in biological systems

Urine, blood, saliva, hair and nail are important biological indicators of bodily exposure to arsenic, and they are often analyzed for arsenic speciation as arsenic could be metabolized into different forms within a few hours after being taken up through respiratory and digestive systems, or dermal exposure. Thioarsenicals have been detected in these biological samples after exposure to different arsenic species, and Table 1 summarized the reported occurrences of major thioarsenicals. Urine is considered as the most useful indicator, because it is the main route of arsenic excretion in biological systems (Marchiset-Ferlay et al., 2012). Recently, some methylated thioarsenicals have been detected in urine samples after long-term consumption of iAs-contaminated drinking water or ingestion of arsenosugars. In an area of arsenic contamination in Bangladesh, $DMMTA^V$ and dimethyldithioarsinic $(DMDTA^V)$ have been identified from the urine of residents (Mandal et al., 2008; Raml et al., 2007). After an oral dosage of iAs, $DMMTA^V$ and $DMDTA^V$ were detected in the urine of hamsters, while $DMMTA^V$ and $MMMTA^V$ were identified as the arsenic metabolites in rats (Chen et al., 2013b; Naranmandura et al., 2007b; Suzuki et al., 2010). After ingestion of arsenosugars, $DMMTA^V$ occurred as trace arsenicals in the urine of male Japanese (Raml et al., 2005, 2006). In wild sheep feeding on algae with high concentrations of arsenosugars, 2-dimethylarsinothioyl acetic acid has been detected in the urine (Helle Rusz et al., 2004).

In addition to urine, thioarsenicals have been detected in blood samples, although blood samples are more difficult to obtain due to the invasive sampling and blood measurement is more difficult to perform because of complex matrix. $DMMTA^V$ was identified in the medium where human red blood cells (RBCs) were incubated, possibly produced from DMA^{III} in the presence of sulfide ions (Naranmandura and Suzuki, 2008). After exposure to iAs^{III} through iAs^{III}-supplemented diets, $DMMTA^V$ and $MMMTA^V$ were found in the plasma and RBCs of female rats (Chen et al., 2013b). After an intravenous injection of $DMMTA^V$ and $DMDTA^V$ at a dose of 0.5 mg As/kg body weight, dimethylthioarsenicals were distributed in major

organs/tissues and body fluids in rats (Suzuki et al., 2007). Small quantities of thio-dimethylarsenoacetate and thio-dimethylarsenoethanol are identified in the serum after ingestion of an oxo-arsenosugar compound (Raml et al., 2009).

Compared to urine and blood, the occurrence of thioarsenicals in other animal samples such as saliva, nail, and hair has been reported in fewer cases. It was reported that thio-dimethylarsenoacetate can be detected in human saliva samples after exposure to Chinese seaweed, with a regular excretion pattern like the manner in urine (Wang et al., 2015a). After intravenous infusion of 10 mg arsenic trioxide for each APL patient, $MMMTA^V$ was identified along with other arsenicals including As^{III}, MMA^{III}, DMA^V, MMA^V and As^V in saliva samples (Chen et al., 2013a). The concentrations of arsenic metabolites in saliva are relatively low leading to the difficulty of analysis. Human nails and hair have been used to identify acute arsenic poisoning (Slotnick and Nriagu, 2006). Through synchrotron X-ray absorption spectroscopy analysis of human nail clippings, it was found that some sulfur-containing trivalent arsenicals composed a significant fraction of total arsenical species (Ponomarenko et al., 2014). The hair and nails are also reliable biological samples used to detect long-term As exposure in animals, but reports of thiolated arsenicals in feathers, fur or hair are rare, possibly due to low extraction efficiencies from keratinous tissues (Ponomarenko et al., 2014).

The detection of thiolated arsenicals in various animal samples may be indicative of broad involvement of thioarsenicals in arsenic metabolism, including in other organisms. In fact, $DMMTA^VGS$ was first detected in cabbages after exposure to DMA^V (Raab et al., 2007). The sulfur analog of As (2,3-dihydroxypropyl-5-deoxy-5-dimethylarsinoyl-β-D-riboside) was identified and quantified in marine shellfish (Conklin et al., 2006). $DMMTA^VGS$ was found as a metabolite in cellular extracts of human multiple myeloma cell lines after darinaparsin exposure (Yehiayan et al., 2014).

It should be noted that this review is primarily focused on thiolated arsenicals, specifically methylated thioarsenicals, during metabolism of arsenic in human and animals. Thioarsenicals in the environment are not covered here, but thiolated arsenic species have been widely detected in natural environments and they play a critical role for arsenic cycling in a sulfide rich environment (Planer-Friedrich et al., 2007; Stauder et al., 2005; Wilkin et al., 2003). While the occurrence, formation, implications, and analysis of thioarsenicals in the environment are briefly mentioned in this paragraph and in Section 1.2, the remainder of the review will focus only on thioarsenicals during biological metabolism of arsenic. Examples of environmental thioarsenicals include the detection of thioarsenates including mono-, di-, tri-, and tetrathioarsenate, as well as methylated arsenic oxy- and thio-anions in geothermal waters of sulfidic Yellow Stone National Park springs (Planer-Friedrich et al., 2007). Two thioarsenical compounds of mono- and dithioarsenate were determined and found to be stable in sterile alkaline solutions collected from Mono Lake, USA (Fisher et al., 2008). In Moira Lake water sample thioarsenates were identified mainly as monothioarsenate (Wallschläger and Stadey, 2007). The formation of environmental thioarsenicals such as thioarsenates (including di- and monothioarsenate) is closely

related to microbial sulfate reduction (Burton et al., 2013), as arsenic is inclined to bind to organic and inorganic sulfur species during microbial sulfate reduction (Couture et al., 2013). It has been suggested that sulfate reducing bacteria (SRB) play an important role in transformation of some inorganic thioarsenic compounds, since they could produce and accumulate sulfide in many environmental scenarios (Burton et al., 2013; Couture et al., 2013; Deplancke et al., 2000). SRB is also confirmed to participate in the thiolation of MMA[V] in human intestinal tract (Rubin et al., 2014).

