APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2001; **15**: 481–489
DOI: 10.1002/aoc.184

Dimethylarsinoylacetate from microbial demethylation of arsenobetaine in seawater

Somkiat Khokiattiwong,[1] Walter Goessler,[2] Søren N. Pedersen,[3] Raymond Cox[4] and Kevin A. Francesconi[3]*
[1]Phuket Marine Biological Center, Phuket 83000, Thailand
[2]Chemistry Institute, Karl-Franzens University Graz, 8010 Graz, Austria
[3]Institute of Biology, University of Southern Denmark, 5230 Odense M, Denmark
[4]Department of Biochemistry and Molecular Biology, University of Southern Denmark, 5230 Odense M, Denmark

The fate of 11 arsenic compounds in microbially enriched seawater was monitored for up to 10 days by HPLC–ICPMS. Most of the arsenicals underwent little or no change in this medium, whereas two of the compounds, arsenobetaine and arsenocholine, were completely degraded. Arsenobetaine ($Me_3As^+CH_2COO^-$), the predominant form of arsenic in marine animals, was transformed within hours, initially to dimethylarsinoylacetate ($Me_2As(O)CH_2COO^-$) and then to dimethylarsinate ($Me_2As(O)O^-$). Arsenocholine behaved similarly but degraded at a slower rate. The identity of the new metabolite, dimethylarsinoylacetate, was confirmed by LC electrospray MS. A repeat experiment with arsenobetaine and dimethylarsinoylacetate, and employing LC electrospray MS to monitor the metabolites, produced results qualitatively identical with those from the first experiment. The rapidity of the degradation processes offers an explanation for the apparent absence of arsenobetaine in natural waters. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: arsenic; dimethylarsinoylacetate; arsenobetaine; seawater; LC electrospray MS; HPLC–ICPMS

Received 23 January 2001; accepted 20 February 2001

* Correspondence to: K. A. Francesconi, Institute of Biology, University of Southern Denmark, 5230 Odense M, Denmark.
Email: kaf@biology.sdu.dk
Contract/grant sponsor: Danida Fellowship Centre.

INTRODUCTION

Arsenic in seawater is present predominantly in inorganic forms, whereas it occurs in marine organisms as organoarsenic compounds.[1] The origin of organoarsenicals has been the subject of considerable research, and various biogenetic schemes have been proposed.[2–4] The degradation of these marine arsenic compounds has also been studied by employing microbial systems derived from a number of marine substrates.[5–12]

Such degradative processes might be expected to play an important role in the biogeochemical cycling of arsenic in the sea. Methylated arsenic compounds, such as arsenosugars in algae and arsenobetaine in animals, must be released to the sea at some point, as a consequence of grazing or predation by animals, or on senescence of the organisms. Yet methylated arsenic compounds do not accumulate in seawater (arsenobetaine has never been detected there) and, outside the photic zone, virtually all the arsenic is present in inorganic forms,[13] indicating that demethylation (and other dealkylation) processes are at work.

Useful information on these degradative processes has been provided by the studies of Hanaoka and coworkers.[5–12] In a series of experiments, they have been able to show that arsenobetaine is converted to arsenocholine, trimethylarsine oxide, dimethylarsinate, or inorganic arsenic depending on the microbial conditions. These studies have used incubation periods of several months and high concentrations of arsenicals (e.g. 800 mg As l^{-1})[7], so that the compounds themselves served as a carbon source for the microbes. Metabolites were usually detected and identified following conversion to their volatile hydrides, and possibly some metabolites that do not form such derivatives may have gone undetected. The current study extends

