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Theoretical Calculations and Reaction Analysis on the Interaction of Pentavalent Thioarsenicals with Biorelevant Thiol Compounds

Noriyuki Suzuki,[†] Hua Naranmandura,[†] Seishiro Hirano,[‡] and Kazuo T. Suzuki*,[†]

Graduate School of Pharmaceutical Sciences, Chiba University, Chuo, Chiba 260-8675, Japan, and Research Center for Environmental Risk, National Institute for Environmental Studies, Tsukuba 305-8506, Japan

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To obtain a rational understanding of the extraordinary interaction of pentavalent thioarsenicals with biorelevant thiol compounds, we carried out *ab initio* calculations on related arsenic compounds and discussed the correlation between the distribution of observed arsenic species in actual reaction systems and the corresponding calculated reaction enthalpies. Previously, it was considered that pentavalent arsenicals do not form thiol conjugates. However, the dimethylmonothioarsinic acid−glutathione conjugate (DMMTA^{V}−GSH) was recentry reported as the first stable conjugate of a pentavalent arsenical with a thiol compound. We carried out detailed analysis of the DMMTA^{V}−GSH formation reaction and demonstrated that this conjugate could be formed nonenzymatically under weakly acidic conditions. On the basis of the *ab initio* calculations, this conjugation was an exothermic reaction ($\Delta H = -4.85$ kcal/mol) and gave the minimum energy point during the reaction sequence of DMMTA^{V} with a thiol compound. However, in the case of dimethylarsinic acid (DMA^{V}), a corresponding oxo acid to DMMTA^{V}, conjugation with a thiol compound is an endothermic reaction ($\Delta H = +0.06$ kcal/mol). The minimum energy point of the reaction sequence of DMA^{V} with a thiol compound was the formation of a trivalent dimethylarsinous acid (DMA^{III})−GSH conjugate. Because the formation of arsenic−sulfur bonds is one of the major mechanisms for arsenic toxicity, these energetic results could account for the extraordinary behaviors and toxicities of thioarsenicals *in vivo* and *in vitro* in comparison with those of the corresponding oxo acids.

Introduction

Arsenic is one of the most important pollutants of concern occurring in the environment, and human health risks such as hyperkeratosis, pigmentation and blackfoot disease (*1, 2*), and lung, skin, and urinary bladder cancers (*3–5*) are increasing in populations affected by arsenic-contaminated drinking water. Although the mechanisms underlying arsenic-induced diseases and cancers are not precisely understood, the binding of arsenicals to biomolecules is considered to be one of the major toxic mechanisms. Trivalent arsenicals are known to have high affinity for the sulfhydryl groups of biomolecules, such as glutathione (GSH[1]) and lipoic acid, and the cysteinyl residues of many enzymes (*1, 2, 6*). Because the sulfhydryl groups of enzymes and cofactors play important roles in many biological processes, the formation of arsenic(III)−sulfur bonds results in various harmful effects. However, it is said that pentavalent arsenicals do not directly bind to sulfhydryl groups. In order to bind to sulfhydryl groups, the reduction of pentavalent arsenicals by bioreductants or reduction systems *in vivo* into more toxic trivalent arsenicals is thought to be necessary.

However, very recently, Raab et al. reported for the first time that sulfide-activated pentavalent arsenic could also bind to the sulfhydryl group of GSH (*7*). They found an unknown arsenic compound during the study of the metabolism of inorganic and methylated arsenicals in cabbage (*Brassica oleracea*) exposed to dimethylarsinic acid (DMA^{V}), and the unkown compound was identified as the dimethylmonothioarsinic acid−GSH conjugate (DMMTA^{V}−GSH, Figure 1) by using HPLC-electrospray mass spectrometry (HPLC-ES MS) and HPLC-inductively coupled argon plasma mass spectrometry (HPLC-ICP MS). In addition, they reported that simply mixing DMMTA^{V} and GSH in water resulted in the formation of the DMMTA^{V}−GSH complex in the same manner as trivalent arsenicals.

