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Speciation of Dimethylarsinous Acid and Trimethylarsine Oxide in Urine from Rats Fed with Dimethylarsinic Acid and Dimercaptopropane Sulfonate

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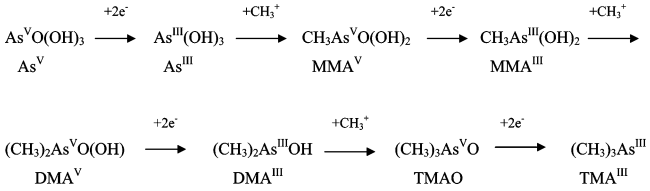
Speciation of arsenic in urine from rats treated with dimethylarsinic acid (DMA^V) alone or in combination with dimercaptopropane sulfonate (DMPS) were studied. Methods were developed for the determination of the methylarsenic metabolites, especially trace levels of dimethylarsinous acid (DMA^{III}) and trimethylarsine oxide (TMAO), in the presence of a large excess of DMA^V. Success was achieved by using improved ion-exchange chromatographic separation combined with hydride generation atomic fluorescence detection. Micromolar concentrations of DMA^{III} were detected in urine of rats fed with a diet supplemented with either 100 μg/g of DMA^V or a mixture of 100 μg/g of DMA^V and 5600 μg/g of DMPS. No significant difference in the DMA^{III} concentration was observed between the two groups; however, there was a significant difference in TMAO concentrations. Urine from rats fed with the diet supplemented with DMA^V alone contained 73 ± 30 μM TMAO, whereas urine from rats fed with the diet supplemented with both DMA^V and DMPS contained only 2.8 ± 1.4 μM TMAO. Solutions containing mixtures of 100 μg/L DMA^V or TMAO and 5600 μg/L DMPS did not show reduction of DMA^V and TMAO. The significant decrease ($p < 0.001$) of the TMAO concentration in rats administered with both DMA^V and DMPS suggests that DMPS inhibits the biomethylation of arsenic.

Biomethylation of arsenic is the major metabolic pathway for inorganic arsenic. The full pathway as seen for many fungi involves first the reduction of a pentavalent arsenic species to a trivalent arsenic species followed by the addition of a methyl group to the trivalent arsenic.[1−3] Specifically, as shown in Scheme 1, inorganic arsenate (As^V) is reduced to arsenite (As^{III}). As^{III} is oxidatively methylated to monomethylarsonic acid (MMA^V). MMA^V is then reduced to monomethylarsonous acid (MMA^{III}) and further methylated to dimethylarsinic acid (DMA^V), Similarly, further steps produce dimethylarsinous acid (DMA^{III}) and trimethylarsine oxide (TMAO), which can be reduced to trimethylarsine (TMA^{III}). The metabolic pathway does not usually proceed beyond the dimethylarsenic species stage for humans and most animals.[3]

Scheme 1. Pathway of Arsenic Methylation, Showing Alternate Steps of Two-Electron Reduction (2e−) and Oxidative Addition of a Methyl Group (CH_3^+)



Biomethylation of arsenic has previously been considered a detoxification process. However, this notion is changing with the findings of trivalent methylation metabolites, MMA^{III} and DMA^{III} in human urine,[4−10] and the numerous studies showing the higher toxicity of these metabolites than the inorganic arsenic species.[11−20]

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It is now believed that the methylation of inorganic arsenic may not be a detoxification mechanism and could be an activation process.[21−23] As a consequence, much attention has been paid to studies of the toxic effects of these metabolites. There is an increasing demand for analytical techniques that are capable of speciating these arsenic metabolites in human and experimental animal samples.