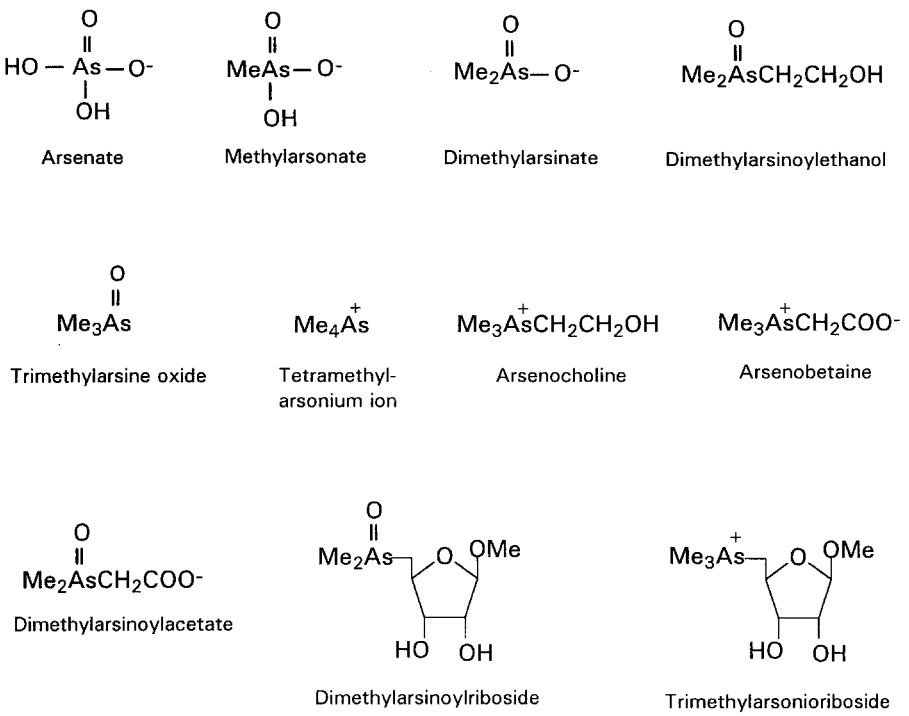

Figure 1 Structures of the 11 arsenic species used in the microbial experiments.

earlier work by examining the fate of 11 arsenic compounds added to microbially enriched seawater at low concentrations (100–750 μg As l^{-1}). Metabolites were determined by high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC–ICPMS) and liquid chromatography (LC) electrospray mass spectrometry (MS).

MATERIALS AND METHODS

Arsenic compounds

The 11 arsenic species (Fig. 1) investigated and their method of preparation were: arsenate (as Na_2HAsO_4); methylarsonate, dimethylarsinate, trimethylarsine oxide, tetramethylarsonium ion, arsenocholine, and arsenobetaine;[14] dimethylarsinoylethanol;[15] dimethylarsinoylacetate;[16] a dimethylarsinoylriboside;[17] and a trimethylarsonioriboside.[18] Stock solutions in water (*ca* 100 μg As ml^{-1}) were prepared, and the arsenic concentrations determined by graphite furnace atomic absorption spectrophotometry. These compounds were also available for use as HPLC chromatographic standards.

Microbial transformations

Experiment 1

Eight shore crabs (*Carcinus maenas*, biomass *ca* 25 g) collected from Odense fjord, Denmark, in July 1998 were maintained in an aquarium containing 18 l of aerated seawater (15 °C, *ca* 20‰) for 2 days, by which time the water had become slightly cloudy because of microbial growth. A complex nutrient mixture (40 ml of sterile MRS medium[19]) was added to stimulate growth, and after 16 h portions (800 ml) of seawater were transferred to 11 plastic beakers (1000 ml capacity). The water in the beakers was aerated with compressed air delivered through a plastic tube. The experiment was carried out at 15 °C, the temperature of the aquarium room. An arsenic compound was added to each beaker to give a concentration of 100 μg As l^{-1}. After 5 min (to ensure thorough mixing), a sample of water (*ca* 3 ml) was removed from the beaker and immediately syringe-filtered (0.45 μm); a portion (100 μl) of the filtrate was transferred to an Eppendorf tube, quickly dried in a centrifugal lyophilizer (Heto

Holton, Allerød, Denmark) and stored at −18 °C along with the remainder of the filtrate. Additional samples from each of the 11 beakers were collected and processed in this manner at $t = 2$ h, 6 h, 24 h, 2 days, 3 days, 5 days, and 10 days. The dried subsamples of filtrate were reconstituted in water (1.00 ml, i.e. tenfold dilution to minimize possible NaCl interference) immediately before HPLC–ICPMS analysis.

Partial purification of arsenic metabolites from arsenobetaine experiment, $t = 2$ h

Seawater (2.8 ml, 0.11 μg As) from the (filtered) 2 h sample from the arsenobetaine experiment was applied to a column of cation-exchange resin (Dowex 50W × 8 H^+, 200 mesh, bed height 80 mm, bed diameter 10 mm). The column was washed with water (75 ml) followed by aqueous ammonia (2.5% w/v). Fractions (1.0 ml) of the ammonia wash were collected and their arsenic content determined by graphite furnace atomic absorption spectrophotometry. The arsenic-containing fractions (which eluted just after the ammonia front) were combined (0.08 μg As) and evaporated to dryness. The residue was redissolved in water (0.50 ml) for analysis by LC electrospray MS.

