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Degradation of a Tetramethylarsonium Salt by Microorganisms Occurring in Sediments and Suspended Substances under both Aerobic and Anaerobic Conditions

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Microbial degradation of a tetramethylarsonium salt during incubation at 25 °C was investigated under both aerobic and anaerobic conditions. Two media (1/5 ZoBell 2216E and inorganic salt medium), added with the sediments or suspended substances as the sources of the microorganisms, were used. Degradation of the tetramethylarsonium salt occurred only in the ZoBell medium: under anaerobic conditions, trimethylarsine oxide and dimethylarsinic acid were derived with the sediments, and dimethylarsinic acid with the suspended substances, the salt degrading more rapidly with the former than with the latter. Small amounts of two metabolites, trimethylarsine oxide and inorganic arsenic(V), was also derived in the aerobically incubated ZoBell medium added with the suspended substances. This result means that the tetramethylarsonium salt is degraded to inorganic arsenic, which is the starting material for arsenic circulation in marine ecosystems, via trimethylarsine oxide and dimethylarsinic acid.

Keywords: Tetramethylarsonium iodide, degradation, sediments, suspended substances, microorganisms, trimethylarsine oxide, dimethylarsinic acid, inorganic arsenic

INTRODUCTION

Various organoarsenic compounds have been identified or confirmed in marine organisms. Among them, arsenobetaine is well known as a compound of which accumulation is ubiquitous in marine animals.[1–3] In order to study arsenic circulation in marine ecosystems, we have investigated the degradation of arsenobetaine by microorganisms occurring in various marine origins, namely sediments,[3–7] macroalgae,[8] mollusk intestine[9] and suspended substances.[10] As a result, the ubiquitous occurrence of microorganisms which can degrade arsenobetaine has been confirmed in marine environments. The degradation activity is higher in microorganisms occurring in the sediments and suspended substances, in which arsenobetaine has been degraded to inorganic arsenic via trimethylarsine oxide and dimethylarsinic acid.

Arsenocholine, which has been considered as a precursor of arsenobetaine,[1,2] has been proved to be converted to arsenobetaine by microorganisms occurring in sediments, and this has been followed by further degradation of arsenocholine to trimethylarsine oxide and dimethylarsinic acid.[11] The bioconversion of arsenocholine to arsenobetaine has also been confirmed in mammals[12,13] and fish.[14]

In addition, trimethylarsine oxide, dimethylarsinic acid and methanearsonic acid, which have been derived as metabolites from arsenobetaine in the degradation experiments so far, have also been investigated with the sedimentary microorganisms.[6] The results from these degradation experiments are summarized as follows: each metabolite, when it is added to the reaction mixture as a starting material, tends to degrade more under anaerobic conditions, and less, or no, degradation occurs under aerobic conditions. On the other hand, arsenobetaine itself has a reversed tendency of degradation, little degradation occurring in anaerobic conditions.

Recently, the tetramethylarsonium salt has been placed on the list of water-soluble organoarsenic compounds which accumulate in marine animals: it has been confirmed in tissues or organs of marine animals belonging to the relatively lower trophic levels, i.e. bivalves,[15,16] sea anemone[17] and sea hare.[17] In the present paper,

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we describe our investigation of the degradation of the tetramethylarsonium ion by micoorganisms occurring in sediments and suspended substances under both aerobic and anaerobic conditions.

MATERIALS AND METHODS

Sources of microorganisms

Bottom sediments were collected with an Ekman grab sampler from the coastal waters of Yoshimi, Shimonoseki, Japan. Suspended substances were collected from the same place by filtration of about two liters of sea water using a membrane filter (pore size 0.22 μm). About 1 g of the sediment or the suspended substances together with the filter was added to each culture medium described below.