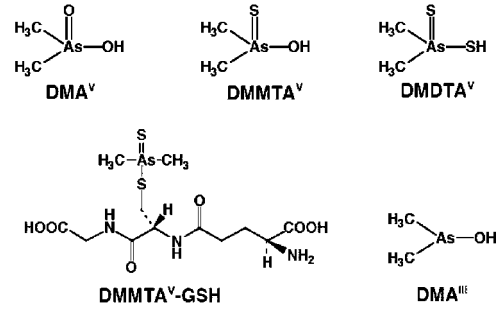

Figure 1. Dimethylarsenicals and dimethylthioarsenicals that have been previously reported.

It is known that such sulfur-containing pentavalent arsenicals as DMMTA^{V} and dimethyldithioarsinic acid (DMDTA^{V}) are formed in the metabolic pathway of arsenic in mammals (*8–11*) and that these two thioarsenicals showed distinct behaviors and toxicities *in vivo* and *in vitro* in comparison with those of the corresponding oxo acids (*12–14*). Both Naranmandura et al. and

* To whom correspondence should be addressed. Dr. Kazuo T. Suzuki, Professor, Graduate School of Pharmaceutical Sciences, Chiba University, Chuo, Chiba 260-8675, Japan. Tel: +81-43-226-2865. Fax: +81-43-226-2865. E-mail: ktsuzuki@p.chiba-u.ac.jp.

[†] Chiba University.

[‡] National Institute for Environmental Studies.

[1] Abbreviations: iAsIII, arsenite; DMA^{III}, dimethylarsinous acid; DMA^{V}, dimethylarsinic acid; GSH, glutathione; DMMTA^{V}, dimethylmonothioarsinic acid; DMDTA^{V}, dimethyldithioarsinic acid; ICP MS, inductively coupled argon plasma mass spectrometry; ES MS, electrospray mass spectrometry; RHF, restricted Hartree−Fock.

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Raml. et al. reported that $DMMTA^V$ showed considerably higher cytotoxicity than nonthiolated DMA^V. Therefore, the interaction of pentavalent thioarsenicals with biorelevant thiol compounds is important from the viewpoint of their toxicity mechanisms, if the similar complexes could be formed in mammal tissues.

Here, we report the results of detailed analysis of the $DMMTA^V$−GSH formation reaction and clarify why this conjugate could be stably isolated, in contrast to DMA^V, which, instead of forming a conjugate with GSH, is reduced to DMA^{III} by GSH.

Experimental Procedures

Synthesis of ^{34}S-DMMTA^V. $Na_2{}^{34}S$ was synthesized by reducing elemental [^{34}S]-enriched sulfur (17 mg (0.5 mmol), 99.90%-enriched, powder) with metallic Na (46 mg (2.0 mmol), lump) in THF at 80 °C under Ar atmosphere for 12 h. ^{34}S-$DMMTA^V$ was obtained by reacting DMA^V and $Na_2{}^{34}S$ according to the procedure in our previous report (*15*). Chemical and isotopic purities were confirmed by HPLC-ICP MS.

HPLC-ICP MS and HPLC-ESI MS Analysis. The separation and detection conditions for arsenic compounds were similar to those described previously (*12, 15, 16*). Briefly, arsenic species were separated on a polymer-based multimode column (Shodex Asahipak GS-220 HQ, 300 mm × 7.6 mm i.d., Showa Denko, Tokyo, Japan) eluted with 50 mM ammonium acetate buffer (pH 6.5 at 25 °C) at a flow rate of 0.6 mL/min. Arsenic in the eluate was monitored with ICP MS (HP4500; Yokogawa Analytical Systems, Hachiouji, Japan) and ES MS (LCMS-2010EV series; Shimadzu, Kyoto, Japan).

Reaction Conditions of DMMTA^V and GSH. $DMMTA^V$ (133 μM, 1 mL) was mixed with GSH (1.33 mM) and incubated at 37 °C for 12 h at various pH in sodium phosphate buffer (0.1 M, pH 1.5 to 9.0), and then the products were subjected to HPLC-ICP MS and HPLC-ESI MS analyses.

