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Formation of diphenylthioarsinic acid from diphenylarsinic acid under anaerobic sulfate-reducing soil conditions

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HIGHLIGHTS

- We studied anaerobic transformation of DPAA under sulfate-reducing soil conditions.
- LC/ICP-MS and LC/TOF-MS were applied to elucidate the metabolite.
- Diphenylthioarsinic acid was newly determined as the metabolite from DPAA.
- Formation of diphenyldithioarsinic acid & subsequent dimerization were also predicted.
- Thionation is a novel metabolic process for DPAA in anaerobic sulfate-reducing soil.

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ABSTRACT

Diphenylarsinic acid (DPAA) is a toxic phenylarsenical compound often found around sites contaminated with phenylarsenic chemical warfare agents, diphenylcyanoarsine or diphenylchloroarsine, which were buried in soil after the World Wars. This research concerns the elucidation of the chemical structure of an arsenic metabolite transformed from DPAA under anaerobic sulfate-reducing soil conditions. In LC/ICP-MS analysis, the retention time of the metabolite was identical to that of a major phenylarsenical compound synthesized by chemical reaction of DPAA and hydrogen sulfide. Moreover the mass spectra for the two compounds measured using LC/TOF-MS were similar. Subsequent high resolution mass spectral analysis indicated that two major ions at m/z 261 and 279, observed on both mass spectra, were attributable to $C_{12}H_{10}AsS$ and $C_{12}H_{12}AsSO$, respectively. These findings strongly suggest that the latter ion is the molecular-related ion ($[M+H]^+$) of diphenylthioarsinic acid (DPTA; $(C_6H_5)_2AsS(OH)$) and the former ion is its dehydrated fragment. Thus, our results reveal that DPAA can be transformed to DPTA, as a major metabolite, under sulfate-reducing soil conditions. Moreover, formation of diphenyldithioarsinic acid and subsequent dimerization were predicted by the chemical reaction analysis of DPAA with hydrogen sulfide. This is the first report to elucidate the occurrence of DPAA-thionation in an anaerobic soil.

1. Introduction

A large amount of phenylarsenic compounds, such as diphenylchloroarsine (CLARK I) and diphenylcyanoarsine (CLARK II), were produced as chemical warfare agents during World Wars I and II. Most of these warfare agents were subsequently dumped into the sea or buried in the earth in several parts of Europe, China and Japan. The compounds have caused water and soil pollution and have become a threat to public health, particularly around the contaminated sites [1–5].

In 2002, adverse health effects due to arsenic (As) ingestion were recorded for several inhabitants in the Kizaki area of Kamisu, Ibaraki, Japan [6]. The persons had drunk well water contaminated

Abbreviations: DMAA, dimethylarsinic acid ($Me_2AsO(OH)$); DMDTA, dimethyldithioarsinic acid ($Me_2AsS(SH)$); DMTA, dimethylthioarsinic acid ($Me_2AsS(OH)$); DMPAO, dimethylphenylarsine oxide (Me_2PhAsO); DPAA, diphenylarsinic acid ($Ph_2AsO(OH)$); DPDTA, diphenyldithioarsinic acid ($Ph_2AsS(SH)$); DPMAO, diphenylmethylarsine oxide (Ph_2MeAsO); DPTA, diphenylthioarsinic acid ($Ph_2AsS(OH)$); ESI, electro-spray ionization; HPLC, high-performance liquid chromatograph; MPDTA, methylphenyldithioarsinic acid; LC/ICP-MS, liquid chromatography/inductively coupled plasma-mass spectrometry; LC/TOF-MS, liquid chromatography/time of flight-mass spectrometry; PAA, phenylarsonic acid ($PhAsO(OH)_2$); PMAA, phenylmethylarsinic acid ($PhMeAsO(OH)$); TIC, total ion chromatograph.

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with DPAA. Also for some rice fields, which had used contaminated well water for irrigation, the rice crop was found to contain phenylarsenicals. This contamination was attributed to the illegal dumping of DPAA. DPAA is a starting chemical for the synthesis of CLARKs. At the same time, DPAA is known as a major As species in soil and ground water contaminated with chemical warfare agents, since DPAA is chemically formed from CLARKs via hydrolysis and oxidation [7,8]. It is, therefore, important to be able to monitor and understand the dynamics of DPAA in the environment.

DPAA was detected in the groundwater in the Kizaki area, and phenylarsonic acid (PAA) and bis(diphenylarsine)oxide were also present but at much lower concentrations [9]. According to Baba et al. [10], DPAA, PAA, phenylmethylarsinic acid (PMAA), dimethylphenylarsine oxide (DMPAO) and diphenylmethylarsine oxide (DPMAO), along with inorganic arsenicals, were detected in contaminated paddy soil collected in the region. Regarding aerobic bacterial cultures capable of degrading DPAA, Nakamiya et al. [11] reported the formation of DPMAO, *cis*, *cis*-muconate and arsenic acid from DPAA by *Kytococcus sedentarius* strain NK0508, which grew using DPAA as the sole carbon source. In addition, monohydroxylated DPAA, PAA and arsenic acid were determined as the metabolites from DPAA in *Ensifer adhaerens* strain L2406 cultures [12]. On the other hand, there have been few studies on the transformation of DPAA under anaerobic conditions. Notably, Arao et al. [13] showed that PAA and MPAA concentrations decreased and DMPAO concentrations increased under flooded conditions in a pot experiment for rice cultivation using contaminated soil collected from the Kamisu region. Maejima et al. [14] confirmed the anaerobic metabolism of DPAA using a model experiment. The two studies suggest that dephenylation and methylation are important anaerobic transformation pathways for DPAA.

