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Characterization of water-soluble organoarsenic compounds in marine sponges[†]

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To provide a better understanding of the arsenic cycle in the marine ecosystem and marine biotechnological viewpoints, the present study is intended to identify arsenic compounds contained in marine sponges. Marine sponges were collected off the coastal waters of Magsaysay Islet within Bohol Sea near Mindanao (Philippines) and in the Seto Inland Sea (Japan) by skin-diving. Water-soluble arsenic compounds were extracted with methanol–water (1: 1, v/v) from freeze-dried samples of 13 marine sponges. The extracts were analyzed using high-performance liquid chromatography (HPLC) with an inductively coupled plasma mass spectrometer (ICP/MS) serving as an arsenic-specific detector. All marine sponges contained arsenobetaine [$(CH_3)_3As^+ CH_2COO^-$], 2,3-dihydroxypropyl 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside (arseno-sugar X), and 3-glycerophosphoryl-2-hydroxy-1-[5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy]propane (arseno-sugar X1), among which arseno-sugarX1 was most abundant in the *Jaspis* spp., *Suberites* spp. and four Japanese sponges. Also, a linear relationship existed between total water-soluble arsenic and arsenobetaine Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: sponge; Philippines; Seto Inland Sea; arsenobetaine; arseno-sugar; HPLC-ICP/MS

INTRODUCTION

Arsenic is a ubiquitous chemical element that is widely distributed in food, water, air and soil. In coastal sea environments, arsenate (V), arsenite (III), monomethylarsinic acid (MMAA) and dimethylarsinic acid (DMAA) usually constitute the predominant forms of arsenic species in oxygenated water. Marine organisms contain higher levels of arsenic than does seawater; small amounts of arsenobetaine,[1] arseno-sugar,[2,3] arsenocholine[4] and tetramethylarsonium[5] have been detected in marine organisms.

Previous studies have specifically addressed arsenic in seaweed. Not only for a better understanding of the arsenic cycle in marine ecosystems, but also from the viewpoint of comparative biochemistry, it is important to identify arsenic compounds contained in taxonomically lower animals. Using high-performance liquid chromatography (HPLC-ICP) analysis, arsenobetaine [$(CH_3)_3As^+CH_2COO^-$ in Fig. 1] was identified as the major arsenic species in three species of sponges, two species of sea anemones, and two species of sea hares.[6] However, information on arsenic species in sponges remains limited. Recently, arseno-sugars have been identified in four species of sponges from the Seto Inland Sea.[4]

The present study examines the identification of chemical forms of arsenic in 13 species of marine sponges from the Bohol Sea, Philippines and Seto Inland Sea, Japan using HPLC-ICP/MS.

MATERIALS AND METHODS

Marine sponges of the genera *Thorecta* sp., *Dysidea* sp., *Theonella* sp., *Acarnthella* sp., *Phyllospongia* sp., *Aaptos* sp.

[†] This paper is based on work presented at the 12th Symposium of the Japanese Arsenic Scientists' Society (JASS) held 5–6 November 2005 in Takizawa, Iwate Prefecture, Japan.

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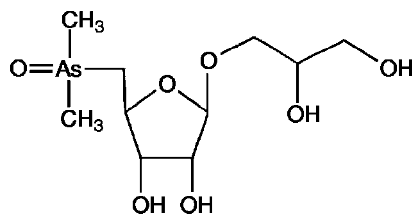
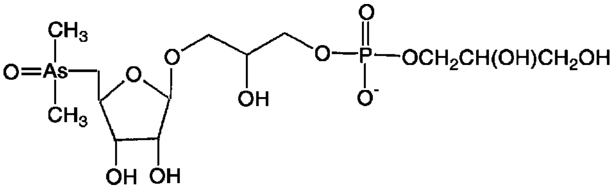
(1) Arsenite AsO_3^{3-} (2) Arsenate AsO_4^{3-}
(3) Monomethyl arsonate $CH_3AsO_3^{2-}$
(4) Dimethyl arsinate $(CH_3)_2AsO_2^-$
(5) Arsenobetaine $(CH_3)_3As^+CH_2COO^-$



(6) 2,3-dihydroxypropyl-5-deoxy-5-(dimethylarsinoyl) -β-ribofuranoside (arseno-sugarX)



(7) 3-glycerophosphoryl-2-hydroxy-1-[5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxypropane](arseno-sugar X1)

Figure 1. Arsenic compounds identified in the present study.

