Bull. Environ. Contam. Toxicol. (1989) 42:829–834

Bulletin of Environmental Contamination and Toxicology

Gill ATPase Activity in *Procambarus clarkii* as an Indicator of Heavy Metal Pollution

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Lake Albufera and the surrounding rice field waters are subjeted to very heavy loads of sewage and toxic industrial residues, including heavy metals, from the many urban and waste waters of this area. The American red crayfish, Procambarus clarkii (Girard, 1852) have a high resistence to toxic effects of heavy metals. In previous reports, we have found that P. clarkii shows a high capacity for cadmium accumulation (Diaz-Mayans et al. 1986a). Data on heavy metal levels in Lake Albufera are not available. However, it is noticeable that crayfish collected in Albufera under 15 days of depuration in our laboratory showed a considerable content of these heavy metals in several tissues.

Since, for most pollutants, uptake from water is the most important route, gills are a primary target organ and may be one of the first organs to exhibit symptoms of sublethal toxicity. Furthermore the crustacean gill has an important role in gas and ionic exchange.

The sublethal effects of heavy metals on gills of fish and aquatic invertebrates have been extensively studied. Some metabolic disturbances and histologic damages have been reported, as well as osmoregulation alterations. However, little work has been done about the effect of heavy metals on Na,K and Mg-ATPases of freshwater invertebrate gills. Na,K-ATPase is the prime mediator of ion transport across cellular membranes and plays a central role in whole body ion regulation in marine and estuarine animals (Towle 1981). Na,K-ATPase has been reviewed and assessed as a potentially useful indicator of pollution stress in aquatic animals; Haya and Waiwood (1983) reported that data base on Na,K-ATPase of aquatic fauna is small and more research, specially on the effects of xenobiotics on it, is required before they can be useful in hazard assessment of pollutants.

The purpose of this study is look for the relation, if any, between crayfish gill ATP-ase activity changes and metal exposure in laboratory. This find would allow us to assay this potential indicator in the field.

MATERIAL AND METHODS

Adult intermolt specimens (males and females) of the crayfish P. clarkii were collected in January 1987 from Lake Albufera (Valencia, Spain) and carried inmediately to the laboratory where they were transferred into 300-L aquaria. They were maintained for 15 days at 20°C and were fed a daily diet of pork liver.

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Ninety crayfish ranging in weight from 20.7 to 30.3 g were divided in three groups of thirty animals each and were exposed to 1 mg Cd^{++}/L($Cl_2Cd.2H_2O$); 100 mg Pb^{++}/L ($(NO_3)_2Pb$) and 0.25 mg Hg^{++}(Cl_2Hg), respectively.

Additional thirty crayfish in the same weight range served as controls and were kept in clean tap water. Conditions in the aquaria were as follows: hardness, 180-300 mg $CaCO_3$/L; alkalinity, 3.8-4.4 mmol/L, chloride concentration < 0.1 mg/L, and pH ranging from 7.0-7.5. The water of experimental aquaria was maintained at 22°C and changed every day to reduce the buildup of metabolic wastes and to keep the concentration of each metal in the nominal level. After 12, 48 and 96 h of heavy metal exposure, six crayfish of each experimental group were transferred to clean water and kept there for an additional 2 h.