1.2. Analysis and identification of thiolated arsenicals

The assessment of metabolism and toxicity studies on arsenic relies on speciation analysis of arsenic metabolites, although earlier pathological studies are often based on total arsenic analysis. Speciation analysis of arsenic in biological matrix is always a challenging task, as it involves a wide range of arsenicals and some of them have poor stability. Speciation analysis of thioarsenicals is not an exception, and care must be exercised during sample storage, pretreatment, extraction, separation of arsenic species, until detection stage.

1.2.1. Stability of thioarsenicals and sample pretreatment
Redox reactions, precipitation, adsorption, and microbial processes may cause the conversion of arsenic species during sample collection, storage, and processing, depending on pH, temperature, oxygen content, light, among other factors. The half-lives of As(GS)$_3$, MMA(GS)$_3$, and DMA(GS) could range from a few minutes to a few hours depending on pH value and glutathione concentration (Raab et al., 2004; Yehiayan et al., 2009). The sulfide-to-oxide conversion may cause significant changes in arsenic speciation for thioarsenicals. In acid-preserved sulfide water, arsenosugar oxide was shown to shift to the sulfide form readily, and thio-arsenosugar could slowly convert back to the oxide form at room temperature (Conklin et al., 2008; Raml et al., 2005). Thioarsenicals show slow conversion of thio-DMA into DMA in human urine samples (Raml et al., 2007). High pH and low temperature are recommended for preservation of thioarsenicals in biological samples (Conklin et al., 2008). Keeping samples in the dark to avoid light-induced reactions could also be helpful for preservation of arsenic speciation. The storage of environmental samples for preservation of thioarsenates is also a challenge (Hollibaugh et al., 2005; Planer-Friedrich and Wallschläger, 2009). Generally, thioarsenical compounds can be preserved under neutral to alkaline conditions (Wallschläger and Stadey, 2007). Flash-freezing could be effective in preservation of thioarsenate species in natural waters (Suess et al., 2009), as thioarsenates remained stable for over 11 days in iron-rich water when anoxic cryopreservation by flash-freezing is employed after addition of neutralized ethylenediaminetetraacetic acid (EDTA)-solution (Suess et al., 2011). An improved preserving method for thioarsenate in iron-rich waters was recently suggested to be under anoxic, ethanolic and cool conditions (Suess et al., 2015).

Extraction is a critical step in arsenic speciation analysis, and various extraction techniques in combination with choices of solvents have been employed in this step. Typical extraction methods include solvent extraction (SE) (Geng et al., 2009),

accelerated solvent extraction (ASE) (Vela et al., 2001), liquid phase microextraction (LPME) (Jiang et al., 2009), pressurized liquid extraction (PLE) (Sanz et al., 2007), solid phase microextraction (SPME) (Jiang et al., 2009; Planer-Friedrich et al., 2006), and enzymatic hydrolysis (EH) (Moreda-Piñeiro et al., 2010). Common solvents for extracting arsenic species in biological samples are water (Raab et al., 2002), methanol–water (Mandal et al., 2004), trifluoroacetic acid (Zhang et al., 2015), and dilute phosphoric acid (Sanz et al., 2007). Comparison studies using water, organic solvents (methanol and trifluoroacetic acid), and enzymes combined with different extraction techniques (vortex, ultrasonic bath, and ultrasonic probe) revealed that ultrasonic probe extraction with water and enzymes was optimal in extraction recoveries and in time efficiency for arsenic in human multiple myeloma cell lines (Yehiayan et al., 2011). Arsenic-containing urinary and blood samples can be extracted by water–methanol mixture with satisfying recoveries for each arsenic species (Raml et al., 2009; Šlejkovec et al., 2008). Combined with centrifugal method, Tris–HCl saline was a choice of extraction buffer for analysis of thioarsenicals in blood of rats (Naranmandura et al., 2010). Recently, the method of triton-X cell lysis on ice was found to be able to maintain arsenic speciation (Hernández-Zavala et al., 2008). Exploring methods with high extraction efficiency and selectivity meanwhile minimizing species conversion and loss through volatilization or adsorption is a continuing task for arsenic speciation analysis in biosamples.

1.2.2. Separation methods
There are a number of options for separation of arsenic species postextraction, including high performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE). HPLC with different modes has often been the method of choice in analysis of biological samples for arsenic speciation (Raml et al., 2006), including ion-exchange, reverse-phase, ion-pairing and micellar column methods. Eight arsenicals including MMMTA[V] were separated by reverse-phase HPLC in saliva samples from APL patients (Chen et al., 2013a). Arsenic–glutathione complexes including As[III](GS)$_3$, MMA[III](GS)$_2$ and DMA[III](GS) were identified by different HPLC modes (Raab et al., 2004). Recently, a reverse-phase HPLC method using C$_8$ column was developed and optimized for analysis of arsenic metabolites in human, with a special focus on thioarsenicals and arsenicals conjugated with GSH, and its application was demonstrated on darinaparsin-treated human cell lines (Stice et al., 2016). Reverse-phase HPLC was also applied to urine samples for separation of thioarsenic compounds, and thio-dimethylarsenoacetate, thio-dimethylarsenoethanol, and DMMTA[V] could be separated within 15 min (Mandal et al., 2008; Raml et al., 2006). SPME fibers coupled to gas chromatography–mass spectrometry (GC–MS) was used to separate and identify volatile arsenic species including (CH$_3$)$_2$AsSCH$_3$ and (CH$_3$)$_2$AsSCl, which revealed the first occurrence of chloro- and thioarsines in a natural environment (Planer-Friedrich et al., 2006). Due to the broad variety of arsenic metabolites in biosamples, it is usually difficult to find a single separation method for a "full-spectrum" analysis of all arsenicals, as co-elution of species could occur. A combination of different separation techniques may be necessary

for a sample, with each separation method targeting a specific group of arsenicals of interest.

