Applied Organometallic Chemistry (1990) 4 239–243

0268-2605/90/030239-05$05.00

Aerobic and anaerobic degradation of several arsenicals by sedimentary micro-organisms

Ken'ichi Hanaoka, Shigeo Hasegawa, Naoki Kawabe, Shoji Tagawa and Toshikazu Kaise*

Department of Food Science and Technology, Shimonoseki University of Fisheries, Yoshimi-cho, Shimonoseki 759–65, Japan, and * Kanagawa Prefectural Public Health Laboratories, Nakao-cho, Asahi-ku, Yokohama 241, Japan

Received November 1989 *Accepted 8 March 1990*

Two media (1/5 ZoBell 2216E and inorganic salt media) were used for degradation experiments. Each arsenical [arsenobetaine, trimethylarsine oxide (TMAO), dimethylarsinic acid (DMA), sodium methanearsonate, sodium arsenate and sodium arsenite] was added to 20 cm^3 of each medium (0.084% as As) in a 30-cm^3 Erlenmeyer flask. The mixtures were kept at 25 °C in the dark for 91 days for aerobic cultivation. For anaerobic cultivation, about 5 cm^3 of liquid paraffin was placed on the surface of each mixture. Under aerobic conditions, arsenobetaine was the only methylarsenical which was rapidly degraded, being converted to several metabolites. In contrast, under aerobic conditions, little or no arsenobetaine (ZoBell or inorganic salt-media respectively) was converted to its metabolites, while all the methylarsenicals other than arsenobetaine were converted to less methylated compounds. The conversion of inorganic arsenic was observed only in the aerobically incubated ZoBell/arsenite mixture; arsenate was derived from it. A peculiar conversion pattern was observed in the aerobically incubated ZoBell/TMAO mixture; DMA derived from TMAO began to convert to TMAO after 14 days of incubation, and TMAO was the only arsenical in the mixture after 35 days.

Keywords: Organic arsenical, inorganic arsenical, microbial degradation, marine micro-organisms, arsenic cycle, methylarsenicals

INTRODUCTION

The ubiquity of arsenobetaine has been demonstrated in marine animals during the past decade (for example being present in Crustacea: western rock lobster;[1] Chondrichthyes: blue shark;[2] Osteichthyes: school whiting;[3] Mollusca: sea cucumber[4]) and this compound is regarded as the final metabolite of arsenic in marine ecosystems. We have also confirmed ourselves that this compound is widely distributed in marine animals independently of their feeding habits and the trophic level to which they belong.[5] To study the arsenic circulation in marine ecosystems, therefore, it is essential to clarify the fate of arsenobetaine after the deaths of animals containing this compound. We previously reported the aerobic degradation of this compound by micro-organisms living in marine sediments[5–7] or those associated with marine macroalgae.[8] Thus, this degradation closes the marine arsenic cycle that begins with the methylation of arsenite on the way to arsenobetaine.

In this study, we deal in detail with the degradation of arsenicals not only arsenobetaine but others also. Trimethylarsine oxide (TMAO), dimethylarsinic acid (DMA), methanearsonic acid (MMA), arsenate and arsenite were used for these degradation experiments by sedimentary micro-organisms in both aerobic and anaerobic environments.

MATERIALS AND METHODS

Sediments

Sediments were collected in September 1988 from the coastal waters of Yoshimi, facing Shimonoseki University of Fisheries, Japan.

Aerobic and anaerobic microbial degradation of arsenicals

Two media [1/5 ZoBell 2216E (g dm^{-3} filtered seawater): peptone 1.0; yeast extract 0.2, pH 7.5, and aqueous solution of inorganic salts (g dm^{-3}): NaCl 30.0; $CaCl_2 \cdot 2H_2O$ 0.2; KCl 0.3;

