Applied Organometallic Chemistry (1988) 2 349–352

0268-2605/88/02409349/$03.50

SHORT PAPER

Arsenate metabolism in aquatic plants

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Received 9 February 1988 *Accepted 26 April 1988*

Of the several types of arsenic metabolic pathways in algae and aquatic higher plants, production of arsenoribosides is predominant and most interesting. Insertion of the ribosyl or adenosyl moiety by transfer from *S*-adenosylmethionine or possibly from adenosylcobalamin is the critical biochemical step which, as yet, has not been experimentally demonstrated. Oceanic or other environmental arsenate (AsO_4^{3-}), absorbed in the plant's quest for phosphate, is fixed by reaction with ATP to yield the phosphoric arsenic anhydride APAs, a short-lived but reducible intermediate which is converted to arsenic(III). The Hill Reaction, or thioredoxin reductase, reduces it to an arsine oxide (HAsO) or arsonous acid [$HAs(OH)_2$] depending upon the water content of its environment. This readily diffusible reagent avidly attacks sulfhydryl groups of proteins to produce arsonous thioesters. The *Sargassum* group of algae appears to process arsenate no further than this. The reduced arsenic may be freed from its sulfur bondage by reaction with 2,3-dimercaptopropan-1-ol (BAL) or dithiothreitol. In experiments with *Sargassum fluitans* and *Sargassum natans* no arsenoribosides were observed. Only protein-bound arsenic was observed. It could be liberated by trituration with dithiothreitol to produce the cyclic arsonous dithioester.

Most diatoms, dinoflagellates and macroalgae as well as freshwater higher plants release such protein-bound arsenic as a result of sequential methylation and adenosylation. Ultimately the products are trialkylarsine oxides, innocuous substances which are slowly or not-at-all metabolized by herbivorous animals or bacteria. Fortunately mammals and most animals also excrete the arsenoribosides readily, unchanged. Arsenic metabolism by a cyanobacterium, *Phormidium* sp., was described by Matsuto *et al.* (*Comp. Biochem. Physiol.*, 1984, 78c:377) as involving two modes of arsenate fixation, reduction, and excretion. We have extended those experiments with *Phormidium persicinum*. We have analyzed algae from 2- and 7-day culture in radioarsenate media. The arsenic products included 80% of arsenolipid, similar if not identical to that formed in the brown algae. The water-soluble products were in low concentration. Insoluble, protein-bound arsonous thioesters accounted for 8% of the fixed arsenic. The mechanism of arsenic depuration in *Phormidium* appears to be primarily lipid-mediated.

Keywords: Arsenic, *Phormidium*, arsenolipid, arsenic metabolism

INTRODUCTION

Absorption of arsenate by aquatic plants assumes importance as the phosphate of their media is depleted. Such depletion occurs in illuminated surface waters of much of the ocean where both ions are absorbed in the plant's quest for phosphate. Once absorbed, the chemical differences between phosphate and arsenate reveal themselves and the plant must adapt or die. Fortunately the problem was solved by cyanobacteria two billion years ago and now all aquatic plants (those having no roots in mineral-rich sediments) possess the metabolic skills for dealing with this problem.

Arsenate differs from phosphate in two important aspects. Its esters are much less stable than those of phosphate, and biological reducing agents can reduce arsenate whilst reduction of phosphate is not possible in such systems.

In studies of arsenate metabolism a primary reaction with adenosine triphosphate (ATP) is shown to yield the unstable phosphoric arsenic anhydride, APAs,[1] whose brief lifetime restricts its metabolic capabilities. Reduction of this evanescent inter-

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$$[\text{Protein}]\text{—SH} + \text{HAs(OH)}_2 \rightarrow [\text{Protein}]\text{—S—}\overset{\overset{\text{H}}{|}}{\text{As}}\text{—OH} + \text{H}_2\text{O} \quad [1]$$

mediate by adenylyl sulfate reductase, or possibly a more specific enzyme,[2] analogously yields adenylyl arsenite. Reductions of arsenite and of methane arsonate to arsonous acid or methylarsonous acid (arsine oxide or methylarsine oxide) proceeds in the presence of chloroplasts in the light (Hill Reaction). The oxidation state of arsenic in these oxides is +1.

