Arsenic Speciation of Arsine-Exposed Blood Samples by High-Performance Liquid Chromatography–Inductively Coupled Plasma Mass Spectrometry and As-Adduct, A Possible Indicator of AsH_3 Exposure

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Abstract

Arsine (AsH_3)-exposed human blood samples were analyzed by high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC–ICP-MS) for arsenic speciation. After exposure of human blood samples to AsH_3 vapor for 90 min at room temperature, partial hemolysis was observed. Plasma samples from these whole blood samples were prepared by centrifugation at 1600 × *g* for 10 min and analyzed by HPLC–ICP-MS. In addition to arsenite [As(III); degraded from AsH_3], an unidentified arsenic species (As-adduct) was detected at a retention time of 1.1 min. Following ultrafiltration of the plasma samples using a molecular weight cut-off of 10 kDa, As-adduct was not detected in the filtrate. To clarify the origin of As-adduct, AsH_3 was added to blank plasma and As(III) was added to both whole blood and hemolyzed blood. Although As(III) was detected in all samples, As-adduct was not detected. These results indicate that As-adduct was derived from erythrocytes during the process of hemolysis by AsH_3 and further suggest that As(III) and plasma ingredients do not contribute to As-adduct production. Therefore, the presence of As-adduct in blood could represent an indicator of acute arsine poisoning.

Introduction

Arsine (AsH_3: CAS No. 7784-42-1) is a poisonous material controlled by the Poisonous and Deleterious Substances Control Law in Japan. Although this substance is a colorless vapor and produced by certain microorganisms under anaerobic conditions (1), it is also synthesized on an industrial scale as a raw material for Ga-As semiconductors. Regarding poisoning cases, AsH_3 is produced by reduction of As contained as an impurity of metals during treatment with acidic solutions, and factory workers are exposed to it. Cases of AsH_3 inhalation have previously been recognized as work-related accidents (2–5). The symptoms of acute AsH_3 intoxication are malaise, headache, sickness, and vomiting, followed several hours later by hemolysis, hematuria, and high fever. Such intoxication sometimes leads to death due to anemia and kidney disorders. The hemolysis observed several hours after inhalation of AsH_3 is a characteristic marker of AsH_3 intoxication. Although the acute toxicity of AsH_3 has been studied with regard to its effects on organs (6–8) and its reactions with erythrocytes and hemoglobin (6,9–13), the mechanism that causes the hemolysis has not been fully elucidated. Furthermore, urinary metabolites after inhalation of AsH_3 have been reported (4,14), but the specification of As compounds in the blood has not been examined sufficiently. Such compounds exist widely under natural conditions, and their toxicities differ significantly depending on their chemical speciation. In particular, it is well known that some kinds of marine products contain large amounts of low-toxicity As compounds, and As compounds are ingested in daily foods. For these reasons, measuring the total As concentrations in biological samples, such as blood and/or urine, is insufficient for clarifying the level of As intoxication, and chemical speciation of As in biological fluids is indispensable (15).

High-performance liquid chromatography–inductively coupled plasma-mass spectrometry (HPLC–ICP-MS) is an analytical method with high sensitivity and a wide dynamic range for quantification that is widely applied to forensic toxicology, environmental analyses (16–18), and food chemistry (19–21).

In the present report, acute intoxication of AsH_3 was simulated by exposing blood samples to AsH_3 vapor. The exposed samples were then analyzed by HPLC–ICP-MS to clarify the chemical specification of their As compounds.

Experimental

Materials

Monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), and tetramethylarsonium (TeMA) were purchased from Tri-Chemical Laboratories

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(Yamanashi, Japan). A standard As solution (1000 mg As/L), certified by the Japan Calibration Service System (JCSS), was obtained from Wako Pure Chemical Industries (Osaka, Japan). Diarsenic trioxide, sodium hydrogen arsenate heptahydrate, and other reagents were of analytical grade and also purchased from Wako Pure Chemical Industries.

Ultrapure water, which was used for the preparation of sample solutions and dilutions, was prepared using a Milli-Q gradient system (Nihon Millipore K. K., Tokyo, Japan).