$FeCl_2 \cdot nH_2O$ 0.01; KH_2PO_4 0.5; K_2HPO_4 1.0; $MgSO_4 \cdot 7H_2O$ 0.5; and NH_4Cl 1.0, pH 7.5] were used for the degradation experiments. For the aerobic experiment, each standard arsenical [arsenobetaine, $(CH_3)_3As^+CH_2COO^-$; trimethylarsine oxide (TMAO), $(CH_3)_3AsO$; dimethylarsinic acid (DMA), $(CH_3)_2AsO_2H$; disodium methanearsonate (MMA), $CH_3AsO(ONa)_2 \cdot 6H_2O$; disodium arsenate (arsenate), $Na_2HAsO_4 \cdot 7H_2O$; and arsenic trioxide (arsenite), As_2O_3 (all with 8.4 mg as As)] and sediment (1 g) was added to each medium (20 cm^3) in a 30-cm^3 Erlenmeyer flask. The flasks were kept static at 25 °C in the dark for 13 weeks under an atmosphere of air. For anaerobic experiments, about 5 cm^3 of liquid paraffin was placed on the surface of each mixture. Mixtures autoclaved at 120 °C for 20 min served as controls for both aerobic and anaerobic experiments.

High-performance liquid chromatography (HPLC)

Filtered aliquots from the mixtures were withdrawn at intervals of a week and diluted with distilled water to 20 times their volume. Arsenic compounds in the diluted aliquots were fractionated with a high-performance liquid chromatograph CCPD 8000 series (Tosoh Co. Ltd) on a TSK gel ODS 120T column (4.6 mm × 250 mm; Tosoh Co. Ltd) with an 11.2 mmol dm^{-3} solution of sodium heptanesulphonate in water/acetonitrile/acetic acid (95:5:6, by vol.) as mobile phase[9] at a flow rate of 0.8 cm^3 min^{-1}. An aliquot of each fraction was injected into the graphite furnace atomic absorption spectrometer (GF AA) as described previously.[5]

Purification and identification of arsenic compounds as aerobic and anaerobic microbial degradation products

Each medium containing microbial degradation products of the added arsenical was centrifuged and the supernatant was applied to a Dowex 50 W-X2 (200–400 mesh) column 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.

The purified metabolite was chromatographed on a cellulose thin-layer plate (Funakoshi Yakuhin Co. Ltd, Avicel SF, 0.1 mm). $SnCl_2$/KI reagent[10] was used for the detection of the spot.

RESULTS

High-performance liquid chromatography

The time course patterns of the added arsenicals and their metabolites in the media are shown in Fig. 1 (aerobic) and Fig. 2 (anaerobic). Their disappearance or appearance was followed by HPLC of aliquots of the diluted media. We assigned the compound of which the HPLC retention time was 3–5 min as peak 1 (P-1), the 5–7 min peak as peak 2 (P-2), that of 8–10 min as peak 3 (P-3) and that of 13–15 min as peak 4 (P-4) in the figures. The retention times of P-2, P-3 and P-4 agreed with those of DMA, arsenobetaine and TMAO, respectively. The retention time of P-1 roughly agreed with that of arsenate, arsenite or MMA: the retention time of arsenate was 150–225 s, that of arsenite was 225–300 s and that of MMA was 200–275 s, so that these three compounds were difficult to separate from each other under the chromatographic conditions used. However, from these retention times, arsenate and arsenite could clearly be separated from each other if there was no MMA in the medium, and arsenate and MMA could also be separated from each other if there was no arsenite in the medium.

Aerobic microbial degradation of arsenicals

Under aerobic conditions, arsenobetaine was the only methylarsenical which was rapidly degraded. It was converted to several metabolites, TMAO, DMA and the less methylated arsenicals (P-1), successively. In contrast, the conversion of arsenicals other than arsenobetaine was observed only in three cases; for the inorganic medium/DMA, the ZoBell medium/arsenite and the ZoBell/TMAO mixtures. DMA in the inorganic salts medium was converted to less methylated arsenicals (P-1), and arsenite in the ZoBell medium was converted to arsenate. The conversion patterns of TMAO and its metabolite in the ZoBell/TMAO mixture were peculiar; DMA derived from TMAO began to reconvert to the original TMAO after 14 days of incubation, and TMAO was the only arsenical in the mixture after 35 days. The conversion observed in the ZoBell/arsenite mixture in which arsenite was converted to arsenate, was the only such case for inorganic arsenic under both conditions.

