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Transport of dissolved amino acids by the mussel, Mytilus edulis: Demonstration of net uptake from natural seawater

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Transport of Dissolved Amino Acids by the Mussel, *Mytilus edulis*: Demonstration of Net Uptake from Natural Seawater

Abstract. High-performance liquid chromatography provides direct evidence for substantial removal of naturally occurring specific amino acids during a single passage of water through the mantle cavity of mussels. This occurs during the few seconds required for passage of the water across the gill, and removal proceeds unabated at ambient substrate concentrations as low as 38 nanomoles per liter.

The total mass of organic material in solution in the oceans is vast, outweighing the total biomass on the earth (1). Marine biologists have speculated for more than a century that this very large potential resource may be available to marine organisms as a nutritional supplement (2). Demonstration of rapid influx of specific radiolabeled substrates into marine invertebrates (3) has kindled interest in this possibility and stimulated work in the field. Studies in the past two decades strongly support the widespread occurrence of uptake and utilization by marine organisms of elements of the dissolved organic pool (4). However, evidence for this conclusion is less direct than is desirable, and considerable controversy still exists about the role of dissolved organic material in invertebrate nutrition (5). Since concentrations of specific organic compounds in the natural pool are extremely low, there is no direct analytical evidence for net entry of organic substrates at the submicromolar levels characteristic of natural waters. Furthermore, it has been impossible to exclude completely a possible role of heterotrophic microorganisms, which have been present in the experimental systems studied to date (6). We report here that net entry of specific organic solutes from natural waters has been established by direct chemical analysis. A significant contribution to the process by contaminant microorganisms was ruled out by the very rapid changes in concentration, which occurred within seconds.

We prepared fluorescent derivatives of amino acids and other amines in solution with p-phthalaldialdehyde (OPA). Derivatives were separated by high-performance liquid chromatography (HPLC). Peaks were monitored with a fluorometer, identified by elution time, and quantified by peak area (7). The technique has a sensitivity in the picomole range. With a sample of 200 μl of seawater, a quantitative measure of specific amino acids present at concentrations as low as 10 nM can be obtained. The linearity of the relation between peak area and concentration was established by chromatography of standards ranging in concentration from 83.5 to 1000 nM. Coefficients of determination (r2) for the three amino acids used were aspartic acid (Asp), .99 (N = 19); serine (Ser), .99 (N = 21); and glycine (Gly), .98 (N = 21).

Specimens of the mussel *Mytilus edulis* were collected locally from Newport Bay in southern California, cleaned with a wire brush, and adapted to room temperature for 24 to 48 hours before use. An animal was placed in 400 ml of medium and allowed to open and begin pumping. The medium was withdrawn and replaced; normally, pumping resumed promptly. Samples were then collected directly from the excurrent siphon of *Mytilus* with a fine plastic cannula positioned with a micromanipulator. Such samples were compared with water samples from the incurrent margin of the animal. Since the animal was unrestrained and pumping normally, differences between incurrent and excurrent samples represented changes that occurred during a single passage of water through the mantle cavity. This procedure has been described in detail (9).

The first two tracings in Fig. 1 are chromatograms obtained from 200-μl samples of natural seawater taken respectively from the incurrent margin and the excurrent siphon of an animal as soon as possible after the onset of pumping. All chromatograms are photographs of original records. Fluorescent derivatives of aspartate, serine, and glycine are labeled. The water was collected from the immediate habitat of the animal, brought to the laboratory, and passed through a 0.2-μm Nuclepore filter to remove particulate matter and microorganisms. Analysis of a small sample of water filtered immediately in the field indicated that the brief period between collection of the water sample and filtration in the laboratory did not result in noticeable changes in amino acid composition. In the case presented in Fig. 1, 63, 84, and 72 percent of aspartate, serine, and glycine were removed by the animal during passage of the water through the mantle cavity.

Fig. 1. HPLC chromatograms of natural seawater and an artificial seawater solution. Tracing 1 is natural seawater collected from the immediate habitat of *Mytilus edulis*. Tracing 2 is the same water after passage through the mantle cavity of the animal. Tracing 3 is an artificial seawater solution containing 0.25 μmol per liter of Asp, Ser, Gly, and GABA. Tracing 4 is the artificial solution after passage through the mantle cavity of the animal. Peaks of the four amino acids are labeled. Peaks A, B, and C are material added by the animal during passage of water through the mantle cavity.
For further observations, a solution of these three amino acids and \( \gamma \)-aminobutyric acid (GABA) was prepared in artificial seawater. This was done to reduce the complexity of the initial medium and to avoid any ambiguity in identification of peaks. Each substrate was present in this artificial medium at an initial concentration of 0.25 \( \mu M \). The GABA was included as a control of the cannulation procedure since preliminary observations indicated that its rate of entry into the animal was considerably lower than that of the other amino acids and its OPA derivative was clearly separated from the other test substrates. Use of the artificial medium also facilitated detection and characterization of any OPA-reactive compounds liberated into the medium by the mussels. Previous work showed that influx of free amino acids is accompanied by liberation of fluorescent-positive material interpreted as unknown primary amines (10).