***Ab Initio* Calculations.** All *ab initio* calculations were carried out using the restricted Hartree–Fock (RHF) theory with the GAMESS quantum calculation package (*17*). We employed the 6-31G(d,p) basis set and the isodensity polarized continuum method (IPCM) (*18*) as an aqueous solvent model for geometry optimizations and subsequent energy calculations. Reaction enthalpies at 37 °C were obtained by thermodynamic parameters calculated at 37 °C.

Results and Discussion

We previously reported that free $DMMTA^V$ and $DMDTA^V$, but not their GSH conjugates, were detected in mammalian tissues including liver, in which the concentration of cytosolic GSH is relatively high (*8*). Meanwhile, Rabb et al. reported that simply mixing $DMMTA^V$ and GSH in water resulted in the formation of the $DMMTA^V$−GSH (*7*). Therefore, we examined the pH dependence of the reaction. First, in order to confirm the formation of the $DMMTA^V$−GSH, a reaction mixture containing $DMMTA^V$ and GSH incubated in phosphate buffer (pH 3) was immediately subjected to HPLC-ICP MS and HPLC-ESI MS analysis on a multimode GS-220 HQ column. In this experiment, we prepared $DMMTA^V$ labeled with ^{34}S (^{34}S-$DMMTA^V$) to discriminate pentavalent and trivalent GSH complexes. The pentavalent complex is considered to possess ^{34}S originating from ^{34}S-$DMMTA^V$, while the trivalent complex is not because the origin of the sulfur atom of the trivalent GSH complex is GSH. As shown in Figure 2a, $m/z = 75$ (^{75}As), and the reaction mixture contained a novel arsenic species (peak 2) distinct from the original $DMMTA^V$ (peak 3). $DMMTA^V$ contained only ^{34}S (Figure 2a, $m/z = 48$ ($^{32}S^{16}O$) and 50 ($^{34}S^{16}O$)), and the novel arsenic species (peak 2) also contained ^{34}S at an equimolar ratio to As, while GSH (peak 1) contained