The arsonous acids or their dehydrated forms, the arsine oxides, react avidly with accessible —SH groups to form arsonous thioesters (Eqn[1]). The nature of the thiol determines the stability and exchange rates of such esters. Natural laboratory rat hemoglobin, for example, contains thioarsonous esters of CYS 93 on up to 4% of its molecules.[3] Within the erythrocyte these esters exchange (migrate), and only small quantities escape from the cell. Such monothioesters of arsonous acid react further and, with a second thiol, form arsonous dithiol esters which are also exchangeable with free thiols. Dithiols with proper spatial relationships, such as lipoic acid, 2,3-dimercaptopropan-1-ol (BAL) or dithiothreitol, form much more stable and difficulty-exchangeable arsonous dithiol esters. Thus, dithiothreitol removes bound arsonous acids from monothiol ester linkages on algal proteins to produce its soluble cyclic arsonous dithiol ester. We have utilized such an extraction and identification of the cyclic diester to characterize the protein-bound arsonous thioester content of algae. The *Sargassum* group, including *S.hizikia*, are such algae. Most diatoms and higher plant aquatic species accumulate little protein-bound arsenic.

When a dithiol of appropriate configuration is an integral part of an essential enzyme system, the metabolic plight of the organism is serious unless removal pathways for arsenic exist. Crucial dithiol enzymes with configurations able to form very stably bound arsonous acid include lipoamide dehydrogenase and thioredoxin reductase. Lipoamide dehydrogenase is required for the utilization of pyruvate in parasites living aerobically. For example, this enzyme system was, no doubt, the target for Ehrlich's Salvarsan. Without efficient lipoamide dehydrogenase, the trypanosome has no opportunity for survival. Now that we begin to understand the biochemical trajectory of Salvarsan it is conceivable that we can utilize the arsonous chemotherapeutic agents effectively.

Extensive studies of biological production of methylated arsenic have led to the impression that methylation is the primary reaction of arsenic. However, absorbed arsenate cannot be methylated until it is reduced and, once reduced to the trivalent state, it reacts immediately with accessible —SH groups of proteins. The arsonous monothiol esters so produced are then subject to methylation and/or adenosylation. The molecular guidance system controlling effective transport and reactive interaction of the arsenic remains unexplored. We suggest in this paper that reduction and probably protein binding of arsenic precedes its alkylation and ultimate release as an arsenoriboside.

It has long been recognized that certain oceanic algae require vitamin B_{12} (cobalamin) for growth. This fact alerted us to the possibility that both *S*-adenosylmethionine and methyl and/or adenosyl cobalamin are involved in release of arsenic from its protein bondage. Both should be involved. Alkylation by carbonium ions does not result in oxidation (valence increase) while alkylation by carbanions effectively reduces the receptor arsenic. The nature of the alkylated product requires two methylations and one adenosylation. The last is a heretofore-undemonstrated reaction. It was predicted by Gulio Cantoni,[4] discoverer of *S*-adenosylmethionine, but so far has not been demonstrated. Following adenosylation the adenosine must be replaced by a phosphatidylglycerol[5] to produce the arsenolipid occurring in most aquatic plants.

This process for detoxicating arsenic must have been developed at an early stage by the cyanobacteria and hence is not a newly acquired environmental adaptation. The genes for processing arsenic must then have been passed from one organism to another, resulting in the apparently universal capability for arsenic detoxication. In a classic research study, Matsuto *et al.*[6] examined the arsenic metabolic products of a species of *Phormidium* isolated from Suruga Bay, Japan, and noted for its tolerance to high concentrations of arsenic. Two modes of arsenate metabolism were revealed, one yielding insoluble bound arsenic and the other giving soluble metabolic products. We have extended the work in our laboratory using a related alga, *Phormidium persicinum*.

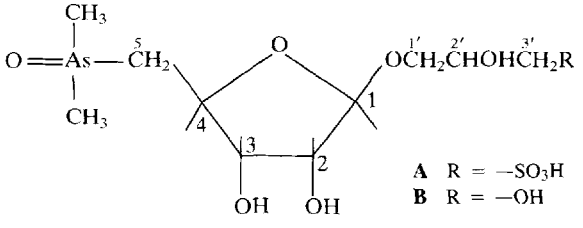
EXPERIMENTAL

To a culture of *Phormidium persicinum* in seawater medium was added 0.10 mCi of $^{74}AsO_4^{3-}$ and il-

lumination continued at the same temperature for 2–7 days. The cells were harvested by centrifugation and washed at 0°C with ice–water followed by rapid centrifugation. Methanol was added to the packed cells and warmed to 50°C prior to two-dimensional chromatography on Whatman No. 4 paper. The total suspension of cells in their extract was applied to the origin of the chromatogram, allowing subsequent measurement of insoluble arsenicals and the water- and lipid-soluble components.

