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SHORT PAPER

Metabolism of trimethylarsine oxide

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Trimethylarsine oxide [$(CH_3)_3AsO$] has been shown to be easily reducible by various biological species, including both aerobic and anaerobic micro-organisms, some skin organisms, soil bacteria, sludge and rumen fluid. The results suggest an enhanced mobility for arsenic owing to facile production of the volatile $(CH_3)_3As$ species.

Keywords: Trimethylarsine oxide, biological reduction, trimethylarsine, micro-organisms, methylation

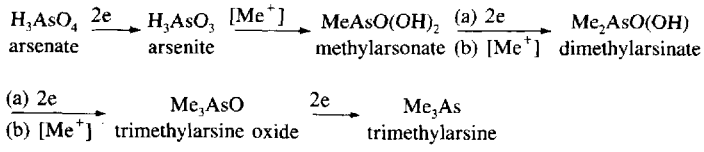
INTRODUCTION

Trimethylarsine oxide, the trimethylated As(V) species, has received scant attention as a possible intermediate in the biological transformation of arsenate to trimethylarsine species. Challenger[1] was the first to postulate that trimethylarsine oxide was the penultimate compound in trimethylarsine biosynthesis (Scheme 1).

Trimethylarsine oxide is found in low concentration in the muscle of plaice[2] and at higher concentration in estuary catfish[3] where it comprises 35% of the total arsenic burden. The major arsenical present in this fish and in most other marine animals is arsenobetaine.[4] The oxide is also found in school whiting which have been deliberately fed arsenate,[3] and it is suggested that the organoarsenical is produced by bacterial action on ingested arsenic compounds in the gut of the fish.

There are numerous reports of the presence of methylarsenicals such as $Me_2AsO(OH)$ derivatives in urine before, and following, the ingestion of arsenate by man,[5–11] dogs,[10,11] etc. The analysis is usually accomplished by using hydride generation techniques so that the species actually detected is Me_2AsH and the presence of $Me_2AsO(OH)$ is inferred, probably correctly, from its known property of reduction to Me_2AsH by borohydride. One study of this type reports the detection of trimethylarsine from human urine treated with borohydride.[5] Presumably this result indicates the presence of trimethylarsine oxide. By the same criterion, the arsine oxide is found in the urine of the rat, mouse and hamster after a single oral administration of arsenate (1 mg of arsenic kg^{-1} body weight);[5] all samples contained arsenate, methylarsanate and dimethylarsinate as well as the arsine oxide.

We have previously shown that cell extracts of the fungus *Candida humicola* produced trimethylarsine oxide from dimethylarsinic acid.[12] Subsequent studies indicated that *C. humicola* readily reduced trimethylarsine oxide to trimethylarsine,[13] and these results led us to investigate trimethylarsine oxide reduction in a variety of natural systems, and with a number of common micro-organisms. We now report that, unlike the preceding steps in trimethylarsine biosynthesis, the biological reduction of trimethylarsine oxide is rapid and seems to be a widespread phenomenon.



Scheme1

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METHODS

Organisms

Veillonella alcalescens, *Streptococcus sanguis* and *Fusobacterium nucleatum* were isolated from human dental plaque. A fluorescent pseudomonad was obtained from seawater. *Propionibacterium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *E scherichia coli* K12 were obtained from the Department of Microbiology's culture collection.

The skin isolates, *B. subtilis*, *E. coli* and the marine pseudomonad were grown in glucose broth (peptone, 5 g; beef extract, 3 g; sodium chloride, 5 g; $Na_2HPO_4.7H_2O$, 0.75 g; dextrose, 2 g; per litre water, pH 7). *S. sanguis* and *S. aureus* were grown in Trypticase–Soy broth (BBL). The media for *V. alcalescens* consisted of: trypticase, 5 g; yeast extract, 3 g; and sodium lactate, 25 cm^3; per litre of water, pH 7. *F. nucleatum* was grown in the medium described by Mayrand *et al.*[14]

Staphylococcus aureus was cultured both aerobically and anaerobically. *F. nucleatum* and *V. alcalescens* were cultured in an anaerobic chamber (Coy Manufacturing, Ann Arbor, Michigan) in a hydrogen:carbon dioxide: nitrogen (10:5:85) atmosphere. The remainder of the organisms were cultured aerobically. The incubation temperature during growth was 37 °C except for the marine pseudomonad which was grown at 20 °C.