Experiment 2

Four shore crabs (*C. maenas* biomass *ca* 18 g) collected from Odense fjord in September 1999 were maintained in an aquarium containing 4 l of aerated seawater (salinity, 18‰) at 15 °C for 2 days. A complex nutrient mixture (MRS medium,[19] 50 ml) was added to stimulate microbial growth. After 24 h, seawater (800 ml) was taken from the aquarium, and a further 10 ml of sterile MRS medium was added with mixing. Three portions (100 ml) of the culture were transferred to sterile bottles (500 ml, Duran); the remaining culture was autoclaved before three portions (100 ml) were similarly transferred to sterile bottles (controls). Portions (1.00 ml) of stock solutions of two arsenic compounds, arsenobetaine (75 μg As ml^{-1}) and dimethyarsinoylacetate (50 μg As ml^{-1}) were filtered (0.20 μm) directly into the two batches of three bottles under sterile conditions in the following manner: arsenobetaine only (75 μg As), dimethylarsinoylacetate only (50 μg As), and a mixture of arsenobetaine (75 μg As) and dimethylarsinoylacetate (50 μg As). The bottles were aerated and stirred at (laboratory) room temperature (21–24 °C). The contents were sampled (2 × *ca* 2 ml) under sterile conditions at $t = 0$, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 10, 24 h, then daily up to day 7. Microbial growth was monitored by following apparent absorbance in a Shimadzu 1601 spectrophotometer for one of the subsamples; the second subsample was filtered (0.2 μm), and the filtrate stored frozen (−20 °C) before analysis by LC electrospray MS. It was not possible to process the filters simultaneously from these samples. Consequently, an identical culture was prepared (arsenobetaine only) in which the $t = 6$ h sample was filtered and the retained biomass and filter membrane were immediately washed with clean seawater (3 ml), then removed from the filter-holder and extracted (with sonication) with Milli-Q water.

Liquid chromatography–mass spectrometry

HPLC–ICPMS

The HPLC system consisted of a Hewlett Packard Series 1100 HPLC (with solvent degasser, binary pump, autosampler, and thermostatic column compartment) (Hewlett Packard, Waldbronn, Germany). Separations were performed on a PRP-X100 anion-exchange column (250 × 4.1 mm, 10 μm) from Hamilton Company (Reno, NV, USA) at 40 °C with a mobile phase (1.5 ml min^{-1}) of 20 mM $NH_4H_2PO_4$ pH 5.6 adjusted with aqueous NH_3; or on a Supelcosil LC-SCX cation-exchange column (250 × 4.6 mm, 5 μm; Supelco, Bellefonte, USA) at 40 °C with mobile phase (1.5 ml min^{-1}) of 20 mM pyridine pH 2.6 adjusted with HCOOH. The outlet of the HPLC column was connected via a 800 mm, 1/16″ PEEK (polyether–ether–ketone) capillary tubing (0.13 mm i.d.) to the Babington-type nebulizer of a Hewlett Packard 4500 inductively coupled plasma mass spectrometer (Hewlett Packard, Waldbronn, Germany). The ion intensities at *m/z* 75 and 77 were monitored. The ICPMS signal was optimized with a solution of the mobile phase containing 20 μg As l^{-1} to give maximum response on the arsenic signal (*m/z* 75). Arsenic compounds were quantified by comparison with standard solutions of arsenate (anion-exchange) or arsenobetaine (cation-exchange). The samples were redissolved in Milli-Q (Millipore, Bedford, MA, USA) water (1.00 ml) immediately before analysis by HPLC–ICPMS.

LC electrospray MS

A Hewlett Packard LC-MSD system consisting of a Series 1100 HPLC (with solvent degasser, binary pump, autosampler, and thermostatic column compartment) and a G1946A MSD single quadru-

Table 1 Retention times of arsenic compounds determined by ion-exchange HPLC–ICPMS

Compound	Retention time (min)	
	Anion-exchange[a]	Cation-exchange[a]
Arsenate	6.24	–
Methylarsonate	3.18	–
Dimethylarsinate	2.29	2.87
Trimethylarsine oxide	1.60	5.06
Tetramethylarsonium ion	1.32	8.30
Arsenocholine	1.34	5.89
Arsenobetaine	1.52	3.47
Dimethylarsinoylethanol	1.59	4.69
Dimethylarsinoylacetate	3.26	2.97
Dimethylarsinoylriboside	2.78	4.80
Trimethylarsonioriboside	1.63	7.70

[a] See Materials and Methods for details.

pole mass spectrometer equipped with an atmospheric pressure ionization (API) LC–MS interface was used for the characterization and quantification of the arsenic compounds. Chromatography was performed with a PRP-X100 anion-exchange column (250×4.1 mm, 10 μm) from Hamilton Company (Reno, NV, USA) at 30 °C equilibrated with a mobile phase comprising 20 mM NH_4HCO_3, pH 10.3 (adjusted with aqueous ammonia) and methanol (9:1). Flow rate was 0.4 ml min^{-1}, and injection volume was 10 μl. Milli-Q water was used in the HPLC mobile phase and HPLC-grade methanol were from Rathburn (Walkerburn, Scotland). Analyses were performed on standard solutions of dimethylarsinoylacetate, arsenobetaine and dimethylarsinate (50–1000 μg As l^{-1} prepared in seawater). The LC electrospray MS analyses were performed using selective-ion monitoring (SIM) in the positive ion mode with variable fragmentor voltages enabling simultaneous detection of $[M + H]^+$ species at *m/z* 181 (dimethylarsinoylacetate), 179 (arsenobetaine), and 139 (dimethylarsinate) at 70 V, and *m/z* 75 (As^+) at 230 V. The technique has been previously described for other arsenic compounds.[20]

RESULTS

Experiment 1: biotransformation of 11 arsenic compounds monitored by HPLC–ICPMS

The retention times of the arsenic compounds in the HPLC–ICPMS analyses are shown in Table 1. Chromatographic analysis of day 1 samples showed that, with the exception of arsenobetaine and arsenocholine, the arsenic compounds remained unchanged during the first day. For arsenobetaine and arsenocholine, however, less than 5% of the starting arsenical remained in solution after 24 h, both being converted mainly into dimethylarsinate. Analysis of day 10 samples showed that small changes had occurred for an additional three compounds: the tetramethylarsonium ion (15% conversion) had partially demethylated to trimethylarsine oxide, and both dimethylarsinoylethanol (11% conversion) and dimethylarsinoylacetate (26% conversion) had been partially degraded to dimethylarsinate.