Jaspis and *Suberites* from Magsaysay Islet in Bohol Sea near Mindanao, Philippines, and *Haliclona permolis, Halichondria japonica, Harichrona white* and *Halichondria okadai* from Aki Nada in Seto Inland Sea were collected by skin-diving. The sponge samples were kept frozen at −20 °C until analysis. Each freeze-dried sponge sample (50–100 mg dry weight) was weighed into the centrifuge tube. To each tube was added 5 ml of methanol–water (1 : 1, v/v); the tube was sonicated for 10 min. Following centrifugation (2000 rpm × 10 min), the extract was removed using a Pasteur pipette. The extraction process was repeated five times for each sample. The extracts were combined, evaporated to dryness, and dissolved in 2 ml of water. The solution was filtered through a 0.5 µm disposable filter unit (Columngard-LCR13; Millipore Ltd), and an aliquot of the solution (25 µl) was injected into an HPLC-ICP/MS system (HP 4500; Yokogawa, Japan).

HPLC-ICP/MS analysis was conducted as reported previously using Inertsil ODS column (4.6 × 250 mm; GL Sciences Inc., Japan). Arsenic compounds were eluted with 10 mM tetraethylammonium hydroxide–4.5 mM malonic acid–0.05% methanol (pH 6.8) Quantitation was performed by comparing the peak areas of respective compounds with that of a known concentration of arsenic compound standards. Interference from Cl [$^{40}Ar^{35}Cl^+$ shows the same m/z, 75, as $^{75}As^+$] was detected by monitoring ion counts at m/z 77 (corresponding to $^{40}Ar^{37}Cl^+$) simultaneously.

The water-soluble arsenic compounds arsenobetaine, arseno-sugar (X) and arseno-sugar (X1) (Fig. 1) were prepared as reported previously.[8]

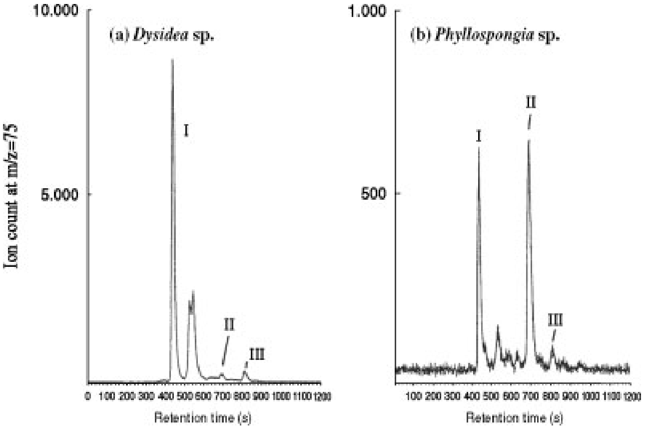


Figure 2. HPLC-ICP/MS chromatograms of the sponge extracts (ion at m/z 75). Column, Inertsil ODS; solvent 10 mm tetraethylammonium hydroxide–4.5 mM malonic acid–0.05% methanol (pH 6.8); flow rate, 0.75 ml/min. (a) *Dysidea* sp.; (b) *Phyllospongia* sp.; (c) standard solutions. The peaks are labeled as: I, arsenobetaine; II, 2,3-dihydroxypropyl 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside (arseno-sugar X); III, 3-glycerophosphoryl-2-hydroxy-1-[5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy] propane (arseno-sugar X1).