The blood samples used in the present study were collected from healthy drug-free volunteers who had not been exposed to AsH_3 or arsenic acid. The study protocol was approved by the Ethical Committee at the National Research Institute of Police Science, and informed consent was obtained from all the volunteers. All blood samples were treated with heparin.

Methods

Preparation of AsH_3-exposed blood samples. AsH_3-exposed blood samples were basically prepared according to the description of the arsenic hydride generator (Figure 1) and its operation in the Japanese Industrial Standard (testing methods for industrial wastewater, JIS K0102-1998-61.1 silver diethyldithiocarbamate absorption spectrometry) (22).

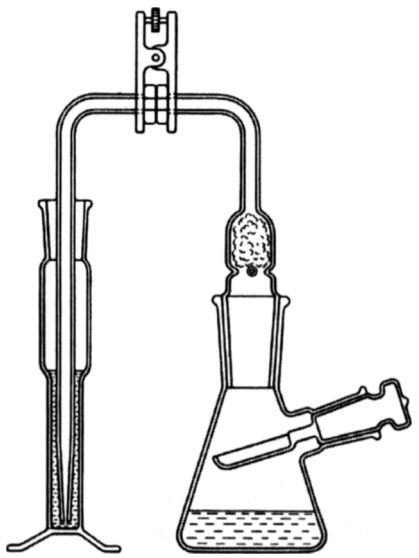

Figure 1. Arsenic hydride generator.

Briefly, 2 mL of As standard solution was placed in an arsenic hydride generator bottle, followed by addition of 3 mL of sulfuric acid and dilution to 40 mL with ultrapure water. Next, 2 mL of concentrated hydrochloric acid/ultrapure water (1:1, v/v), 15 mL of potassium iodide (200 g/L), and 5 mL of $Sn(II)Cl_2$ (4% hydrochloric acid solution) were added and the bottle was shaken, before being left for 10 min. An introduction tube and an AsH_3 absorption tube containing 5 mL of blood were then connected to the arsenic hydride generator bottle. After the connection, 3 g of Zn was immediately added, and the apparatus was dipped in a water bath (25°C). After 10 min, the AsH_3 absorption tube was separated from the arsenic hydride generator bottle and incubated at room temperature (23°C) for 90 min. The operations until this step were conducted in a draft chamber with a scrubber system. Schema of the protocol for AsH_3 generation and AsH_3 exposure of blood samples are shown in Figure 2.

Separation of erythrocytes and plasma was carried out by centrifugation at 1600 × *g* for 10 min. Deproteinization of samples was performed using an ultrafiltration cartridge (ULTRACENT-10; molecular weight cut-off: 10 kDa) supplied by TOSOH (Tokyo, Japan). The filtrates and sample solutions were diluted with ultrapure water prepared using the Milli-Q system.

HPLC–ICP-MS conditions. Speciation of the arsine compounds was conducted by HPLC–ICP-MS, which consisted of an on-line combination of an 1100 series HPLC and 4500plus type ICP-MS (Agilent Technologies, Tokyo, Japan). The conditions of the analysis are summarized in Table I.

Results and Discussion

Although hemolysis was not observed immediately after exposure of blood to AsH_3, it was detected after incubation at room temperature for 90 min.

Partial hemolysis was visually observed in the exposed blood samples, consistent with the reported symptoms of acute AsH_3-

