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Free Amino Acids in Mantle Tissues of the Bivalve *Amblema plicata*: Possible Relation to Environmental Stress

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One problem in assessing pollutant effects on freshwater ecosystems is recognizing subtle signs of stress in perturbed systems. If contaminants or abnormal conditions affect homeostatic mechanisms that control concentrations of biochemicals in organisms, measurements of affected metabolites in normal and stressed organisms may provide a means for evaluating degraded aquatic habitats. Changes in amino acid ratios (e.g. glycine: taurine) and amounts of marine bivalves have been observed in response to a variety of pollutants (JEFFRIES 1972; BAYNE et al. 1976; ROESIJADI & ANDERSON 1979; ROESIJADI 1979). The changes of free amino acid patterns in marine bivalves resulting from environmental stresses led us to examine the hypothesis that free amino acid patterns in tissues of freshwater bivalves can reflect adverse conditions in stream habitats. Objectives of this investigation were to determine the composition and concentrations of free amino acids in mantle tissues of Amblema plicata and to examine this hypothesis for A. plicata in selected polluted and relatively unpolluted Missouri streams. This species was chosen because it is robust, long lived (up to 30 years), and widespread in clean and polluted streams of the United States (STANSBERRY 1971; BURCH 1975; IMLAY 1980). Mussels are attractive bioindicator organisms for freshwater ecosystems because they normally do not migrate extensively within or from their native streams (ISELY 1914; IMLAY 1980), and as filter feeders they are exposed to particulate components that can sorb toxic materials, as well as to dissolved substances in water (CHAISEMARTIN 1977).

EXPERIMENTAL

Specimens of A. plicata 6 to 14.5 cm long were sampled manually from four streams in Missouri. The mussels were frozen on dry ice at the sampling sites and stored at -7°C until analysis. Each was thawed individually under cold running tap water, the shell opened, and a mantle excised from one side with a scalpel. For Cedar Creek and Gasconade River mussels, both mantles were removed and analyzed and the results averaged. The mantle tissue was weighed and then homogenized with water (4:1 mL

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water:g wet wt) for 1.5 min with a Sorvall Omnimixer. A Teflon-glass tissue grinder accessory was used for mantles weighing less than 1 g and a steel blade attachment for larger tissues. Particulate material was removed by centrifugation at ambient temperature for 10 min at 240 g. Two mL of the supernatant were combined with 1.2 mL ethanol in another vial, to precipitate high molecular weight proteins (JEFFRIES 1972), and refrigerated. After 2 h, the ethanol-water solution was centrifuged for 10 min at 800 g and 1 mL of supernatant was fortified with 0.025 μmol norleucine as an internal standard and acidified with 1 drop of 6N HCl. A 5-μL sample of this solution was analyzed on an amino acid analyzer equipped with a fluorometric detector (HARE 1975).

Soft tissues and sections of shells of five mussels each from the Big and Bourbeuse Rivers were analyzed for lead, cadmium, zinc, and arsenic by atomic absorption. Analyses were done by Carborundum Co., Lexington, Massachusetts. Soft tissues that remained after the mantle was removed were freeze-dried and homogenized before analysis.

RESULTS AND DISCUSSION

During December 1978, mussels were collected from Cedar Creek (Callaway County) and Gasconade River (Maries County) in central Missouri. Cedar Creek has a history of exposure to acid coal mine drainage, which has detrimentally affected biota in the stream (DIEFFENBACH 1974; PARSONS 1977) and resulted in several fish kills during the periods following overflows from coal strip-mine lakes (CZARNEZKI 1979). In contrast, the Gasconade River is a clear, relatively unpolluted Ozark stream containing diverse biological populations (CLIFFORD 1966).

