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Metabolism in mice of arsenic compounds contained in the red alga *Porphyra yezoensis*

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Arsenic compounds in the red alga *Porphyra yezoensis* were purified by gel filtration on Bio-Gel P-2; chromatographic behavior suggested that they were arsenic-containing ribofuranosides (arsenosugars). When partially purified arsenosugars were orally administered to mice, 86% and 13% of the arsenic administered were excreted in feces and urine, respectively, within 48 h. Arsenic compounds excreted in feces were identified as arsenosugars by gel filtration and high-performance liquid chromatography, while those excreted in urine were identified as methylarsonic acid, dimethylarsinic acid and arsenobetaine. On the other hand, upon intravenous administration of arsenosugars, a large part of the arsenic administered was excreted rapidly in urine; after 72 h, 92% of the arsenic administered was recovered in urine and 6% in feces. Similarly to the case of oral administration, methylarsonic acid, dimethylarsinic acid and arsenobetaine were detected in urine.

Keywords: Arsenic, arsenosugars, red alga, *Porphyra yezoensis*, mice, excretion, feces, urine

INTRODUCTION

Marine algae contain appreciable amounts of arsenic, chiefly as water-soluble organic compounds, similarly to marine animals. Among water-soluble arsenic compounds in marine algae, those of the brown alga *Ecklonia radiata* were first isolated and identified as arsenic-containing ribofuranosides (arsenosugars) by Edmonds and Francesconi.[1] Since then, arsenosugars have been found as major arsenic compounds in four brown algae, *Hizikia fusiforme*,[2] *Laminaria japonica*,[3] *Sphaerotrichia divaricata*[4] and *Sargassum thunbergii*,[5] and one green alga, *Codium fragile*.[6] During the preparation of this manuscript, arsenic compounds of the red alga *Porphyra tenera* were also identified as arsenosugars.[7] Although the algal species examined were limited in number, arsenosugars appear to be major arsenic compounds in marine algae. Since some marine algae are frequently utilized as foodstuffs, especially in Japan, it is important to clarify the fate of arsenosugars in mammals from the viewpoint of food hygiene. Before the discovery of arsenosugars. Fukui *et al.*[8] investigated the metabolism in humans of arsenic compounds from two species of brown algae, *L. japonica* and *H. fusiforme*, and found that the bulk of the arsenic ingested is excreted rapidly in the urine. Arsenic compounds of *L. japonica* were later identified as arsenosugars[3] and those of *H. fusiforme* as arsenosugars and inorganic arsenate.[2] Therefore, at least the results obtained with *L. japonica* suggest the fate of arsenosugars ingested by humans.

The present paper deals with the identification of arsenosugars in the red alga *Porphyra yezoensis*, a principal material of 'Nori' (dried laver), and the metabolism of arsenosugars in mice, which differs remarkably from that in humans.[8]

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MATERIALS AND METHODS

Materials

Fresh specimens of *P. yezoensis* were obtained from Chiba Nori Culture Station and kept at −20°C until use. Dried specimens of two species of brown algae, *L. japonica* and *H. fusiforme*, were purchased from a retail supplier, ground to powder and kept in a desiccator at room temperature until use. Male mice (ddY strain) weighing about 20 g were purchased from Sankyo Lab. Service (Tokyo, Japan); metabolic cages (type MM-ST) from Sugiyama-Gen Co. (Tokyo, Japan); Bio-Gel P-2 from Nippon Bio-Rad Laboratories (Tokyo, Japan); a pre-packed column of Nucleosil 10SA from Chemco Co. (Tokyo, Japan). Nitric acid, perchloric acid and

sulfuric acid used for wet digestion were Super Special Grade and the other reagents were Analytical Grade.

Determination of arsenic

As described in our previous paper,[9] solid samples were digested with a mixture of nitric acid, perchloric acid and sulfuric acid and their arsenic concentrations were determined on an inductively coupled argon plasma emission spectrometer (ICP; Jarrell–Ash AtomComp Series 800). In the case of aqueous samples, arsenic concentrations were directly estimated on the ICP without wet digestion.