The tissue for enzyme analysis was taken by removing the carapace and excising the gills. The seventh and eight gill pairs were routinely used in order to avoid differences between anterior and posterior gill filaments. The ATPase assay was modified from that of Neufeld and Pritchard (1979), Tucker (1979) and Haya et al. (1980). Gill filaments were homogenized in a ice cold-solution of 250 mM sucrose and 5 mM Na_2EDTA. A 0.2 mL aliquot of the crude homogenate was added to 0.6 mL of the assay medium and preincubated for 5 min at 37°C. Then the reaction was started by adding 0.2 $MgCl_2$-ATP solution. The final concentration in the assay mixture was 50 mM NaCl, 20 mM KCl, 4 mM Cl_2Mg, 6 mM Na_2ATP and 90 mM pH 7.6 Tris buffer. To determine Na,K ATPase and Mg-ATPase activities the reaction was run with and without a final concentration of 10^{-2} M of ouabain for 20 min at 37°C. The reaction was stopped by adding 40 µL of 1 M citric acid. Samples were centrifuged at 13,000 rpm (42 g) for 10 min. The inorganic phosphate (Pi) liberated from ATP in the supernatant was determined by the method of Heinoen and Lathi (1981). All assays were done in triplicate using a sample blank for each animal. The amount of inorganic phosphate liberated in the reaction $ATP \rightarrow ADP + P_i$ was expressed as the number of µmoles P_i/mg protein/hr. The protein content of the homogenate was determined by a Coomassie blue Dye-binding method (Bradford 1976), using bovine serum albumin as standard.

Statistical comparisons were made between exposure times for all parameters examined using an ANOVA test at the 0.05 significance level. The analyses were performed on a Apple Macintosh™ systems using Stat Works™ and CLR ANOVA subprograms. For those parameters which showed stastically differences, Tuckey test was used to compare means.

RESULTS AND DISCUSSION

The Na,K-ATPase, Mg-ATPase and Total ATPase activities in gills of controls and crayfish exposed to 0.25 mg Hg/L are presented in Table 1. The Na,K-ATPase activity in gill tissue went up as the exposure time increased. On the contrary, the Mg-ATPase activity in the same tissue showed a no statistically significant tendency to decrease with exposure period increasing. The Total-ATPase activity after 96-h exposure decreased respect to the controls. However significant differences were not found when the values were analyzed by the ANOVA test ($p > 0.05$). After 12-h exposure, both Na,K-ATPase and Mg-ATPase activities showed no variation with respect to the controls (see Table 2). These activities increased after 48-h exposure and decreased to 13 and 26% respectively when the exposure-time was 96-h. However, the differences were not significatives ($p > 0.05$). Table 3 shows the gill Na,K-ATPase, Mg-ATPase and Total-ATPase activities of control and treated

crayfish after several times exposure. Time exposure to lead did not significantlly affect Na,K-ATPase activity; however ANOVA test shows significant differences($p < 0.05$) in Mg-ATPase activity.The ulterior Tuckey test only indicated significant differences between means corresponding to 12 and 48-h exposure.

Table 1. Gill ATPase activity of control and mercury treated animals at several exposure times.

Exposure time (h)	Na,K-ATPase	Mg-ATPase	Total-ATPase
control	0.86±0.66	1.31±1.35	2.17±1.91
12	0.71±0.43	1.73±1.01	2.46±0.67
48	0.99±0.35	1.46±0.92	2.45±1.10
96	1.06±0.52	0.80±0.57	1.86±0.82

Enzyme activity are reported as µmol Pi/mg protein/h.
Each value represents the mean ± SD ($n \geq 5$).

Table 2. Gill ATPase activity of control and cadmium treated animals at several exposure times.

Exposure time (h)	Na,K-ATPase	Mg-ATPase	Total-ATPase
control	0.71±0.37	0.50±0.25	1.21±0.43
12	0.71±0.29	0.50±0.17	1.12±0.48
48	0.93±0.41	0.58±0.39	1.57±0.75
96	0.62±0.30	0.37±0.28	1.01±0.49

Enzyme activity are reported as µmol Pi/mg protein/h.
Each value represents the mean ± SD ($n \geq 5$).

Table 3. Gill ATPase activity of control and lead treated animals at several exposure times.

Exposure time (h)	Na,K-ATPase	Mg-ATPase	Total-ATPase
control	1.15±0.23	0.66±0.28	1.62±0.32
12	0.81±0.40	0.57±0.14	1.37±0.48
48	0.87±0.40	1.09±0.40	1.96±0.78
96	1.02±0.30	1.02±0.27	2.03±0.26

Enzyme activity are reported as µmol Pi/mg protein/h.
Each value represents the mean ± SD ($n \geq 5$).