Free amino acid levels in mantle tissues were significantly ($P < 0.01$, Student's t test) higher in mussels from Cedar Creek than in those from Gasconade River (Table 1). Because of their large range in size (shell length, 6.0 - 14.5 cm; mean 10.4 cm), we examined Cedar Creek mussels for relations between free amino acid concentrations in the mantle and shell lengths. A significant negative linear correlation ($r = -0.859$, $P < 0.01$) described by the equation: μmol total free amino acids/g wet wt = 3.59 - (0.164) (shell length in cm) was observed for Cedar Creek mussels. Since Gasconade River mussels were smaller (shell length, 6.8 - 8.8 cm; mean 7.7 cm) than those from Cedar Creek, size-corrected amino acid concentration differences between the two groups of mussels would be even greater than indicated by the data in Table 1. The relative composition of amino acids differed less than total concentrations (Table 2). All SE ranges for individual amino acids from the two rivers overlapped, except for glutamic acid, which was higher in mussels from Cedar Creek than in those from Gasconade River; and aspartic acid, phenylalanine, and histidine, which were relatively lower in Cedar Creek mussels (Table 2). A compound eluting at the

Table 1. Total free amino acids (± SE) extracted from mantle tissues of Amblema plicata collected from four Missouri streams.

Type of pollution studied, month of specimen collection, and stream	Amino acids (μmol/g wet wt)		No. Samples
	Avg.	SE	
Coal acid mine drainage December 1978			
Cedar Creek	1.89**	0.24	8
Gasconade River (control)	0.59**	0.06	13
Lead mine tailings May 1979			
Big River	1.64*	0.14	12
Bourbeuse River (control)	1.24*	0.10	9

**$P < 0.01$
* $P < 0.05$, Student's *t* test on log-transformed data.

retention time of taurine (taurine RT) was found in all mussels examined, but its identity was not verified because it occurred at low levels and did not change in relative composition in stressed animals. Taurine occurs less commonly in freshwater invertebrates than in marine species (AWAPARA 1962). Likewise, relative levels of glycine did not appear to differ in mussels from the two rivers.

On the assumption that increased amino acid levels in mantle tissues of *A. plicata* from Cedar Creek over those from Gasconade River were due to stress from acid mine drainage, we hypothesized that free amino acid levels in mantle tissues of *A. plicata* may also be increased if the animals are exposed to other types of contaminants. As one test of this hypothesis, we sampled mussels in May 1979 from the Big and Bourbeuse Rivers, which are tributaries of the Meramec River in east central Missouri. The middle section of Big River is located adjacent to currently inactive lead mining sites. Lead mine tailings have been polluting the river ever since a large dike around a mine-settling basin failed in 1977-78 (WHITLEY 1979; BUCHANAN 1980). *A. plicata* was not found in the river immediately below the spill site, but was abundant about 50 km downstream, near House Springs, Missouri. The Bourbeuse River is not polluted with trace metals but is in an agricultural basin and carries more suspended material than do Ozark streams such as the Big and Gasconade Rivers (MISSOURI WATER POLLUTION BOARD 1964; CLIFFORD 1966). Mussels from the Big River had shell lengths

TABLE 2

Relative composition of free amino acids (mean mole percent of total ± SE) extracted from mantle tissues of Amblema plicata collected from four Missouri streams.