Although there are differences between human and animals and between various animal species,[24−28] the majority of experimental animals have been found to excrete arsenic efficiently, usually as DMA^V. There is a much lower concentration of MMA^V in animal urine compared with human urine. Animals such as the marmoset monkey,[24] chimpanzee,[25] and guinea pig[26] are reported to be extremely poor methylators of inorganic arsenic. These animals have very little MMA^V excreted in their urine presumably because of a lack of arsenic methyl transferase enzymes. Rats and mice, however, are efficient methylators of arsenic.[27,28]

Urinary excretion is the major pathway for elimination of arsenic compounds from the human and animal body.[24−32] While inorganic As^{III}, As^V, MMA^V, and DMA^V have been frequently detected in animal urine samples, the presence of the trivalent methylarsenic metabolites has only been reported recently.[28] Rats were treated with either 100 μg/g of DMA^V or a mixture of 100 μg/g of DMA^V and 5600 μg/g of dimercaptopropane sulfonate (DMPS).[28] Urine samples from the rats were collected for detailed analysis of arsenic speciation. Speciation of trace levels of intermediate arsenic metabolites in the presence of a large excess of DMA^V presented an analytical challenge. This paper describes a method to deal with this challenge, with an emphasis on the speciation of TMAO and DMA^{III} in rat urine samples. DMA^{III} is a key metabolic intermediate in the pathway from DMA^V to TMAO. The results on speciation of these arsenicals suggest an inhibitory effect of DMPS on the biomethylation of arsenic.

EXPERIMENTAL SECTION

Standards and Reagents. The source of MMA^{III} was the solid oxide (CH_3AsO) and that of DMA^{III} the iodide [$(CH_3)_2AsI$]. The precursors were prepared following literature procedures[33,34] and were kept at +4 or −20 °C when not in use. Dilute solutions of the precursors were prepared fresh in deionized water to form $CH_3As(OH)_2$ (MMA^{III}) and $(CH_3)_2AsOH$ (DMA^{III}), respectively. MMA^{III} and DMA^{III} solutions were prepared immediately prior to use. TMAO [$(CH_3)_3AsO$] was obtained from Tri Chemical Laboratory (Yamanashi, Japan). Solutions of other standard arsenic compounds, As^{III}, As^V, DMA^V (Aldrich, Milwaukee, WI), and MMA^V (Chem Service, West Chester, PA), were prepared by appropriate dilutions with deionized water from 1000 mg/L stock solutions, as described previously.[35−38]

DMPS was purchased from Heyltex (Houston, TX). Sodium dihydrogen phosphate and sodium borohydride were obtained from Aldrich (Milwaukee, WI). High-performance liquid chromatography (HPLC) grade methanol, sodium hydroxide, hydrochloric acid, and nitric acid were from Fisher (Pittsburgh, PA). A sodium borohydride solution (1.3%) in 0.1 M sodium hydroxide was prepared fresh daily. These reagents were of analytical grade or better. All solutions were prepared using deionized water (18 MΩ; Millipore, Bedford, MA).

Instrument and Methods. Arsenic speciation was carried out by using HPLC separation with hydride generation atomic fluorescence detection. The HPLC system consisted of a Gilson (Middleton, WI) HPLC pump (model 307), a Rheodyne six-port sample injector (model 7725i) with a 20 μL sample loop, and an analytical column. HPLC separation was carried out at room temperature. Mobile-phase solutions were filtered through a 0.45 μM membrane and sonicated in an ultrasonic bath for 15 min prior to use.

A polymer-based, strong anion-exchange column (150 × 4.1 mm; PRP-X100, Hamilton, Reno, NV) was used to separate TMAO from other arsenic species. A mobile phase contained 5 mM phosphate buffer (pH 8.2) and 5% methanol in deionized water. The flow rate of the mobile phase was 0.8 mL/min.

