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REVIEW

The origin of arsenobetaine in marine animals

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Trimethyl(carboxymethyl)arsonium zwitterion (arsenobetaine) is virtually ubiquitous in marine animals consumed by man. Experimental work on the transformation of arsenate to arsenobetaine in the marine environment is reviewed. Current evidence favors the conversion of arsenate to dimethyl(ribosyl)arsine oxides by algae, and the microbially mediated transformation of dimethyl(ribosyl)arsine oxides to arsenobetaine or to its immediate precursors in the sediments. Information about the transfer of arsenobetaine from the sediments to marine animals is lacking.

Keywords: Arsenobetaine, dimethyl(ribosyl)-arsine oxides, dimethyl(2-hydroxyethyl)arsine oxide, arsenate, trimethylarsine oxide, arsenic metabolism, marine animals

INTRODUCTION

The quaternary arsenic compound trimethyl (carboxymethyl)arsonium zwitterion [arsenobetaine, $(CH_3)_3As^+CH_2COO^-$] is virtually ubiquitous in marine animals, particularly in those contributing to the human diet.[1] The biosynthesis of arsenobetaine, which has to date been found only in marine animals, from the arsenate in seawater[2] is not fully understood.

ANIMALS

First, it is necessary to determine whether the animals are absorbing arsenic from ambient water by ingestion or during passage across the gills, or from the food. Several authors[3–5] have indicated that food rather than water is the source of arsenic. If arsenic is absorbed from the water, the arsenic could be in the form of arsenate (which is then converted to arsenobetaine in the animal body) or in the form of arsenobetaine or some organic precursor of arsenobetaine, in which case a very rapid flux of arsenobetaine through the water from its site of origin (possibly sediments) to animal tissue must exist, because such compounds have not been detected in seawater.[2,6] A rapid flux has been suggested to account for at least part of the methylmercury burden of fishes,[7] because methylmercury is found in seawater,[8] if at all, only in traces. The very fast absorption of arsenobetaine from spiked seawater by mussels, *Mytilus edulis*, might be taken as supporting evidence for such a pathway.[9] However, preliminary results[6] suggest that arsenobetaine is absorbed less readily by rock lobsters and fishes from spiked seawater.

Fishes exposed to arsenate do convert arsenate to organic arsenic compounds.[10–12] Penrose showed, however, that the compound synthesized by the brown trout, *Salmo trutta*, from arsenate was not the same as the compound (presumably arsenobetaine) present in witch flounder, *Glyptocephalus cynoglossus*. Oral administration of arsenate to the school whiting, *Sillago bassensis*, and the estuarine catfish, *Cnidoglanis macrocephalus*, led to an accumulation of trimethylarsine oxide in fish tissues and no detectable increase in the concentration of arsenobetaine. Very little arsenate was absorbed and converted to trimethylarsine oxide and little or no arsenate was retained.[12] As suggested by Penrose,[10] the gut flora of the fishes was probably responsible for the conversion. Interestingly, estuarine catfish contain trimethylarsine oxide as a 'natural' arsenic compound in addition to arsenobetaine.[12] This fish takes mouthfuls of sediment when seeking food and inevitably some of the sediment finds its way into the gut together with food organisms. Inorganic arsenic in the sediment may then be subjected to bacterial action in the fish gut. Trimethylarsine oxide was identified in four species of Baltic Sea fish by Norin *et al.*[13] These authors considered that the trimethylarsine oxide had been formed not from arsenate but by decomposition of arsenobetaine, because more

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trimethylarsine oxide was present in fish that had been kept frozen than in fresh fish. Corroborating evidence for the decomposition of arsenobetaine in frozen fish is not available.