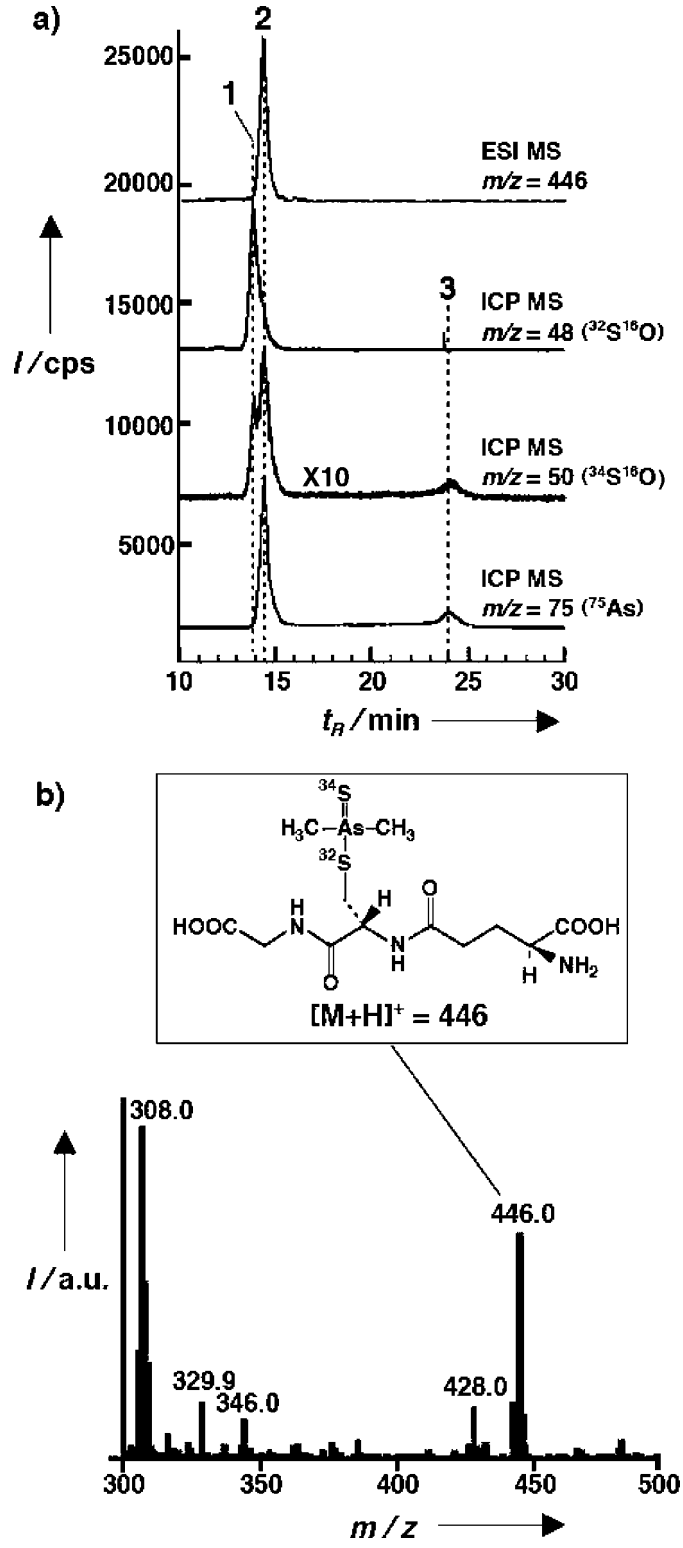

Figure 2. (a) HPLC-ICP MS and HPLC-ESI MS chromatograms of the reaction mixture of ^{34}S-$DMMTA^V$ and GSH. Detection methods and monitoring mass numbers are shown in each chromatogram. Peak 1, GSH; peak 2, ^{34}S-$DMMTA^V$−GSH; peak 3, ^{34}S-$DMMTA^V$. (b) ESI MS spectrum of peak 2.

^{32}S and ^{34}S in the ratio of natural abundance (94.9% and 4.3%, respectively). For the interference-free detection of S, the signals of S were detected as that of SO^+ by introducing O_2 gas into the mass spectrometer (*19, 20*). This result shows that this novel arsenic species contains pentavalent thioarsenic. Peak 2 also showed a signal at $m/z = 446$ in the ESI MS spectrum that agreed with $[M + H]^+$ of ^{34}S-$DMMTA^V$−^{32}S-GSH (Figure 2a

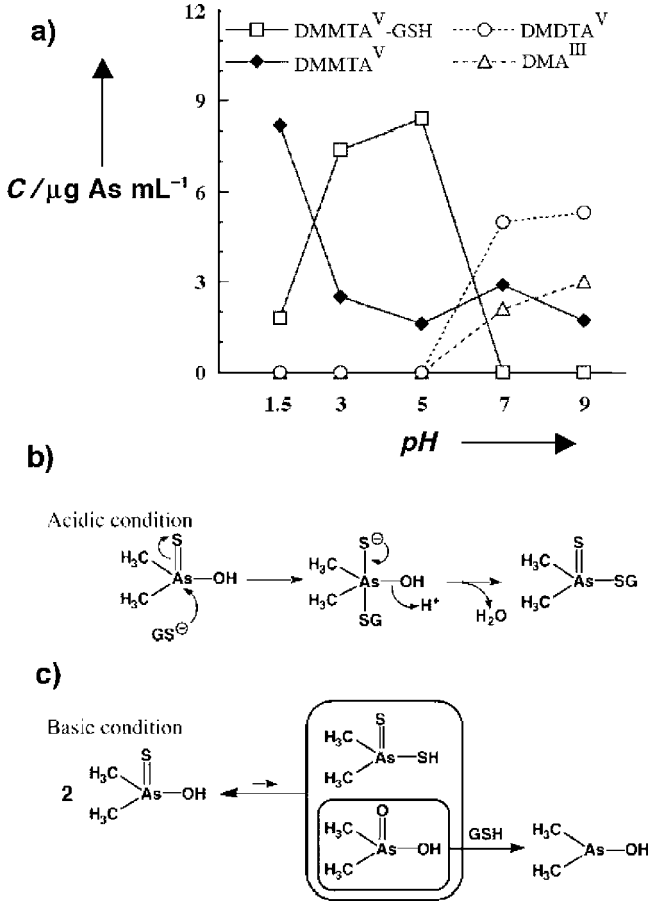

Figure 3. (a) pH dependence of the formation of DMMTA^V−GSH and the hydrolysis of DMMTA^V. (b and c) Proposed schemes of the reaction under acidic or basic conditions.

and b). In this experiment, the formation of DMMTA^V−GSH was confirmed, as reported by Rabb et al. (*7*).