Two silica-based anion-exchange columns (250 × 4.60 mm; Phenosphere, 5 μm SAX 80R, Phenomenex, Torrance, CA), after modification with DMPS, were used for separation of DMA^{III} from the large excess of DMA^V. One of the columns was previously used, and the other was new. A mobile phase consisted of 20 mM phosphate and 5% methanol (pH 5.8), and its flow rate was 0.8 mL/min. The columns were pretreated with DMPS, by 10 repeated injections of a 50 μM DMPS solution as a sample onto the column, followed by washing with 50 mL of 5% methanol in deionized water. The treated column provided the desired separa-

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Table 1. Concentration of DMA^{III} in Rat Urine Samples Collected on Day 2 and TMAO and DMA^{V} in 24 h Rat Urine Samples Collected on Day 8 of the Experiment[a]

treatment group	DMA^{III} concn (μM)	TMAO concn (μM)	DMA^{V} concn (μM)
control	nd ($n = 8$)	0.2 ± 0.3 ($n = 10$)	0.2 ± 0.1 ($n = 10$)
100 μg/g DMA^{V}	1.38 ± 0.88 ($n = 8$) (0.6−3.0)	73 ± 30 ($n = 10$) (34−124)	66 ± 3 ($n = 10$) (51−77)
100 μg/g DMA^{V} and 5600 μg/g DMPS	0.93 ± 0.49 ($n = 9$) (0.5−2.1)	2.8 ± 1.4 ($n = 10$) (1.2−4.9)	507 ± 31 ($n = 10$) (376−688)

[a] nd: not detectable (concentration below the detection limit of 0.027 μM for DMA^{III}). *n*: number of rats. The results of DMA^{III} and TMAO are mean ± standard deviation from replicate analyses of urine samples from these rats. The values in the parentheses represent the range. The spot urine samples for the analysis of DMA^{III} were collected at 7:00−9:00 am on day 2 of the experiment. The 24 h urine samples for the analysis of TMAO and DMA^{V} were collected on day 8 of the experiment.

tion and was stable for 50 analyses before repeated treatment was needed.

A hydride generation atomic fluorescence detector (model Excalibur 10.003, P.S. Analytical, Kent, U.K.) was used for arsenic detection. HPLC effluent directly met with a flow of hydrochloric acid (10%, 10 mL/min) and sodium borohydride (1.3%, 3 mL/min). Volatile arsines generated were carried by a continuous flow of argon (250 mL/min) to the atomic fluorescence detector. A computer with Varian Star Workstation software and an analog-to-digital converter board was used for data acquisition and processing. For the determination of TMAO, a lower concentration of hydrochloric acid (5%) was used because a higher sensitivity was achieved for TMAO at the lower acid concentration.

Rats and Urine Samples. Thirty female F344 rats, 4 weeks old, were purchased from Charles River Breeding laboratories (Raleigh, NC). The animals were housed in polycarbonate cages (5/cage) on dry corncob bedding in a room with a targeted temperature of 22 °C, humidity of 50%, and 12 h light/dark cycle as described previously.[28] They were fed pelleted Certified Rodent Diet 5002 (PMI Nutrition International, Inc., St. Louis, MO). Food and water were available ad libitum throughout the study. Following quarantine, rats were randomized into three groups of 10 each: group 1 was fed basal diet, group 2 was fed the basal diet supplemented with 100 μg/g of DMA^{V}, and group 3 was fed the diet supplemented with 100 μg/g of DMA^{V} and 5600 μg/g of DMPS. The rat diets supplemented with DMA^{V} and DMPS were obtained from Dyets, Inc. (Bethlehem, PA), that mixed the desired amounts of DMA^{V} and DMPS into Certified Purina 5002 and then pelleted the diets.

Fresh void urine samples were collected separately from each rat for the quantification of DMA^{III}. After the three groups of rats were given the specified diet for 1 day, urine samples were collected from each rat at 7:00−9:00 a.m. the following morning. All urine samples were immediately frozen in liquid nitrogen and kept frozen on dry ice during transportation. The frozen urine samples were thawed at room temperature and were analyzed for DMA^{III} within 48 h of sample collection (results are shown in Table 1). To confirm the presence of DMA^{III} in rat urine, repeated fresh void rat urine samples were collected for analysis on study day 71 and day 175. The small volume of urine samples from each rat (~200 μL) was only sufficient for the quantification of DMA^{III}, and other arsenic species in these samples were not quantified.