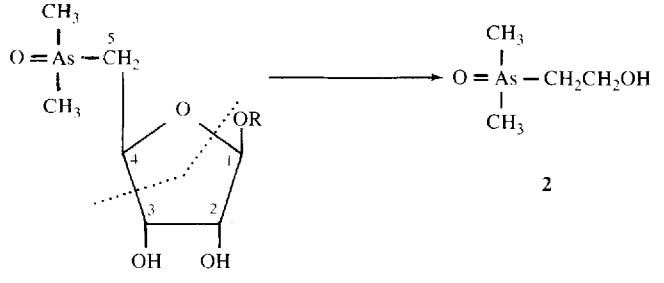
ALGAE

If arsenobetaine is not synthesized *de novo* by marine animals from ingested arsenate, then the transformation of arsenic at earlier stages of the food chain, perhaps facilitated by primary producers, must be responsible for the production of arsenobetaine. Marine algae do indeed contain substantial quantities of arsenic[1] in concentrations similar to those in marine animals. In general, the arsenic concentrations are higher in brown algae (10–40 mg kg^{-1} wet weight) than in red or green algae (1–12 mg kg^{-1} wet weight). Although some brown algae of the family Sargassaceae were reported to contain inorganic arsenic,[14–16] the bulk of the arsenic in algae appears to be in the form of dimethyl(5-ribosyl)arsine oxides (arsenoribosides) (Scheme 1), five of which (**1a–1e**) differing only in the aglycone moiety have so far been identified.[16–19] The arsenolipids reported[20,21] to be present in some algae may be derived from the arsenoriboside **1e** by acylation of the two free hydroxyl groups of the terminal glycerol residue.[17]

Arsenobetaine has not yet been identified in algae. Conversion of arsenoribosides to arsenobetaine requires the cleavage of the C_3–C_4 bond of the sugar ring (Scheme 1), oxidation of the $-CH_2OH$ group thus formed to a carboxyl group, reduction of the arsine oxide, and methylation of the resulting arsine (Scheme 2). Cooney and Benson reported[22] that organic arsenic compounds (probably arsenoribosides) biosynthesized by and contained in the unicellular alga *Dunaliella tertiolecta* were not metabolized to arsenobetaine by the American lobster *Homarus americanus*, although the native arsenic compound in *H. americanus* was shown to be arsenobetaine.[23] Studies by Klumpp and Peterson[20] of a short, macroalga-based food chain lacking a detrital stage also demonstrated the absence of arsenobetaine in snails feeding on *Fucus spiralis*, although the properties of the main arsenic compounds in the alga indicated[17] that arsenic may be present as arsenoribosides. These observations support the suggestion by Edmonds and Francesconi[24] that a microbially mediated stage, probably occurring within sediments, is necessary for the generation of arsenobetaine from arsenoribosides.

SEDIMENTS AND WATER

The transformations of arsenic compounds by microorganisms, particularly bacteria, in the marine en-



1 2

1a R = $-CH_2CH(OH)CH_2OH$

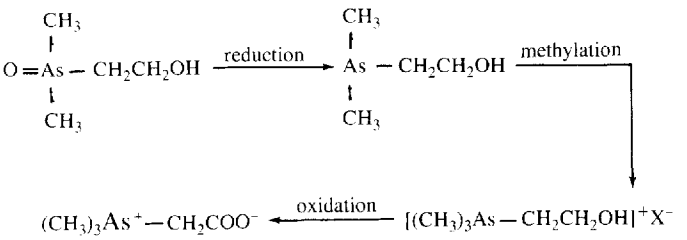
1b R = $-CH_2CH(OH)CH_2SO_3H$

1c R = $-CH_2CH(OH)CH_2OSO_3H$

1d R = $-CH_2CH(NH_2)CH_2SO_3H$

1e R = $-CH_2CH(OH)CH_2O\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}OCH_2CH(OH)CH_2OH$