Time course data were then obtained for the experiments with arsenobetaine and arsenocholine. The chromatograms (Fig. 2) clearly show that arsenobetaine was rapidly removed from the seawater, and that two products were formed with retention times of 2.30 and 3.33 min. The peak at 2.30 min was assigned to dimethylarsinate. The peak at 3.33 min was tentatively assigned to dimethylarsinoylacetate; this assignment was later confirmed by LC electrospray MS (see below). The total quantity of arsenic in solution decreased at first (to 35% of the initial value after 6 h), but then increased and reached the initial value after 10 days. By this time, however, the arsenobetaine originally present in solution had been completely transformed into dimethylarsinate. Arsenocholine behaved in a manner similar to that displayed by arsenobetaine, except that the initial uptake from water was slower. Presumably, the first step in the

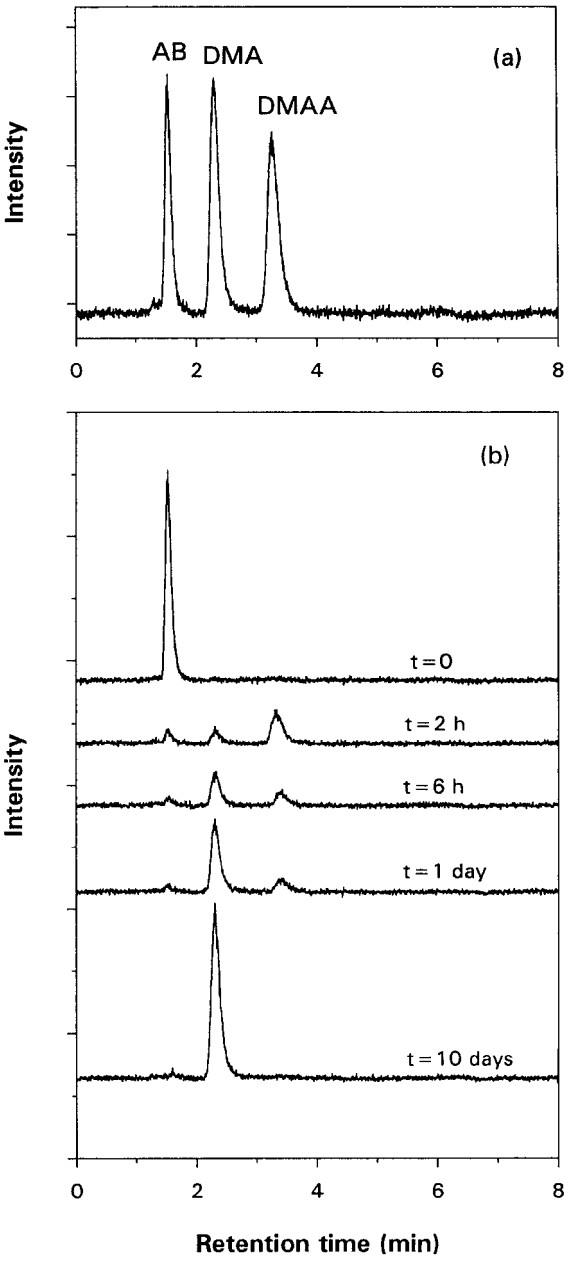

Figure 2 Anion-exchange chromatograms (HPLC–ICPMS) for: (a) three standard arsenic species each 10 μg As l^{-1}: AB = arsenobetaine, DMA = dimethylarsinate, DMAA = dimethylarsinoylacetate; (b) arsenic species in microbially enriched seawater (tenfold dilution in water) at various times after addition of arsenobetaine. Chromatographic conditions were: PRP-X100 anion-exchange column (250 × 4.1 mm, 10 μm) at 40 °C with a mobile phase of 20 mM $NH_4H_2PO_4$ pH 5.6 adjusted with aqueous NH_3; flow rate was 1.5 ml min^{-1}; 20 μl injection.