Another set of urine samples were collected on day 8 after the rats had been administered with the specified diets. The rats were housed in individual metabolic cages. A 24 h urine sample was collected from each rat. These urine samples were kept frozen on dry ice during transportation and stored at −20 °C until analysis. The frozen urine samples were thawed at room temperature and were analyzed for TMAO and DMA^{V} concentrations within 1 month of sample collection (results are shown in Table 1).

RESULTS AND DISCUSSION

Determination of DMA^{III}. We have previously developed a method for the determination of the six individual arsenic species, As^{V}, As^{III}, MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III}, in human urine samples.[8] It involved ion-pair HPLC separation of the arsenic species followed by hydride generation atomic fluorescence spectrometry (HGAFS) detection of trace amounts of arsenic. The method has been successfully used for the speciation of arsenic in urine samples from highly exposed populations in Guizhou (China),[7,8] Romania,[5] and Inner Mongolia (China).[4] The six target arsenic species were well resolved, and the elution order was As^{III}, MMA^{III}, DMA^{V}, MMA^{V}, DMA^{III}, and As^{V}.

To investigate the possibility that DMA^{III} might be present in the urine of animals, rats were fed with a diet containing 100 μg/g of DMA^{V}. The concentrations of DMA^{V} in the urine from these rats peaked at 5000 μg/L when the samples were analyzed by using the HPLC−HGAFS method as described previously.[8] This high concentration of DMA^{V} presents a problem for the determination of DMA^{III} at trace levels because the small DMA^{III} peak was masked by the tail of the much larger DMA^{V} peak. One approach to solving this problem would be to modify the chromatographic separation so that DMA^{III} (trace amounts) is eluted before the large excess of DMA^{V}.

We have previously observed that the chromatographic retention behavior of MMA^{III}, DMA^{III}, and DMA^{V} could be affected by micromolar levels of DMPS.[39] In addition, DMPS is present in the urine samples from the rats fed with a diet that contained DMPS. It is necessary to achieve a consistent chromatographic retention time for the arsenic species in urine samples regardless of whether DMPS is present in the sample. Therefore, we decided to use DMPS to modify the column for the separation of DMA^{III} in the presence of a large excess of DMA^{V}. The pretreatment of the column with DMPS involves 10 replicate injections (20 μL each) of 50 μM DMPS. With this DMPS treatment, the silica-based anion-exchange column enables consistent separation of arsenic species. The column is stable for approximately 50 analyses before another DMPS treatment is needed. Importantly, the desired elution order (DMA^{III} before DMA^{V}) is achieved, allowing for the determination of trace levels of DMA^{III} in the presence of much higher concentrations of DMA^{V} in the same

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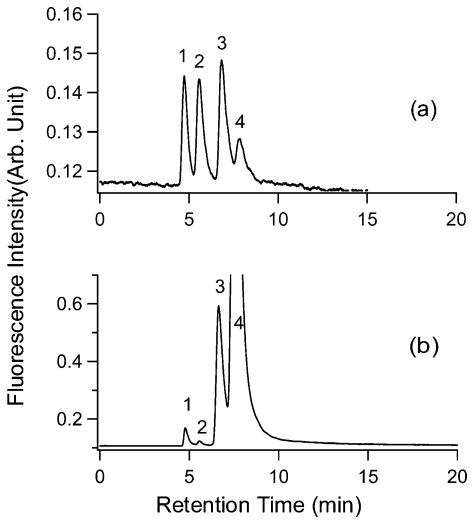

Figure 1. HPLC−HGAFS chromatograms of a standard solution containing six arsenic species in deionized water (a) and arsenic species in a rat urine sample (b). The urine sample was collected on day 2 of the experiment and was analyzed for DMA^{III} within 48 h after collection. A previously used anion-exchange column (250 × 4.60 mm, Phenosphere, 5 μm, SAX) was modified with DMPS and was used for separation. The mobile phase contained 20 mM phosphate and 5% methanol (pH 5.8), and its flow rate was 0.8 mL/min. Peak 2 is DMA^{III}, and peak 4 is DMA^{V}. Peak 1 represents MMA^{III} and As^{III}. TMAO, MMA^{III}, and As^{V} coelute under peak 3.