Extraction and fractionation on Bio-Gel P-2 of water-soluble arsenic compounds

Wet samples (310 g) of *P. yezoensis* were extracted three times with three volumes of 50% aqueous methanol. The extract was evaporated to remove methanol and defatted three times with an equal volume of ether. The aqueous solution thus obtained (water-soluble arsenic fraction) was evaporated to dryness and dissolved in 500 cm^3 of 0.1 mol dm^{-3} ammonium bicarbonate (NH_4HCO_3). After removal of insoluble materials by centrifugation, the supernatant (10 cm^3 each) was applied to a Bio-Gel P-2 column (2.5 cm × 95 cm). Elution was achieved with 0.1 mol dm^{-3} NH_4HCO_3 at a flow rate of about 30 cm^3 h^{-1} (Fig. 1). Fractions of 8 cm^3 were collected and determined for arsenic. Arsenic-containing fractions were pooled, evaporated to dryness and used for animal experiments as partially purified arsenic compounds.

Water-soluble arsenic fractions were similarly prepared from dried samples of *L. japonica* and *H. fusiforme* and separately subjected to gel filtration. A mixture of seven standard arsenic compounds, arsenate (AN), methylarsonic acid (MAA), dimethylarsinic acid (DMA), arsenobetaine (AB), arsenocholine (AC), trimethylarsine oxide (TMAO) and tetramethylarsonium iodide (TEMA), was also chromatographed on Bio-Gel P-2 (Fig. 1).

Treatment of mice

The partially purified arsenic compounds from *P. yezoensis* were dissolved in 0.01 mol dm^{-3} phosphate buffer containing 0.15 mol dm^{-3} NaCl (pH 7.0) at a concentration of 550 on

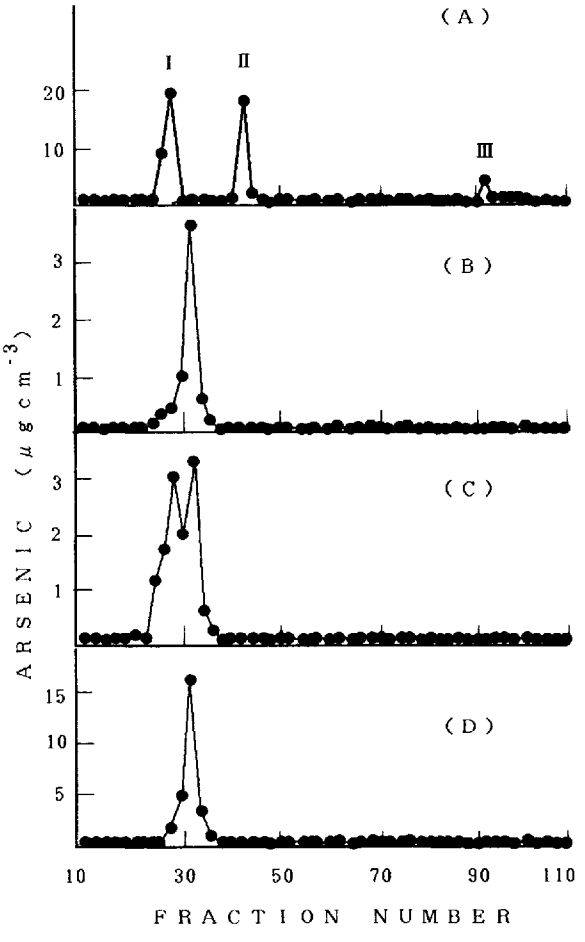

Figure 1 Gel filtration of standard arsenic compounds (A), and arsenic compounds in *Laminaria japonica* (B), *Hizikia fusiforme* (C) and *Porphyra yezoensis* (D). Column, Bio-Gel P-2 (2.5 cm × 95 cm); solvent, 0.1 mol dm^{-3} NH_4HCO_3. Fractions of 8 cm^3 were collected at a flow rate of about 30 cm^3 h^{-1}. Peak I, arsenate + methylarsonic acid + dimethylarsinic acid; peak II, arsenobetaine + arsenocholine + trimethylarsine oxide; peak III, tetramethyarsonium iodide.