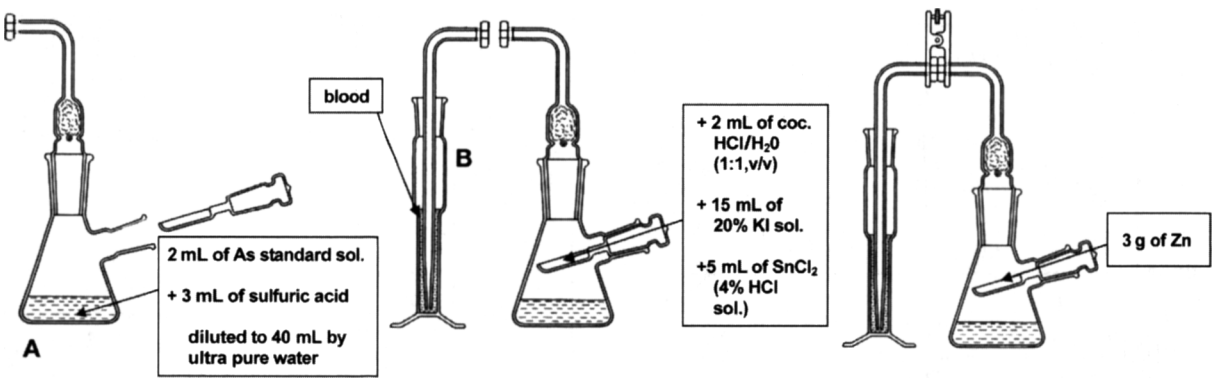

Figure 2. Schema of the method for generating arsenic hydride and preparation of AsH_3-exposed blood: A, arsenic hydride generator bottle and B, AsH_3 absorption tube.

poisoned patients. AsH_3 is absorbed into the blood through the mucosae of the lungs and airway during respiration. In the present study, AsH_3 was directly added to blood samples by bubbling of AsH_3 gas. The solubility of AsH_3 in water is low at 0.7 g/L. Although the amount of AsH_3 absorption could not be defined, it was considered that a small amount of AsH_3 was absorbed by the blood, reacted with erythrocytes, and caused a slow rate of hemolysis. For the purpose of determining the compound related to the hemolysis, the chemical speciation of As and its metabolites was examined by HPLC–ICP-MS. The HPLC–ICP-MS chromatograms at *m/z* 75 of standard solutions of arsenous acid [As(III)], arsenic acid [As(V)], MMA, DMA, TMAO, and TeMA are shown in Figure 3.

All the compounds were clearly discriminated by their retention times under these conditions.

After the addition of AsH_3, plasma fractions were obtained by centrifugation, and analyzed for their chemical speciation of As and its metabolites by HPLC–ICP-MS. The chromatogram at *m/z* 75, in addition to As(III), indicated an undetermined As compound (designated As-adduct) at 1.1 min (Figure 4A). After ultrafiltration of the plasma fractions, the filtrates were analyzed under the same conditions. On the HPLC–ICP-MS chromatogram at *m/z* 75, only As(III) was observed (Figure 4B).

This result suggests that the molecular weight of the unidentified As-adduct was over 10 kDa. Because As(III) was observed in both samples, but not detected in control blood and plasma samples, As(III) was considered to be a product of AsH_3 decomposition. AsH_3 has a strong reduction ability, and is known to be easily oxidized and decomposed by light and moisture.

In order to examine the origin of As-adduct, AsH_3 was added

Table I. Operating Conditions of HPLC–ICP-MS

Agilent 1100 series HPLC	
Column:	Excelpak G1836A/101 (150 mm x 4.6-mm i.d.)
Mobile phase:	2.0 mM phosphate buffer/0.2 mM EDTA* (pH 6.0)
Flow rate:	1.0 mL/min
Temperature:	ambient
Injection volume:	50 µL
Agilent 4500plus ICP-MS	
RF power:	1.2 kW
Plasma gas:	Ar at 15 L/min
Auxiliary gas:	Ar at 1.0 L/min
Carrier gas:	Ar at 1.1 L/min
Sampling depth:	8.0 mm
Dwell time:	0.5 s
Monitoring mass:	*m/z* 75 (As) and 35 (Cl)

* EDTA: ethylenediamine tetraacetic acid.