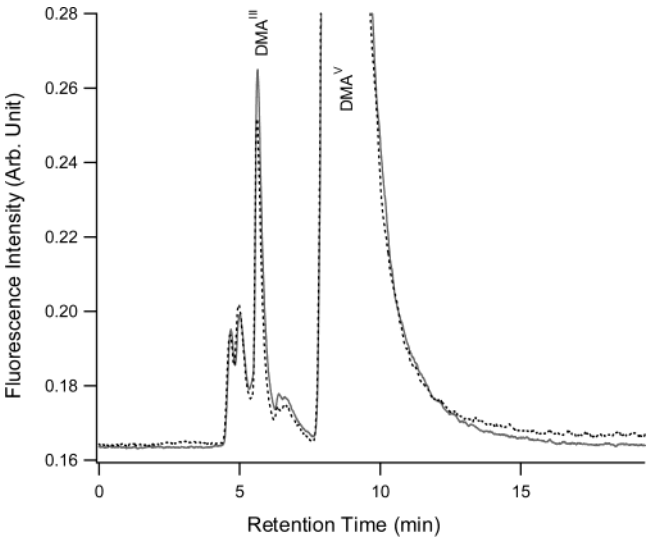

Figure 2. Chromatograms showing speciation analyses of DMA^{III} in a rat urine sample (dotted line) and the urine sample spiked with a DMA^{III} standard (solid line). A new anion-exchange column (Phenosphere, 5 μm, 250 × 4.60 mm) modified with DMPS was used for separation. The mobile phase contained 20 mM phosphate and 5% methanol (pH 5.8), and its flow rate was 0.8 mL/min. The rat was fed with a diet supplemented with 100 μg/L DMA^{V}. The urine sample was collected from the rat on day 2 of the experiment. The amount of DMA^{III} spiked to the urine sample (solid line) is equivalent to a final concentration of ~10 μg/L.

urine sample. Furthermore, the retention time of arsenic species on the DMPS-treated column is not affected by the presence of DMPS in the urine sample.

Figure 1 shows chromatograms from preliminary analyses of seven arsenic species in water (Figure 1a) and in a rat urine sample (Figure 1b). DMA^{III} is clearly resolved from a large excess of DMA^{V}. The chromatogram of the rat urine sample (Figure 1b) was acquired from the direct analysis of the original urine sample without any sample treatment or dilution. The fact that DMA^{III} is eluted before DMA^{V} makes it possible to determine trace levels of DMA^{III} in the presence of excess DMA^{V}. The concentration of DMA^{V} in the experimental rat urine samples was as high as 1000-fold that of DMA^{III}. The high DMA^{V} concentration did not show interference with the determination of DMA^{III}. The detection limit of DMA^{III} was 2.0 μg/L (~0.027 μM).

A DMA^{III} standard was spiked into urine samples to confirm the chromatographic peak identity of DMA^{III} in the urine sample. Figure 2 shows chromatograms obtained from the analyses of a urine sample (dotted trace) and the same urine sample spiked with a DMA^{III} standard (solid trace). The urine sample was collected from a rat that was fed with the mixture of 100 μg/g of DMA^{V} and 5600 μg/g of DMPS in the diet. Co-injection of the urine samples with a DMA^{III} standard (~10 μg/L) showed the same retention time for DMA^{III} in the urine and standard, suggesting the presence of DMA^{III} in the urine sample.

