Bull. Environ. Contam. Toxicol. (1985) 35:810–815

Bulletin of Environmental Contamination and Toxicology

Free Amino Acid Pattern in Blue Mussel (*Mytilus edulis*) Exposed to Crude Oil

Juhani Soini and Pirjo Rantamäki

Department of Biochemistry, University of Turku, SF-20500, Turku, Finland

Levels of free amino acids in the tissues of aquatic organisms change under environmental stress (Jeffries 1972; Bayne et al. 1976; Roesijadi and Anderson 1979). In molluscs special attention has been paid to the molar taurine:glycine ratio. However, the results do not seem to be generally applicable (Jeffries 1972; Bayne et al. 1976; Roesijadi and Anderson 1979; Widdows et al. 1982). Less attention has been paid to the levels of other amino acids and to the metabolic background of the phenomenon. Roesijadi and Anderson (1979) found a significant decrease in the levels of arginine, lysine and threonine in Macoma inquinata exposed to marine sediments contaminated by oil. Lysine concentration decreased also in the mollusc Protothaca staminea exposed to crude oil. In this experiment also the concentrations of alanine, aspartic acid and glutamic acid decreased (Augenfeld et al. 1980-81).

In this study we investigated changes of free amino acid levels in different tissues of Mytilus edulis exposed to low concentrations of crude oil in a short term study. This investigation was accomplished in the northern area of the Baltic Sea, in the Archipelago of Turku. In this area the salinity of the sea water varies between 6-7 o/oo. Mytilus edulis lives there in its extreme conditions and it is therefore especially sensitive to disturbances in its environment. It is also known that salinity has effect on the content of free amino acid pools in molluscs (Bricteux-Gregoire et al. 1964; Gilles 1972). Therefore we wnted to clear out if the model of the changes of free amino acid levels in Mytilus edulis differs from those of mussels which live in the water of high or very fluctuating salinity.

MATERIALS AND METHODS

The blue mussels were collected at the end of January in an uncontaminated area (60° 7.1′N; 21° 49.0′E). After harvesting they were kept in the cold room at 5° in sea water (salinity 6.5 o/oo; pH 7.8). The water was continuously aerated. To diminish the effect of individual differences, 15 mussels of the same size (30 mm length) were selected. They were kept in 4 litres of sea water under slight aeration and without external food supply. The cultivation vessels were kept in an ice bath at 5°. In 48 hours experiments Russian crude oil was added to a concentration of 25 mg per litre. Thus, the con-

centration of dissolved oil was in the beginning of the experiment 0.7 ppm and at the end of the cultivation period 0.2 ppm. The amount of the dissolved fraction was determined with the method of Ahnhoff and Eklund (1977). In 240 hours experiments 20 mg of crude oil was added daily into the cultivation vessel. The volume of the sea water was kept constant by adding distilled water.

At the end of the exposure period, the gills and mantle were removed and cooled immediately in an ice bath. The gills and the mantles were freeze-dried separately and total dry weights were determined. 0.5 ml of 0.5 µM phenylmethylsulphonyl fluoride and 1 ml of LPF-DL buffer, pH 3.06 (Hitachi Ltd.) per 100 mg dry weight of lyophilisate were added. The tissue was homogenized in a Potter-Elvehjem homogenisator and heated in a boiling water bath for 10 min. The homogenate was centrifuged for 40 min 120 000 x g. The volume of the supernatant was measured. The free amino acids in the supernatant were quantified with a Hitachi Model KLA-5 amino acid analyzer by using the ligand physiological fluid analysis method (Hitachi Ltd.). Norleucine was used as an internal standard. Hamilton P-AN and P-B amino acid mixtures of known concentrations were used as standards.

The remaining pellet was used for total hydrolysis of tissue proteins in order to determine the composition of bound amino acids. The pellet was washed twice with distilled water, dried, and weighed. The protein hydrolysis was performed by using 6 N hydrochloric acid (FAO 1976). Tryptophan was determined according to Graham et al. (1947) and cysteine by the method of Schram et al. (1954). The amino acid content of the hydrolysate was determined on a Hitachi Model KLA-5 amino acid analyzer.