1.2.3. Detection methods

The commonly used technique for detection of arsenic species is element-specific detection system such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), and particularly inductively coupled plasma mass spectrometry (ICP-MS) (Mandal et al., 2001; Xie et al., 2006). For better determination of arsenicals, complementary techniques are often utilized along with these instruments, as exemplified by HPLC–hydride generation (HG)–AFS (Gong et al., 2001; Le et al., 2000a, 2000b) and pH-HG–cryotrapping (CT)–AAS (Del Razo et al., 2001; Devesa et al., 2004; Valenzuela et al., 2005). ICP-MS has high selectivity and low detection limits and allows for the determination of arsenic species at concentrations varying by orders of magnitude, but it cannot provide molecular structural information. Electrospray ionization (ESI)-MS, which is capable of providing structural information for unknown peaks, has often been used together with ICP-MS post-HPLC separation to aid in peak resolution and identification. A parallel use of HPLC–ICP-MS with HPLC–ESI-MS

revealed the new arsenic metabolite, $DMMTA^V$, in sheep's wool after ingesting arsenosugar (Hansen et al., 2004). The ESI-MS was used to identify a major unknown peak after HPLC–ICP-MS analysis of urine samples and confirmed the structure of thioarsenicals (Helle Rusz et al., 2004; Raml et al., 2007). By using HPLC–ICP-MS and ESI-MS, $DMMTA^V$GS was determined and verified in cabbage (Raab et al., 2007), and as a metabolite in cellular extracts of DMA^{III}(GS)-treated human multiple myeloma cell lines (Yehiayan et al., 2014). A combination of ion chromatography (IC)–ICP-MS, liquid chromatography (LC)–ESI-MS and ESI-MS–MS helped in identification of other thioarsenicals including $MMMTA^V$, $DMMTA^V$ and $DMDTA^V$ in urine (Adair et al., 2007; Drobna et al., 2009; Naranmandura et al., 2013). In addition to ICP-MS and ESI-MS, GC-MS has also been used for identification of thioarsenicals such as sulfur-arseno compound $((CH_3)_2AsSCH_3)$ (Kösters et al., 2003). Additionally, spectroscopic methods, including Raman (Wood et al., 2002) and X-ray absorption spectroscopy (Burton et al., 2013; Suess et al., 2009; Xiao et al., 2015), can provide useful information on arsenic speciation, e.g., confirmation of the species formed in arsenite-sulfide model solutions. Relatively comprehensive reviews on arsenic
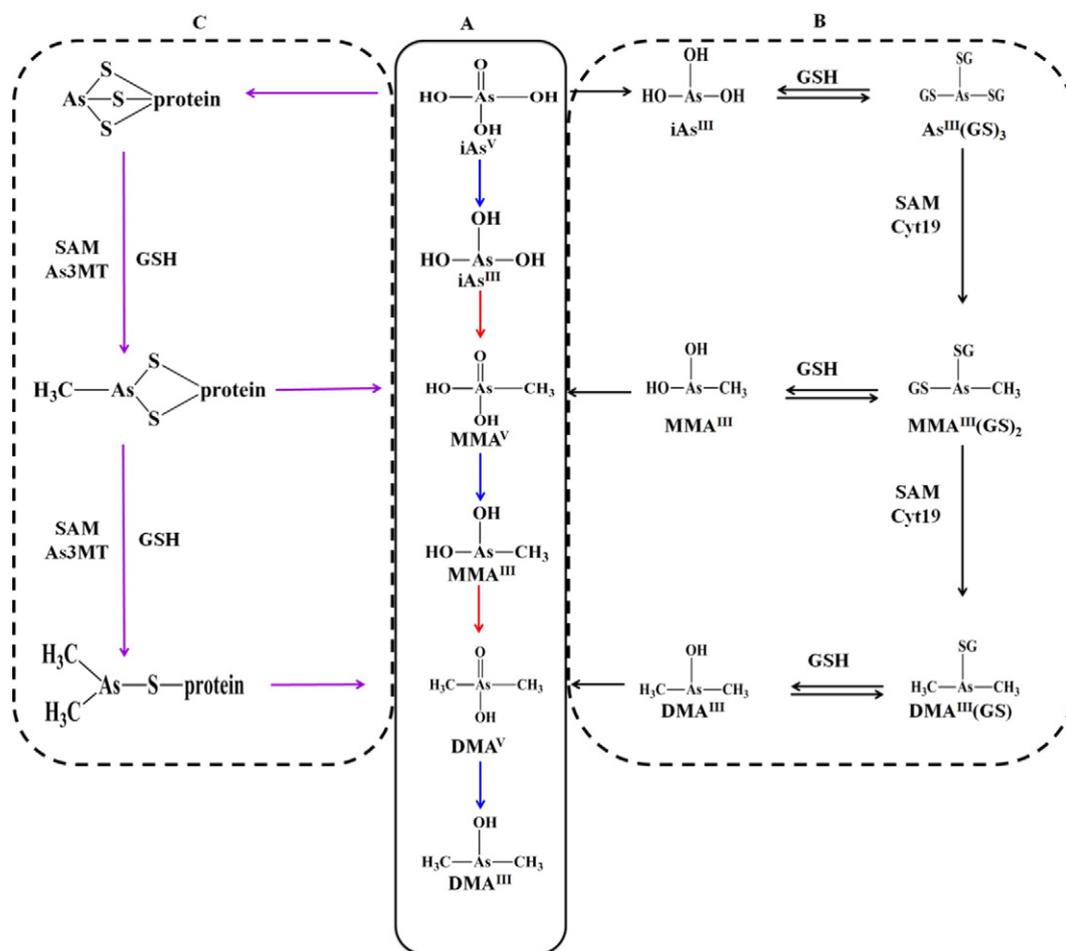

Fig. 2 – Three proposed arsenic metabolic pathways. (A) Oxidative methylation pathway, where red arrows show methylation while blue for reduction (Challenger, 1945); (B) Reductive methylation through As–GSH complexes (Hayakawa et al., 2005); and (C) Reductive methylation through As–protein complexes (Naranmandura et al., 2006).

speciation methods can be found in recent publications (McKnight-Whitford and Le, 2006; Nearing et al., 2014). Despite the recent progress in identification and determination of thioarsenicals, there are still difficulties in analyzing thioarsenicals in biological matrices due to the complexity of arsenic metabolism, the homophyly of oxoarsenicals and thioarsenicals (Hansen et al., 2004), and the lacking of standards of thioarsenicals. Many thioarsenical standards have to be synthesized in individual laboratories and, in some cases, the synthesis is very difficult due to instability of the compound like DMMTA^{III}.