Having established a method for the speciation of DMA^{III} in the presence of excess DMA^{V}, we further demonstrated its application to studies of the arsenic metabolism of experimental animals. Thirty rats were randomly divided into three groups (10 in each group). The control rats were fed with a basal diet. The diet for the second group of rats was supplemented with 100 μg/g of DMA^{V}. The third group of rats was fed with the diet supplemented with both 100 μg/g of DMA^{V} and 5600 μg/g of DMPS. An objective of the study was to examine how DMPS affects the metabolism and toxicity of DMA^{V}. DMPS is a chelating agent that has been used for the treatment of acute arsenic and heavy-metal poisoning.[40,41]

Figure 3 shows representative chromatograms from the analyses of urine samples collected from rats on day 2 of the experiment. Chromatogram a was from the analysis of a urine sample from a control rat under the basal diet. DMA^{III} was not detectable in this group of rats. Chromatogram b was obtained from a urine sample from the second group of rats that were fed with 100 μg/g of DMA^{V} in the diet. DMA^{III} was detected in this urine sample and in urine samples from the other seven rats in this group (data not shown). Chromatogram c was obtained from a urine sample from the third group of rats. Their diet was supplemented with 100 μg/g of DMA^{V} and 5600 μg/g of DMPS. DMA^{III} was also present in urine samples from this group of rats.

The procedure of urine collection and analysis was repeated three times during study day 1, day 71, and day 175 from rats fed the control diet, the diet with 100 μg/g of DMA^{V}, and the diet with the mixture of 100 μg/g of DMA^{V} and 5600 μg/g of DMPS. DMA^{III} was present in all urine samples from the DMA^{V} treatment group and the DMA^{V} plus DMPS treatment group (data not shown).

The concentrations of DMA^{III} in urine samples from the rats are summarized in Table 1. The concentrations of DMA^{III} in the DMA^{V} and the DMA^{V} plus DMPS treatment groups were at the micromolar levels. There was no significant difference ($p = 0.3$

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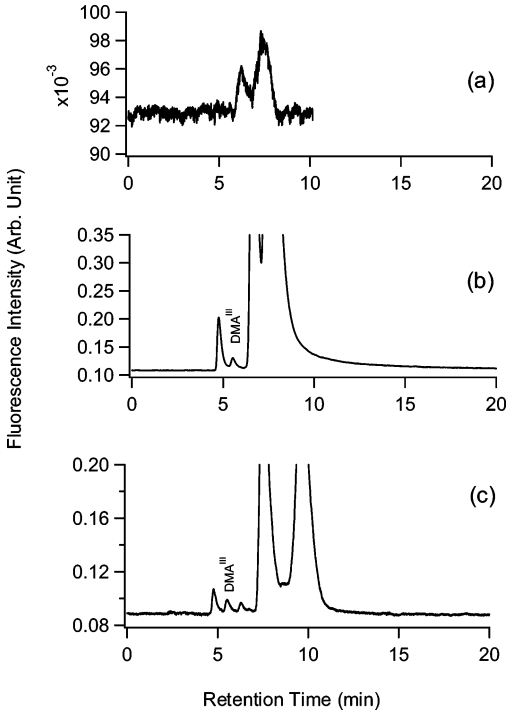

Figure 3. Typical chromatograms showing the analysis of DMA^{III} in urine samples from a control rat (a), a rat fed with 100 μg/g DMA^{V} (b), and a rat fed with 100 μg/g DMA^{V} in combination with 5600 μg/g DMPS (c). The analysis of samples a and b was carried out using the same conditions as those shown in Figure 1. The analysis of sample c was carried out using the same conditions as those shown in Figure 2.