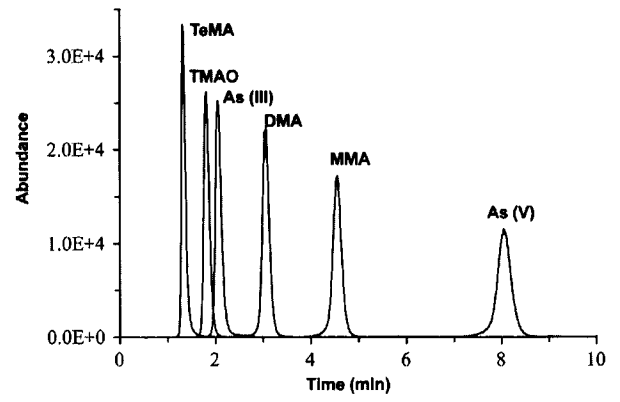

Figure 3. HPLC–ICP-MS chromatograms at *m/z* 75 of arsenic standards (100 ng As/mL each). Abbreviations: TeMA, tetramethylarsonium; TMAO, trimethylarsine oxide; As(III), arsenite; DMA, dimethylarsinic acid; MMA,monomethylarsonic acid; and As(V), arsenate.

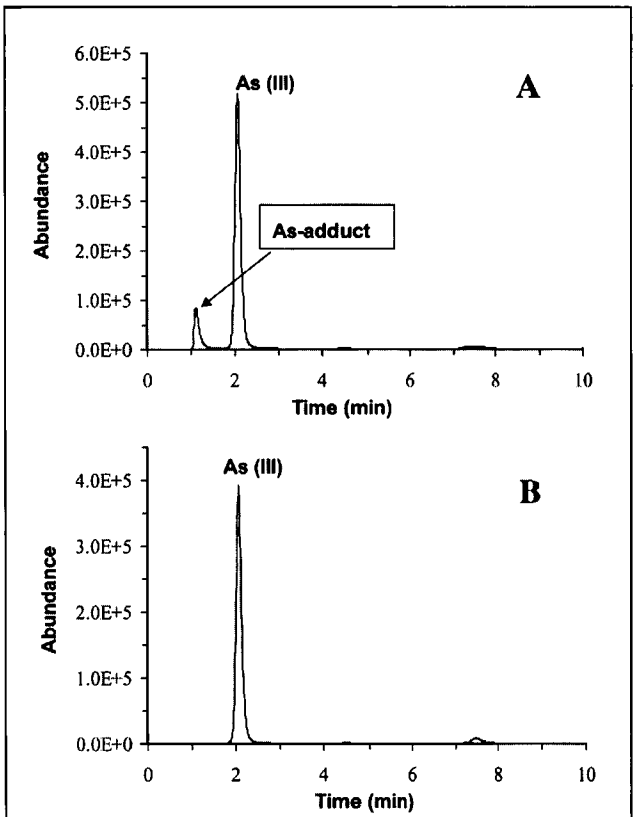

Figure 4. HPLC–ICP-MS chromatograms at *m/z* 75 of human blood plasma after incubation of whole blood and AsH_3 before ultrafiltration (A) and after ultrafiltration (B).

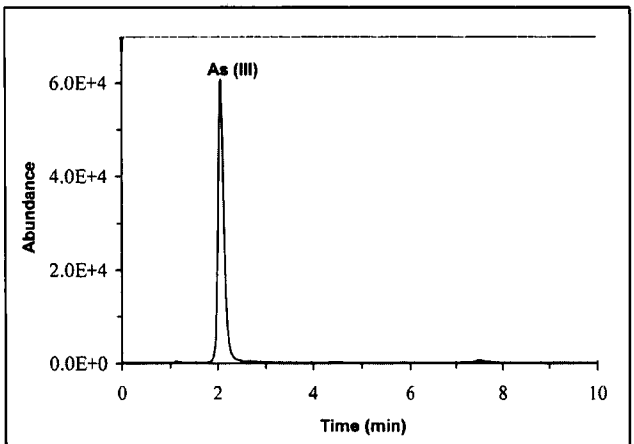

Figure 5. HPLC–ICP-MS chromatogram at *m/z* 75 of human blood plasma after incubation with AsH_3.

to a blank plasma fraction prepared by centrifugation of blank blood in the same manner as its addition to blood samples. However, HPLC–ICP-MS analysis did not detect the presence of any As-adduct (Figure 5).

