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Rapid determination of arsenic species in freshwater organisms from the arsenic-rich Hayakawa River in Japan using HPLC-ICP-MS

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ABSTRACT

Speciation analyses of water-soluble arsenicals from freshwater and biological samples collected from the Hayakawa River (Kanagawa, Japan), which contains a high concentration of arsenic, were performed using high performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC-ICP-MS). River water contained only arsenate, which is a pentavalent inorganic arsenical. The water bug *Stenopsyche marmorata* contained inorganic arsenicals accounting for 77% of the water-soluble arsenicals, followed by oxo-arsenosugar-glycerol, which is a type of dimethylarsinoylriboside (arsenosugar). The freshwater green macroalga *Cladophora glomerata* contained oxo-arsenosugar-glycerol and oxo-arsenosugar-phosphate as 64% of the water-soluble arsenicals. Production of the same types of arsenosugars was confirmed in the freshwater green microalga *Chlamydomonas reinhardtii* CC125 experimentally exposed to arsenate. The muscle tissues of all freshwater fish and crustaceans analyzed contained arsenobetaine, oxo-arsenosugar-glycerol, and/or oxo-arsenosugar-phosphate in various concentrations. Seven freshwater fish (*Cobitis biwae, Leuciscus hakonensis, Phoxinus lagowski steindachneri, Plecoglossus altivelis, Rhinogobius* sp. CB, *Rhinogobius* sp. CO, *Sicyopterus japonicus*) and the crustacean *Macrobrachium nipponense* contained arsenobetaine in their muscle tissues as the predominant form, contributing up to 80% of the water-soluble arsenicals, while the freshwater fish *Anguilla japonica* muscle tissues primarily contained dimethylarsinic acid as 77% of the water-soluble arsenicals, followed by arsenobetaine. The freshwater fish *Zacco platypus* muscle tissues predominantly contained oxo-arsenosugar-phosphate, accounting for 51% of the water-soluble arsenicals, followed by dimethylarsinic acid and arsenobetaine. These biological samples possessed non-extractable arsenical(s) accounting for more than 50% of the total arsenic concentration.

1. Introduction

Arsenic is ubiquitous in the biosphere and undergoes uptake and bioaccumulation through food chains, followed by alkylation to form a variety of arsenicals in organisms. Arsenic circulation in the marine environment has been extensively studied. Marine organisms contain much higher concentrations of arsenic (<10–100 mg kg^{-1} dry weight [DW]) than seawater (1.0–1.8 μg L^{-1}), and inorganic arsenicals are biotransformed into organoarsenicals through marine food chains (Francesconi and Edmonds, 1997). Arsenosugars are its predominant forms in marine algae (McSheehy et al., 2002), while arsenobetaine is most abundant in marine fish and crustaceans (Peshut et al., 2008). In marine bivalves and sponges, arsenosugars and arsenobetaine have been identified as the primary arsenicals (Shibata and Morita, 1992; Yamaoka et al., 2001).

In contrast to the large number of reports on arsenicals in marine organisms, there are fewer data on arsenic speciation in freshwater organisms. Certain river waters in Japan contain much higher arsenic concentrations than seawater (0.25–7.7 μg L^{-1}), because hot-spring waters containing large amounts of arsenic (10–5000 μg L^{-1}) mix with river water in some areas (Kanamori and Sugawara, 1965). Mt. Hakone is famous as a hot-spring area in Japan, and Owakudani Valley is one source of the hot springs. The hot-spring waters containing high concentrations of arsenic from the Owakudani Valley flow into the Hayakawa River. Kaise et al. (1997) found that the major arsenic species in river water,

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freshwater algae, and freshwater fish from the Hayakawa River were inorganic arsenicals, dimethylated arsenicals, and trimethylated arsenicals, respectively. Shiomi et al. (1995) and Slejkovec et al. (2004) reported that arsenobetaine was a major arsenical in freshwater fish, while Zheng and Hintelmann (2004) found that arsenobetaine was present only in trace amounts in freshwater fish, and Lawrence et al. (1986) did not detect arsenobetaine at all in freshwater fish. Koch et al. (2001) and Soeroes et al. (2005) reported that arsenosugars predominated in some freshwater fish, and that arsenobetaine was present as only a minor arsenical. Schaeffer et al. (2006) found that the dominant arsenic species in freshwater algae, mussels, and fish from a river were oxo-arsenosugars, and that the freshwater mussels and fish contained only traces of arsenobetaine. Thus, there is little consensus on the major arsenic species present in freshwater organisms, and it is important to understand arsenic behavior in freshwater ecosystems from the standpoint of environmental assessment.

In the present study, freshwater and biological samples were collected from the Hayakawa River, which contains a high level of arsenic from the hot springs at Mt. Hakone. The chemical forms of water-soluble arsenicals were analyzed by high performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC-ICP-MS). Experimentally, the freshwater green microalga *Chlamydomonas reinhardtii* CC125 (*C. reinhardtii* CC125) was also exposed to arsenate and arsenic metabolites in the cells were analyzed to confirm biotransformation of arsenic. The results suggest that the hot-spring water and river water contained only arsenate, and also that the water bug collected from the river contained arsenate as the predominant water-soluble arsenical. On the other hand, the freshwater green algae, crustaceans, and fish contained oxo-arsenosugar-glycerol and/or oxo-arsenosugar-phosphate at variety of concentrations and, moreover, the crustaceans and fish contained arsenobetaine as a primary water-soluble arsenical.