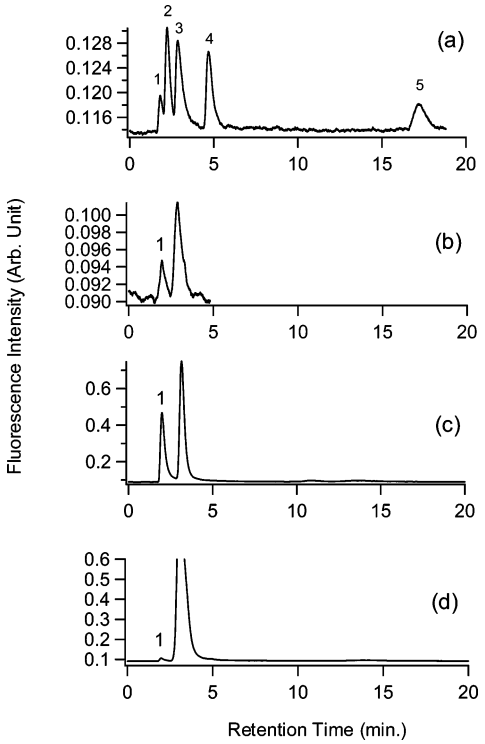

Figure 4. Typical chromatograms showing the presence of TMAO in urine samples from three groups of rats (b−d) in comparison with standard arsenic species in water (a). The control rats were fed a basal diet (b). The second group of rats were fed the diet supplemented with 100 μg/g DMA^{V} (c). The third group of rats were fed the diet supplemented with 100 μg/g DMA^{V} and 5600 μg/g DMPS (d). Urine samples were collected for 24 h from the rats on day 8 of the experiment. An anion-exchange column (PRP-X100, 150 × 4.1 mm) was used for separation. The mobile phase contained 5 mM phosphate and 5% methanol (pH 8.2), and its flow rate was 0.8 mL/min. Peak 1 represents TMAO. Peaks 2, 4, and 5 correspond to As^{III}, MMA^{V}, and As^{V}, respectively. DMA^{V}, DMA^{III}, and MMA^{III} coelute under peak 3.

using Mann Whitney test) in the DMA^{III} concentration between the rats in the DMA^{V} alone group and the DMA^{V} plus DMPS treatment group. No DMA^{III} was detected in any of the control urine samples (below detection limit of 2 μg/L, or ~0.027 μM).

To confirm that the DMA^{III} detected in the rat urine is a consequence of metabolism by the rats, we have analyzed the DMA^{V} that was added to the rat diet. There was no DMA^{III} in the rat diet. In addition, there was no detectable DMA^{III} in a solution containing 100 μg/L DMA^{V} and 5600 μg/L DMPS.

DMA^{III} was only found in fresh void urine analyzed within 48 h after collection. As demonstrated previously,[42] DMA^{III} in human urine is oxidatively unstable. It is readily oxidized to DMA^{V}. Thus, the amounts of DMA^{III} detected in these rat urine samples probably represent a small fraction of DMA^{III} that was in the fresh void urine.

Our recent work indicates that DMA^{III} readily binds to rat hemoglobin.[43] The binding of DMA^{III} to hemoglobin in red blood cells may explain that only a small amount of DMA^{III} is excreted into the urine.

Determination of TMAO. Further methylation of DMA^{III} would result in the formation of TMAO (Scheme 1). To examine whether TMAO is formed in the rats, we further developed a method for the determination of TMAO. This method involves the separation of TMAO from other arsenic species using an anion-exchange column (PRP-X100) with 5 mM phosphate buffer (pH 8.2) and 5% methanol as the mobile phase. Detection of TMAO at trace levels is achieved by HGAFS. Figure 4 shows the analyses of arsenic standards (a) and rat urine samples (b−d) from three treatment groups. Chromatograms reveal the presence of TMAO in all of the rats tested.

Unlike DMA^{III}, TMAO is much more stable in the urine samples. TMAO remained unchanged in the urine samples at −20 °C for 1 month, the storage duration tested in this study. Thus, the measured concentrations of TMAO represent the actual concentrations of TMAO in the urine samples.

Concentrations of TMAO and DMA^{V} in 24 h rat urine samples collected on study day 8 are summarized in Table 1. These are the major arsenic species found in the rat urine samples. In the rats fed with the diet supplemented with 100 μg/g of DMA^{V}, their urine samples contained 34−124 μM (73 ± 30 μM) TMAO. However, in the rats fed with the diet supplemented with both 100 μg/g of DMA^{V} and 5600 μg/g of DMPS, the concentration of TMAO in their urine decreased to 1.2−4.9 μM (2.8 ± 1.4 μM). The concentrations of TMAO in the two treatment groups are significantly different ($p < 0.001$).