RESULTS AND DISCUSSION

During the exposure periods all the mussels were alive.

The free amino acids listed in Table 1 represent 94.1% of the total free amino acid content in the gill tissue and in Table 2 93.2% in the mantle tissue of Mytilus edulis calculated on the basis of 2 days control experiments. The total content of free amino acids in the gills was determined to be 140.1 µmol/g dry tissue, and in the mantle 104.3 µmol/g dry tissue. Large differences in total free amino acid contents can be noted between different marine organisms. It is also a known fact that the amounts of free amino acids are greater in organisms living in high salinity than in low salinity organisms. Thus, the free amino acid content in Mytilus edulis grown in the North Sea is ca. ten times as great as in our experiments (Widdows et al. 1982). The value in Amblema plicata is 0.59-1.24 µmol/g wet tissue (Gardner et al. 1981), and in Protothaca staminea 85.5 µmol/g dry weight (Augenfeld et al. 1980-81). Kluytmans et al. (1977) have shown that the free amino acid pool excluding ammonia in the adductor muscle in Mytilus edulis grown anaerobically in North Sea water is 500 µmol/g dry weight.

The results in Tables 1 and 2 show that the total amount of the 12 tested free amino acids increase during the oil treatment except in 2 days experiment in the mantle tissue. The E/C ratio in 2 days

experiment done with the gill tissue was 1.7 and in 20 days experiment 1.6. In the mantle tissue, the E/C ratio after 2 days was 1 and after 20 days 2. The increase of free amino acids after oil exposure suggests that proteolysis had occurred. As a comparison, the molar percentages of gill tissue amino acids after protein hydrolysis are presented in Table 3.

Table 1. Free amino acids, ammonia, and urea in the gills of Mytilus edulis grown in crude oil contaminated sea water for 2 and 20 days. Values are μmoles per gram of dry weight of tissue. C = control; E = exposed.

	2 days		20 days	
Amino acid	C	E	C	E
OH-LYS	7.7	0.1	7.9	3.0
LYS	0.9	1.1	0.1	59.2
ARG	2.8	1.7	6.3	5.6
THR	2.3	4.1	2.7	9.2
SER	2.8	6.8	6.9	9.9
ASX	13.4	3.3	21.9	12.4
GLX	20.0	45.2	28.4	24.0
PRO	4.9	2.0	4.6	1.9
GLY	2.0	14.2	28.0	36.9
ALA	42.6	57.7	40.0	37.0
TAU	26.6	47.3	37.9	45.9
ORN	6.4	41.5	13.5	83.4
Sum	132.4	225.0	198.2	328.4
NH_3	16.8	207.5	6.6	29.8
UREA	143.6	305.5	495.9	937.6

Table 2. Free amino acids, ammonia, and urea in the mantle of Mytilus edulis grown in crude oil contaminated sea water for 2 and 20 days. Values are μmoles per gram of dry weight of tissue. C = control; E = exposed.

	2 days		20 days	
Amino acid	C	E	C	E
OH-LYS	5.5	4.5	5.0	3.7
LYS	3.0	2.8	0.5	20.1
ARG	8.0	7.7	10.9	18.1
THR	2.3	1.2	3.8	10.0
SER	3.4	1.5	5.5	10.6
ASX	8.6	2.8	19.2	38.1
GLX	13.1	25.8	23.1	42.0
PRO	1.0	2.0	4.1	4.7
GLY	8.0	2.2	33.6	37.1
ALA	21.8	13.1	35.2	65.1
TAU	17.3	27.9	16.3	51.8
ORN	5.2	3.0	9.6	36.8
Sum	97.2	94.5	166.8	338.1
NH_3	11.2	25.7	14.3	35.4
UREA	233.8	321.4	191.7	463.2

Table 3. Amino acid content of proteins in the gill. Numbers are molar percentages of individual amino acids.