Next, we analyzed the products of the reaction of DMMTA^V (133 μM, 10 μg As/mL) with GSH (1.33 mM, 10 equivalents to arsenic), which were incubated at 37 °C for 12 h at various pH in sodium phosphate buffer (0.1 M, pH 1.5 to 9.0). The analytical methods for other arsenic compounds except DMMTA^V−GSH were reported in our previous work (*15*). As shown in Figure 3a, the formation of DMMTA^V−GSH was promoted under weakly acidic conditions because the dehydration reaction from the intermediate illustrated in Figure 3b could be accelerated under acidic conditions. Since the p*K*a value of the thiol group of GSH (8.65) (*21*) is lower than those of simple alkanethiols (ca. 10), the nucleophilicity of GSH is considered to be sufficient for this reaction under weakly acidic conditions. Under strongly acidic conditions, the formation of DMMTA^V−GSH was inhibited in response to the defect of the nucleophilicity of GSH. In contrast, under neutral or basic conditions, DMA^{III} and DMDTA^V were formed instead of DMMTA^V−GSH. This phenomenon could be explained as follows. In the presence of the OH^- ion, an equilibrium could be assumed in which DMMTA^V was hydrolyzed to DMA^V, and another DMMTA^V molecule reacted with the liberated sulfide ion to afford DMDTA^V (Figure 3c). However, as DMMTA^V is stable even under basic conditions in the absence of a reducing agent, it is considered to be favorable in this equilibrium. However, DMA^V formed by hydrolysis was immediately reduced to DMA^{III} in the presence of GSH; therefore, DMA^{III} and DMDTA^V were accumulated in the reaction system. These results show that DMMTA^V−GSH is produced rapidly under

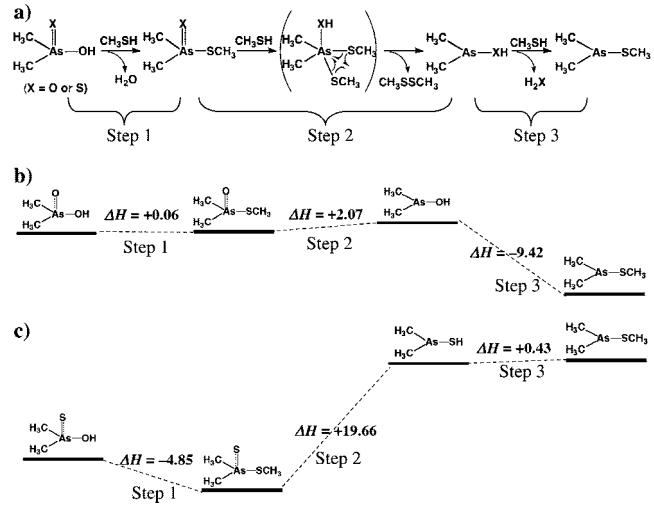

Figure 4. Potential energy diagrams for the reaction of pentavalent arsenicals with methanethiol. The proposed sequential reactions are presented in a, and enthalpy changes (ΔH, kcal/mol) of each step are presented in b (DMA^V) and c (DMMTA^V).

weakly acidic conditions, but under neutral or basic conditions, hydrolysis of DMMTA^V and subsequent reduction is predominant. In fact, *in vivo*, free DMDTA^V and DMA^{III} conjugated with proteins were detected as the two major arsenic species in rat liver and kidneys exposed to DMMTA^V (*12*).

