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The degradation of arsenobetaine to inorganic arsenic by sedimentary microorganisms

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Abstract

Two growth media containing arsenobetaine [$(CH_3)_3\,As^+CH_2COO^-$] were mixed with coastal marine sediments, the latter providing a source of microorganisms. The mixtures were kept at 25 °C in the dark and shaken for several weeks under an atmosphere of air. The disappearance of arsenobetaine and the appearance of two metabolites were followed by HPLC. The HPLC-retention time of the first metabolite agreed with that of trimethylarsine oxide [$(CH_3)_3AsO$]. The second metabolite was identified as arsenate (As(V)) using hydride generation/cold trap/GC MS analysis and thin layer chromatography. This is the first scientific evidence showing that arsenobetaine is degraded by microorganisms to inorganic arsenic via trimethylarsine oxide. The degradation of arsenobetaine to inorganic arsenic completes the marine arsenic cycle that begins with the methylation of inorganic arsenic on the way to arsenobetaine.

Introduction

Various arsenic compounds occur in marine ecosystems, a fact that has been proved by the isolation and identification of each arsenic compounds from many organisms during the past decade (Edmonds & Francesconi, 1988; Maher & Butler, 1988). A report by Edmonds *et al.* (1977) on the occurrence of arsenobetaine in the tail muscle of lobster, stimulated a number of subsequent studies in this field. Resulting from these studies, a widely accepted hypothesis has been advanced to explain the conversion of arsenic in marine food chains. This involves the uptake from seawater, concentration and conversion of inorganic arsenic to organoarsenic compounds by phytoplankton or algae. The organoarsenicals produced, such as arsenosugars, are further metabolized via the food chain and bioaccumulated as arsenobetaine in marine animals.

We have also confirmed the ubiquity of arsenobetaine in marine organisms (Hanaoka & Tagawa, 1985a, b; Hanaoka *et al.*, 1986, 1987a, b). In recent years, we have been more interested in the circulation of arsenic in marine ecosystems (Hanaoka *et al.*, 1987c, 1988, 1989, 1991; Kaise *et al.*, 1987) rather than the bioconversion of inorganic arsenic to arsenobetaine. In particular, we have been most interested in the fate of arsenobetaine after the death of marine animals.

In the present study, we show that arsenobetaine is degraded to inorganic arsenic via trimethylarsine oxide by sedimentary microorgan-

isms under aerobic conditions. A tentative arsenic cycle is proposed of marine ecosystems on the basis of the results.

Experimental details

The sediments were collected from the coastal waters of Yoshimi facing the Shimonoseki University of Fisheries in August and November, 1989. Two media were used for the degradation experiments. These were (a) ZoBell medium ($\frac{1}{5}$ ZoBell 2216E (g dm^{-3} filtered sea water): peptone 1.0; yeast extract 0.2), and (b) inorganic salt medium (aqueous solution of inorganic salts at pH 7.5 (g dm^{-3}): sodium chloride (NaCl) 30.0; calcium chloride ($CaCl_2 \cdot 2H_2O$) 0.2; potassium chloride (KCl) 0.3; iron (II) chloride ($FeCl_2 \cdot nH_2O$) 0.01; phosphates (KH_2PO_4) 0.5 and (K_2HPO_4) 1.0; magnesium sulphate ($MgSO_4 \cdot 7H_2O$) 0.5; and ammonium chloride (NH_4Cl) 1.0). Synthetic arsenobetaine [$(CH_3)_3As^+CH_2COO^-$, 50 mg] and sediment (1.0 g) were added to each medium (25 cm^3) in a 50-cm^3 Erlenmeyer flask.

The flasks containing the mixtures were kept at 25 °C in the dark and shaken for 9 or 12 weeks under an atmosphere of air. Mixtures autoclaved at 120 °C for 20 min served as controls. Filtered aliquots from the mixtures in the flasks were withdrawn at intervals of several days and diluted with distilled water to 20 times their volume. The diluted aliquots were then analyzed for arsenic compounds by high performance liquid chromatography (HPLC).

The arsenic compounds in the diluted media samples were separated using HPLC (Toyo Soda Co., CCP 8000 series, TSK Gel ODS-120T column, 4.6 mm × 250 mm) with a 11.2 mmol dm^{-3} solution of sodium heptanesulphonate in water/acetonitrile/acetic acid (95:5:6 by volume) (Stockton & Irgolic, 1979) as the mobile phase at a flow rate of 0.8 cm^3 min^{-1}. Fractions were collected and an aliquot (20 mm^3) of each fraction was injected into the graphite furnace atomic absorption spectrometer as described previously (Hanaoka *et al.*, 1988). Arsenite, arsenate and methanearsonic acid did not separate under these conditions.