55 μg As cm^{-3}. Before administration, mice were kept without both food and water for 24 h. The partially purified arsenic compounds were administered orally (p.o.) once only to a group of three mice at 5.5 μg As g^{-1} body weight. Another group of three mice was injected intravenously (i.v.) with the arsenic compounds (0.55 μg As g^{-1} body weight). Each group was housed in a metabolic cage and given food and water *ad libitum*. Urine and feces were collected at intervals indicated in Fig. 2.

Analysis of arsenic metabolites in urine and feces

To the urine (4–5 cm^3) collected was added an equal volume of 10% perchloric acid. Insoluble matter was removed by centrifugation. The supernatant obtained was regarded as a water-soluble arsenic fraction. The water-soluble arsenic fraction of the feces was prepared by the same method as adopted for algal samples. Both water-soluble arsenic fractions were separately analyzed by gel filtration on Bio-Gel P-2 under the same conditions as described above. They were also subjected to a high-performance liquid chromatography (HPLC)–ICP system developed by Shiomi *et al.*[10] In brief, a Nucleosil 10SA column (0.46 cm × 25 cm) was used with 0.05 mol dm^{-3} pyridine–formic acid buffer (pH 3.1). The eluate was directly introduced into the nebulizer of the ICP and arsenic concentrations were recorded at 10-s intervals. The seven standard arsenic compounds were used for comparison.

RESULTS

Behavior of water-soluble arsenic compounds on Bio-Gel P-2

When a mixture of the standard arsenic compounds was subjected to gel filtration on Bio-Gel P-2, three arsenic peaks (peaks I, II and III) were observed at fractions 28, 42 and 92; peak I was attributable to a mixture of AN, MAA and DMA, peak II to a mixture of AB, AC and TMAO and peak III to TEMA (Fig. 1A). On the other hand, the water-soluble arsenic fraction of *L. japonica* gave one arsenic peak at a different position (fraction 32 in Fig. 1B) from those of the peaks I to III, and that of *H. fusiforme* two arsenic peaks at fractions 28 and 32 (Fig. 1C). Previously, arsenosugars have only been detected in *L. japonica*[3] while AN, together with arsenosugars, has been found in *H. fusiforme*.[2] Therefore, the arsenic peak at fraction 28 observed with *H. fusiforme* is assignable to AN and the peak at fraction 32 (observed with both algae) to arsenosugars.

The arsenic content of the samples of *P. yezoensis* used in this study was estimated to be 4.9 μg As g^{-1}. About 80% of the total arsenic was found in the water-soluble arsenic fraction and the rest in the residue. As shown in Fig. 1D, the water-soluble arsenic fraction gave only one arsenic peak at fraction 32 when chromatographed on Bio-Gel P-2. As compared with the chromatographic behaviors of the arsenosugars of *L. japonica* and *H. fusiforme*, the arsenic compounds of *P. yezoensis* were thus assumed to be arsenosugars as well.

Excretion of arsenic in urine and feces

Following p.o. administration of partially purified arsenosugars from *P. yezoensis*, the greater part of the arsenic administered was rapidly excreted in feces (Fig. 2A). After 48 h, as much as 86% of the arsenic administered was recovered in feces, while only 13% was found in urine. The rapid excretion of the arsenic administered was also observed with the case of i.v. administration of arsenosugars (Fig. 2B). In this case, however, the bulk of the arsenic administered appeared in urine. After 72 h, 92% of the arsenic administered was recovered in urine and 6% in feces.