This result suggests that As-adduct was not produced by the plasma fraction but by erythrocytes. Furthermore, to examine the relationship of As(III) to the production of As-adduct, As(III) was added to blank blood, incubated at room temperature for 90 min, and centrifuged. HPLC–ICP-MS was performed using the obtained plasma fraction, but only As(III) was detected on the chromatogram at *m/z* 75 (Figure 6) and no hemolysis was detected.

These results indicate that the plasma fraction and As(III) are not involved in the production of As-adduct. To examine the interaction of As(III) with constituents from erythrocytes, As(III) was added to hemolyzed blank blood prepared by dilution with ultra-pure water. Only As(III) was detected in the blood by HPLC–ICP-MS (Figure 7).

This result suggests that As(III) and erythrocyte constituents are not involved in the production of As-adduct.

Summarizing these experiments, an unidentified As compound (As-adduct) was produced by the addition of AsH_3 to whole blood samples. This As-adduct is a reaction product of erythrocytes and has a molecular weight of over 10 kDa. Plasma components and As(III), which arises as a product of AsH_3 decomposition, do not appear to be involved in the production of As-adduct. Recently, it was reported that As-adduct with hemoglobin and globin chains could not be produced after addition of AsH_3 in vitro (23).

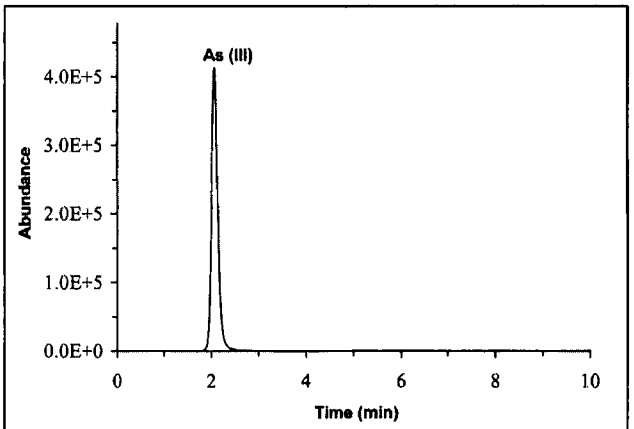

Figure 6. HPLC–ICP-MS chromatogram at *m/z* 75 of human blood plasma after incubation of whole blood and $NaAsO_2$ (10 µg AS/mL).

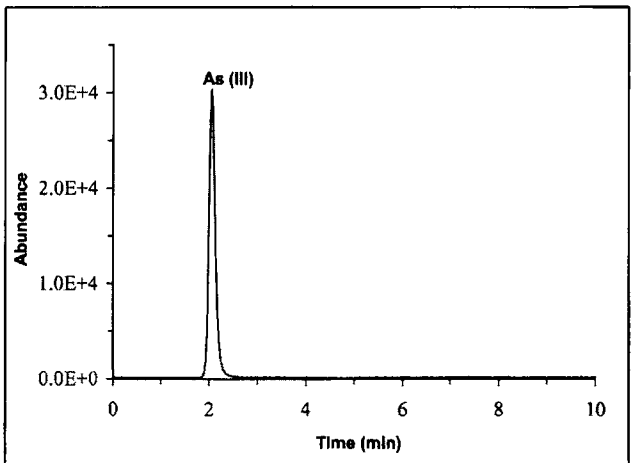

Figure 7. HPLC–ICP-MS chromatogram at *m/z* 75 of human blood plasma after incubation of hemolyzed blood and $NaAsO_2$ (10 µg AS/mL).

Conclusions

In the present study, arsenic speciation of AsH_3-exposed human blood was carried out by HPLC–ICP-MS. Exposure of whole blood to AsH_3 led to hemolysis and As-adduct production by a reaction with erythrocytes, but not with plasma. Furthermore, this adduct was not produced by As(III) and erythrocytes. The molecular weight of the adduct was over 10 kDa. Although the structure and origin of the As-adduct could not be clarified, it represents a possible marker for acute AsH_3 intoxication using blood samples.

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