Culture methods

Two culture media which have been used for the microbial degradation experiments so far were used also in this study: 1/5 ZoBell 2216E ($g\,l^{-1}$ filtered seawater: peptone 1.0; yeast extract 0.2, pH 7.5) and an aqueous solution of inorganic salts at pH 7.5 [$g\,l^{-1}$: sodium chloride (NaCl) 30.0; calcium chloride ($CaCl_2.2H_2O$) 0.2; potassium chloride (KCl) 0.3; iron(II) chloride ($FeCl_2.nH_2O$) 0.01; phosphates (KH_2PO_4) 0.5 and (K_2HPO_4) 1.0; magnesium sulphate ($MgSO_4.7H_2O$) 0.5; and ammonium chloride (NH_4Cl) 1.0]. Sediments or the suspended substances were added to each medium (25 cm^3) containing tetramethylarsonium iodide [$(CH_3)_4As^+I^-$, 50 mg] in a 50 cm^3 Erlenmeyer flask. The flasks for the aerobic incubation were shaken at 25 °C in the dark under an atmosphere of air, while those for the anaerobic one were kept static at 25 °C in the dark after being covered with 5 cm^3 of liquid paraffin. Mixtures with or without liquid paraffin autoclaved at 120 °C for 20 min served as controls for both conditions. At intervals of several days of incubation, 0.1 cm^3 of the mixtures in the flasks was withdrawn and added to 2.0 cm^3 of water.

Tetramethylarsonium iodide and its metabolites in the diluted mixtures were fractionated with a high-performance liquid chromatograph (HPLC—Tosoh Co., CCP 8000 series) using a TSK Gel ODS-120T column (4.6 mm × 250 mm) with a 11.2 mmol dm^{-3} solution of sodium heptanesulfonate in water–acetonitrile–acetic acid (95:5:6, by vol.) as mobile phase.[18] Portions of 10, 20 or 50 μl of each eluted fraction was analyzed using a graphite furnace atomic absorption (GF AA) spectrometer serving as the arsenic-specific detector as described previously.[3]

Purification and identification of metabolites

About three-quarters of each culture mixture in which metabolites were detected was taken from the flask. After being filtered, each of the mixtures was applied to a Dowex 50W-×8 cation-exchange column (H^+ form, 100–200 mesh, 2.0 cm × 53 cm) and eluted with water (500 cm^3), 2 mol dm^{-3} pyridine (500 cm^3) and 1 mol dm^{-3} HCl (500 cm^3), successively. For further purification, the arsenic-containing fractions were concentrated and placed on a Dowex 50W-×2 column (pyridinium form, 200–400 mesh, 1.0 cm × 35 cm) equilibrated with 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1) and eluted with the same buffer, 0.1 mol dm^{-3} pyridine and 1 mol dm^{-3} HCl, successively. Each arsenic-containing fraction was pooled and freeze-dried.

The purified metabolites were chromatographed on a cellulose thin layer (Avicel SF, 0.1 mm, Funakoshi Yakuhin Co. Ltd). $SnCl_2$–KI reagent[19] and iodine vapor were used for the detection of spots (Table 1, below).

Fast atom bombardment (FAB) mass spectrometry (FAB mass, JEOL JMS DX-300 mass spectrometer equipped with fast atom bom-

Table 1 R_f values of metabolite-1, metabolite-2 and metabolite-3 on thin-layer chromatography

	R_f value				
	Solvent system:[a]				
Sample	1	2	3	4	5
Metabolite-1	0.87	0.76	0.56	0.23	0.71
Dimethylarsinic acid	0.87	0.75	0.54	0.22	0.70
Metabolite-2	0.88	0.80	0.60	0.42	0.77
Trimethylarsine oxide	0.88	0.81	0.61	0.43	0.76
Metabolite-3	0.53	0.00	0.54	0.00	0.28
Inorganic arsenic (V)	0.53	0.00	0.53	0.00	0.28

[a] Solvent systems: 1, ethyl acetate–acetic acid–water (3:2:1); 2, chloroform–methanol–25% aq. ammonia (3:2:1); 3, 1-butanol–acetone–formic acid–water (10:10:2:5); 4, 1-butanol–acetone–25% aq. ammonia–water (10:10:2:5); 5, 1-butanol–acetic acid–water (4:2:1).

bardment, xenon atoms at 6 keV) and a combination of gas-chromatographic separation with hydride generation followed by a cold-trap technique and selected ion monitoring mass spectrometric analysis (hydride generation/cold trap/GC MS/SIM) were used for the confirmation of the structure of the purified metabolites. The method was described previously.[20]

RESULTS

Degradation of the tetramethylarsonium ion by sedimentary microorganisms

No conversion of tetramethylarsonium ion was observed in the inorganic salt medium added with the sediment under either aerobic or anaerobic conditions throughout the incubation period. On the other hand, in the ZoBell medium, its degradation and the formation of two metabolites were observed under anaerobic conditions (Fig. 1): these metabolites, labelled metabolite-1 and metabolite-2, appeared in the medium on days 12 and 78 of incubation, respectively. The retention times of metabolite-1 and metabolite-2 agreed with those of dimethylarsinic acid and trimethylarsine oxide, respectively. The recovery of tetramethylarsonium ion decreased relatively rapidly after 42 days of incubation, followed by an increase of metabolite-1. After 78 days of incubation the recovery of metabolite-1 was more than that of the remaining tetramethylarsonium.