TRP	1.44	SER	5.29	CYS	1.63
HIS	0.24	GLX	10.22	MET	2.28
OH-LYS	0.36	OH-PRO	0.87	ILE	4.32
LYS	5.77	PRO	3.85	LEU	6.73
ARG	2.52	GLY	9.85	TYR	3.36
ASX	8.89	ALA	7.45	PHE	3.61
THR	5.29	VAL	9.13		

Varying results have been obtained in investigations of free amino acids in Mytilus edulis. Aarset and Zachariassen (1982) have shown that no changes occur in free amino acid concentrations in body fluid and muscle of mussels grown in oil-contaminated sea water. The studies of Widdows et al. (1982) indicate that low hydrocarbon concentrations caused no significant changes in serine and threonine concentrations. In some other marine bivalves, a chemical stress causes a significant decrease in free amino acid concentrations, e.g. in Macoma inquinata (Roesijadi and Anderson 1979) and Protothaca staminea (Augenfeld et al. 1980-81). However, in the case of a fresh water bivalve Amblema plicata, contrasting observations have been made (Gardner et al. 1981).

In several investigations concerning the effects of chemical stress on bivalves, the glycine content has been reported to decrease with time under stress e.g. (Jeffries 1972; Augenfeld et al. 1980-81). In our experiments, the glycine content in the mantle tissue (Table 2) decreased in the 2 days experiment but showed a slight increase in the 20 days experiment. A marked increase was found in the gill tissue (Table 1) in both 2 and 20 days experiments. It must be noted that the glycine content increased even in the control experiments. The taurine concentration increased markedly in the 2 and 20 days experiments both in the gill and mantle tissues. Similar observations have been made by Widdows et al. (1982). Jeffries (1972) has proposed that the molar ratio of taurine to glycine is an index of stress in bivalves. Several authors have reported the increased molar taurine:glycine ratios. In our experiments, an increased ratio can be seen in mantle tissue: in the 2 days experiment the taurine:glycine ratio was strongly enhanced.

Both in gill and mantle tissues the lysine concentrations were constant in 2 days experiments but were markedly elevated in 20 days experiments, whereas the hydroxylysine content decreased in both tissues. Thus the ratio lysine:hydroxylysine increased strongly in 20 days experiments. This increased ratio can be considered as a possible indicator of the presence of oil hydrocarbons in the environment.

The concentration of ornithine was clearly increased in both 2 and 20 days experiments in the gill tissue, and in 20 days experiments in the mantle tissue. The accumulation of ornithine might be due to inhibition of either carbamylphosphate synthetase or ornithine transcarbamylase. Another possibility is that hydrocarbons cause

an activation of ornithine cycle without affecting the activities of carbamylphosphate synthetase or ornithine transcarbamylase. There is a slight but insignificant decrease in arginine content in the gill tissue in the 2 and 20 days experiments and in the mantle tissue in the 2 days experiment. In the 20 days experiment the arginine content in the mantle tissue was clearly increased.

The presence of free ornithine and its increase reveals that ornithine cycle enzymes are present in gill and mantle tissues in Mytilus edulis. Andrews and Reid (1972) could not with certainty detect the ornithine cycle in mantle tissue of Mytilus edulis. In our experiments, the amount of free ornithine in the mantle tissue was increased in the 20 days experiment. Whether this is due to some kind of activation by hydrocarbons, remains to be studied more deeply by us.

In conclusion, it seems that the brackish water Mytilus edulis differs from commonly studied sea water mussels in some respects under oil exposure. One reason probably is that our test organism lives in its natural environment in extreme conditions (cold water, low salinity), and hence its enzyme systems are very sensitive to chemical changes in water. The results described above were similar in repeated experiments. The amino acid metabolism of this organism exposed to oil hydrocarbon stress seems to be a very promising area to study.

Acknowledgements. We thank the Archipelago Sea Research Institute of the University of Turku for the collection of the mussel specimens and the Science Research Council of Finland for the financial support of this work.