RESULTS AND DISCUSSION

Arsenic metabolism in *Phormidium persicinum* appears similar to that in the brown algae. However, it is mediated by different compounds having similar physical properties. We have measured paper chromatographic R_f values and electrophoretic mobilities at pH 6.0 of the products of 2-day and 7-day metabolism of [^{74}As]arsenate in the light. Of these, 90% was arsenolipid and 8% was insoluble (protein-bound) arsenic for both samples. The soluble products were arsenate, arsenite (AsO_2^-) and methanearsonate ($CH_3AsO_3^{2-}$). Cacodylate [$(CH_3)_2AsO_2^-$] was not observed. *Phormidium* differed from most algae in that the amounts of the water-soluble **A** and **B** produced (Scheme 1), identified by Edmonds and Francesconi,[7] were very small compared with the arsenolipid. This is quite the opposite of the situation in diatoms where the amount of lipid is usually very small while that of the water-soluble derivatives is high.



Scheme 1

The lipid product was highly unsaturated as evidenced by its oxidative polymerization on the paper and consequent difficulty of elution. It was deacylated and the products chromatographed two-dimensionally on paper. The products of deacylation of the *Phormidium* lipid included five arsenical derivatives. The major (80%) product exhibited the R_f and electrophoretic mobility of **C**, the deacylation product of the ubiquitious diatom (etc.) lipid (**C** = arsenoribosylglycerophosphorylglycerol, Scheme 1, R = $-OP(O^-)(OH)OCH_2CH(OH)CH_2OH$. Some cleavage of this compound was revealed by the presence of 5% of **B** (arsenoribosylglycerol). Minor products were anionic with high chromatographic R_f values, similar to that of **B**. They have not been observed in the brown algae. One must conclude that the *Phormidium* lipid is, in the main part, identical to the widely recognized arsenolipid but it also contains some minor unknown components.

In the medium, recovered from the 7-day arsenate fixation, three compounds were observed in addition to residual [^{74}As]arsenate. Their electrophoretic mobilities and chromatographic positions suggested their identities as **A**, **B** and cacodylate. The slightly higher electrophoretic mobility of the **A** product (—41 versus —31) precludes confidence in the tentative identification prior to repetitive experiments.

CONCLUSIONS

These experiments with *Phormidium* offer further evidence for the importance of algal membrane lipids in arsenic detoxication. Our experiments with the diatom, *Chaetoceros gracilis*, revealed considerable transfer of membrane arsenolipid activity from one cell to another.[8] *Phormidium* could well be utilizing such a mechanism for transfer of its arsenic to other algae or bacteria. Future experiments with labeled *Phormidium* in diatom cultures, where centrifugal separation of the components is practical, could establish the reality of such a transfer process.

Another mode of arsenic excretion proposed earlier[9] involves transfer of the arsenolipid to the external lipid layer of the cell plasmalemma whereupon the hydrophilic arsenoribosyl moiety is exposed to the environment and degradative extracellular enzymes. The nature of many of the excretion products of diatoms is consistent with such a process.

Direct excretion of compound A, the arsenoribosylglycerol sulfate ester, by algae has been reported for diatoms.[10] It is clear that this process cannot occur to any important extent in cultures of *Phormidium persicinum*.

REFERENCES

1. Knowles, F C *Arch. Biochem. Biophys.*, 1986, 251:767
2. Muschinek, G, Alscher, R and Anderson, L E *J. Exptl Bot.*, 1987, 38:1069

3. Knowles, F C and Benson, A A *Trends Biochem. Sci.*, 1983, 8:178
4. Cantoni, G L Adenosylmethionine. In: *The Biochemistry of Adenosylmethionine*, Salvatore, F, Borek, E, Zappia, V, Williams-Ashman, H G and Schlenk, F (eds), Columbia University Press, New York, 1977, p 560
5. Benson, A A and Maruo, B *Biochim. Biophys. Acta*, 1958, 27:189
6. Matsuto, S, Kasuga, H, Okumoto, H and Takahashi, A *Comp. Biochem. Physiol.*, 1984, 78C:377
7. Edmonds, J and Francesconi, K A *Nature (London)*, 1981, 289:602
8. Katayama, M and Benson, A A *Int. Congr. Biochem. Abstr.*, 1985,
9. Benson, A A *J. Am. Oil Chem. Soc.*, 1987, 64:1309
10. Benson, A A Algal excretion of the arsenoribosylglycerol sulphate ester. In: *Proc. Conf. Biol. Alkylation of Heavy Elements*, Craig, P J and Glocking, F (eds.), R. Soc. Chemistry, London, 1988, pp 132 – 134