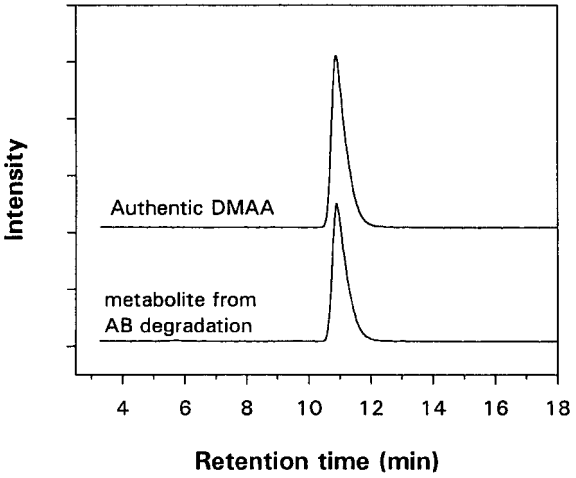

Figure 3 Anion-exchange chromatograms (LC electrospray MS) of partially purified metabolite from arsenobetaine (AB) incubation ($t = 2$ h) and authentic dimethylarsinoylacetate (DMAA) detected by positive ion SIM mode at *m/z* 181 ($[M + H]^+$). Chromatographic conditions were: PRP-X100 anion-exchange column (250 × 4.1 mm, 10 μm) at 30 °C with a mobile phase comprising 20 mM NH_4HCO_3, pH 10.3 (adjusted with aqueous ammonia) and methanol (9:1); flow rate was 0.4 ml min^{-1}; 10 μl injection volume. For these two chromatograms, the electrospray mass spectrometer was programmed to begin measuring ions at $t = 3$ min (chromatographic solvent front was 5.5 min).

process following uptake by the microbes involved oxidation to arsenobetaine.[21]

Confirmation of dimethylarsinoylacetate as a metabolite of arsenobetaine

The arsenic metabolites in the 2 h arsenobetaine sample were separated from the sea-salt by passage through a cation-exchange column. The arsenic-containing fraction that was eluted from the column with aqueous ammonia was then analysed by LC electrospray MS at *m/z* 181 ($[M + H]^+$ of the proposed metabolite, dimethylarsinoylacetate). The chromatogram was identical to that of authentic material (Fig. 3).

Experiment 2: biotransformation of arsenobetaine and dimethylarsinoylacetate monitored by LC electrospray MS

The transformation of arsenobetaine into dimethylarsinoylacetate was an unexpected result, because it had not been reported in the many related studies conducted so far.[5–12] For this reason the microbial

experiment was repeated with arsenobetaine and dimethylarsinoylacetate. On this occasion, autoclaved controls were also examined to establish that the changes observed were microbially mediated. Furthermore, LC electrospray MS was used to monitor the biotransformation processes by simultaneously determining the arsenic (*m/z* 75) and protonated molecular species for arsenobetaine (*m/z* 179), and dimethylarsinoylacetate (*m/z* 181) (Fig. 4). Dimethylarsinate (*m/z* 139) was also able to be monitored, although it was not fully chromatographically resolved from dimethylarsinoylacetate (retention times were 9.48 min and 9.98 min for dimethylarsinate and dimethylarsinoylacetate respectively).

Arsenobetaine disappeared rapidly from seawater concomitant with the slow formation of dimethylarsinoylacetate in the water (Fig. 4). The form of arsenic in the filtered biomass at $t = 6$ h, determined in a separate but identically prepared culture containing arsenobetaine, was >90% arsenobetaine. The concentration of dimethylarsinoylacetate reached a peak at about 3 h and thereafter slowly decreased. By 48 h neither arsenobetaine nor dimethylarsinoylacetate was present in the seawater (Fig. 4); all of the arsenic had been released back into the water as dimethylarsinate (Fig. 5). This behaviour was qualitatively identical to that observed for arsenobetaine in experiment 1. The time course data for the degradation of arsenobetaine, dimethylarsinoylacetate, and a mixture of the two compounds are shown in Fig. 6. The pattern for dimethylarsinoylacetate degradation was consistent with that found in experiment 1; in both cases the metabolite dimethylarsinate was found in the water only in the latter phase of the experiment. The changes occurring in the mixture of arsenobetaine and dimethylarsinoylacetate matched the changes taking place in the individual cultures. The arsenic compounds added to the autoclaved samples remained unchanged after 48 h (samples from longer incubation periods were not measured): initial and final concentrations (single LCMS analysis) were 734 and 740 μg As l^{-1} for arsenobetaine, and 460 and 520 μg As l^{-1} for dimethylarsinoylacetate.

The apparent absorbance of the microbial cultures was recorded to provide an index of biomass. The values increased during the first 10 h and then declined, presumably because of lysis of the cells, to an absorbance less than the inital value. The apparent absorbance of the autoclaved samples remained stable for the first 48 h.