Recently, we reported enhanced transformation of DPAA to three unknown As species under sulfate-reducing conditions [15]. The unknown metabolites were eluted after DPAA under reverse phase chromatographic conditions, suggesting that the unknowns could have more hydrophobic character than DPAA. Since the unknown As metabolites were found only in the soils amended with sulfate, hydrogen sulfide, generated by sulfate-reducing bacteria, was predicted to play a key role in the metabolism [16].

Using liquid chromatography/inductively coupled plasma-mass spectrometry (LC/ICP-MS) and liquid chromatography/time of flight-mass spectrometry (LC/TOF-MS), we seek to elucidate the chemical structure of the major unknown As metabolite reported in the previous work [15]. The chemical reaction between DPAA and hydrogen sulfide was also examined and the chemical characteristics of the products were compared with those of the unknown As species formed from DPAA under sulfate-reducing soil conditions.

2. Experimental

2.1. Reagents

High-performance liquid chromatograph (HPLC) grade acetonitrile was purchased from Sigma–Aldrich (MO, USA). Phosphoric acid (85%), formic acid and LC/MS grade methanol were from Wako Pure Chemical industries (Osaka, Japan). The following arsenicals were used as analytical standards for As speciation analysis: 60% arsenic acid; DPAA and PAA were purchased from Wako Pure Chemical industries; PMAA and DPMAO were purchased from Hayashi Pure Chemical Industry (Osaka, Japan). A mixed As standard solution for LC/ICP-MS, containing 0.5 mg-As L^{-1} arsenate, 0.5 mg-As L^{-1} PAA, 0.2 mg-As L^{-1} PMAA, 0.4 mg-As L^{-1} DPMAO and 0.5 mg-As L^{-1} DPAA was prepared with ultrapure water purified using a PURELAB Ultra (Organo, Tokyo, Japan). Other reagents were purchased from Wako Pure Chemical industries.

2.2. Soil and rice straw samples

Fallow upland soil (sand dune Regosol) was collected from the Ikarashi field in Niigata University, Niigata, Japan (N37.8714, E138.9456) in 2011. The soil properties are described in Guan et al. [15]. Before the experiments, the soil was air-dried and passed through a 2-mm mesh stainless steel sieve. The rice straw sample was collected from the Shindori station of the Field Center for Sustainable Agriculture and Forestry, Niigata University, Niigata, Japan (N37.8556, E138.9594) in 2007. An air-dried rice straw sample was used after it was finely ground and passed through a 0.5-mm mesh stainless steel sieve.

2.3. Soil culture and DPAA analysis

DPAA-spiked soil cultures were prepared in 100 mL Erlenmeyer flasks, as described in Guan et al. [15]. Briefly, each flask received 20 g soil with 30 mL deionized water supplemented with 10.7 μg-As (g dry soil [gds])$^{-1}$ DPAA and was closed with a double rubber plug. In addition, 3.5 mg-C gds^{-1} rice straw and 425 μg-S gds^{-1} K_2SO_4 were added as a carbon and sulfur source, respectively. The soil cultures in triplicate were incubated statically in the dark at 30 °C for 3 weeks. DPAA concentrations in the soil cultures were determined using HPLC and the initial was 9.24 μg-As gds^{-1} (the recovery was 86.4%). The details of the sample preparation and the HPLC conditions are described in Guan et al. [15].

2.4. Chemical reaction between DPAA and hydrogen sulfide

The chemical reaction between DPAA and hydrogen sulfide was examined in accordance with Stauder et al. [16]. Namely, 0.5 mL 50 mM Na_2S was added to 5 mL 0.1 mM DPAA solution and the pH of the solution was subsequently adjusted to approximately 7.0 by adding dilute hydrochloric acid. The reaction solution was analyzed using LC/ICP-MS (X series 2, Thermo Fisher Scientific, MA, USA) after the solution was left standing at ambient temperature for approximately 4 days and, in addition, the As metabolites, formed from DPAA in the soil culture, prepared as above, were measured. The analytical conditions were same as those described in Guan et al. [15] except that the injection volume was 1 μL.

2.5. Identification of unknown As species using LC/TOF-MS

The structure of the unknown As species was identified by LC/TOF-MS (1200 series; Agilent Technology, CA, USA, combined with JMS-T100LP; JEOL, Tokyo, Japan). The LC/TOF-MS was operated in the positive electro-spray ionization (ESI) mode. A reversed phase column (SUPELCO Discovery C18, 5 μm, 4.6 mm i.d. ×150 mm, Sigma–Aldrich), held at 40 °C, was used for separation. The mobile phase, a mixture of ultrapure water (PURELAB Ultra, Organo) and methanol (1:1) containing 0.1% formic acid, was pumped at a flow rate at 1 mL min^{-1}. A flow splitter, connected between the HPLC and TOF-MS, was used to reduce the flow rate into the TOF-MS to 0.2 mL min^{-1}. The details of TOF-MS operating parameters are summarized in Table 1. Data acquisition was performed in the total ion chromatograph (TIC) mode.

Table 1
Operating conditions of TOF–MS.

Mass range	*m*/*z* 50–800
Cone voltage	80 V
Drying gas temperature	250 °C
Capillary voltage	2.0 kV
Detector voltage	2000–2300 V