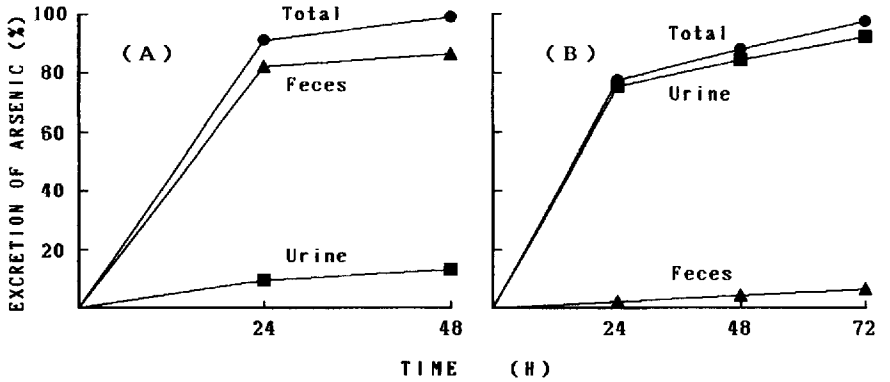

Figure 2 Excretion of arsenic in feces and urine following p.o. administration (A) or i.v. administration (B). The sum of the arsenic excreted in feces and urine is expressed as 'total'.

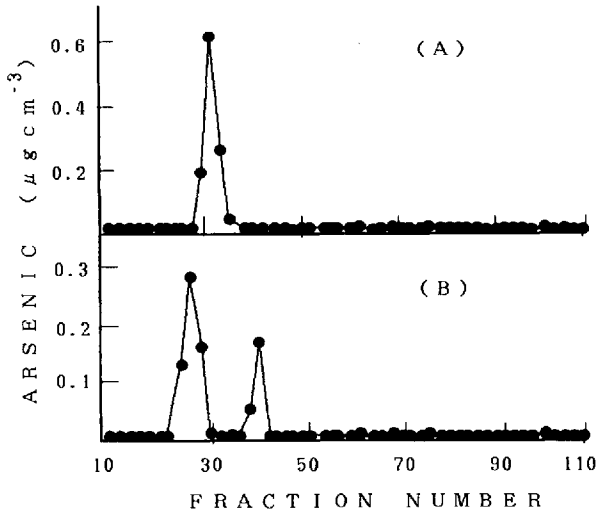

Figure 3 Gel filtration of arsenic compounds excreted in 0–24-h feces after p.o. administration (A) and the 0–24-h urine after i.v. administration (B). The chromatographic conditions are the same as given in the legend for Fig. 1.

Chemical forms of arsenic excreted in feces and urine

Arsenic compounds recovered in feces after i.v. administration were not analyzed due to the small quantities of arsenic involved. As shown in Fig. 3A, the water-soluble arsenic fraction of the 0–24-h feces collected after p.o. administration afforded one arsenic peak at fraction 32 in gel filtration on Bio-Gel P-2. This peak apparently corresponded to that of arsenosugars (see Fig. 1). The identity of the arsenic compounds in the feces with arsenosugars was also supported by HPLC–ICP (Figs 4B and C). On the other hand, no arsenosugars but three arsenic compounds, MAA, DMA and AB, were detected in urine, irrespective of the administration route. As typical results, the chromatographic behavior of the arsenic compounds in the 0–24-h urine collected after i.v. administration are shown in Figs 3B and 4D. In gel filtration, the arsenic compounds in the urine were separated into two peaks at fractions 28 and 42 (Fig. 3B); the former peak agreed with that of a mixture of AN, MAA and DMA and the latter with that of a mixture of AB, AC and TMAO (see Fig. 1A). Analysis by HPLC–ICP clearly evidenced the presence of three arsenic compounds in the urine (Fig. 4D); as compared with the behavior of the standard arsenic compounds (Fig. 4A), these three compounds were identified as MAA, DMA and AB.

DISCUSSION

It was found that gel filtration on Bio Gel P-2 was very effective in distinguishing arsenosugars from the other seven arsenic compounds (Fig. 1). Although a few kinds of arsenosugars are contained in *L. japonica* and *H. fusiforme*, the arsenosugars of these two brown algae were eluted in a single peak at the same position. Furthermore, the arsenosugars of *P. yezoensis* also afforded one arsenic peak at the same position as those of the two brown algae. As shown in Fig. 4B, the arsenosugars of *P. yezoensis* seemed to be separable from the standard arsenic compounds by HPLC on Nucleosil 10SA. However, both arsenosugars of the two brown algae were eluted in a relatively wide range from Nucleosil 10SA and hence were not clearly distinguishable from the standard arsenic compounds. For a quick separation of arsenic compounds including arsenosugars, Shibata and Morita[11] recently developed a new method using ion-pair HPLC. In order to distinguish arsenosugars from other arsenic compounds in a short time, the method developed by Shibata and Morita appears to be superior to that using Bio-Gel P-2. However, gel filtration on Bio-Gel P-2 could be performed as a simple and easy method for the preparation of a relatively large amount of arsenosugars from biological samples, especially marine algae, or even for the speciation of arsenic in biological samples.