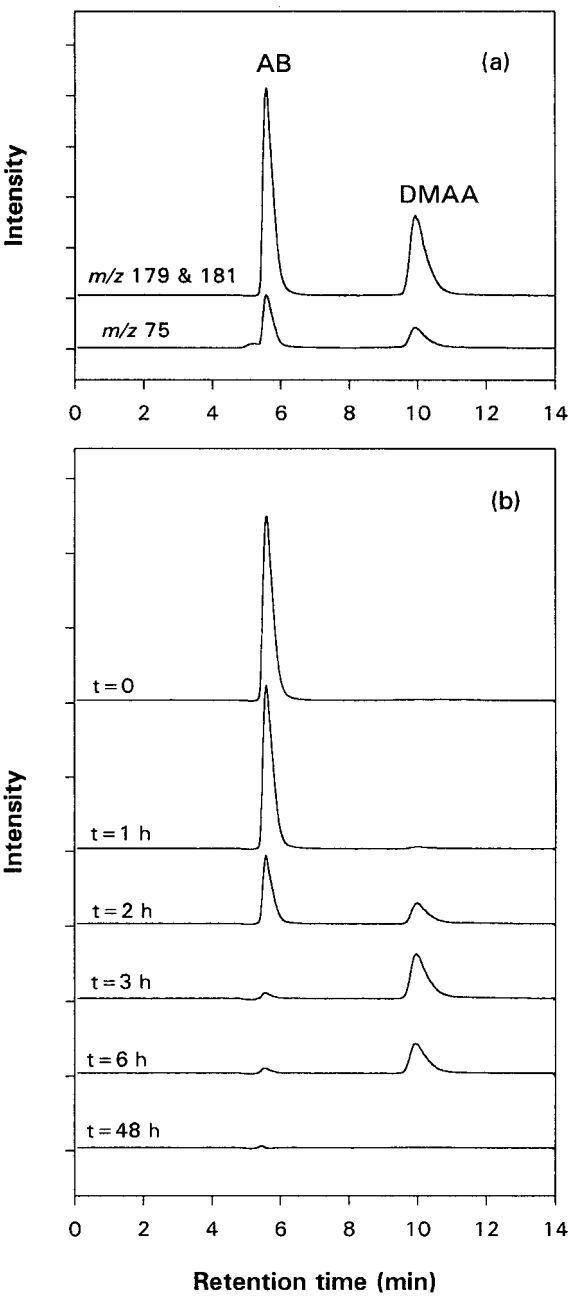

Figure 4 Anion-exchange chromatograms (LC electrospray MS) of: (a) standard arsenobetaine (AB) and dimethylarsinoylacetate (DMAA) detected simultaneously at *m/z* 75 (As^+), and at *m/z* 179 ($[AB + H]^+$) and *m/z* 181 ($[DMAA + H]^+$); (b) microbially enriched seawater at various times after addition of arsenobetaine detected at *m/z* 179 ($[AB + H]^+$) and *m/z* 181 ($[DMAA + H]^+$). Chromatographic conditions were as shown in Fig. 3, except that a pre-column was not used.

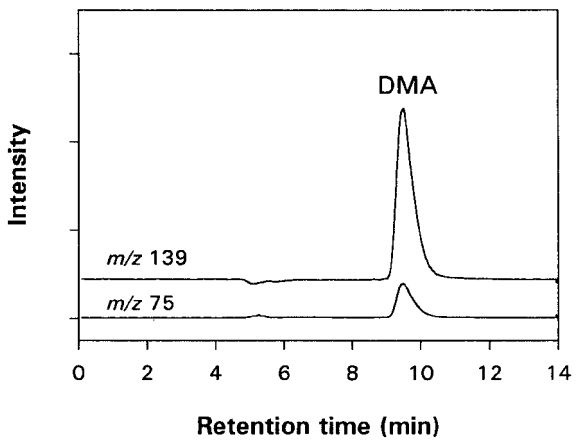

Figure 5 Anion-exchange chromatograms (LC electrospray MS) of microbially enriched seawater at $t = 48$ h after addition of arsenobetaine detected simultaneously at *m/z* 75 (As^+) and *m/z* 139 ($[DMA + H]^+$). Chromatographic conditions were as shown in Fig. 3, except that a pre-column was not used.