In order to prove the oxidation of arsenite to

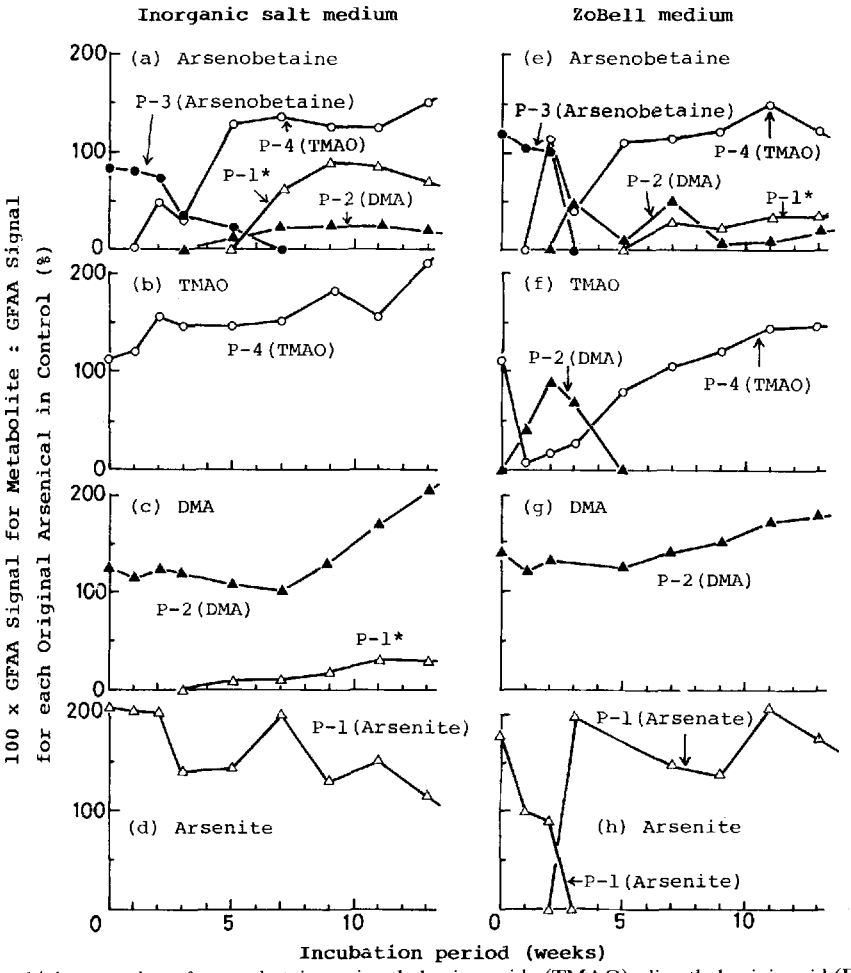

Figure 1 The microbial conversion of arsenobetaine, trimethylarsine oxide (TMAO), dimethylarsinic acid (DMA) and arsenite in an inorganic salts medium and a ZoBell medium under aerobic conditions. Methanearsonate (MMA) and arsenate were *not* converted in both media (not shown).
* MMA, arsenate and/or arsenite.

arsenate [Fig. 1(h)] to be not a chemical conversion but a microbial one, sediment was collected again in July 1989 from the same place, added to the ZoBell/arsenite mixture and incubated for 13 weeks under the same conditions. In this experiment, the mixture autoclaved at 120 °C for 20 min after the addition of the sediment was also kept at 25 °C, serving as a control. As a result, only the arsenite in the mixture which had not been autoclaved was converted to arsenate after 21 days: the conversion of arsenite to arsenate was therefore a microbial one.

In order to confirm the methylation of the DMA, degradation product of TMAO, the TMAO (P-4) derived from the DMA (P-2) in the ZoBell/TMAO mixture was subjected to purification and identification by TLC. After centrifugation of the mixture, the supernatant was subjected to cation-exchange chromatography (Dowex 50 W-X2; pyridinium form). Almost all the arsenic was eluted with 0.1 mol dm^{-3} aqueous pyridine and the arsenic-containing fractions were pooled and analysed by TLC. The purified arsenical gave a single spot positive to $SnCl_2$/KI reagent in some solvent systems, and as Table 1 snows, the R_f value of P-4 derived from DMA was the same as that of TMAO.