2. Formation pathways of thiolated arsenicals

2.1. Reductive/oxidative biomethylation of arsenic

Arsenic metabolism in biological systems has been extensively studied, and biomethylation has been identified as the most important process with respect to arsenic metabolism and toxicity. There have been three arsenic metabolic pathways proposed by Challenger in 1945, Hirano's group in 2005, and Suzuki's group in 2006, respectively (Challenger, 1945; Hayakawa et al., 2005; Naranmandura et al., 2006). Detailed comparisons among these pathways of arsenic metabolism have been summarized and discussed recently (Carter et al., 2003; Cullen, 2014; Rehman and Naranmandura, 2012; Wang et al., 2015b), and are beyond the scope of this review as thioarsenicals are the focus here. A brief description was given below to these three arsenic metabolic pathways (Fig. 2) before the focus was moved to formation pathways of thioarsenicals.

The classical pathway of arsenic metabolism is that inorganic arsenic compounds in mammal's body undergo a series of successive and repetitive oxidative methylation and reductive elimination reactions: iAs^{V}-iAs^{III}-MMA^{V}-MMA^{III}-DMA^{V}-DMA^{III}, with S-adenosylmethionine (SAM) being the source of CH$_3^+$ in mycological methylation (Challenger, 1945; Cullen, 2014). This pathway suggests that oxidative methylation of iAs^{III} directly produces MMA^{V}, followed by reduction of MMA^{V} to MMA^{III}. Subsequently, MMA^{III} is oxidatively methylated into DMA^{V} which undergoes a reduction step to form DMA^{III}. The Challenger pathway involves accepting a CH$_3^+$ group during oxidative methylation of trivalent arsenic species, which is chemically feasible as suggested by Cullen when using chemistry principles to analyze the plausibility of the proposed arsenic metabolic pathways (Cullen, 2014). As a matter of fact, the Challenger pathway, an analog of the Meyer reaction that is an uncatalyzed oxidative addition reaction used to prepare MMA^{V} from iAs^{III} and methyl halide, can be fully modeled by using the trimethylsulfonium ion as methyl donor and sulfur dioxide as the reducing agent (Antonio et al., 1979; Cullen, 2014; Quick and Adams, 1922). The second pathway of arsenic metabolism described that GSH is a mandatory component for arsenic methylation, and that As–GSH complexes, including arsenic triglutathione (As(GS)$_3$) and monomethylarsenic diglutathione (MMA(GS)$_2$), are the substrates for the enzyme arsenic methyltransferase (AS3MT) (Hayakawa et al., 2005). In this pathway, instead of the oxidative methylation of iAs^{III}, MMA^{III} to MMA^{V}, DMA^{V} respectively, trivalent MMA(GS)$_2$ and DMA(GS) are the methylation products of As(GS)$_3$ and MMA(GS)$_2$ by AS3MT, followed by formation of MMA^{V} and DMA^{V} (Hayakawa et al., 2005). This pathway, proposed from a biological point of view, is a reductive methylation pathway that requires accepting a CH$_3^-$ group, which, from the chemical point of view, does not seem to be very likely (Cullen, 2014). The third pathway follows the reductive methylation scheme and suggests that in the presence of glutathione and S-adenosylmethionine, inorganic arsenic is metabolized in the body by bounding to proteins in a trivalent form during the successive reductive methylation by AS3MT (Naranmandura et al., 2006; Rehman and Naranmandura, 2012). This pathway is based on the fact that a sulfhydryl functional group from proteins should be physiologically more stable than that of GSH, and therefore trivalent arsenic compounds have higher affinity to the thiol groups of proteins in comparison to glutathione (Bogdan et al., 1994; Styblo and
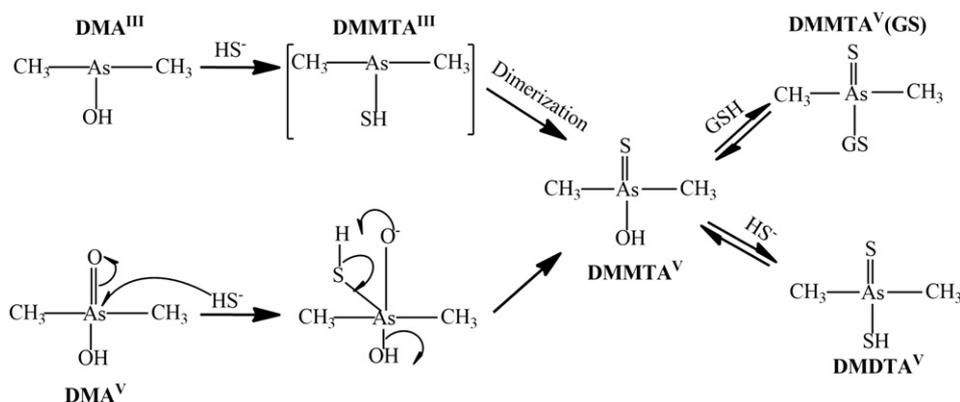
Fig. 3 – A scheme showing formation of thioarsenicals such as DMMTA^{V}, DMMTA^{V}GS and DMDTA^{V} from DMA^{III} or DMA^{V}. Note that DMA^{III} and DMA^{V} are common arsenic metabolites and could be formed during metabolism of inorganic arsenic species (Mandal et al., 2008; Naranmandura and Suzuki, 2008; Naranmandura et al., 2007b; Raml et al., 2007; Suzuki et al., 2004a) or organic arsenicals like darinaparsin (Stice, 2014; Yehiayan et al., 2014).

Thomas, 1997; Suzuki et al., 2004b). It should be noted that the latter two pathways suggest that DMA^V and MMA^V should be end-products (instead of intermediates) of arsenic biotransformation, since trivalent arsenic, whether in glutathione or protein complex forms, undergoes reductive methylation without being oxidized (Naranmandura et al., 2007b). These two pathways involve accepting a CH_3^- group and appear to have problem against chemistry principle (Cullen, 2014), although they seem to agree with the observation that trivalent arsenicals were demonstrated to be rapidly taken up by the organs/tissue and bound to cellular proteins, instead of excretion (Hippler et al., 2011).