Each mixture shaken for 9 weeks (ZoBell medium) or 12 weeks (inorganic salt medium) was centrifuged and the supernatant was placed on a Dowex 50W-X2 column (1 × 50 cm) equilibrated with 0.1 mol dm^{-3} pyridine-formic acid buffer (pH 3.1) and eluted with the same buffer and 0.1 mol dm^{-3} pyridine successively.

Each purified metabolite was analysed using a combination of gas chromatographic separation with hydride generation followed by a cold trap technique and selected ion monitor mass spectrometry (Kaise *et al.*, 1988). Each metabolite was also chromatographed on a cellulose thin layer plate (Funakoshi Yakuhin Co. Ltd., Avicel SF, 0.1 mm). Five solvent systems were used for development: ethyl acetate/acetic acid/water (3:2:1), chloroform/methanol/25% aqueous ammonia (2:2:1), 1-butanol/acetone/formic acid/water (10:10:2:5), 1-butanol/acetone/25% aqueous ammonia/water (10:10:2:5), and 1-butanol/acetic acid/water (4:2:1). Detection of the spot occurred during $SnCl_2$-KI reagent (Tagawa, 1980).

Results

Microbial conversion of arsenobetaine

Figure 1 shows the reduction of arsenobetaine and subsequent production of the two metabolites in each media with time. In both media, the disappearance of arsenobetaine and the appearance of two metabolites were followed by HPLC. The recovery of these arsenic compounds is expressed as the percentage of the graphite furnace AAS signal obtained for a metabolite to the signal for arsenobetaine in the control. The compound with an HPLC-retention time of 20–22 min was labelled metabolite-1, and that with a retention time of 2.5–4 min as metabolite-2. The retention time of metabolite-1 was the same as that of trimethylarsine oxide and that of metabolite-2 the same as arsenite, arsenate and methanearsonic acid. In both media, the arsenobetaine was

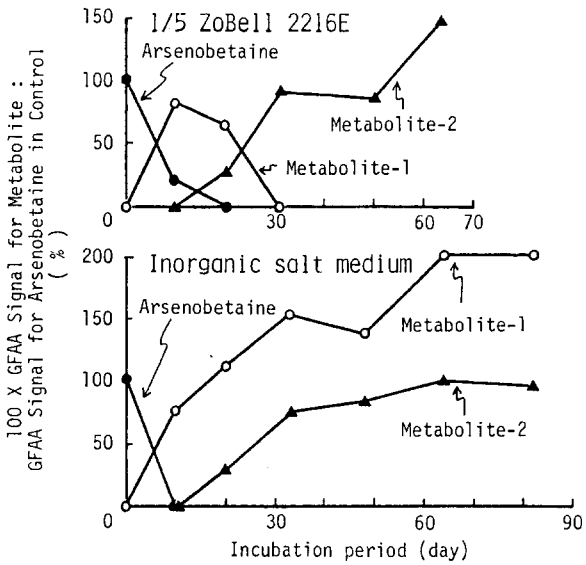

Fig. 1. The conversion of arsenobetaine to two metabolites (metabolite-1 and metabolite-2) by sedimentary microorganisms in ZoBell medium and an inorganic medium.

eventually completely converted to metabolite-1 within 10–20 days of incubation. Production of metabolite-2 commenced between 10–20 days after incubation. In the ZoBell medium, metabolite-2 was the only arsenic compound present, all arsenobetaine and metabolite-1 having been used up (Fig. 1).

Isolation and identification of metabolite-2

The supernatant from each medium was analysed using cation exchange chromatography (Dowex 50W-X2). Metabolite-2 was eluted with 0.1 mol dm^{-3} pyridine-formic acid buffer and the metabolite-1 which was retained on the column was eluted with 0.1 mol dm^{-3} pyridine. Metabolite-2 was isolated from the salts added to the media and four trace amounts of other arsenicals compounds by repeated chromatography.

Each purified sample of metabolite-2 was analysed using hydride generation/cold trap/GC MS/SIM and thin layer chromatography. The results of the analysis of metabolite-2 from the ZoBell medium are shown in Fig. 2. The metabolite-2 from the inorganic salt medium showed a similar chromatogram. Since only arsine (AsH_3) was detected in this analysis, it was confirmed that metabolite-2 was inorganic arsenic. Further, the thin layer chromatography of each sample of metabolite-2 produced only a single spot positive to $SnCl_2$-KI reagent, and each R_f value was the same as that of arsenate in five solvent systems tested (Table 1).