Scheme 1



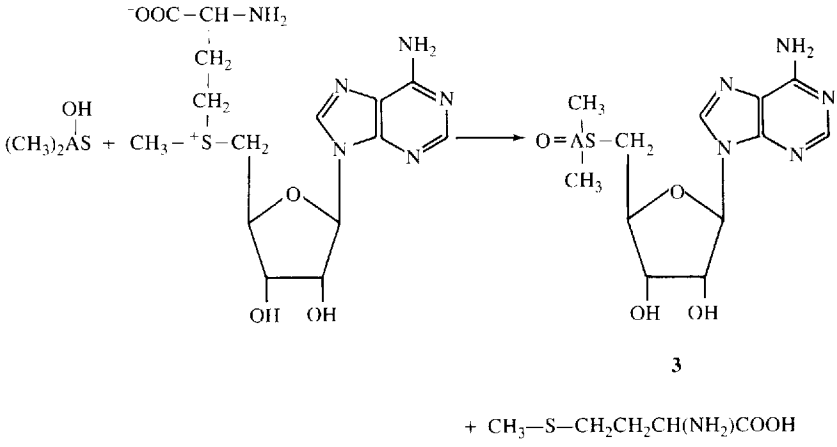
Scheme 2

vironment have been explored by several investigators. Although arsenate predominates in oxic waters, arsenite is always present at greater than thermodynamic equilibrium concentrations.[2,25,26] Microorganisms are known to transform arsenate in seawater to arsenite and simple methylated arsenic compounds.[27–29] Microbes also play a role in the demethylation and oxidation of methylated arsenic compounds in seawater.[30,31] Thus, all reactions in the sequence arsenate ⇌ arsenite ⇌ $CH_3AsO(OH)_2$ ⇌ $(CH_3)_2AsO_2H$ have been shown to involve biological mediation.

It is not unreasonable, therefore, to consider the possibility that arsenobetaine is produced by bacterial action from arsenate in marine sediments or, less likely, in the water column. No evidence to support this suggestion is available. However, the formation of arsenobetaine in sediments by microbial transformation of algal arsenoribosides has received support from experimental work. When fragments of the brown kelp *Ecklonia radiata* were allowed to decompose anaerobically in the presence of seawater and beach sand, the algal arsenoribosides were quantitatively converted to dimethyl(2-hydroxyethyl)arsine oxide **2** (Scheme 1). This compound could occupy a key position in the biosynthesis of arsenobetaine.[32] Although dimethyl(2-hydroxyethyl)arsine oxide has not yet been identified in the natural environment, the compound is simply and speedily produced in the laboratory under conditions much like those found in anaerobic sediments and beach deposits of kelp. The conversion of the arsine oxide to arsenobetaine (Scheme 2) by a similar replication of natural conditions has yet to be demonstrated in the laboratory. Thus it is still not known whether the conversion of dimethyl(2-hydroxyethyl)arsine oxide to arsenobetaine occurs in sediments with the arsenobetaine thus formed becoming available to the food web through detritovores or whether dimethyl(2-hydroxyethyl)arsine oxide is released into the water column, absorbed by marine animals, and then rapidly converted to arsenobetaine.

BIOSYNTHESIS OF ARSENOBETAINE

The most plausible route to arsenobetaine has algal arsenoribosides and their microbial breakdown product, dimethyl(2-hydroxyethyl)arsine oxide, as intermediates. Any discussion of the origin of arsenobetaine must consider the processing of arsenate by algae and the biosynthesis of the arsenoribosides. It is tempting to look to the biochemistry of metabolically important neighbors of arsenic in the Periodic Table, such as phosphorus and nitrogen, for suggestions concerning the transformation of arsenic compounds in algae. It has been suggested[33] that arsenate is taken up by cells because of its similarity to the essential phosphate. Competitive uptake with phosphate is claimed[34] to lead to arsenate toxicoses in phytoplankton at arsenate concentrations only a little above ambient. On the other hand, independent mechanisms for arsenate and phosphate absorption were demonstrated for phytoplankton[35] and macroalgae[36] at close to normal concentrations. However, the chemical structure of arsenobetaine suggests that arsenic metabolism may parallel nitrogen rather than phosphorus metabolism with the possibility that arsenobetaine may arise by a pathway analogous to that for glycine or betaine, or at least that arsenobetaine may be formed by oxidation of arsenocholine, which may be produced analogously to choline. Such an idea was used by Phillips and Depledge[37] to account for the formation of arsenoribosides and arsenobetaine. These authors employ as their starting compound arsenoethanolamine (2-hydroxyethylarsine) without suggesting how it is derived. It seems most unlikely that arsenic analogues of ethanolamine, glycine or serine exist, even transiently, in living cells. It is more likely that the algal arsenoribosides are biosynthesized by the mechanisms outlined initially by Challenger[38,39] for the methylation of inorganic arsenic by micro-organisms. Repeated reduction and methylation by *S*-adenosylmethionine[40] converts arsenate to methylarsonic acid and then to dimethylarsinic acid. Aspects of this postulated



3

+ CH_3—S—$CH_2CH_2CH(NH_2)COOH$

Scheme 3

mechanism were recently refined by Cullen *et al.*[41,42] However, the reduction of dimethylarsinic acid and the methylation of the resulting methylarsenic compound containing trivalent arsenic to a trimethylarsine derivative observed with micro-organisms does not occur in the biosynthesis of arsenoribosides; instead, the adenosyl group of *S*-adenosylmethionine is transferred to the trivalent arsenic compound (Scheme 3). The key intermediate would thus be **3**, which has yet to be detected in algae. Hydrolytic removal of the adenine residue followed by glycosylation of available algal metabolites would then give rise to the arsenoribosides **1a**–**1e**.