2.2. Formation pathways of thioarsenicals from trivalent methylated oxoarsenicals

With the discovery of new thioarsenical compounds such as $DMMTA^V$, $MMMTA^V$ and $DMDTA^V$ in mammal's urine (Raml et al., 2007; Suzuki et al., 2010), blood (Naranmandura and Suzuki, 2008; Naranmandura et al., 2007b) and saliva (Wang et al., 2015a), an unavoidable question is how these thioarsenicals are formed in biological systems. Thioarsenicals were sometimes regarded as the product of the intestinal flora of animals, as they were detected in the urine and feces of animals exposed to DMA^V (Kubachka et al., 2009b). However, the broad detection of thioarsenicals in urine and hair of sheep feeding on arsenic in the nature (Helle Rusz et al., 2004), as well as in urine of human exposed to arsenic-contaminated water or ingestion of arsenosugar (Raml et al., 2005, 2007), suggests that thioarsenicals could be common metabolites of arsenic exposure and formation pathways of thioarsenicals should be included in arsenic metabolism. Although it remains largely unknown on how these thioarsenicals are produced in biological bodies, studies have shown that thioarsenicals could be generated from DMA^{III} and DMA^V under different physiological conditions probably through reactions with certain forms of sulfides, and these studies have proposed some pathways of thioarsenicals formation and are described below (Naranmandura et al., 2008; Suzuki et al., 2004a). A conceptual model of pathways of DMMTA-^{V}GS formation from DMA^{III} and DMA^V was provided by Yehiayan et al. and shown in Fig. 3 (discussed below), which involves the formation of such thioarsenicals as $DMMTA^V$ and $DMDTA^V$ and could serve as a source of information on thioarsenicals formation (Yehiayan et al., 2014).

A number of studies have reported the formation of thioarsenicals from DMA^{III} (Naranmandura et al., 2008; Suzuki et al., 2004b). In rats, DMA^{III} was transformed into $DMMTA^V$ in the presence of a sulfide ion in the liver supernatant (Suzuki et al., 2007). In the presence of human RBCs, DMA^{III} is taken up after being released from hepatocytes and transformed to $DMMTA^V$ and $DMDTA^V$ (Naranmandura and Suzuki, 2008). In the liver homogenate containing thiocyanate and sulfur transferase, DMA^{III} reacts with HS^- and converts to $DMMTA^V$ by desulfurization of sulfur bond (Kurosawa et al., 2016). In gastrointestinal microbiota of rats, MMA^{III} is reported to convert into $MMMTA^V$ and $DMMTA^V$ and then absorbed into the bloodstream and finally excreted into bile and urine (Bu et al., 2011). Compared to DMA^V, DMA^{III} can be easily converted into $DMMTA^V$ and $DMDTA^V$, and the yield ratio of $DMMTA^V$ and $DMDTA^V$ is determined by the stoichiometry of DMA^{III} and the thiol group as well as the reaction time (Naranmandura and Suzuki, 2008; Naranmandura et al., 2006; Suzuki et al., 2004b).

The pathways of $DMMTA^V$ and $DMDTA^V$ formation from DMA^{III} remain unclear, although it has been proposed that DMA^{III} reacts with free sulfide ions or sulfane sulfur (Naranmandura et al., 2008; Suzuki et al., 2004b). The sources of sulfides in biological body mainly include free sulfide HS^-, sulfide and thiol compounds bound protein $-S-SH$, sulfide and dithiol binding state (protein $-S-S-SH$), which are deemed to be the products of intestinal flora and/or the metabolic products of sulfur-containing amino acids in liver and other organs (Stice et al., 2016; Suzuki et al., 2004b; Yehiayan et al., 2009, 2014). Controversially, $DMMTA^{III}$ was hypothesized to be an intermediate produced by the reactions between DMA^{III} and sulfide, which could be further oxidized and/or thiolated to $DMMTA^V$ and $DMDTA^V$. In addition, it was speculated that $DMMTA^{III}$ might be produced by different forms of DMA^{III}, such as DMA^{III} conjugates with glutathione or protein molecules, as from theoretical consideration or in vitro experiments DMA^{III}-SG was thought to be able to generate $DMMTA^{III}$ (Diaz-Bone et al., 2009; Mandal et al., 2008; Naranmandura and Suzuki, 2008; Naranmandura et al., 2006; Suzuki et al., 2007; Yehiayan et al., 2014). Despite of the assumption that $DMMTA^{III}$ might be produced from DMA^{III} reactions with sulfides and/or from DMA^{III} conjugates with glutathione or proteins, there is much debate on the detection and identification of this extremely unstable intermediate and its involvement in thioarsenicals formation. To make things more complicated, the exact processes behind the transformation from this trivalent thiolated arsenic intermediate to pentavalent thioarsenicals remain unclear, even if $DMMTA^{III}$ was proposed to be involved. One of the pathways in the transformation from $DMMTA^{III}$ to $DMMTA^V$ was assumed to involve a series of reactions containing DMA^{III} attack on the $DMMTA^{III}$ dimer, nucleophilic attack of sulfide and disproportionation proposed by our group (Fig. 3) (Yehiayan et al., 2014).

More recently, a sulfur transferase enzyme, rhodanese, was identified to catalyze the sulfur atom-addition reaction from DMA^{III} to $DMMTA^V$ (Kurosawa et al., 2016; Shimoda et al., 2015), which can convert CN^- to SCN^-. The activity of rhodanese in the liver of humans is known to be much lower than some other mammals, such as bovine, mouse, and dog. However, the concentration of rhodanese in the kidneys of human is twice as much as in the liver. Therefore, DMA^{III} may readily be transformed to $DMMTA^V$ as a substrate of rhodanese in the kidney except in the blood and liver.