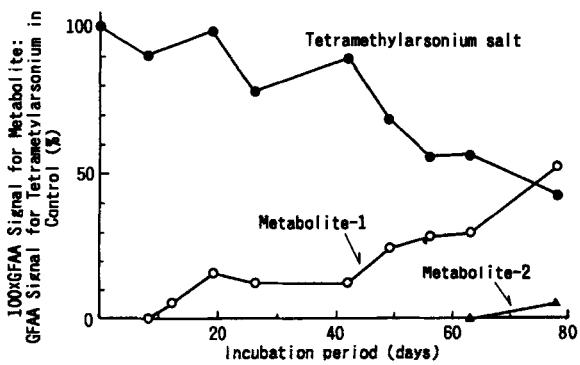

Figure 1 Microbial conversion of tetramethylarsonium ion and the formation of metabolite-1 and metabolite-2, in anaerobically incubated ZoBell medium added with sediments. The HPLC retention times of metabolite-1 and metabolite-2 agreed with those of dimethylarsinic acid (300–375 s) and trimethylarsine oxide (825–900 s), respectively.

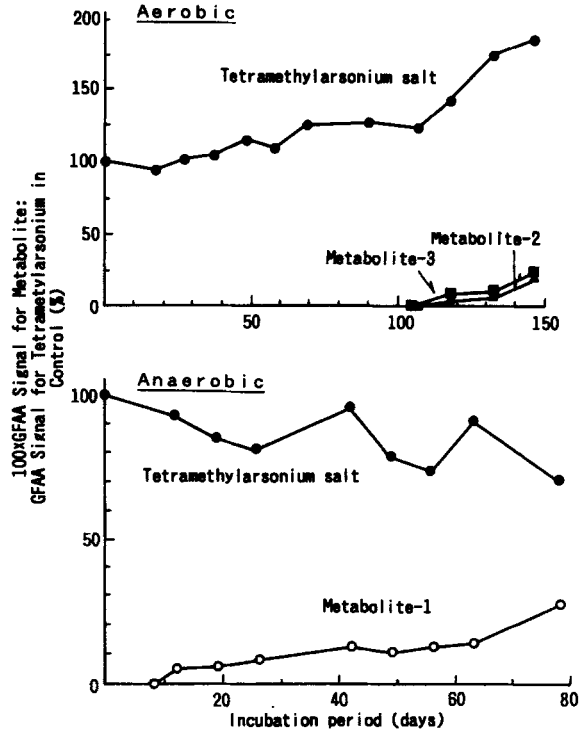

Figure 2 Microbial conversion of tetramethylarsonium ion and the formation of metabolites in ZoBell medium added with suspended substances. Metabolite-2 and metabolite-3 were aerobically (upper) and metabolite-1 was anaerobically (lower) derived during the incubation. The HPLC retention times of metabolite-1, metabolite-2 and metabolite-3 agreed with those of dimethylarsinic acid (300–375 s), trimethylarsine oxide (825–900 s) and inorganic arsenic(V) (150–225 s), respectively.

Degradation of the tetramethylarsonium ion by microorganisms occurring in suspended substances

No conversion occurred here again in the inorganic salt medium under both conditions. Tetramethylarsonium ion degradation and the formation of metabolites observed in both the aerobic and anaerobic ZoBell medium added with the suspended substances is shown in Fig. 2. The metabolites were labelled metabolite-2 and metabolite-3 under aerobic conditions and metabolite-1 under anaerobic conditions. The retention time of metabolite-3 agreed with that of inorganic arsenic(V). The conversion activity was higher under anaerobic conditions than under aerobic ones. The extent of degradation of tetramethylarsonium ion to metabolite-1 was smaller in the suspended substances–ZoBell medium

mixture than in the sediments–ZoBell medium mixture (Fig. 1).

