Arsenic Depuration *via* the *Tridacna* Gill Membrane

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Arsenate absorbed by zooxanthellae in *Tridacna* species is converted to water-soluble arsenical products which accumulate in the kidney with concentrations up to 2%. Kinetic uptake experiments with radioarsenate revealed rapid labeling in the algae and in the membrane lipids of the gills. Since the arsenic content of the gills is low, it is concluded that turnover is rapid and that the gills are the site of arsenic depuration in these clams. The amphipathic lipid nature of the gill arsenicals suggests membrane lipid mediation of the process.

Introduction

The tridacnid clams rely upon photosynthetic algal symbiosis for part of their energy requirement. Hence their gills probably absorb more phosphate in order to provide for their algae than do the gills of other bivalves. Comparable concentrations of arsenate in tropical surface waters lead to concomitant uptake of these isomorphous ions in the algal quest for phosphate. Arsenate is rapidly metabolized by a nearly universal mechanism in aquatic plants [1].

Arsenic concentrations of 2066 ppm in giant clam *(Tridacna maxima)* kidney [2] indicated presence of arsenical compounds at concentrations near 1 mM. The biological and chemical bases for this phenomenon are not yet clear, but its investigation has revealed what appears to be a novel and important avenue for arsenic excretion.

The arsenic content of most marine organisms is a hundredfold lower than that of *Tridacna* kidney. Gills of *Tridacna,* for instance, contained only 5 to 20 ppm arsenic [2]. Similarly, oceanic phytoplankton accumulated only 20−80 ppm arsenic in spite of their rapid uptake of radioarsenate *in vitro* [2]. It is concluded in this report that arsenic excretion, or depuration, mechanisms must function extremely rapidly in Nature.

Arsenate, absorbed with the isomorphous phosphate ion by algae in the low nutrient surface areas of the oceans, where both occur in concentrations near 2×10^{-8} molar, is rapidly detoxicated by reduction, methylation and ribosylation. The predominant arsenical products are 5-dimethylarsinoyl-5-deoxyribosides of glycerol and its 3-O-sulfate ester [3]. They are produced by all aquatic plants [1, 4] and not by animal tissues. It is these compounds which accumulate in the kidney of the giant clam. Very little arsenite, methanearsonate, cacodylate, or other toxic intermediates accumulate in these algae or in the clam tissues. Not yet understanding the reasons for failure of *Tridacna* kidneys to excrete these arsenicals which are readily released by mammalian kidneys, we have discerned what appears to be an important mechanism for excretion of compounds of this type by *Tridacna*. Such mechanism may function in algae and other organisms as well.

The initial study [2] implicated the gills as a major site of arsenic excretion by *Tridacna*. After 24 h in radioarsenate, equal activities of [74]As-arsenic were found in the kidney and gills even though the ratio of arsenic content in these organs is as high as 2066, 1004 ppm to 5.2 ppm. The observed transfer of algal radioarsenical products to gill tissue suggested a central role for the gill in arsenic depuration. Studies reported here provide further support for this mechanism in *Tridacna*.

Materials and Methods

Tridacna

Small specimens of *T. maxima* and *T. crocea* were collected on Davies Reef (18°51′S) in the Great Barrier Reef and maintained under diffused daylight in cascade aquaria with flowing coastal seawater. Small specimens were selected in order

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to limit the use of radioarsenate and its dilution. They were superficially cleaned of red calcareous algae with an electric rotary wire brush. The larger species, *T. gigas*, *T. derasa* and *Hippopus hippopus* function similarly in that their zooxanthellae produce the same products which accumulate in their kidneys.

Arsenate-[74]As uptake and products

One to three small (7–10 cm) *Tridacna* were maintained under diffused ambient light in 4-liter beakers containing 2 l of aerated filtered seawater. After 24 h, 0.1 to 0.4 mCi of "carrier-free" (<1 µg As/mCi) arsenate-[74]As was added to the medium at "$t = 0$" and air bubbling continued with adequate cheesecloth covering to reduce airborne contamination. Initial experiments revealed greater radioactivity fixed by the clam shell than by the animal. This was reduced by careful cleaning of the shells. Such mechanical cleaning, either by hand or by motor-driven wire brush, appeared to have little deleterious effect on the animal. The considerable variability in the observed radio-arsenic incorporation rates was a function of the individual animal's extension of its mantle and resumption of its normal photosynthetic metabolism and transport processes which was not possible to control.