2.3. Formation pathways of thioarsenicals from pentavalent methylated oxoarsenicals

In addition to DMA^{III}, it was reported that DMA^V could be transformed into thioarsenicals. In fact, the reasoning of thioarsenicals formation through DMA^V pathway was based on the chemical reactions of MMA^V and DMA^V with sulfides which have shown to be able to produce $MMMTA^V$ and $DMMTA^V$ and been used to prepare standards of thioarsenicals. Due to the commercial unavailability of $MMMTA^V$, $DMMTA^V$ and $DMDTA^V$ standards, laboratories have used reactions of pentavalent methylated oxoarsenicals and sulfides to produce standards of thioarsenicals. In the presence of

sulfide and solution pH ≤ 8, the two methylarsine oxides, DMA^V and MMA^V, can be converted into their respective sulfide forms DMMTA^V and MMMTA^V (Conklin et al., 2008). Another way is through further hydroxylation of the oxygen-bridged dimethylthioarsinic anhydride (Fricke et al., 2007). MMMTA^V as standard can be synthesized by adding the molar ratio of MMA-^{V}:Na$_2$S:H$_2$SO$_4$ of 1:2:3 gradually (Bu et al., 2011).

Formation of thiolated arsenicals from pentavalent methylated oxoarsenicals through *in vitro* incubation has been reported, lending support to this pathway. DMMTA^V was produced by incubation of DMA^V in the cecal content containing anaerobic microflora of a mouse *in vitro* (Kubachka et al., 2009a), and DMA^{III} was not detected in this reaction mixture implying the substitution reactions of sulfur and oxygen atoms. MMMTA^V was produced from MMA^V by human colon microbiota, and the experimental results revealed that MMMTA^V formation was also from pentavalent arsenicals *in vivo* depending on human gut microbiota (Conklin et al., 2008; Van de Wiele et al., 2010). Similar reactions of DMMTA^V production from DMA^V occurred in the anaerobic microbiota of mouse cecum (Kubachka et al., 2009b). In the conceptual scheme of thioarsenicals formation pathways in Fig. 3, it was proposed that DMMTA^V could be produced through direct reaction of DMA^V with sulfides without involvement of trivalent intermediates (Stice, 2014; Yehiayan et al., 2014).

In addition to DMA^{III} and DMA^V, other formation pathways of thioarsenicals may be present. For example, six arsenicals including two methyl-thioarsenicals MMDTA^V and DMDTA^V were produced after the incubation of iAs^V with intestinal flora, implying that inorganic pentavalent arsenicals could be converted into related thioarsenicals (Pinyayev et al., 2011). Based on the detection of thioarsenicals including thio-dimethylarsenoacetate and thio-dimethylarsenoethanol in the urine of human volunteer after ingestion of oxo-arsenosugar, a study implied that there may be a new formation pathway of thioarsenicals from arsenosugar (Raml et al., 2005).

2.4. Theoretical calculation methods on stability and formation of thioarsenicals

Arsenic biochemistry involves a variety of metabolites of diverse molecular structures and in many cases the key intermediates are unstable and difficult to detect, computational chemistry could be a useful tool in studying arsenic metabolism. Computational chemistry methods have been widely used for calculations of the stability of molecules, rates and equilibria of reactions, physiochemical properties of substances and interactions of substrates with proteins (Lewars, 2003). For studying the properties of arsenic compounds by theoretical methods, it is important to select a suitable combination of functional/basis set with quantum chemical method in order to reduce the overall cost of the calculation for a given accuracy (Xu and Truhlar, 2011). Density functional theory (DFT) is often the choice of methods in the investigation of arsenic transformation in biological systems. Although there are some deficiencies of DFT such as an approximate exchange functional and near-degeneracy errors (Ghosh, 2006; Lundberg and Siegbahn, 2005), it still provides good accuracy with less cost in exploring the molecular structures and spectra of As-containing biological systems (Teixeira et al., 2007; Toulouze et al., 2012; Zampella et al., 2012). B3LYP is the most

widely used hybrid functional, which displays the highest accuracy among DFT functionals in benchmark tests and is fast enough to treat rather large systems (Jensen, 2009; Siegbahn, 2006).

Computational studies have been conducted to explore the structure and spectra of arsenic compounds and the interactions of arsenicals with biorelevant thiol compounds (He and Guo, 2015; Suzuki et al., 2008). Several density functional methods have been used to study the molecular structure vibrational and absorption spectra of arsenical thiolate complexes (Liu et al., 2012), providing physicochemical properties and basic information of spectra. A DFT calculation combined with Raman spectroscopy illustrated the mechanism of As^{III} adsorption onto a cysteine-rich biomaterial at a molecular level (Teixeira et al., 2007). Structure, stability and vibrational spectra of As(OH)$_3$, AsO(OH)$_3$, As(SH)$_3$, AsS(SH)$_3$ and their conjugate bases in aqueous solution have been calculated recently at CBSB7 B3LYP level (Tossell and Zimmermann, 2008), providing useful information on simulating aqueous chemistry of arsenic compounds. In predicting the structure and spectra of arsenic complexes with biomolecules, a systemic study has been performed to evaluate the accuracy of different basis set combinations. The research showed that the combination of generalized gradient approximation to the exchange-correlation functional of density functional theory with basis sets of triple-zeta valence with polarization (SOGGA with TZVP) can successfully predict the optimal structures and theoretical absorption spectra of As^{3+}-binding β domain of rabbit liver metal-lothioneines-2 (He and Guo, 2015).

Quantum chemical methods should be able to lend help toward an improved understanding of the metabolism and formation pathways of thiolated arsenicals, although theoretical calculations directly related to thioarsenicals are scarce. A computational study of thioarsenicals in comparison to their oxo analogs by means of *ab initio* calculations was reported, revealing that the reaction of H$_2$S with oxoarsenicals leading to the formation of thioarsenicals was easier than its reverse reaction (Orthaber et al., 2012). A theoretical calculation was carried out and demonstrated that DMMTA^{V}GS can be formed nonenzymatically under weakly acidic conditions based on the thermodynamics and that thioarsenicals are more toxic than the corresponding oxo acids (Suzuki et al., 2008). It is expected that the theoretical calculation method would be used more widely as a new powerful tool for the study of sulfur-containing arsenic compounds.