Purification of the metabolites

The anaerobic ZoBell medium mixture added with the sediment and the anaerobic ZoBell medium mixture added with the suspended substances were subjected separately to cation-exchange chromatography using Dowex 50W–×8 (H^+ form). Metabolite-1 and metabolite-2 derived in the anaerobic ZoBell–sediment mixture were eluted with 2 mol dm^{-3} pyridine. Metabolite-1 was further purified by using Dowex 50W–×2 (pyridinium form), being eluted with 0.1 mol dm^{-3} pyridine–formic acid buffer. On the other hand, metabolite-3 and metabolite-2 derived in the aerobic ZoBell–suspended substances mixture were eluted with water and 2 mol dm^{-3} pyridine from Dowex 50W–×8 (H^+ form), respectively. Each metabolite was further purified using Dowex 50W–×2 (pyridinium form), metabolite-3 being eluted with 0.1 mol dm^{-3} pyridine–formic acid buffer and metabolite-2 with 0.1 mol dm^{-3} pyridine. These purified metabolites were subjected to analyses to confirm the structure.

Identification of the metabolites

The purified metabolite-1, metabolite-2 and metabolite-3 were subjected to thin-layer chromatogaphy. Their R_f values agreed with those of dimethylarsinic acid, trimethylarsine oxide and inorganic arsenic(V) in five different solvent systems (Table 1). Hydride generation/cold trap/GC MS/SIM analysis of a portion of the freeze-dried metabolite-1 without hydrolysis with sodium hydroxide gave only dimethylarsine, indicating metabolite-1 to be dimethylarsinic acid. The same analysis of metabolite-3 gave only arsine without hydrolysis with sodium hydroxide, indicating metabolite-3 to be inorganic arsenic(V). FAB mass spectra of metabolite-2 and synthetic trimethylarsine oxide are shown in Fig. 3. Both are essentially the same, showing peaks at $m/z=137$ $(M+1)^+$ and $m/z=273$ $(2M+1)^+$. Besides these peaks, adduct ions were also shown in the spectrum of metabolite-2, i.e. at $m/z=159$ $(M+Na)^+$ and $m/z=175$ $(M+K)^+$. The peak at $m/z=211$ corresponds to $(M+As)^+$. This peak was attributed to contamination with trace amounts of metabolite-3.

From the information from HPLC, thin-layer chromatography and hydride generation/cold trap/GC MS/SIM analysis, metabolite-1 and metabolite-3 were confirmed as dimethylarsinic acid and inorganic arsenic(V), respectively. On the other hand, metabolite-2 was confirmed to be trimethylarsine oxide on the basis of the results from HPLC, thin-layer chromatography and FAB mass spectrometry.

DISCUSSION

In this study, the degradation of tetramethylarsonium iodide by microorganisms occurring in sediments or suspended substances was clearly demonstrated. With both sediments and suspended substances, dimethylarsinic acid was derived as a major metabolite in the anaerobically incubated ZoBell medium. This degradation pattern was analogous to that of trimethylarsine oxide under the same conditions where it degraded only anaerobically to dimethylarsinic acid as sole metabolite.[6] Thus, the tendency for methylarsenicals (trimethylarsine oxide, dimethylarsinic acid and methanearsonic acid) to degrade more under anaerobic conditions than under aerobic conditions[6] has applied to the degradation of the tetramethylarsonium ion. Although the mechanistic meaning of this tendency of degradation is still unknown, it may be that these methylarsenicals were degraded mainly by microorganisms in anaerobic zones such as bottom sediments rather than in the aerobic water column under natural conditions.

No degradation was observed in the inorganic salt medium. In the degradation experiments so far, it is suggested that the tendency for a medium to be more suitable for a degradation may not depend on the abundance of organic substances or carbon sources in incubation mixtures, but on the microflora present. Degradation never occurred in the inorganic salt medium in this study, from whatever origin. This means that microorganisms occurring in the sediments or the suspended substances may not be able to use this compound as sole carbon source, because there were no carbon sources other than the tetramethylarsonium salt in the inorganic salt medium. Conclusions here, however, will be drawn from degradation experiments with these sources collected over various seasons.

We have postulated previously an arsenic cycle in marine ecosystems[7,20] through linking the

generally accepted hypothesis on the bioconversion of organoarsenicals to our results from their degradation experiments so far: arsenobetaine which is bioconverted from inorganic arsenic in seawater is degraded to the original inorganic arsenic via trimethylarsine oxide, dimethylarsinic acid and methanearsonic acid. It was not clear at the present stage how tetramethylarsonium ion participates in this arsenic cycle in marine ecosystems. However, as in the aerobically incubated Zobell–suspended substances mixture, inorganic arsenic(V) was derived together with trimethylarsine oxide (Fig. 2). Although their formation rates were much lower than those derived from arsenobetaine,[3–7] the same microbial degradation route to inorganic arsenic as for arsenobetaine was confirmed for this compound: tetramethylarsonium iodide is degraded to inorganic arsenic via trimethylarsine oxide and dimethylarsinic acid by microorganisms occurring in the marine environment.