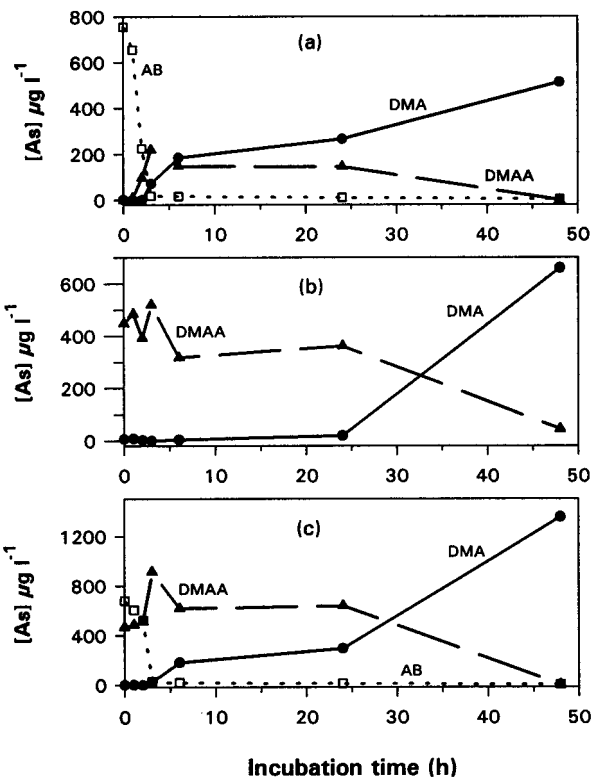

Figure 6 Time course degradation of arsenobetaine (AB) and dimethylarsinoylacetate (DMAA) added to microbially enriched seawater: (a) AB added at 750 $\mu g_{As}\ l^{-1}$; (b) DMAA added at 500 $\mu g_{As}\ l^{-1}$; and (c) a mixture of AB (750 $\mu g_{As}\ l^{-1}$) and DMAA (500 $\mu g_{As}\ l^{-1}$). DMA = dimethylarsinate. Arsenobetaine was not detected in (b).

DISCUSSION

Hanaoka *et al.*[8] studied the degradation of trimethylarsine oxide, dimethylarsinate, methylarsonate, arsenite and arsenate under both aerobic and anaerobic conditions. With aerobic microbes, changes occurred only under certain experimental conditions; methylarsonate and arsenate were not changed under any of the conditions tested. The mixed cultures of aerobic microbes in our study were unable to metabolize trimethylarsine oxide, dimethylarsinate, methylarsonate or arsenate. The anaerobic conditions reported by Hanaoka *et al.*[8] elicited demethylation of trimethylarsine oxide, dimethylarsinate and methylarsonate, but did not substantially degrade arsenobetaine. Edmonds *et al.*[15] found that dimethyarsinoylribosides (arsenosugars) degraded to dimethyarsinoylethanol under anaerobic microbial conditions. In our study with aerobes, the two arsenosugars tested were unchanged. Probably, the mixed and variable nature of the predominant microbes in the different studies accounts for the different results. Future studies in this interesting area should focus on isolation of the specific organisms responsible for the observed diverse biotransformations.

Arsenobetaine is readily taken up by marine animals, including fish,[16] mussels,[21] and crustaceans,[22] and accumulated unchanged to very high concentrations. Microbes also accumulate arsenobetaine,[9,23] but its fate in microbial systems is variable (see Ref.[8] for a review). When microbes from marines sediments were cultured aerobically with arsenobetaine the major metabolite was initially trimethylarsine oxide, which, in turn, degraded to inorganic arsenic.[7] Interestingly, aerobic microbes from suspended particles were able to reduce arsenobetaine to arsenocholine,[6] and other microbes associated with deep-sea particles were shown to degrade arsenobetaine to dimethylarsinate, trimethylarsine oxide and arsenate.[11] In further experiments, it was shown[9] that, although arsenobetaine was degraded in the culture medium, the microbes contained this arsenical unchanged throughout the incubation period. This result suggested that the microbes utilized at least some of their accumulated arsenobetaine as a carbon source, and excreted the arsenic metabolites, mainly arsenate, back into the medium. The authors note,[9] however, that extracellular degradation of arsenobetaine to arsenate could not be discounted. A similar result was obtained in our study: the one sample of microbes analysed showed arsenobetaine to be the major arsenical (>90%) at $t = 6$ h, even

though it was unlikely to be present in the culture medium at this stage.

Microbes associated with animal tissue have also been shown to degrade arsenobetaine, and such conditions might more closely match those in our experiments. For example, microbes associated with the gill of a clam *Meretrix lusoria* degraded arsenobetaine to trimethylarsine oxide apparently quantitatively after 60 days incubation.[12] The authors note that tetramethylarsonium ion, a significant natural constituent of *M. lusoria* gill,[24] was not formed in their experiments.

The results from our study differ qualitatively from those reported in all the aforementioned studies. Our experimental approach was to investigate biotransformations in seawater under conditions approximating those likely to be found in natural environments. Hence, we used a natural mixed microbial population of marine origin (microbes associated with shore crabs and/or seawater), and we added arsenic compounds at low concentrations (100 to 750 μg As l^{-1}). This last factor was in contrast to the experimental design of Hanaoka and co-workers, where arsenic compounds at concentrations of several hundred milligrams of arsenic per litre (e.g. Ref. 8) were used as the carbon source for microbes. This factor may explain the differences in the reported transformations of arsenobetaine.

Possible processes at play in the transformation of arsenobetaine into dimethylarsinoylacetate and dimethylarsinate observed in the current study are described in the following. Arsenobetaine is quickly taken up by the microbes, where it is retained mostly unchanged. A small amount is demethylated to dimethylarsinoylacetate, which is then excreted. As the carbon source in the medium decreases with time, however, the accumulated arsenobetaine is utilized as a source of the carboxymethyl group to yield dimethylarsinate, which is excreted.