3. Biological implications

3.1. Protein binding of pentavalent thioarsenicals

Binding of arsenic species to proteins may be a critical process with respect to arsenic metabolism, toxicity, and therapeutic effects. Traditionally, based on Pearson's hard and soft acids and bases (HSAB) theory, pentavalent arsenic is a hard acid which is unlikely to bind to sulfur-containing biomolecules (Pearson, 1963). The arsenic species including iAs^V, iAs^{III}, monomethylarsonic acid (MMA^V), and dimethylarsinic acid (DMA^V) were investigated for their interaction with glutathione and cysteine by ESI-MS (Park and Butcher, 2010). Trivalent arsenicals have a strong binding affinity for the thiol groups of

biomolecules and form the arsenic-sulfur compounds *in vitro* and *in vivo* (Naranmandura et al., 2006). The protonated arsenic–thiol complexes, protonated noncomplexed thiol compounds, sodium bound cluster ions, and proton bound cluster ions were detected, indicating that DMA^V cannot bind to the GSH and cysteine and would be reduced by GSH (Kurosawa et al., 2016).

However, the detection of thioarsenicals, specifically $DMMTA^V$GS, may have changed the traditional view of pentavalent arsenicals being unable to bind to proteins. Dimethylarseninothioly glutathione complex generated from $DMMTA^V$ binding to the cysteinyl residue of glutathione is detected firstly in cabbage (Raab et al., 2007) and later in DMA^{III}(GS)-treated human multiple myeloma cell lines (Yehiayan et al., 2014), indicating that pentavalent arsenic-containing sulfur could interact with cysteinyl residues in polypeptides and proteins. Moreover, $DMMTA^V$ could bind to rat hemoglobin, indicating the high possibility for interaction of pentavalent As with sulfhydryls on proteins and peptides (Naranmandura and Suzuki, 2008). The possible reason could be that arsenic seemed to become softer when it is presented as its sulfide, as the two methyl groups help stabilize the molecule (Raab et al., 2007). Theoretical calculations about the interaction of pentavalent thioarsenicals with biorelevant thiol compounds were carried out by Suzuki et al. (2008). They stated that the reaction of $DMMTA^V$ with thiol compound (methanethiol) is exothermic, while the one of DMA^V with methanethiol is endothermic. They also demonstrated the possibility of the formation of the $DMMTA^V$-thiol conjugate in any organisms *via* a nonenzymatic reaction in acidic organelles.

3.2. Relation of thioarsenicals to arsenic toxic effects

Arsenic toxicity is highly dependent on its valence state and chemical species, and the toxicity is also dependent on the animal species and cell types involved. Traditionally, a general view of toxicity of arsenic species was that pentavalent arsenicals were less toxic than their trivalent counterparts (Nakamuro and Sayato, 1981; Schwerdtle et al., 2003). This was probably related to the less efficient uptake of pentavalent arsenicals by cells, tissues, and organs and thus lower accumulation rate, compared to trivalent species (Hirano et al., 2004; Styblo et al., 2000). Methylated arsenicals used to be regarded as less toxic than iAs, as DMA^V and MMA^V were initially observed as main methylation products, until the later detection of trivalent methylation products DMA^{III} and MMA^{III}. Probably due to the strong affinity for sulfur ligands, MMA^{III} was observed to exhibit higher toxicity than nonmethylated iAs^{III} (Kubachka et al., 2009b; Naranmandura et al., 2006; Styblo et al., 2000). Similarly, as thioarsenicals at pentavalence state (*e.g.*, $DMMTA^V$) were proved to be able to bind to thiol groups (from GSH and proteins), studies have shown that $DMMTA^V$ exhibited unusually high toxicity for a pentavalent species, underscoring the importance of thio-arsenicals in arsenic toxicity (Naranmandura et al., 2007a, 2009).

$DMMTA^V$ has been reported to be potentially cytotoxic to many human cells. In cultured A431 human epidemoid carcinoma cells, $DMMTA^V$ (LC50 = 10.7 μM) seemed to be much more toxic than its oxo counterpart DMA^V (LC50 = 843 μM), comparable to trivalent species iAs^{III} (LC50 = 5.49 μM) and DMA^{III} (LC50 = 2.16 μM) (Naranmandura et al., 2007a). For EJ-1 human urinary bladder carcinoma cells, $DMMTA^V$ (LC50 = 16.7 μM) even showed a higher cytotoxicity than iAs^{III} (LC50 = 112 μM) (Naranmandura et al., 2009), with the cytotoxicity of arsenic compounds approximately in the order of DMA^{III}, $DMMTA^V$ > iAs^{III} ≫ iAs^V > $MMMTA^V$ > MMA^V, DMA^V and $DMDTA^V$ (Naranmandura et al., 2011). In A549 human lung adenocarcinoma epithelial cells, a study showed the strong and similar cytotoxicity of $DMMTA^V$ and DMA^{III}(GS) (Bartel et al., 2011; Leffers et al., 2013). The high cytotoxicity of $DMMTA^V$ was probably because $DMMTA^V$ could bind to sulfur groups and be taken up by cells efficiently and then immediately hydroxylated to DMA^V in the present of GSH. Inside the cells reactive oxygen species (ROS) would be produced through the redox equilibrium between DMA^V and DMA^{III} in the presence of GSH, which may cause deoxyribonucleic acid (DNA) damage by disrupting tumor suppressor genes and enhancing the expression of proto-oncogenes (Naranmandura et al., 2007a, 2011).

In addition to cytotoxicity, $DMMTA^V$ also shows high genotoxicity. In human hepatocarcinoma cells line and Syrian hamster embryo cells, $DMMTA^V$ was proved to induce chromosomal aberrations when inducing apoptosis (Ochi et al., 2008). The research showed that $DMMTA^V$ caused abnormalities in mitotic spindle organization and centrosome integrity, leading to genotoxic cell apoptosis. As $DMMTA^V$ was observed to reduce the p53 protein expression remarkably in a time-dependent manner, DNA damage caused by $DMMTA^V$ could be induced by the formation of highly reactive oxygen species (hROS) and repair Protein p53 and downstream protein p21 (Naranmandura et al., 2011). In cultured human utrothelial (UROtsa) cells, $DMMTA^V$ showed cell cycle distribution and genotoxicity, cellular bioavailability induction of apoptosis, and response to oxidative stress (Ebert et al., 2014). The research showed that $DMMTA^V$ disturbed the H_2O_2-induced poly(adenosine diphosphate [ADP]-ribosyl)ation at a low concentration, but whether there is conversion of $DMMTA^V$ intracellular was not investigated in this study.