The conversion of dimethyl(2-hydroxyethyl)arsine oxide (**2**) to arsenobetaine requires reduction followed by methylation to a quaternary arsenic compound. It is interesting to note that quaternary arsonium compounds (tetra-alkylarsonium compounds) were not observed as metabolites after administration of inorganic arsenic to micro-organisms,[38,39] animals[43,44] or man.[45] Challenger's and subsequent studies[46] suggest that the conversion of trimethylarsine oxide to trimethylarsine is the final stage of the methylation pathway involving micro-organisms. Most mammals respond to administered inorganic arsenic by methylation, with methylarsonic acid or dimethylarsinic acid as the end products. However, several reports[47,48] claim the production of trimethylarsine oxide from dimethylarsinic acid administered to mice and hamsters. Alkylation does not appear to proceed beyond the trialkyl stage in marine algae.

Cullen *et al.*[41,42] considered the mechanism of the reduction of pentavalent arsenic compounds by micro-organisms, i.e. the source of the electron pair at each reduction step. In the presence of an excess of thiol groups, the end-product of each reduction is a trivalent arsenic species bonded to a thiolate. Trimethylarsine cannot react with thiol groups. If oxidative methylation under enzymatic control occurs in cell membranes at specific sites, at which the trivalent arsenic compound must be held by interaction with a thiol group, trimethylarsine cannot be held, will be released, and will not be available for further methylation. Certainly, there is no chemical reason why trimethylarsine should not be further methylated to the tetramethylarsonium ion. The failure to observe quaternary arsonium compounds after the administration of inorganic arsenic to a range of organisms makes if difficult to explain the ubiquity of arsenobetaine in marine animals. Recently, tetramethylarsonium ion was identified as a natural product in bivalve[49,50] and gastropod[51] molluscs and in a holothurian.[50] As yet no suggestions about the formation and source of this quaternary arsenic compound have been published.

Trimethyl(ribosyl)arsonium compounds have not yet been detected in the few species of algae examined. However, such arsenic compounds may exist in algae at very low concentrations and may decompose to arsenocholine in a reaction anologous to the decomposition of dimethyl(ribosyl)arsine oxide (Scheme 1). Arsenocholine could then be oxidized to arsenobetaine. For trimethyl(ribosyl)arsonium compounds to account for the universal predominance of arsenobetaine requires a very high degree of selectivity favoring the passage and accumulation of breakdown products of ribosylarsonium compounds through the food web. For example, the coastal ecosystem of Western Australia supporting the

arsenobetaine-containing rock lobster[52] and school whiting[53] has as its major primary producers *Ecklonia radiata* and *Sargassum* sp. These brown algae contain dimethyl(ribosyl)arsine oxides[17,54] but not trimethyl(ribosyl)arsonium compounds in detectable amounts. Consequently, if arsenobetaine is indeed derived from algal ribosylarsine oxides, then the addition of a third methyl group to dimethyl(ribosyl)arsine oxide is more likely than the selective accumulation of decomposition products derived from undetectably low levels of trimethyl(ribosyl)arsonium compounds.

Finally, it is interesting to consider whether arsenobetaine is completely retained by marine animals, or whether the observed arsenobetaine concentrations are a consequence of absorption and excretion rates. No function has been suggested for arsenobetaine, but it may serve as an adventitiously utilized and unimportant osmolyte.[55] If arsenobetaine is excreted by marine animals, it may be eliminated unchanged or as a metabolite (tetramethylarsonium ion, trimethylarsine oxide). No results have been reported on this aspect of arsenobetaine metabolism.

SUMMARY

The dimethyl(ribosyl)arsine oxides present in marine algae and dimethyl(2-hydroxyethyl)arsine oxide, their anaerobic degradation product, are the most likely candidates as intermediates for the production of arsenobetaine from oceanic arsenate in marine food webs. Several important questions must be answered to explain the pathway in its entirety. The biosynthesis of arsenobetaine by marine animals or by microbial activity without the intervention of algae is currently not supported by experimental evidence.

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