Anaerobic microbial degradation of arsenicals

As shown in Fig. 2, under aerobic conditions the opposite conversion pattern of arsenicals from that under aerobic conditions was observed:

either no (inorganic medium) or a little (ZoBell medium) arsenobetaine was converted to its metabolites, while all the methylarsenicals other than arsenobetaine were converted to less methylated compounds. TMAO was converted to DMA, DMA to less methylated arsenicals (P-1), and MMA to arsenate. Thus, every arsenical was cleaved by sedimentary micro-organisms to liberate methyl groups. The conversion of inorganic arsenic, arsenate and arsenite, was not observed in either of the media.

DISCUSSION

There was a clear difference in the conversion pattern of arsenicals between aerobic and anaerobic conditions. Under aerobic conditions, arsenobetaine was converted to its metabolites to a much greater extent than the other methylarsenicals, and vice versa under anaerobic conditions. Thus, the tendency that the extent of degradation of arsenobetaine is much greater in aerobic environments than in anaerobic ones was clearly demonstrated in this study. Aerobic environments may therefore be suitable for arsenobetaine to be acted on by micro-organisms. With regard to other arsenicals with methyl groups, TMAO, DMA and MMA, however, it was more difficult to understand in which environment it was more feasible for them to undergo degradation. For example, TMAO derived from arsenobetaine was relatively rapidly converted to DMA under aerobic conditions [Fig. 1(a)], while

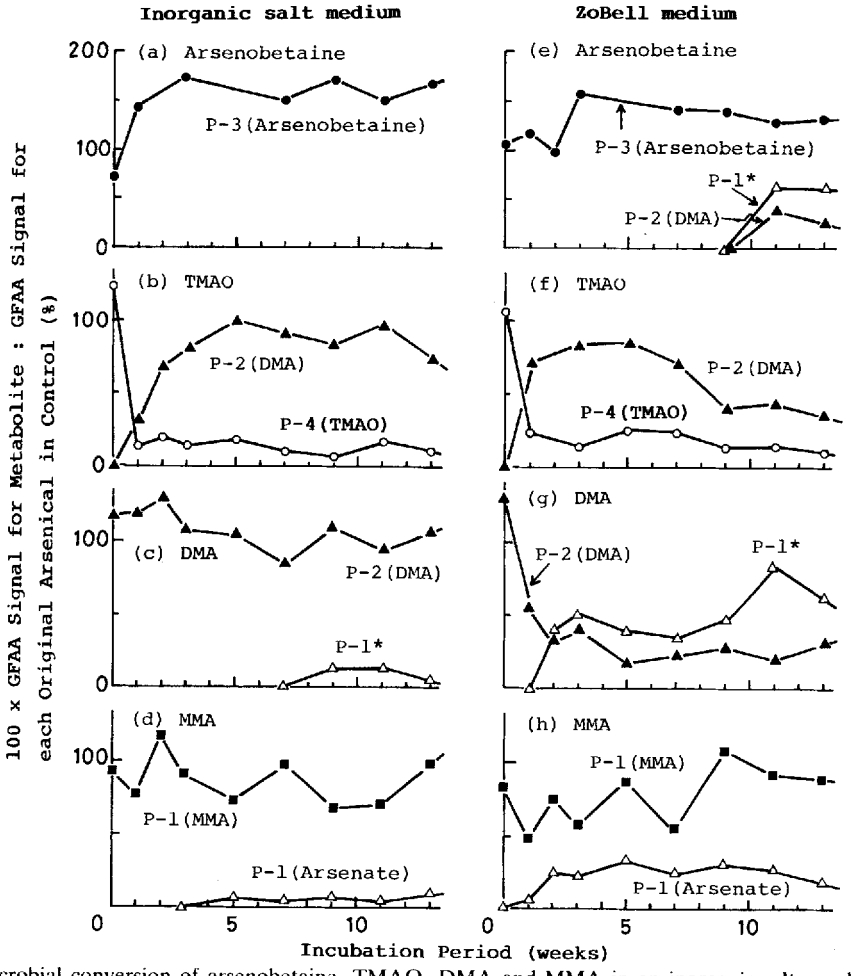

Figure 2 The microbial conversion of arsenobetaine, TMAO, DMA and MMA in an inorganic salts medium and a ZoBell medium under anaerobic conditions. Arsenite and arsenate were *not* converted in both media (not shown).
* MMA, arsenate and/or arsenite.

Table 1 R_f values in thin-layer chromatography of P-4 derived from DMA in the ZoBell/TMAO mixture.