Tracings 3 and 4 in Fig. 1 are chromatograms of samples of the artificial medium, taken respectively from the incurrent margin and excurrent siphon of an actively pumping animal. This procedure was repeated several times. Percentages of the substrates removed during passage of water through the mantle cavity of four animals are shown in Table 1. In another experiment, concentrations of the substrates were compared in current and incurrent samples taken at 15-minute intervals after the onset of pumping. Figure 2 presents the data for aspartate. Notice that passage through the mantle cavity at 45 minutes after observations were initiated reduced the aspartate concentration from 38 to 12 nM. Thus 68 percent of the substrate was removed even at this very low ambient concentration. There was no discernible decrease in efficiency of removal as aspartate ambient concentration decreased from 250 nM down to the limit of resolution of the analytical procedure.

The peaks A, B, and C in the fourth tracing of Fig. 1 were present in all samples taken from the excurrent siphon of animals. They represent material released by the animal during passage of water through its mantle cavity. As expected, these peaks appear in the bulk medium in which the animal is placed and increase in height with the passage of time. Mytilus was placed in seawater that contained no organic substrate. Tracing 5 in Fig. 3 is a chromatogram of a sample taken 45 minutes after the animal began pumping. Appearance of these peaks occurs independent of substrate entry; tracing 6 in Fig. 3 is a chromatogram of the artificial medium 45 minutes after exposure to an animal. Peaks A, B, and C increase in size for the 5 hours during which observations were made; no other peaks appear during this time.

Chromatogram 7 in Fig. 3 was obtained by injecting a solution containing 4 nmoles of \( \text{NH}_4 \text{Cl} \) in 200 \( \mu l \) of artificial seawater into the HPLC. The presence of three peaks whose elution time corresponds to that of peaks A, B, and C strongly suggests that ammonia is the principal excretory product being added to the medium during passage through the animal (11). The possibility that some other material that coelutes with one of the ammonia peaks is being excreted is not excluded, but there is no evidence to support this.

The rapid removal of specific amino acid substrates observed in this work is consistent with reports of influx of \( ^{14} \text{C} \)-labeled amino acids and disappearance of fluorescamine-positive amines in mussels (10). Ammonia is a major excretory product of bivalves (12) and reacts to form fluorescent derivatives with both OPA and fluorescamine (13). Its appearance in the medium is consistent with the slow excretion of "unknown amines" previously reported. Rapid removal of amino acids from the medium is observed only if the animal is actively pumping. Close examination of scanning and transmission electron micrographs of Mytilus gill provides no morphological evidence of any bacterial association; autoradiograms of gills exposed for very brief periods to radiolabeled amino acids show intense labeling within the cells of the gill epithelium (14). Since dramatic decreases in concentration occur in a period of seconds during passage of water across the gill, no significant role can reasonably be assigned to heterotrophic microorganisms in removal of substrate in these observations.

Rapid removal of naturally occurring amino acids from seawater is not confined to Mytilus, nor is it limited to bivalve mollusks. Preliminary observations indicate that the ascidian Styela montereyensis removes half to two-thirds of specific amino acids from natural seawater during passage of the water through the branchial basket. The work reported here strongly supports inferences of a nutritional role of dissolved organic material in marine organisms based on less direct observations (2, 4). The use of HPLC separation combined with fluorescent detection of substrates

<table>
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<tr>
<th>Animal</th>
<th>Asp</th>
<th>Ser</th>
<th>Gly</th>
<th>GABA</th>
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Fig. 2. Removal of aspartate from an artificial seawater solution containing Asp, Ser, Gly, and GABA at an initial concentration of 0.25 \( \mu M \) for each substrate. 1 Samples of the medium taken from the incurrent margin of the animal; 2 samples taken with a fine plastic cannula from the excurrent siphon.

Fig. 3. HPLC chromatograms 5 and 6 are samples of the medium taken 45 minutes after the onset of pumping by an animal. Tracing 5 represents a medium of artificial seawater to which no organic substrate was added initially. Tracing 6 is artificial seawater containing Asp, Ser, Gly, and GABA (initial concentrations, 0.25 \( \mu M \)). The same animal was used to obtain chromatograms 5 and 6. Tracing 7 is the chromatogram obtained when 4 nmoles of \( \text{NH}_4 \text{Cl} \) in 200 \( \mu l \) of artificial seawater was derivatized and injected into the HPLC.
allows quantitative assessment of the input of specific solutes from the natural pool of dissolved organic material and should greatly facilitate assessment of the importance of this transport pathway in energy flow in marine communities.

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References and Notes
8. Separation was obtained on a 10-μm C18 column at a flow rate of 1.5 ml/min and a pressure of approximately 2000 pounds per square inch. Two eluent buffers were employed isocratically. The first was phosphate pH 5.0 mixed 1:2 by volume with methanol. The second was used 7 minutes after derivatization and 5 minutes after injection and consisted of phosphate pH 4.5 mixed 2:3 by volume with methanol. Analysis time was approximately 30 minutes. Peak areas were estimated by a digital integrator.
11. The reaction of OPA with ammonia appears not to have been studied. The product of OPA and primary amines is reported by S. S. Simons, Jr., and D. F. Johnson [J. Am. Chem. Soc. 99, 7098 (1976)] to be an isoindole. As a result of the additional N–H hydrogen, the reaction of NH3 with OPA would be expected to be more complex than the reaction with primary amines; more than one product is apparently produced.
14. Unpublished observations from our laboratory.
15. This work was supported in part by NSF grants PCM 78-09576 and OCE 78-09017.

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