Trimethylarsine reduction

The preparation of trimethylarsine oxide and the assay for the reduction of trimethylarsine oxide to trimethylarsine are described in detail elsewhere.[12]

Cells were harvested from a 24 h culture, washed with phosphate-buffered saline (PBS) (sodium chloride, 8 g; potassium chloride, 0.2 g; KH_2PO_4, 0.2 g; $Na_2HPO_4.7H_2O$, 2.17 g; per litre distilled water, pH 7) and resuspended in PBS at a cell concentration of 0.2 g wet weight cells cm^{-3}. A 1.0 cm^3 cell suspension was then incubated at 37 °C with trimethylarsine oxide (12 mmol dm^{-3}). Trimethylarsine in the headspace was determined by gas chromatography and in some cases by cautiously sniffing a sample of the head space. The latter is a sensitive qualitative test. The rate of production of trimethylarsine (nmol min^{-1} g^{-1} wet weight cells) was then calculated. Values are listed in Table 1.

Table 1 The rate of trimethylarsine production from trimethylarsine oxide

Organism	Rate of production[a] (nmol min^{-1} g wet cells)$^{-1}$
S1[c]	127
S2[c]	170
S6[c]	20
S. sanguis	10
Marine pseudomonad	585[b]
E. coli	100
F. nucleatum	3
V. alcalescens	142
B. subtilis	138
S. aureus (aerobic)	81
S. aureus (anaerobic)	208
S1 an[c]	3
S4 an[c]	5

[a] Determined at 37 °C unless otherwise indicated. [b] Determined at 20 °C. [c] S1, S2, S6 are aerobes and S1 an, S2 an are anaerobes isolated from human skin (identity unknown)

RESULTS

Of the organisms tested, the marine pseudomonad showed the highest rate of trimethylarsine oxide reduction (Table 1). More detailed experiments were performed with this organism in order to determine the optimum conditions for the reduction. Each of the assay parameters (temperature, pH, cell concentration and trimethylarsine oxide concentration) were varied in turn from standard values of temperature, 30 °C; pH 7.0; cell concentration, 0.04 g wet weight cells cm^{-3}; trimethylarsine oxide concentration, 10 mmol dm^{-3}.

Under the standard assay conditions the rate of production of trimethylarsine from the marine pseudomonad was usually between 500 and 600 nmol min^{-1} g^{-1} cells. Variations between different batches of cells sometimes caused deviations from this range; therefore for each parameter studied the same batch of cells was used.

At temperatures below 10 °C the rate of production of the arsine is low. Increasing the incubation temperature above 10 °C causes an increase in the rate up to a maximum of 4680 nmol min^{-1} g^{-1} at 40 °C. At higher temperatures the rate decreases and at 60 °C is only 400 nmol min^{-1} g^{-1}.

The pH profile of the reduction was broad, optimum activity of 520 units occurring over the range 6.5–7.5. At pH values 4.2 and 9.5 the activity was less than

10% of this value, and at pH > 10 no activity was detected.

Over the range of trimethylarsine oxide concentration, 0–0.24 mmol dm^{-3}, the rate of trimethylarsine formation is directly proportional to the trimethylarsine oxide concentration. Similarly, when expressed as nmol trimethylarsine min^{-1}, the rate of reduction is directly proportional to cell concentration.

Trimethylarsine oxide reduction in natural systems

The production of trimethylarsine from trimethylarsine oxide in several natural systems was studied. Trimethylarsine oxide was added to samples of fresh river water, sea sediment, soil, sewage sludge and rumen fluid. After incubation at appropriate temperatures the presence of trimethylarsine was determined using gas chromatography or by cautious sniffing.

Water collected from the surface of the Fraser River, British Columbia, Canada was mixed with trimethylarsine oxide (final concentration 10 mmol dm^{-3}) and incubated for 24 h at 25 °C. Head space analysis revealed 4.5 nmol of trimethylarsine per cm^3 had been produced in the head space.