These results suggest that metabolite-2 was arsenate and provide the first evidence that arsenobetaine is degraded to inorganic arsenic by sedimentary microorganisms.

Discussion

In similar experiments previously reported by us (Kaise *et al.*, 1987; Hanaoka *et al.*, 1988), metabolite-1 was identified as trimethylarsine oxide using thin layer chromatography, ^{1}H and ^{13}C NMR spectrometry and FAB mass spectrometry. It was demonstrated that arsenobetaine is degraded to inorganic arsenic via trimethylarsine oxide by sedimentary microorganisms. This result is slightly different to that obtained using microorganisms associated with marine macroalgae

Table 1. Rf values in TLC of Metabolite-2 and reference arsenic compounds

Sample	Rf value				
	Solvent system				
	1	2	3	4	5
Metabolite-2 (Inorganic medium)	0.51	0.00	0.59	0.00	0.30
Metabolite-2 (Zobell medium)	0.51	0.00	0.59	0.00	0.30
Arsenate	0.51	0.00	0.58	0.00	0.31
Arsenite	0.27	0.32	0.36	0.13	0.20
Methanearsonic acid	0.65	0.11	0.60	0.00	0.62
Trimethylarsine oxide	0.88	0.83	0.57	0.50	0.72
Arsenobetaine	0.78	0.73	0.61	0.44	0.73

Solvent systems; 1, ethyl acetate/acetic acid/water (3:2:1); 2, chloroform/methanol/aq. ammonia (28%) (2:2:1); 3, 1-butanol/acetone/formic acid (85%)/water (10:10:2:5); 4, 1-butanol/acetone/aq. ammonia (28%)/water (10:10:2:5); 5, 1-butanol/acetic acid/water (4:2:1).