RESULTS AND DISCUSSION

Chromatograms of the HPLC-ICP/MS analysis of *Suberites* sp. are shown in Fig. 2. As shown in Table 1, water-soluble organoarsenics were detected in all marine sponges. The major water-soluble arsenic species in *Suberites* sp.were identified as As(V), DMAA, arsenobetaine and arseno-sugar derivatives (X and X1) by comparison with the standards. Several other peaks existed in the chromatograms, but they were not sufficiently identified. Their concentrations were relatively low, that is less than 15 µg/g dry weight in all sponges except for *Theonella* sp., *Aaptos* sp. and *Dysidea* sp. We inferred that the ability to accumulate arsenic differs from species to species. All sponges contained three arsenic compounds in their extracts, as shown in Table 1. It is noteworthy that the concentration of arsenobetaine in *Theonella* sp. and *Aaptos* sp. was much higher than that in *Dysidea* sp. The meaning of this specific characteristic remains unclear. After discovery of arsenobetaine in the tail muscle of the western rock lobster, much work has been done to elucidate major arsenic species in marine animals.[4]

Shiomi *et al.*[6] reported an analysis of arsenic species in three Japanese sponges using HPLC-ICP analysis. Yamaoka *et al.*[7] found a major amount of arsenobetaine and significant amounts of arseno-sugars in four Japanese sponges (*Halichondria okadai, Halichondria japonica, Haliclona permollis* and *Haliclona* white) using HPLC-ICP-MS analysis.[7] In contrast, *Phyllospongia* sp. from the Philippines contained 2,3-dihydroxypropyl,5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside (arseno-sugar X) as major water-soluble arsenic compounds. The most abundant arsenic compound

Table 1.

	Arsenic concentration (μg/g dry weight)				
Species	Water-soluble arsenic	Arsenobetaine	Arsenosugar(X1)	Arsenosugar (X)	Other Compounds
Thorecta sp.	6.2(100)	3.0(49)	0.9(14)	1.1(17)	1.2(19)
Dysidea sp.	24.8(100)	15.2(61)	0.9(4)	0.5(2)	8.1(33)
Theonella sp.	157.0(100)	136.6(87)	0.01(0)	2.2(1)	18.2(12)
Acanthella sp.	6.1(100)	1.7(28)	1.3(22)	0.7(11)	2.4(39)
Phyllospongia sp.	4.4(100)	1.5(34)	0.02(0)	2.1(48)	0.7(17)
Aaptos sp.	112.5(100)	98.1(87)	0.05(0)	0.2(0)	14.2(13)
Biemna fortis	1.0(100)	0.13(12)	0.02(2)	0.13(12)	0.8(76)
Jaspis sp.	6.1(100)	0.6(9)	2.7(47)	1.0(17)	1.7(27)
Subertes sp.	10.3(100)	1.7(17)	6.1(59)	1.3(13)	1.1(10)
Haliclona permolis	13.0(100)	3.3(26)	8.1(61)	1.1(9)	0.5(4)
Halichondria japonica	3.4(100)	1.1(32)	1.4(41)	0.2(4)	0.8(23)
Halichona white	0.8(100)	0.2(19)	0.4(49)	0.1(6)	0.2(26)
Halichondria okadai	5.5(100)	0.5(10)	4.2(76)	0.3(5)	0.5(9)

[a] Values in parentheses are percentages.
[b] Water-soluble arsenic represents the sum of all arsenic species obtained by HPLC.

in *Subertes* sp., *Jaspis* sp. and Japanese sponges (*Halichondria okadai, Halichondria japonica, Haliclona permollis* and *Haliclona* white) was 3-glycerophosphoryl-2-hydroxy-1-[5-deoxy-5-(dimethylarsinoyl) -β-ribofuranosyloxy] propane (arseno-sugar X1). Generally, arseno-sugars have been identified in brown alga *Ecklonia radiata*,[9] green algae, red algae, microalgae[3,7,10,11] and labyrinthulids.[15] On the other hand, relationships between the quantities of arseno-sugar (X) and arseno-sugar (X1) among different sponge species are unclear.