Dissection

The radiolabeled clams were quickly rinsed in fresh seawater, chilled in freezing seawater, and dissected. Kidney, gills, mantle and adductor muscle were rinsed in ice water and placed in polystyrene tubes for radioactivity measurement in the gamma scintillation well counter.

Separation of products

Radiolabeled gill extracts (one-half of total gill radioactivity) were chromatographed two-dimensionally on Whatman No. 4 paper and radioautographs prepared. They indicated that the soluble components included equal activities of lipid and water-soluble compounds. TLC separation of the lipids on silica gel plates revealed at least six distinct arsenolipids, one of which (5%) corresponded to the chromatographic position of the algal arsenolipid [5], Fig. 1.
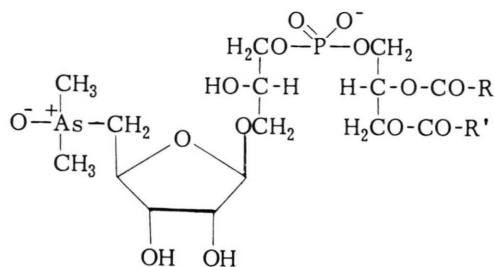

Fig. 1. Structure of the arsenolipid of aquatic plants, phosphatidylglycerol-[3-β-D-5'-dimethylarsinoyl-5'-deoxyriboside [5, 12].

In vitro tissue fixation of radioarsenate

Freshly dissected strips of mantle were incubated in light and dark with radioarsenate in seawater. The light fixation (128,000 cpm) was 2.4 times greater than the dark fixation (52,000 cpm). Similar fixation by an equal weight of fresh gill tissue was 39,000 cpm.

Radiophosphate fixation by Tridacna maxima

Small *T. maxima* were exposed 60 min and 22 h to radiophosphate-^{32}P followed by dissection and measurement of radioactivity, Table II.

Results

Radioarsenic contents of kidney and gill tissue and their ratios as a function of time of exposure of the animal to radioarsenate are presented in Table I. Fixation of radiophosphate, Table II was predominantly in the mantle after 60 min and only 20% greater in the mantle than in kidney or gills after 22 h.

Discussion

Arsenate uptake with production of apparently inocuous arsenoribosides occurs only in the algal zooxanthellae of the *Tridacna* mantle and in other such symbiotic systems. Animal tissues or bacteria have not been observed to produce such compounds. For this reason, the mantle tissue of the clam became labeled most rapidly in our experiments with either radioarsenate or radiophosphate. The photosynthetic energy of the zooxanthellae appears to be essential for uptake of phos-

Table I. Kinetics of arsenic incorporation in organs of *Tridacna maxima*.

Time of [74]As exposure and day	[74]As Incorporation (cpm × 10⁻³)				Gill/kidney ratios	
	Gills	Kidney	Mantle	Muscle	[74]As	Spec. act.[10]
15 min L	4.32	0.94	6.32	2.7	4.6	
35 min L[1]	14.8	2.72	8.05	–	5.5	
1 h L[2]	19.3	7.98	20.1	–	2.4	
3 h L[3]	26.6	31.2	204	11.6	0.85	
3 h L[4]	171	54.8	118	55.3	3.1	
10 h L–24 h DL[5]	32.7	117	14.6	–	0.28	
3 da LDLDL[6]	125	412	46.8	128	0.30	0.55
3 da LDLDL[7]	129	876	104	160	0.15	0.30
4 da LDLDLDL[8]	545	2350	1210	358	0.22	
6 da LD, 19 h rinse	2080	1400	1620	534	1.49	
Ultimate[9] (ppm As)	24.8, 5.2 ppm	1004 ppm	26 ppm	25 ppm	0.002	0.01

