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Taurine and Glycine in the Gills of the Clam *Protothaca staminea* Exposed to Chlorinated Seawater

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The levels of free amino acids in the tissue of molluscs have been reported to fluctuate as a function of environmental stress (JEFFRIES 1972, BAYNE *et al.* 1976, ROESIJADI and ANDERSON in press). Particular attention has been paid to the molar ratio of taurine to glycine which JEFFRIES (1972) proposed as an index of stress. Taurine:glycine ratios have been reported to increase in clams and mussels subjected to environmentally or experimentally-induced stress. At the present time the biological basis for the increase in the ratio is not particularly well defined, as JEFFRIES (1972) reported an increase in taurine coincident with a decrease in glycine, while BAYNE *et al.* (1976) and ROESIJADI and ANDERSON (in press) found no change in taurine and a decrease in glycine. Observations by the latter investigators that the glycine changes were correlated with either a negative scope for growth (assimilated calories - respired calories) or a decline in dry weight condition index suggested that alterations in energy metabolism were involved.

In the present study, individuals of the clam *Protothaca staminea* were exposed to chlorinated seawater for 26 days and the free amino acid levels of the gill tissue were measured. This experiment represents yet another condition of stress and a fourth bivalve species with respect to the available literature on the taurine:glycine ratio in stressed molluscs. Chlorine is commonly used as either a biocide or disinfectant in coastally located electric power plants or domestic waste treatment systems, respectively, and has been reported to adversely impact marine organisms (CAPUZZO *et al.* 1976).

MATERIALS AND METHODS

Clams between 5.2 and 5.8 cm were collected from the intertidal zone at Sequim Bay, Washington, and held for 8 days in a flowing seawater system containing Crystal Amber #8 Aqua sand (Monterey Beach Co., Monterey, Ca.).

Exposures were conducted in 97ℓ aquaria constructed for

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laminar flow and containing Crystal Amber #8 Aqua sand 9 cm in depth for substrate. Continuous flows of sodium hypochlorite (Mallinkrodt) in deionized water solution and unfiltered Sequim Bay seawater were delivered to the tanks as described previously (ROESIJADI *et al.*, in press). Seawater flow was 1.5ℓ/min. and toxicant flow did not exceed 6.4 mℓ/min. Exposures were for 26 days.

Parameters measured routinely during the exposure for water quality included total residual oxidants (TRO), seawater and toxicant flow rates, ammonia, dissolved oxygen, temperature, salinity and pH. Analyses were conducted as already described (ROESIJADI *et al.*, in press). Values are summarized in Table 1.

At the end of exposure, clams were frozen whole at -80°C. After thawing at room temperature, gills were excised and free amino acids were analyzed as described in ROESIJADI and ANDERSON (in press) by AAA Laboratory, Mercer Island, Wa.

RESULTS AND DISCUSSION

One of the primary targets for the activity of oxidants such as chlorinated seawater on marine organisms most likely are exposed and sensitive body surfaces. Since gills of bivalves are directly in contact with the external environment and involved in essential processes such as gaseous and solute exchange and ciliary feeding, it was felt that gills would be a suitable organ for the study of the effects of chlorination. The higher levels of chlorination in this study (75 and 155 µg/ℓ TRO) have been shown to be acutely toxic to several marine species (THATCHER 1978).

The results of the present study demonstrated an alteration in the free glycine content in the gills of *Protothaca staminea* exposed to chlorinated seawater. Consistent with observations reported by BAYNE *et al.* (1976) and ROESIJADI and ANDERSON (in press), free glycine levels declined significantly ($p<0.05$) (Table 2). Mean values were 11.72 µM/g for controls (average of 0 da and 26 da values), 8.47 µM/g at 26 µg/ℓ TRO, 8.05 µM/g at 75 µg/ℓ TRO, and 5.92 µM/g at 155 µg/ℓ TRO. Taurine and total free amino acid concentrations and taurine:glycine ratios, however, were not found to be significantly different when 26 da control values were compared to values for exposed clams sampled at the same time.