CONCLUSIONS

Tetramethylarsonium iodide was aerobically degraded to inorganic arsenic by microorganisms occurring in suspended substances and anaerobically so dimethylarsinic acid by microorganisms occurring in both sediments and suspended substances. Although anaerobic conditions were suitable for the degradation of the tetramethylarsonium ion, the degradation route of this compound under aerobic conditions to inorganic arsenic is essentially the same as that of arsenobetaine. This

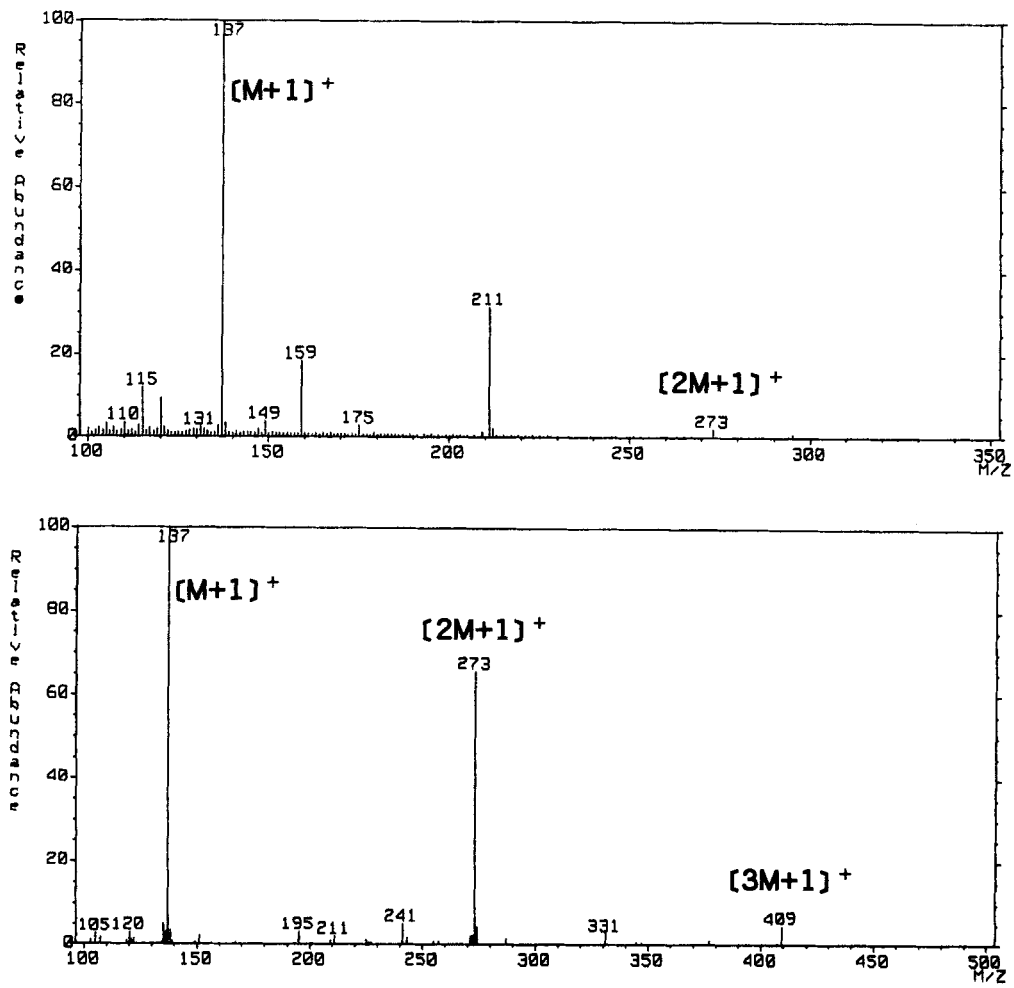

Figure 3 FAB mass spectra of metabolite-2 (above) and synthetic trimethylarsine oxide (below).

degradation occurred in the ZoBell medium but not in the inorganic salt medium, indicating that the microorganisms investigated in this study cannot degrade tetramethylarsonium ion without other carbon sources being present.

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