REFERENCES

Aarset AV, Zachariassen KE (1982) Effects of Oil Pollution on the Freezing Tolerance and Solute Concentration of the Blue Mussel Mytilus edulis. Marine Biology 72:45-51

Ahnhoff M, Eklund G (1977) Report on the Chemistry of Sea Water XX. Department of Analytical and Marine Chemistry, Chalmers University of Technology and University of Göteborg, Sweden

Andrews TR, Reid RGB (1972) Ornithine Cycle and Uricolytic Enzymes in Four Bivalve Molluscs. Comp Biochem Physiol 42B:475-491

Augenfeld JM, Anderson JW, Woodruff DL, Webster JL (1980-81) Effects of Prudhoe Bay Crude Oil-Contaminated Sediments on Protothaca staminea (Mollusca: Pelecypoda): Hydrocarbon Content, Condition Index, Free Amino Acid Level. Marine Environmental Research 4:135-143

Bayne BL, Livingstone DR, Moore MN, Widdows J (1976) A Cytochemical and a Biochemical Index of Stress in Mytilus edulis L. Marine Pollution Bulletin 7:221-224

Bricteux-Gregoire S, Duchateâu-Bosson G, Jeuniaux C, Florkin M (1964) Constituants osmotiquement actifs des muscles adducteurs de Mytilus edulis adaptée à l'eau de mer ou à l'eau saumâtre. Archives Internationales de Physiologie et de Biochemie 72:116-123. In: Bayne BL (ed) Marine Mussels Their Ecology and Physiology. Cambridge University Press, Cambridge, p. 241

FAO (1976) Nutritional Studies No. 24. Amino Acid Content of Foods and Biological Data of Proteins, 3d ed, Malta

Gardner WS, Miller III WH, Imlay MJ (1981) Free Amino Acids in Mantle Tissues of the Bivalve Amblema plicata: Possible Relation to Environmental Stress. Bull Environ Contam Toxicol 26:157-162

Gilles R (1972) Osmoregulation in Three Molluscs: Acanthochitona discrepans (Brown), Glycymeris glycymeris (L) and Mytilus edulis L. Biological Bulletin 142:25-35. Marine Biological Laboratory, Woods Hole, Mass. In: Bayne BL (ed) Marine Mussels Their Ecology and Physiology. Cambridge University Press, Cambridge, p 241

Graham CE, Smith EP, Hier SW, Klein D (1947) An Improved Method for the Determination of Tryptophane with p-Dimethylaminobenzaldehyde. J Biol Chem 168:711-716

Hitachi Ltd. Manual of Model KLA-5 Amino Acid Analyzer. Tokyo, Japan

Jeffries HP (1972) A Sress Syndrome in the Hard Clam Mercenaria mercenaria. J Invertebr Pathol 20:242-251

Kluytmans JH, de Bont AMT, Janus J, Wijsman TCM (1977) Time Dependent Changes and Tissue Specificities in the Accumulation of Anaerobic Sea Mussel Mytilus edulis L. Comp Biochem Physiol 58B:81-87

Roesijadi G, Anderson JW (1979) Condition Index and Free Amino Acid Content of Macoma inquinata Exposed to Oil-Contaminated Marine Sediments. In: Vernberg WB et al (eds) Marine Pollution: Functional Responses; Proceedings of the Symposium on Pollution and Physiology of Marine Organisms, Georgetown, SC, USA, Nov 14-17 1977. Academic Press, Inc New York, USA, 1979, pp 69-84

Schram E, Moore S, Bigwood E (1954) Chromatographic Determination of Cysteine as Cysteic Acid. Biochem J 57:33-37

Widdows J, Bakke T, Bayne BL, Donkin P, Livingstone DR, Lowe DM, Moore MN, Evans SV, Moore SL (1982) Responses of Mytilus edulis on Exposure to the Water-Accommodated Fraction of North Sea Oil. Marine Biology 67:15-31

Received November 15, 1984; accepted January 25, 1985.