Previously, it was considered that pentavalent arsenicals do not form thiol conjugates. To gain a rational understanding of the abnormality of DMMTA^V, we carried out *ab initio* calculations of DMA^V, DMMTA^V, and related compounds. We assumed a reaction system involving pentavalent arsenicals and methanethiol (methanethiol was employed instead of GSH to shorten the calculation time) as shown in Figure 4a, according to the reduction mechanism proposed by Martin et al. (*22*). This proposed mechanism begins with a nucleophilic attack on pentavalent arsenicals by the first thiol to give a pentavalent arsenic-SCH_3. The second thiol then attacks this complex to produce the trivalent arsenicals and CH_3SSCH_3 via the $(CH_3)_2As(XH)(SCH_3)_2$ intermediate. We calculated the enthalpy change (ΔH) at 37 °C of each step in aqueous solvent by using the RHF/6-31G(d,p) basis set and the IPCM method (*18*). Energetic results of the reaction of DMA^V and DMMTA^V with methanethiol are given in Figures 4b and c, respectively. In these calculation methods, it is hard to consider the pH effects that we discussed in Figure 3. Nevertheless, we consider that calculations of enthalpy changes are helpful to understand the diference of reactivity between two arsenic species with thiol compounds.

Figure 4b indicates that the reduction of DMA^V with methanethiol is indeed an endothermic reaction, while the subsequent complexation of DMA^{III} with methanethiol is an exothermic one ($\Delta H = -9.42$ kcal/mol) and works as a driving force for this series of reactions. Enthalpy change in the formation of DMA^V−SCH_3 indicates a slightly endothermic reaction, and this DMA^V−SCH_3 immediately reacts with another CH_3SH molecule. This is why DMA^V−thiol conjugates could not be isolated in the presence of excess thiol compounds. However, as shown in Figure 4c, the enthalpy change in the formation of DMMTA^V−SCH_3 indicates an exothermic reaction ($\Delta H = -4.85$ kcal/mol), while the reaction with the next CH_3SH molecule is a highly endothermic one ($\Delta H = +19.66$ kcal/mol). As a result, DMMTA^V−SCH_3 gives the minimum energy point during the reaction sequence. This potential energy diagram provides a rational understanding for the stability of

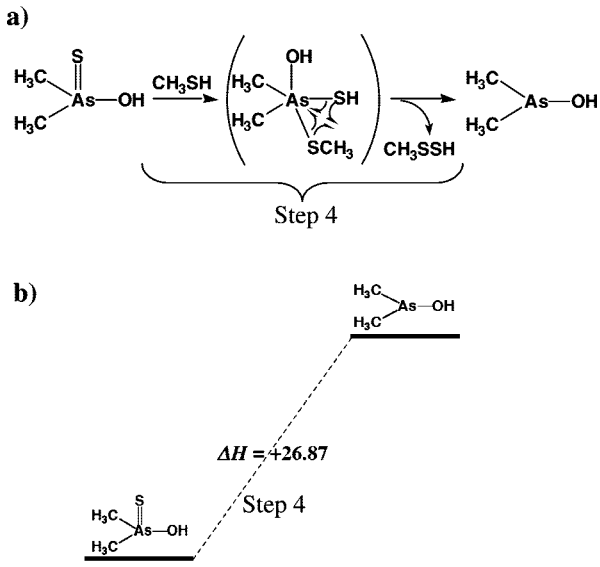

Figure 5. Potential energy diagrams for the CH_3SSH elimination reaction. The proposed reaction is presented in a, and an enthalpy change (ΔH, kcal/mol) of this reaction is presented in b.

$DMMTA^V-SCH_3$. Although another reaction pathway could be assumed in which CH_3SSH is eliminated from the $(CH_3)_2As(SH)(OH)(SCH_3)$ intermediate to directly afford DMA^{III} (Figure 5a), this pathway is also highly endothermic ($\Delta H =$ +26.87 kcal/mol, Figure 5a) and considered to be not favorable.

The results presented in this work could be the key to understanding the physiological behavior of thioarsenicals. For example, it is surmised that $DMMTA^V$ is hydrolyzed into DMA^V and undergoes further reduction, rather than forming complexes with thiol groups under physiological conditions in mammalian cells, as previously reported (*12*). However, this result also demonstrates the possibility of the formation of the $DMMTA^V$−thiol conjugate in any organisms via a nonenzymatic reaction in acidic organelles, or via some type of enzymatic reaction.

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