It is generally considered that marine algae contain arsenosugars as major arsenic compounds.[1–7] In this study the red alga *P. yezoensis* was also assumed to contain arsenosugars as major arsenic compounds. However, the kind of arsenosugars contained in *P. yezoensis* is still unclear. Shibata *et al.*[7] recently detected two kinds of arsenosugars in the red alga *P. tenera* (belonging to the same genus as *P. yezoensis*). From the viewpoint of comparative biochemistry, it is of interest to examine whether *P. yezoensis* contains the two kinds of arsenosugars found in *P. tenera*, or other kinds of arsenosugars.

It is significant that there was a distinct difference in the metabolism of p.o. administered arsenosugars between mice and humans. The greater part of arsenosugars which had been administered p.o. to mice was excreted in feces without biotransformation, suggesting that the arsenosugars were not absorbed from the gastrointestinal tract of mice. On the other hand, when extracts of two species of marine brown algae, *L.*

japonica and *H. fusiforme*, were ingested by humans, about 100% and 51% of the arsenic ingested was excreted in urine, respectively.[8] Aside from the results with *H. fusiforme*, which contains AN together with arsenosugars, those with *L. japonica*, in which no arsenic compounds other than arsenosugars are found, indicate that arsenosugars are absorbed by the gastrointestinal tract of humans and excreted in urine, probably after conversion to other arsenic compounds.

It should be also pointed out that the results obtained with the p.o. administration of arsenosugars to mice differ remarkably from the previous findings for other arsenic compounds. Following p.o. administration to mammals, most arsenic compounds, including inorganic and organic compounds, are excreted, with or without biotransformation, much more in urine than in feces.[12–17] The exception has been known only for the case of p.o. administration of MAA.[18]