3.3. Thioarsenicals and therapeutic effects of arsenic

Arsenic compounds are being used as medicines for the treatment of different conditions that may not respond to other agents. In addition to inorganic arsenic trioxide that has been approved by the United States Food and Drug Administration (FDA) to treat APL (Chen et al., 2007; Nicolis et al., 2009; Wang and Chen, 2008; Zhou et al., 2005), some organic arsenicals such as salvarsan and melarsoprol are now in use, due to their unique effect, to treat some conditions, *e.g.*, syphilis and trypanosomiasis for the former and East African and West African trypanosomiasis for the latter (Gibaud and Jaouen, 2010; Lloyd et al., 2005). In addition, as alternatives for arsenic trioxide due to its high toxicity, organic arsenicals such as darinaparsin and GSAO are in clinical trials being investigated for treatment of hematological cancers (Gibaud and Jaouen, 2010; Stice, 2014). Just like the toxicity of arsenic that is regulated by arsenic metabolism, the therapeutic effects of arsenical medicines could be related to their

metabolic processes inside biological systems and the intermediates produced during these processes.

While the metabolic pathways of arsenical medicines are not well understood and thus the exact mechanisms underlying the therapeutic effects of arsenic medicines are difficult to be identified, the detection of metabolic intermediates, such as thiolated arsenicals, could provide helpful information for this type of investigations. A recent review on therapeutic use of arsenic and the related protein binding during arsenic metabolism covered arsenic-based drugs, imaging of cellular events, capture and purification of arsenic-binding proteins, and biosensing of arsenic, providing an improved understanding toward metabolism and therapeutic effects of arsenic medicines (Chen et al., 2015). Darinaparsin might be an example for possible involvement of thioarsenicals in therapeutic effects of arsenic drugs. As a new effective drug against APL, darinaparsin is considered to induce mitochondrial damage, antiangiogenic and cause apoptosis in multiple myeloma cell lines (Apostolia Maria et al., 2009; Matulis et al., 2009). There is much debate on the uptake, metabolism and mechanism of action of darinaparsin. We found thiolated arsenicals, including $DMMTA^V$ and $DMMTA^VGS$, to be major metabolites (along with DMA^V) in $DMA^{III}(GS)$-treated human multiple myeloma cell lines (Stice et al., 2016; Stice, 2014; Yehiayan et al., 2014). As the speciation of $DMA^{III}(GS)$ (e.g., free or GS-bound form) is controlled by the dynamic equilibrium process between DMA^{III} and GSH depending on the concentration of exogenous GSH, it has been postulated that $DMA^{III}(GS)$ could enter the cells in the form of DMA^{III} at low GSH concentrations and/or as S-(dimethylarsenic) cysteine ($DMA^{III}(Cys)$) after $DMA^{III}(GS)$ was processed by the enzyme γ-glutamyl transpeptidase (γ-GT) on the cell surface (Garnier et al., 2013; Matulis et al., 2009; Stice, 2014). The thioarsenicals, $DMMTA^V$ and $DMMTA^VGS$, were proposed to be formed inside the cells through DMA^{III} and/or DMA^V pathways (Fig. 3) (Yehiayan et al., 2014). In consideration of high toxicity of $DMMTA^V$, these thiolated metabolites could be related to the therapeutic effects of darinaparsin, and the involvement of thiolated metabolites in the metabolism and therapeutic effects of arsenical drugs warrants further investigation.

4. Concluding remarks

Thiolated arsenicals, such as $DMMTA^V$, $DMMTA^VGS$, $MMMTA^V$, and $DMMTA^V$, are a new class of metabolites of important relevance to arsenic metabolism, as summarized here with respect to their occurrences and analysis in biological systems, possible formation pathways, and biological implications on arsenic toxicity, and therapeutic effects. The increasing detection of thiolated arsenicals in various animal biomarkers may be indicative of broad involvement of thioarsenicals in arsenic metabolism, with more occurrences of thioarsenicals after exposure of different arsenic species being expected. There remain difficulties in analysis of thioarsenicals, due to the broad variety of arsenic metabolites in animal samples, the complexity of sample matrices, the instability of metabolic intermediates, and the lacking of standards of thioarsenicals. A combination of different analytical procedures each targeting

different groups of arsenicals may be helpful for better identification and determination of arsenic metabolites. Although studies have shown that thioarsenicals could be produced from DMA^{III} and DMA^V under different physiological conditions, much remains unknown on the formation pathways of thioarsenicals and further studies are definitely called for to examine the currently proposed pathways. The identification of $DMMTA^VGS$, a pentavalent thioarsenical GSH conjugate, may have changed the traditional view of pentavalent arsenicals being unable to bind to proteins, and further studies on the binding of pentavalent thioarsenicals to polypeptides and proteins are warranted. Probably because of the ability of binding to thiols (from GSH and proteins), pentavalent thioarsenicals (e.g., $DMMTA^V$) exhibited unusually high cytotoxicity and genotoxicity for a pentavalent arsenic species, underscoring the importance of thioarsenicals in arsenic toxicity. The detection of thiolated metabolites (e.g., $DMMTA^V$ and $DMMTA^VGS$) of anticancer arsenic drug darinaparsin suggests that thioarsenicals, as being highly toxic, could be related to the therapeutic effects of darinaparsin, and the involvement of thiolated metabolites in the metabolism and therapeutic effects of arsenical drugs warrants further investigation. Quantum computational chemical methods may be used as a novel tool to study arsenic metabolism including thioarsenicals, and these theoretical calculation methods would be used more widely and help elucidate the formation and metabolism of thioarsenicals.

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