The fate of dimethylarsinoylacetate in the water is more speculative. An unusual (but reproducible) property of this compound is that it degrades very slowly for the first 24 h (<10%); thereafter it degrades quickly, and it is completely converted after 48 hours (Fig. 6b). Possibly, this observed lag period reflects the time required for induction of the biotransforming enzyme(s). Alternatively, the microbes initially present in the culture may not be involved directly in the conversion of dimethylarsinoylacetate to dimethylarsinate. In this regard it is interesting that the viable culture appeared to change at $t > 10$ h (as shown by the decrease in optical density). This may have resulted, after 24 h, in a transient predominance of microbes different from those originally present, and these microbes might have facilitated the conversion of dimethylarsinoylacetate to dimethylarsinate. It is likely that time course data on the arsenic species in the biomass would shed considerable light on the precise processes and kinetics involved, and appropriate analyses will be incorporated in our future work in this area.

Finally, the rapid microbial decomposition of arsenobetaine demonstrated here provides a ready explanation for the apparent absence of this arsenical in seawater. In addition, the observed conversion of arsenobetaine into dimethylarsinoylacetate is relevant to hypotheses about the origin of arsenobetaine. Dimethylarsinoylacetate was proposed[15] as a possible intermediate in the biogenesis of arsenobetaine *via* dimethylarsinoylethanol produced by anaerobic degradation of arsenosugars. Consequently, the presence of these two metabolites in marine samples might be taken as support for such a pathway. However, dimethylarsinoylethanol has never been identified as a naturally occurring compound, and there has been only one report of dimethylarsinoylacetate as a constituent of marine samples.[25] In light of the laboratory experiments reported here, the presence of dimethylarsinoylacetate in marine samples would not necessarily constitute support for a biogenetic pathway for arsenobetaine based on arsenosugars.

Acknowledgements We thank the Danida Fellowship Centre for financial support.

REFERENCES

1. Edmonds JS, Francesconi KA, Stick RV. *Nat. Prod. Rep.* 1993; **10**: 421.
2. Edmonds JS, Francesconi KA. *Experientia* 1987; **43**: 553.
3. Phillips DJH, Depledge MH. *Mar. Environ. Res.* 1985; **17**: 1.
4. Edmonds JS. *Bioorg. Med. Chem. Lett.* 2000; **10**: 1105.
5. Hanaoka K, Tagawa S, Kaise T. *Appl. Organomet. Chem.* 1991; **5**: 435.
6. Hanaoka K, Koga H, Tagawa S, Kaise T. *Comp. Biochem. Physiol.* 1992; **101**: 595.
7. Hanaoka K, Tagawa S, Kaise T. *Hydrobiologia* 1992; **235–236**: 623.
8. Hanaoka K, Tagawa S, Kaise T. *Appl. Organomet. Chem.* 1992; **6**: 139.
9. Hanaoka K, Uchida K, Tagawa S, Kaise T. *Appl. Organomet. Chem.* 1995; **9**: 573.

10. Hanaoka K, Dote Y, Yosida K, Kaise T, Kuroiwa T, Maeda S. *Appl. Organomet. Chem.* 1996; **10**: 683.
11. Hanaoka K, Kaise T, Kai N, Kawasaki Y, Miyasita H, Kakimoto K, Tagawa S. *Appl. Organomet. Chem.* 1997; **11**: 265.
12. Kaise T, Sakurai T, Saitoh T, Matsubara C, Takadaoikawa N, Hanaoka K. *Chemosphere* 1998; **37**: 443.
13. Andreae MO. *Deep-Sea Res.* 1978; **25**: 391.
14. Geiszinger A, Goessler W, Kuehnelt D, Francesconi K, Kosmus W. *Environ. Sci. Technol.* 1998; **32**: 2238.
15. Edmonds JS, Francesconi KA, Hansen JA. *Experientia* 1982; **38**: 643.
16. Francesconi KA, Edmonds JS, Stick RV. *Sci. Total Environ.* 1989; **79**: 59.
17. Francesconi KA, Edmonds JS, Stick RV, Skelton BW, White AH. *J. Chem. Soc. Perkin. Trans. I* 1991; 2702.
18. Francesconi KA, Edmonds JS, Stick RV. *Appl. Organomet. Chem.* 1994; **8**: 517.
19. De Man JC, Rogosa M, Sharp ME. *J. Appl. Bacteriol.* 1960; **23**: 130.
20. Pedersen SN, Francesconi KA. *Rapid Commun. Mass Spectrom.* 2000; **14**: 641.
21. Gailer J, Francesconi KA, Edmonds JS, Irgolic KJ. *Appl. Organomet. Chem.* 1995; **9**: 341.
22. Hunter DA, Goessler W, Francesconi KA. *Mar. Biol.* 1998; **131**: 543.
23. Randall K, Lever M, Peddie BA, Chambers ST. *Biochim. Biophys. Acta* 1995; **1245**: 116.
24. Shiomi K, Kakehashi Y, Yamanaka H, Kikuchi T. *Appl. Organomet. Chem.* 1987; **1**: 177.
25. Larsen EH. *Fresenius. J. Anal. Chem.* 1995; **352**: 582.