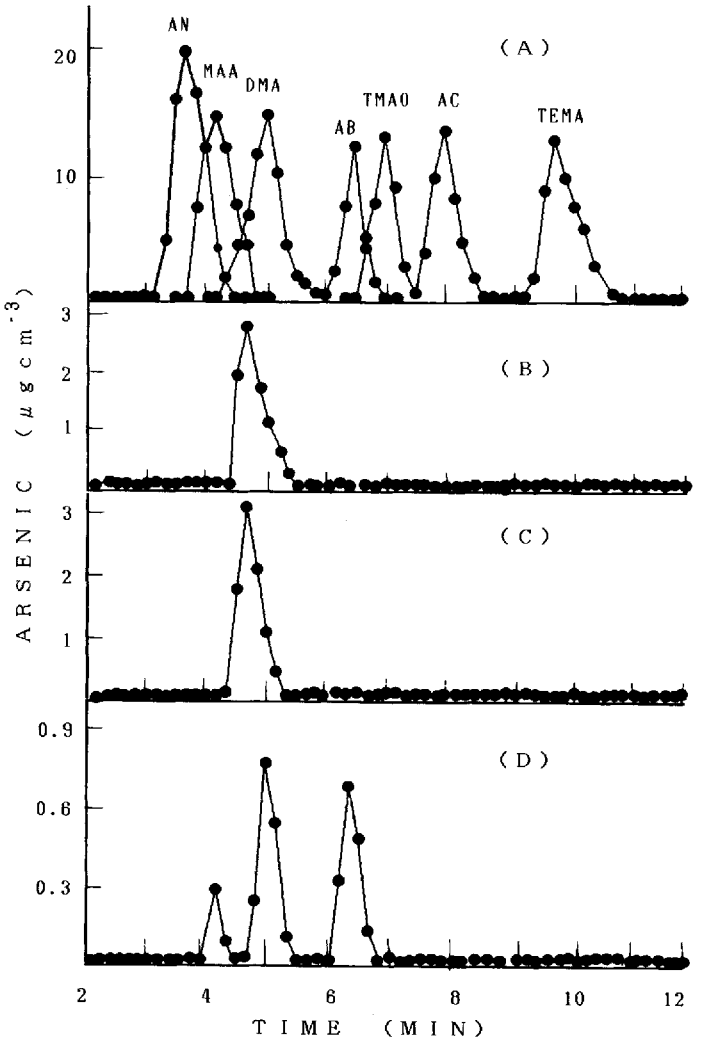

Figure 4 HPLC of standard arsenic compounds (A), arsenosugars of *P. yezoensis* (B) and arsenic compounds excreted in the 0–24-h feces after p.o. administration (C) and the 0–24-h urine after i.v. administration (D) monitored by ICP. Column, Nucleosil 10SA (0.46 cm × 25 cm); solvent, 0.05 mol dm^{-3} pyridine–formic acid buffer (pH 3.1); flow rate, 1 cm^3 min^{-1}. Abbreviations of the standard arsenic compounds: AN, arsenate; MAA, methylarsonic acid; DMA, dimethylarsinic acid; AB, arsenobetaine; AC, arsenocholine; TMAO, trimethylarsine oxide; TEMA, tetramethylarsonium iodide.

In the present study the arsenic compounds excreted in urine were identified as MAA, DMA and AB by HPLC–ICP. It is still unknown, however, how these three arsenic compounds are transformed from arsenosugars in mice. Further study should be directed to the elucidation of the metabolic pathway of the three arsenic compounds from arsenosugars.

REFERENCES

1. Edmonds, J S and Francesconi, K A *Nature (London)*, 1981, 289: 602
2. Edmonds, J S, Morita, M and Shibata, Y *J. Chem. Soc. Perkin Trans. 1*, 1987, 577
3. Shibata, Y, Morita, M and Edmonds, J S *Agric. Biol. Chem.*, 1987, 51: 391
4. Jin, K, Shibata, Y and Morita, M *Agric. Biol. Chem.*, 1988, 52: 1965
5. Shibata, Y and Morita, M *Agric. Biol. Chem.*, 1988, 52: 1087
6. Jin, K, Hayashi, T, Shibata, Y and Morita, M *Appl. Organomet. Chem.*, 1988, 2: 365
7. Shibata, Y, Jin, K and Morita, M *Appl. Organomet. Chem.* 1990, 4: 255
8. Fukui, S, Hirayama, T, Nohara, M and Sakagami, Y *Shokuhin Eiseigaku Zasshi*, 1981, 22: 513
9. Shiomi, K, Kakehashi, Y, Yamanaka, H and Kikuchi, T *Appl. Organomet. Chem.*, 1987, 1: 177
10. Shiomi, K, Orii, M, Yamanaka, H and Kikuchi, T *Nippon Suisan Gakkaishi*, 1987, 53: 103
11. Shibata, Y and Morita, M *Anal. Sci.*, 1989, 5: 107
12. Vahter, M, Marafante, E and Dencker, L *Sci. Total Environ.*, 1983, 30: 197
13. Marafante, E, Vahter, M and Dencker, L *Sci. Total Environ.*, 1984, 34: 223
14. Shiomi, K, Horiguchi, Y and Kaise, T *Appl. Organomet. Chem.*, 1988, 2: 385
15. Yamauchi, H and Yamamura, Y *Toxicol. Appl. Pharmacol.*, 1984, 74: 134
16. Yamauchi, H and Yamamura, Y *Toxicology*, 1985, 34: 113
17. Yamauchi, H, Takahashi, K, Yamamura, Y and Kaise, T *Toxicol. Environ. Chem.*, 1989, 22: 69
18. Yamauchi, H and Yamamura, Y *Nippon Eiseigaku Zasshi*, 1985, 40: 544