Marine sponges are known to contain symbiotic microalgae and bacteria.[12] A possible source of the arseno-sugars may be symbiotic microalgae. Furthermore, from the standpoint of metabolism and cycling of arsenic in the marine food web, it is especially interesting that a fairly large amount of arsenobetaine and arseno-sugars was detected in sponges that feed on microalgae containing arseno-sugar derivatives.[11] No correlation was found between arsenosugar and arsenobetaine in 13 marine sponges from Table 1. Figure 3 shows the relationship between total water soluble arsenic and arsenobetaine in 13 marine sponges near Mantigue Island in the Sea of Mindanao (Philippines) and in the Seto Inland Sea (Japan). Total water-soluble arsenic and arsenobetaine in marine sponges showed a linear relationship. These results suggested that arsenic concentrations in marine sponges are more influenced by arsenobetaine produced by the sponges themselves than by arseno-sugars that are produced by microalgae.[11] However, no correlation was found between total water-soluble arsenic and arseno-sugar (X + X1) in marine sponge (Table 1). The total water-soluble arsenic concentration in *Theonella* and *Aaptos* was about 100 mg/kg dry weight, indicating that these sponges accumulate arsenic easily. *Dysidea*, which accumulates bromine,[12] exhibits higher total water-soluble arsenic concentrations than other sponges. However, in most sponges aside from *Subertes* and *Haliclona* *permollis*, the value was not more than 10 mg/kg dry weight.

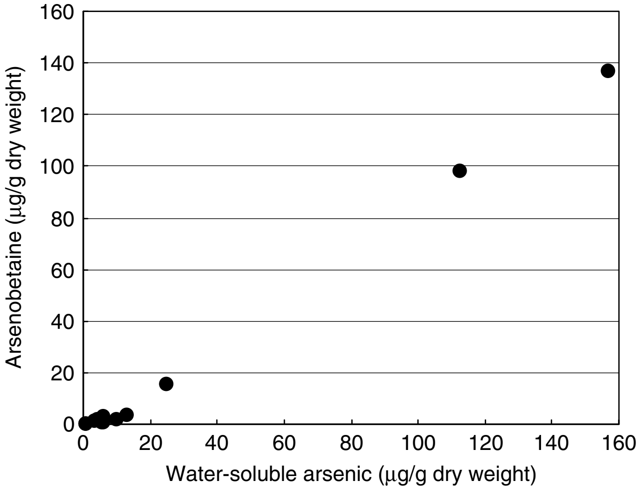


Figure 3. Relationship between water-soluble arsenic and arsenobetaine concentrations in 13 marine sponges near Magsaysay Islet in Bohol Sea (Philippines) and in the Seto Inland Sea(Japan). This data obtained from Table 1.

More information regarding variations in season, location, size, age and symbiotic microorganisms is necessary to elucidate the arsenic compounds in marine sponges.

In coastal regions, arsenic (V) is usually found as predominant form of arsenic species in oxygenated seawater. Maeda *et al.*[16] documented reduced and methylated arsenic for several microalgae. Shibata *et al.*[11] found that microalgae contain arsenosugar. Generally zooplankton do not contain arsenosugar and detectable arsenobetaine. We presume that arsenosugar in sponge is made by similar processes of simbiotic microalgae in sponges. The present study engenders the conclusion that arsenic (V) is reduced and biomethylated to arseno-sugar compounds and arsenobetaine in these marine sponges. Acute toxicity decreases in the following order: As (III) > As (V) > methylarsonicacid > arseno-sugar > arsenobetaine. This suggests that acute toxicity of arsenic compounds diminishes with progressive methylation.[13] Arsenobetaine and arseno-sugar compounds have low toxicity: these compounds were administered orally to mice at a dose of 10 g/kg of body weight, causing no toxic symptoms.[14] Marine sponges might contribute to biological detoxification and to control of arsenic in natural waters or industrial polluted waters if marine sponges take up inorganic arsenic compounds efficiently in the water and convert that arsenic into nontoxic arseno-sugar compounds and arsenobetaine.

$$\mathrm{As(V)} \longrightarrow \mathrm{As(III)} \longrightarrow \mathrm{MMAA} \longrightarrow \mathrm{DMAA} \longrightarrow \text{arsenosugar} \longrightarrow \text{arsenobetaine}$$

In conclusion, this equation shows the detoxification process of arsenic in marine sponge.

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