Taurine was the most abundant free amino acid in clam gills, accounting for over 50% of the total free amino acid pool. It is interesting to note that taurine levels in gills of control clams sampled at 0 da, the day of initiation of

TABLE 1

TROs, applied chlorine concentrations, ammonia, dissolved oxygen, temperature, salinity and pH during exposure. Measurements were made on Mondays, Wednesdays and Fridays (n = 12) for all parameters. Flow rates were checked daily. ($\bar{x} \pm 1$ S.D.)

Parameter	Control	Treatment 1	Treatment 2	Treatment 3
TRO (µg/ℓ)	≤ 2	26 ± 6	75 ± 12	155 ± 18
Applied chlorine concentration (µg/ℓ)	-	129 ± 5	263 ± 8	465 ± 17
Ammonia (µg $N\text{-}NH_3$/ℓ)	23 ± 11	18 ± 6	18 ± 12	11 ± 5
Dissolved oxygen (µg/mℓ)	8.5 ± 0.2	8.5 ± 0.2	8.5 ± 0.1	8.5 ± 0.2
Temperature (°C)	10.2 ± 0.3	10.1 ± 0.4	10.0 ± 0.4	10.1 ± 0.4
Salinity (°/₀₀	30	30	30	30
pH	8.2	8.2	8.2	8.2

TABLE 2

Taurine, glycine and total free amino acid concentration (μMoles/gram wet weight) and taurine:glycine ratios in gills of *Protothaca staminea* (x ± 1 S.E.M., n = 5).

Amino Acids	0 da control	26 da control	26 μg/ℓ	75 μg/ℓ	155 μg/ℓ	f Sample
Glycine	11.98 ± 2.69	11.46 ± 1.88	8.47 ± 0.67	8.05 ± 1.40	5.92 ± 0.32	3.47*
Taurine	63.05 ± 5.96	34.01 ± 3.89	39.81 ± 7.52	40.79 ± 3.75	27.83 ± 3.28	1.49 n.s.
Total	94.20 ± 9.20	64.89 ± 4.80	77.97 ± 8.89	70.66 ± 5.34	55.35 ± 3.44	2.83 n.s.
Taurine: Glycine	5.80 ± 0.64	3.50 ± 1.12	4.69 ± 0.90	5.66 ± 1.05	4.65 ± 1.05	0.85 n.s.

*Significantly different at $p<0.05$; analysis of variance; comparisons made with 26 da control and treatment groups.

n.s. Differences not significant at $p<0.05$; analysis of variance; comparisons made with 26 da control and treatment groups.

exposure, and after 26 da were found to be reduced by 29 µM/g. The reduction of total free amino acids of 29 µM/g in the same controls was due to the decline in taurine. Thus, alterations in metabolism of controls during the exposure had a profound influence on gill taurine concentrations. Glycine did not show such changes in controls.

This study has provided additional evidence that significant declines in free glycine levels are probably correlated with stress in marine bivalves. In accordance with the studies of BAYNE *et al.* (1976) and ROESIJADI and ANDERSON (in press), taurine levels did not exhibit such a relationship. With the studies conducted to date, (JEFFRIES 1972, BAYNE *et al.* 1976, ROESIJADI and ANDERSON, in press; this paper), levels of free glycine have consistently been reduced in response to stress. It appears that understanding glycine metabolism may provide useful information regarding the nature of bivalve stress responses. The usefulness of the taurine:glycine ratio, however, was not evident in the present study.

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REFERENCES

BAYNE, G. L., D. R. LIVINGSTONE, M. N. MOORE, and J. WIDDOWS: Mar. Pollut. Bull. 7, 221 (1976).
CAPUZZO, J. M., S. A. LAWRENCE and J. A. DAVIDSON: Water Research 10, 1093 (1976).
JEFFRIES, H. P.: J. Invert. Pathol. 20, 242 (1972).
ROESIJADI, G. and J. W. ANDERSON: Marine Pollution: Functional Responses. Proceedings of symposium on Pollution and Physiology of Marine Organisms, Georgetown, S. C., Nov. 14-18, 1977. Academic Press, New York (in press 1978).
ROESIJADI, G., D. M. JACOBSEN, J. R. BRIDGE and E. A. CRECELIUS: Mar. Environ. Res. (in press 1978).
THATCHER, T. O.: In: Water Chlorination/Environmental Impact and Health Effects, 2, (ed. by R. L. Jolley, H. Gorcher, D. H. Hamilton, Jr.) Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, pg 341-350 (1978).