[1] Bright sunlight, radioarsenate added at 800 h; dissected at 835 h.
[2] Diffuse sunlight, 1300–1400 h.
[3] Same [74]As solution and conditions as 2; dissected at 1600 h.
[4] Diffuse sunlight, 1100–1400 h.
[5] Cloud-diffused bright light 700 h; three rinses filtered fresh seawater at 1700 h, remained over-night and dissected at 1700 h.
[6] Unbrushed clam placed in [74]As seawater at 1000 h; chilled and dissected at 1500 h.
[7] Fresh unbrushed clam, swabbed twice with seawater solution containing 5×10^{-4} M arsenate and 10^{-3} M phosphate with intermediate seawater rinse and maintained in fresh seawater 30 min before adding radioarsenate solution at 1700 h. After 15 h the arsenate-phosphate treated clamshell surface radiated 17 cps while the untreated control, 6, radiated >1000 cps. After 7 more h in light the two radiated 500 and 1020 cps, respectively. The highest surface activity corresponded to areas overgrown with red calcareous algae.
[8] Same beaker of labeled seawater as 3. Clam chilled and dissected at 1400 h.
[9] Based upon arsenic content of gill and kidney tissues, ppm dry weight.
[10] Corresponds to ratios of cpm/gram wet weight tissue for experiments where appropriate data were available.

Table II. Radiophosphate fixation by *Tridacna maxima* (cpm × 10⁻³).

Time of radiophosphate exposure	Gills	Kidney	Mantle
60 min L [32]P	590	150	7255
22 h LDL [32]P	382	452	501

phate and arsenate. The fact that the arsenicals accumulating in the *Tridacna* kidney are dimethyl-arsinoylribosides means that they are derived from the animal's zooxanthellae and not directly from the gills, the probable port of entry for arsenate and phosphate for the animal. The zooxanthellae lie in the mantle of the clam where they are ex-posed to ambient light. They lie in the "blood space" of the animal, and their excretion products, such as the arsenoribosylglycerol sulfate ester [6], are released to the circulation system. These, there-fore, have free access to both kidney and gill tis-sues. In short-term experiments radioarsenicals from the algae, or arsonous thiol esters [1] of ex-posed protein sulfhydryl groups produced by *in situ* reduction of absorbed arsenate rapidly accumulate in the gills. The activities recorded in Table I reveal very high gill activity in the briefer exposures to radioarsenate and diminished gill/kidney radioactivity ratios in the longer experi-ments. These whole organ activities varied as a result of differing size of the organ and state of de-velopment of the individual experimental animal. The ultimate gill/kidney arsenic ratio is that of the arsenic contents of the two organs, 0.005. From these radioarsenic values, the 5.47 gill/kidney ratio corresponds to a ratio of 2200 in the specific radio-activities of arsenic in the gill compared to that in the kidney at $t = 35$ min. The specific activity ratios in Table I are the ratios of radioactivity per unit wet weight of tissue. Rapid labeling of the gill tissues and their low total arsenic content leads to very high specific radioactivities (cpm/mol arseni-cal) and indicates fast turnover of arsenic in the gill.

The lipid solubility and chromatographic prop-erties of the rapidly metabolized soluble arsenicals

of gill tissue indicated that they are amphipathic membrane lipids like the phospholipids. As such, their hydrophilic moieties would be exposed to seawater where the dimethylarsinoylribosyl group may be cleaved oxidatively or by sulfurolysis to release the arsenoribosylglycerol sulfate ester [6] and other possible products to the sea. It appears that arsenic depuration in *Tridacna* and very possibly by algae and other organisms involves conversion of arsenical metabolites to amphipathic membrane lipids which may be exposed to seawater and released by extramural enzyme activities. Direct transfer of arsenolipid from algae to bacterial membranes has been observed [7] but there is yet no precedent for conversion of arsenoribosides to arsenolipids in animal membranes such as the gill. We have not determined that the amphipathic arsenolipids of the *Tridacna* gill actually possesses arsenoribosyl or related components. We are presuming that they are constructed from the predominant circulating water-soluble arsenicals excreted by the zooxanthellae or released from their storage reservoir in the kidney. One experiment, 5, Table I, intended to indicate rate of gill depuration was inconclusive because of its flush period being too long to preclude equilibration with the large kidney pools.

The kidney apparently provides a reservoir for arsenical compounds. The calcareous concretions of *Tridacna* and of other molluscan kidneys [8] may play a role in adsorption of arsenicals as they do with other heavy metal ionic materials [9]. One is reminded of the concentric calcareous concretion structure of the bezoar stones of Rennaissance pharmacy [10] which can absorb small amounts of arsenate and other substances [11].

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