2. Experimental

2.1. Materials

Water was prepared with a Milli-Q water system (18.2 MΩ cm^{-1}). Methanol (HPLC grade), hydrogen peroxide (special grade), and nitric acid (ultrapure grade) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Sodium 1-butane sulfonate and tetramethylammonium hydroxide pentahydrate were obtained from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Malonic acid (analytical grade) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium arsenate (Na_2HAsO_4), sodium arsenite ($NaAsO_2$), methylarsonic acid ($CH_3AsO(OH)_2$), dimethylarsinic acid ($(CH_3)_2AsO(OH)$), arsenobetaine ($(CH_3)_3As^+CH_2COO^-$), trimethylarsine oxide ($(CH_3)_3AsO$), tetramethylarsonium iodide ($(CH_3)_4As^+I^-$), and arsenocholine bromide ($(CH_3)_3As^+CH_2CH_2OHBr^-$) were obtained from Tri Chemical Laboratories Inc. (Yamanashi, Japan). Oxo-arsenosugar-glycerol was synthesized following the procedures described in McAdam et al. (1987). Brown macroalga *Fucus* extract containing four types of oxo-arsenosugars was prepared as described by Madsen et al. (2000). The structures of these oxo-arsenosugars are shown in Fig. 1. Certified reference materials (CRMs) such as NMIJ 7402-a cod-fish tissue and NIES No.9 Sargasso were utilized to evaluate analytical methods. Freshwater green microalga *C. reinhardtii* CC125 (wild-type mt^+) strain was provided by Dr. Mikio Tsuzuki (Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan).

2.2. Collection of water and biological samples from the Hayakawa River

Sample collection was carried out in the Hayakawa River (Kanagawa, Japan) in May and June, 2005. The river water contains a much higher arsenic concentration than the environmental water quality standard in Japan (10 μg L^{-1}) (Kaise et al., 1998). The collected samples were: hot-spring water, river water, a water bug (*Stenopsyche marmorata*), a green macroalga (*Cladophora glomerata*), an omnivorous crustacean (*Macrobracbium nipponenese*), a herbivorous fish (*Plecoglossus altivelis*), and eight omnivorous fish (*Anguilla japonica*, *Cobitis biwae*, *Leuciscus hakonensis*, *Phoxinus lagowski steindachneri*, *Rhinogobius* sp. CB, *Rhinogobius* sp. CO, *Sicyopterus japonicus*, *Zacco platypus*).

Water samples were cryopreserved at −84 °C. Biological samples were rinsed with water and green macroalga and water bug were freeze-dried. The muscle tissues of fish and crustaceans were isolated by scalpel from the skin, viscera, bones, and carapaces and freeze-dried. Freeze-dried samples were homogenized by pestle and kept in a powdered state until extraction.

2.3. Experimental exposure of freshwater algal cells to arsenate

The green microalga *C. reinhardtii* CC125 was cultured mixotrophically in tris-acetate-phosphate (TAP) medium. Cultures in flasks were agitated on a gyratory shaker (120 rpm) at 27 °C with a 12 h light and 12 h dark cycle at 35 μmol photons m^{-2} s^{-1} of fluorescent

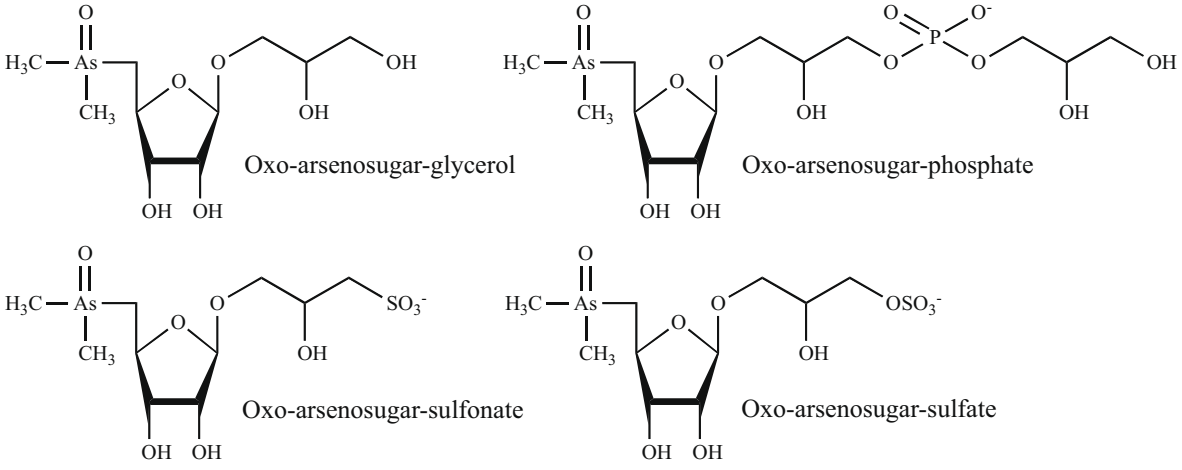

Fig. 1. Structures of oxo-arsenosugars relevant to this study.