Sublethal concentrations of mercury, cadmium and lead, did not cause notable effects on ATPase activities of P. clarkii gills after 12, 48, and 96-h. exposure. It is

well known, when considering freshwater and marine fishes and crustaceans, the gill appears to be the most likely site of attack by heavy metals. These pollutants induced alterations on gills osmoregulation and respiratory functions in crustaceans (Bouquegneau and Gilles 1979).

In previous reports we have found that sublethal concentrations of mercury, cadmium and lead, caused both oxygen uptake and gill morphological alterations in P. clarkii (Diaz-Mayans et al. 1986b; Torreblanca et al. 1987). Tucker and Matte (1980) found in in vitro experiments with gill whole homogenates from rock crab that 10 ppm of Cd caused an inhibition of Na,K-ATPase (37% respect the control values), and Mg-ATPase was inhibited up to 13% by 1 ppm of Cd. On the contrary, in in vivo experiments with Homarus, exposure to 6 ppb Cd resulted in 25% increase in Mg-ATPase activity, but Na,K-ATPase activity was the same as for the control (Tucker 1979).

Our results indicate a unclear effect of cadmium on ATPase activities in gill of P. clarkii. In relation to mercury effect, these findings would agree with other authors. We have found no significant effect of sublethal concentrations of this metal on ATPase activity in gill of P. clarkii. Schmit-Nielsen et al (1977) reported that sublethal concentrations of methylmercury for 8 days did not affect gill Na,K-ATPase activity in Pseudopleuronectes americanus. Sublethal concentrations of both methylmercury and Cl_2Hg did not cause significative alterations on Na,K-ATPase activity of trout gills (Lock et al. 1981).

Tucker and Matte (1980) did not find inhibition by in vitro lead exposure in gill tissue of rock crab. We have observed that lead exposure up 48-h increased the Mg-ATPase activity significantly, but not Na,K-ATPase activity, to values corresponding to 12-h exposure. These results would indicate that Mg-ATPase is more sensitive than the Na,K-ATPase in gills of P. clarkii. This is true not only in the case of lead exposure but in all heavy metals investigated here.

On the basis of the results given in this work and that obtained by other authors, the ATPase activities do not seem valuable parameters in the determination of the physiological damage caused by the heavy metal treatment at sublethal concentrations when this enzymatic activity is the only indicator utilized.

In our opinion, there are a lot of other complementary assays that provide more accurate data in the matter of evaluation of the heavy metal effects on the crustacean gills. Between the metals tested in this work, only the lead gave significant alterations in the ATPase activity; even in this case, when the same or lower lead concentrations were used, remarkable variations in oxygen consumption are observed. Likewise, there are important structural alterations in the P. clarkii gill tissue after exposition to sublethal concentration of lead (Torreblanca et al. 1987); the gill filament sewelling may be due to disorders in the osmoregulation processes, that could not be attributed to a direct action of the metal on the Na,K,-ATPase activity.

Environmental organic pollutants usually affect the Na,K-ATPase by decreasing its activity (Haya and Waiwood 1983); otherwise the effect of heavy metals is not defined and depends on multiple factors as the nature of the heavy metal or the type of the salt used, the length of the intoxication period and the species studied (Bouquegneau and Gilles 1979). This fact makes us question the validity of ATPase activities as a parameter in the assesment of heavy metal environmental pollution. We are in agreement with Giesy et al. (1983) and Haya et al. (1983) in the proposing a non-specific multitest assay to study the toxic stress produced by

pollutants. These procedures could include the ATPase activity modification, but complimented by other biochemical, histological and metabolical tests.

Acknowledgements.We acknowledge the support given by the Conselleria de Cultura de la Generalitat Valenciana with a fellowship granted to A. Torreblanca, as well, the support given by the Ministerio de Educacion y Ciencia with a fellowship to J.J. Del Ramo.

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Received December 24, 1987; accepted July 5, 1988