Amino Acid	Cedar Creek (N = 8)		Gasconade River (N = 13)		Big River (N = 12)		Bourbeuse River (N = 9)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Taurine RT	1.6	0.2	1.7	0.2	2.2	0.4	2.4	0.4
Aspartic acid	3.7	0.6	4.9*	0.4	3.4	0.4	3.3	0.2
Threonine	8.0	1.3	9.3	1.0	8.2	0.7	7.8	0.7
Serine	14.2	1.9	16.8	1.7	15.3	1.5	14.7	1.4
Glutamic acid	17.5	2.7	11.1*	1.0	16.0	1.3	15.2	1.4
Glycine	15.0	2.1	12.9	1.4	12.0	1.1	13.7	1.0
Alanine	17.3	1.9	15.3	1.7	15.2	1.2	17.8	1.8
Valine	5.9	0.8	7.2	0.8	6.3	0.6	6.3	0.5
Methionine	1.2	0.2	1.2	0.2	1.6*	0.2	1.1	0.2
Isoleucine	2.9	0.4	3.0	0.5	3.2	0.4	3.2	0.3
Leucine	3.2	0.5	3.0	0.5	3.7	0.5	3.5	0.4
Tyrosine	0.7	0.2	0.9	0.2	0.8	0.2	0.8	0.1
Phenylalanine	5.8*	0.6	7.5	0.7	8.9	1.0	7.4	0.5
Histidine	2.5	0.3	4.7*	0.5	2.5	0.2	2.2	0.2
Arginine	0.5	0.3	0.5	0.1	0.6	0.1	0.5	0.2

* Standard error ranges do not overlap with those for same amino acids in mussels from other rivers. Serine values include concentrations of glutamine and asparagine which coelute with serine on the amino acid analyzer. Samples were not analyzed for cystine, lysine, tryptophan, proline, or hydroxyproline.

of 11.0 ± 1.1 cm (mean ± SE) as compared with 10.0 ± 1.0 cm for those sampled from Bourbeuse River. Mussels from Big River had significantly ($P < 0.01$, Student's *t* test) higher levels of lead in both soft tissues [160 μg (g dry wt)$^{-1}$, SE = 59(5)] and shells [7.9 μg (g dry wt)$^{-1}$, SE = 0.8 (5)] than did specimens from the Bourbeuse River [soft tissues: 6.4 μg (g dry wt)$^{-1}$, SE = 0.2 (5); shells 2.9 μg (g dry wt)$^{-1}$, SE = 0.3(5)]. Cadmium levels were also elevated in soft tissues of Big River mussels [5.9 μg (g dry wt)$^{-1}$, SE = 0.3(5)] as compared to levels for Bourbeuse River mussels [0.72 μg (g dry wt)$^{-1}$, SE = 0.08(5)].

Significantly higher ($p < 0.05$, one-tailed Student's *t* test on log transformed data) free amino acid concentrations were found in the metal-contaminated Big River mussels than in the Bourbeuse River controls, but differences were not as pronounced as in the Cedar Creek-Gasconade River comparison (Table 1). Relative compositions of amino acids were similar in mussels from the two rivers; SE ranges overlapped for means of all the respective compounds except methionine, which appeared to be slightly higher in Big River mussels (Table 2).

Total concentrations (Table 1) and relative composition (Table 2) of free amino acids in mussel mantles from both the Big and Bourbeuse Rivers were more similar to those in Cedar Creek mussels than to those from the Gasconade River. A posteriori consideration of the control river suggests that the Bourbeuse River may have been a more stressed environment in spring than was the Gasconade in winter. Relative frequency of die-offs suggests that the reproductive period may be a more stressful season for Missouri mussels than other times of the year (A. Buchanan personal communication). Although we have no direct evidence for toxic organic contamination, the agricultural influence increases the possibility of exposure to pesticides in the Bourbeuse (SCHMITT & WINGER 1980).

In agreement with previous comparisons between marine and freshwater invetebrates (AWAPARA 1962; GARDNER & MILLER 1980), total levels of free amino acids in mantle tissues of *A. plicata* were substantially lower than have been reported for marine bivalves (100 to 600 μmol/g wet wt; JEFFRIES 1972; ROESIJADI & ANDERSON 1979). Glycine and taurine, which occur at high levels in marine invertebrates, were not elevated in *A. plicata* and did not show measurable compositional differences in animals from polluted and control streams. In contrast with marine bivalve studies in which stressed animals had lower concentrations of most amino acids than controls, *A. plicata* sampled from streams contaminated with acid mine drainage and trace metals had higher concentrations of free amino acids in their tissues than did those collected from control streams.

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