	R_f value				
	Solvent system:[a]				
Sample	1	2	3	4	5
TMAO	0.83	0.85	0.59	0.44	0.63
P-4 derived from DMA	0.84	0.85	0.59	0.44	0.64

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

TMAO added to the media as a starting material was not converted to its metabolite under the same conditions [Fig. 1(b)]. On the whole, the methylarsenicals which were derived from arsenobetaine as metabolites under aerobic conditions [Fig. 1(a), (e)] and which were added to the media as starting materials under anaerobic conditions [Fig. 2(b)–(d), (f)–(h)] were rapidly cleaved with the liberation of methyl groups while those added to the media as starting materials under aerobic conditions [Fig. 1(b), (c), (f), (g)] were hard to cleave. In spite of these complications, however, these results were very interesting for considering the degradation or conversion of arsenicals in marine ecosystems. In marine environments), when dead animals, their residues or faeces are decayed by micro-organisms, the arsenobetaine contained in them is possibly converted to less methylated arsenicals mainly in water or on the bottom surface but not in the anaerobic interior of the sediment.

The phenomenon of reconversion of DMA derived from TMAO to the original TMAO [Fig. 1(f)] is not necessarily strange. The concept that arsenic metabolism by micro-organisms consists of degradations and syntheses of arsenicals is quite reasonable. We, however, have not previously observed these methylations *in vitro*. The degradation experiment, in which arsenobetaine was used as a starting material, may be unsuitable for a study to deal with the microbial methylation of arsenicals. In marine ecosystems, however, there may be various arsenic metabolites to be acted upon by various micro-organisms. Therefore, the present result *in vitro*, that the methylation of arsenicals is merely a minor phenomenon, might not be applicable to the field. At the present stage we only conclude that, in marine ecosystems, micro-organisms which have the ability to degrade the various arsenicals are ubiquitous both in aerobic and anaerobic environments.

CONCLUSIONS

The conversion pattern of arsenicals (arsenobetaine, trimethylarsine oxide, dimethylarsinic acid, methanearsonic acid, arsenate and arsenite) is different between aerobic and anaerobic conditions. Under aerobic conditions, arsenobetaine is converted to its metabolites to a much greater extent than the other methylarsenicals, and in anaerobic ones, vice versa. With the inorganic arsenicals, arsenate is derived from arsenite under aerobic conditions. Micro-organisms which have the ability to convert or degrade various arsenicals may be ubiquitous under both conditions.

Acknowledgements We thank Dr H Fujisawa, Dr M Murakami and Dr B Kimura, Shimonoseki University of Fisheries, for helpful microbiological suggestions. We also thank Mr H Koga and Mr K Onda, students of Shimonoseki University of Fisheries, for their technical assistance.

REFERENCES

1. Edmonds, J S, Francesconi, K A, Cannon, J R, Raston, C L, Skelton, B W and White, A H *Tetrahedron Lett.*, 1977, 18: 1543
2. Kurosawa, S, Yasuda, K, Taguchi, M, Yamazaki, S, Toda, S, Morita, M, Uehiro, T and Fuwa, K *Agric. Biol. Chem.*, 1980, 44: 1933
3. Edmonds, J S and Francesconi, K A *Mar. Pollut. Bull.*, 1981, 12: 92
4. Shiomi, K, Shinagawa, A, Yamanaka, H and Kikuchi, T *Nippon Suisan Gakkaishi*, 1983, 49: 79
5. Hanaoka, K, Yamamoto, H, Kawashima, K, Tagawa, S and Kaise, T *Appl. Organomet. Chem.*, 1988, 2: 371
6. Hanaoka, K, Matsumoto, T, Tagawa, S and Kaise, T *Chemosphere*, 1987, 16: 2545
7. Kaise, T, Hanaoka, K and Tagawa, S *Chemosphere*, 1987, 16: 2551
8. Hanaoka, K, Ueno, K, Tagawa, S and Kaise, T *Comp. Biochem. Physiol.*, 1989, 94B: 379
9. Stockton, R A and Irgolic, K J, *Environ. Anal. Chem.*, 1979, 6: 313
10. Tagawa, S *Nippon Suisan Gakkaishi*, 1980, 46: 1257