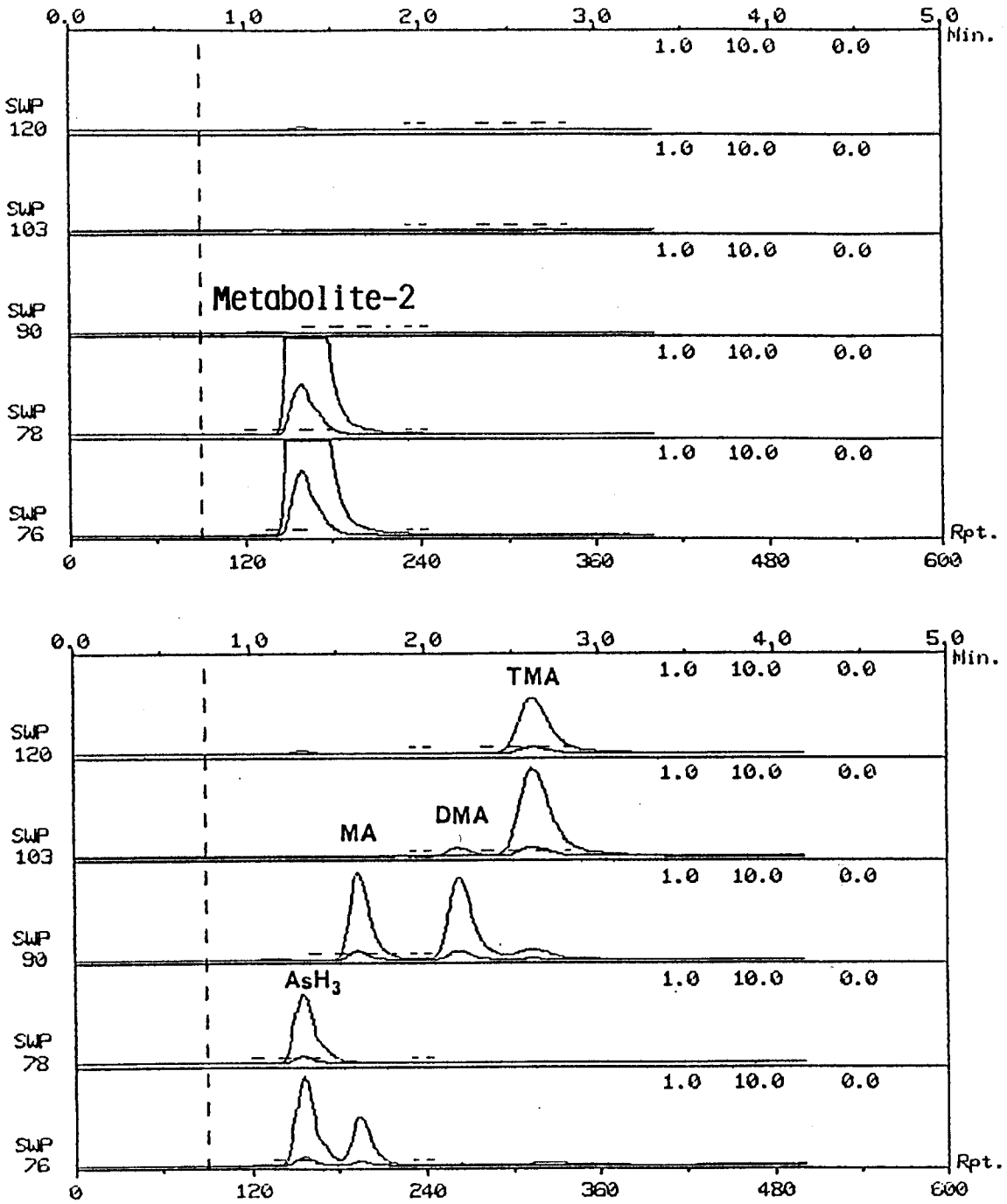

Fig. 2. The SIM chromatograms of metabolite-2 (the upper figure) and those of arsine (AsH_3), methylarsine (MA), dimethylarsine (DMA) and trimethylarsine (TMA) volatalized from each standard arsenicals (the lower figure).

(Hanaoka *et al.*, 1989), where arsenobetaine was converted to trimethylarsine oxide and/or dimethylarsinic acid, but not to inorganic arsenic. These results suggest that sedimentary microorganisms may play a major role in the degradation of arsenobetaine to inorganic arsenic in the field,

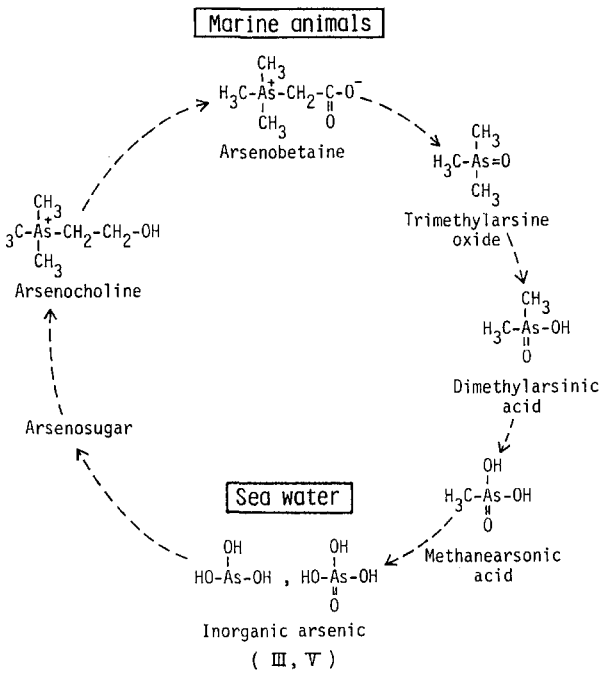

Fig. 3. A tentative cycle of arsenic in marine ecosystems.

although arsenobetaine-decomposing microorganisms are probably ubiquitous in marine systems.

The conversion of arsenic compounds depends upon whether the conditions are aerobic or anaerobic. The *in vitro* experiments performed with methylarsenicals (arsenobetaine, trimethylarsine oxide, dimethylarsinic acid and methanearsonic acid) and sedimentary microorganisms under anaerobic conditions, showed that little or no arsenobetaine was converted to its metabolites, though the methylarsenicals other than arsenobetaine were converted to less methylated arsenic compounds (Hanaoka *et al.*, 1990). This suggests that the degradation of arsenobetaine to trimethyarsine oxide probably takes place mainly in aerobic environments such as the water column or at the surface of the bottom sediments. The further degradation of trimethylarsine oxide to less methylated arsenicals may occur by the microorganisms in both aerobic and anaerobic environments.

A complete arsenic cycle can now be formed by linking the results of this study with the generally accepted hypothesis on the biosynthesis of arsenobetaine (Fig. 3). Arsenobetaine, which is derived via the food chain and accumulated in marine animals, is degraded step by step to inorganic arsenic by microorganisms after the death of those animals. Dimethylarsinic acid and methanearsonic acid are possible intermediate degradation products. Arsenocholine, considered to be the precursor of arsenobetaine, may also occur as an intermediate degradation product.

Conclusions

Arsenobetaine was degraded to inorganic arsenic via trimethylarsine oxide by sedimentary microorganisms. From this and other evidence, it is postulated that there is a marine arsenic cycle which begins with the methylation of inorganic arsenic on the way to arsenobetaine and terminates with the complete degradation of arsenobetaine to inorganic arsenic.

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