The concentration of DMA^{V} in the combined DMA^{V} and DMPS treatment group is much higher than that in the DMA^{V} treatment

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group. This reflects the increased total urinary arsenic excretion due to the administration of DMPS, a finding consistent with the observed increases in the total arsenic concentration in human urine samples following the administration of DMPS.[4] The concentrations of As^{V}, As^{III}, and DMA^{V} in the urine samples from all three groups of rats are minor (2 orders of magnitude lower than the concentration of DMA^{V}).

To examine whether the in vitro reduction of TMAO by DMPS could be responsible for the decrease in the TMAO concentration, an aqueous solution containing 100 μg/L TMAO and 5600 μg/L DMPS was analyzed repeatedly for arsenic speciation. This ratio of TMAO and DMPS is the same as that used in the rat diet. Speciation analysis did not show a reduction of TMAO over a period of 48 h. Likewise, no reduction of DMA^{V} was observed in a solution containing 100 μg/L DMA^{V} and 5600 μg/L DMPS. Therefore, the dramatic decrease of the TMAO concentration in the rats that were treated with both DMA^{V} and DMPS suggests that DMPS may inhibit the formation of TMAO in vivo. It is possible that DMPS inhibits the pathway of arsenic methylation from DMA^{V} to TMAO.

Two steps are involved in the methylation of DMA^{V} to TMAO (Scheme 1): the reduction of DMA^{V} to DMA^{III}, which may be mediated by a reductase, and the oxidative addition of a methyl group to DMA^{V}, which may require a methyl transferase. DMPS could presumably interfere with these enzymatic mediated reactions. Alternatively, DMPS could form complexes with DMA^{III}, reducing the availability of DMA^{III} as a substrate to accept a methyl group. Both of these actions would result in the decrease of TMAO formation.

Aposhian et al.[4,41] reported that administration of DMPS to people who were exposed to high levels of arsenic in drinking water resulted in large decreases in the concentration and percentage of urinary DMA^{V}. They pointed out that DMPS inhibited the methylation of MMA^{V} to DMA^{V}. Our results, focusing on the subsequent step of arsenic methylation, from DMA^{V} to TMAO, are consistent with these earlier findings.

A striking difference between humans and rats in arsenic methylation is the differences in the end methylation products. While this and previous studies have shown that rats produce TMAO,[28,44−47] much less is known about the formation of TMAO in humans. Only two studies have reported the presence of TMAO in human urine.[45,48] Francesconi et al.[48] detected traces of TMAO in urine collected from a volunteer after a single ingestion of 1220 μg of arsenic in the form of a dimethylated arsenosugar. Arsenic speciation analysis was carried out using ion-exchange chromatography with inductively coupled plasma mass spectrometry detection. TMAO accounted for only 0.5% of the total arsenic excreted. Marafante et al.[45] studied arsenic species in urine samples from a volunteer following the ingestion of a high dose of DMA^{V} (8 mg of arsenic). Arsenic species were determined using hydride generation followed by cold-trapping gas chromatography with atomic absorption detection. They found small amounts of TMAO in the urine samples up to 3 days after the ingestion of DMA^{V}. Le et al.[7,8] did not find TMAO in human urine samples collected from people who were exposed to arsenic in their drinking water, although their method was able to detect low levels of TMAO (detection limit of 2 μg/L). There is no other report on the determination of TMAO in human samples from the general population.

While the previous arsenic speciation techniques have assisted studies of arsenic metabolism, the analytical techniques that we report here for speciation of DMA^{III} and TMAO also enable the study of arsenic methylation in rats. The dosing of rats with DMA^{V} alone or in combination with DMPS provides an opportunity to observe DMPS inhibiting the methylation of DMA^{V} to TMAO. Results of this study along with previous findings on the methylation of MMA^{V} to DMA^{V} suggest that DMPS inhibits arsenic methylation. Understanding the mechanism of arsenic methylation and the factors affecting arsenic methylation is important for a better interpretation of arsenic toxicity and carcinogenicity because of the dramatic differences in toxicity of various arsenic methylation products.[11−19,49]

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