Sea sediments

Sea sediments from different locations in Rupert Inlet, in Holberg Inlet and Quatsino Sound on the west coast of British Columbia (BC) were provided by Dr J.A.J. Thompson of the Institute of Ocean Sciences, Sidney, BC, Canada. Sediment (2 g) was placed in each of two 14 cm^3 vials, and trimethylarsine oxide added to a final concentration of 10 mmol dm^{-3}. The vials were sealed and one set of samples was incubated at 4 °C and another at 25 °C. Trimethylarsine production by all samples was too low to be detected by using gas chromatography. The presence of trimethylarsine detected by smell is shown in Table 2. Trimethylarsine was not detected in samples incubated at 4 °C. Sample F, which had produced noticeably more trimethylarsine after both 10 and 40 days, was of considerably higher organic content (5%) compared with other samples (A: 0.41% and B: 0.39%) for which these data were available.

Table 2 Production of trimethylarsine from sediments treated with trimethylarsine oxide[a]

Sample	Origin	Incubation for 10 days	Incubation for 40 days
A	Rupert Inlet	?	+
B	Rupert Inlet	?	+
C	Rupert Inlet	—	+
D	Holberg Inlet	?	?
E	Holberg Inlet	+	?
F	Quatsino Sound	++	++++

[a] —, Not detected; ?, uncertain; +, detectable with number indicating relative strength (Incubation at 25 °C).

Rumen fluid

Rumen fluid was obtained from a fistulated steer. The ability of this fluid to reduce trimethylarsine oxide to trimethylarsine was tested by incubating 1.0 cm^3 of the fluid with trimethylarsine oxide (to a final concentration of 10 mmol dm^{-3}) in a sealed 14-cm^3 vial at 37 °C. As well as the usual aerobic incubation system, an anaerobic system was set up in which a mixture of hydrogen (10%), carbon dioxide (5%) and nitrogen (85%) replaced head-space air. Trimethylarsine production was monitored by gas chromatography. The results are given in Table 3. Rumen fluid which had been boiled for 20 min prior to incubation with trimethylarsine oxide at 37 °C did not produce trimethylarsine after an aerobic 24-h incubation with the oxide at 36 °C. After 50 h, trimethylarsine was present at a level of 0.7 nmol cm^{-3} head space in this control.

Table 3 Production of trimethylarsine from rumen fluid treated with trimethylarsine oxide

	Trimethylarsine (nmol cm^{-3} head space)	
Incubation time (h)	Aerobic	Anaerobic
3	0	2.9
5	1.2	—
25	12.6	12.5
50	36.2	20.0

Sewage sludge

Sewage sludge (1 cm^3) was incubated at 25 °C with trimethylarsine oxide (final concentration, 10 mmol dm^{-3}) in a sealed 14 cm^3 vial. After three

days the odor of trimethylarsine was detected. When boiled sludge replaced fresh sludge no detectable trimethylarsine was produced from triemthylarsine oxide.

DISCUSSION

It can be concluded from these experiments that trimethylarsine oxide is readily reduced by a number of aerobic and anaerobic organisms. Some human skin organisms can do this reasonably rapidly and the odor of trimethylarsine becomes immediately apparent if some of the oxide is placed on the skin. Not surprisingly, this activity is reflected in samples obtained from natural sources; samples with intense biological activity, such as rumen fluid and sewage, rapidly reduced the oxide. Soil reduced trimethylarsine oxide but less actively than sludge. Marine sediments produced trimethylarsine only when the organic concentration reached 5% of the weight of the sample.

The speed with which trimethylarsine oxide is reduced by a variety of micro-organisms probably explains why it has not been found in many arsenic-containing systems. The rate-limiting step in the biological synthesis of trimethylarsine occurs prior to trimethylarsine oxide, thus ensuring that this product will not accumulate.

Trimethylarsine has been reported to be present in low concentration in prawns and some other food.[15] This could be formed by reduction of the oxide either chemically,[16–18] or by microbial action of the type described in this paper.

It has been suggested that the formation of the volatile trimethylarsine oxide by atmospheric oxidation of trimethylarsine constitutes a means of converting the toxic arsine to a less mobile and less toxic form.[19] However, present results do not support this and bring out the important principle that any methylation of arsenic enhances the mobility of this element because more organisms then seem to be able to transform the product further, finally producing volatile and mobile trimethylarsine.

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