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Impact of mercury on the activity pattern of a marker enzyme in a freshwater bivalve

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Abstract Sessile benthic organisms are universally accepted as bio-indicators of environmental pollution and lysosomal membrane is often the target of injury by xenobiotics resulting in destabilization. Effect of mercury on the activity pattern of the marker enzyme, Acid Phosphatase in the gill and hepatopancreas of the freshwater mussel, *Lamellidens corrianus* (Lea) at 24, 72, and 168 h, post-exposure discussed. ACP activity was determined as given in Sigma Technical Bulletin (No. 104) and μmol of *p*-nitrophenol liberated/mg protein/h calculated. The work aimed to study the duration of exposure and concentration of the metal influence, the direction of the response of the enzyme in the two tissues studied and results show that the concentration of the metal as well as the period of exposure do influence the enzyme activity and may have predictive value as a biomarker of impending population changes. Higher concentration of the metal is assumed to have induced stress proteins like metallothioneins.

Keywords Acid phosphatase · Heavy metals · Hepatopancreas · *Lamellidens corrianus* · Metallothioneins

1 Introduction

Mussels are considered ideal aquatic monitors of chemicals as they are able to concentrate chemicals from ingested particulate matter or from water siphoned through the gills (Pipe et al. 1999) Gill is the main interface between the organism and its environment, and is more susceptible to oxidative stress than the digestive gland as inhibition of antioxidants are more pronounced in the gill (Cossu et al. 1997). Hepatopancreas often act as 'sink' for all contaminants (Everard and Denny 1984). The appropriate use of biomarkers in sentinel organisms provide the first set of tools with which we can measure the actual effects of the chemicals on the biota in the field (Svendsen 2004). Understanding how biomarkers relate to each other on exposure to particular contaminants in different species is the key to their widespread application in environmental management (Brown et al. 2004). Biomarker-based techniques to play a major role in the overall effort of environmental monitoring and protection. It is suggested that biomarkers can be used to devise rapid, effective screening assays, which can complement other testing techniques by significantly reducing the number of samples that may require a more elaborate, definitive or specific evaluation (STAP 2003). In this context, biomarker-based techniques to play a major role in the overall effort of environmental monitoring and protection. There is a considerable body of evidence indicating that lysosomal integrity and functions are compromised in bivalves following exposure to diverse environmental contaminants (Lowe and Pipe 1994). The mechanism(s) causing this alteration in membrane stability is not well understood, but it may involve direct effects of chemicals on the membrane or the increased frequency of secondary lysosomes in toxicant-stressed cells (Myers et al. 1992). The ability of mollusk to concentrate high amount of heavy metals without any apparent bad effects could make these animals very dangerous to their predators (Carpene 1993).

Acid phosphatase is a lysosomal marker enzyme and can be used as a reliable tool for the biological assessment of heavy metal pollution. The potential of this biomarker in freshwater biomonitoring has been demonstrated in zebra

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mussels by Giambrini and Cajaravalli (2005). In the present study, acid phosphatase activity pattern in the gill and hepatopancreas of the freshwater mussel, *Lamellidens corrianus* (Lea) exposed to three sublethal concentrations of mercury for 24, 72, and 168 h. was investigated. One of the key functions of the biomarkers is to provide an early warning signal of significant biological effects with sub-organismic (molecular, biochemical, and physiological) responses preceding those that occur at higher levels of biological organization.

The main objectives of this investigation were to ascertain:

1. Time-response factor in enzyme activity.
2. Dose-response factor in enzyme activity.

2 Materials and methods

Specimens of the freshwater mussel, *L. corrianus (*Lea) of the size group 5.5 ± 1 cm, collected from a freshwater body near Aluva, 20 km from Cochin were brought to the laboratory with minimum disturbance and acclimated for 96 h in flat bottomed, large-sized, square, fiber glass tanks containing aged, well-aerated, de-chlorinated tap water of pH 7–7.5 and temperature 27–28°C. During acclimation, water was changed at regular intervals of 24 h without handling the mussels which were fed with the green algae, Ps*enedesmus* sp.

Specimens of Lamellidens corrianus maintained as above was exposed to three sublethal concentrations of 75, 150, and 300 ppb of mercury. Standard solution of mercury was prepared by dissolving the mercuric chloride HgCl2 (AR Grade) in water. A total of 24 mussels were exposed to each sublethal concentration of the metal, selected on the basis of LC_{50} studies described elsewhere (Rajalekshmi and Mohandas 1993). Eight animals were harvested from each concentration at 24, 72, and 168 h time period. An identical number of mussels maintained in metal-free medium under identical conditions served as the control. Experimental medium was replenished at every 24 h and metal concentrations were maintained at their respective levels.

At each time-period, eight mussels were sacrificed from each group of the experimental and control, the gill and hepatopancreas were removed immediately. After rinsing in chilled glass distilled water, tissue samples were accurately weighed and homogenized. A 5% homogenate was then centrifuged in a refrigerated centrifuge, supernatant collected, deep frozen. Enzyme activity as well as protein concentration was determined within 2 h.

ACP activity was determined as given in Sigma Technical Bulletin No. 104 with slight modifications (Anon 1963). The protein concentrations in the tissue extracts were determined following the method of Lowry (Lowry et al. 1951). From this µmol of *p*-nitrophenol liberated/mg protein/h was calculated.

All data were statistically analyzed using students *t*-test (Croxton et al. 1985).

3 Results and discussion

Gill tissue enzyme activity had been significantly high at 24 and 72 h in 75 ppb exposed mussels and at 72 and 168 h in 150 and 300 ppb mercury exposed mussels (see Table 1). The hyper enzyme activity in 75 ppb exposed mussels can be considered as an immediate response to stress. 75 ppb being a relatively lower concentration, metallothionein produced during the earlier period might have been sufficient to sequester the metal accumulated, and hence the enzyme activity which remained high during the earlier period dropped to the level of control during the course of the experiment. Neosynthesis of metallothionein represents a specific response of the organism to pollution by heavy metals (Lehtonen and Leinio 2003) and this response varies widely from almost instantaneous to years (Svendsen 2004). A close and complex relationship exist between lysosomal response and antioxidant responses to metals, and exposure to copper and mercury greatly decreased lysosomal membrane stability (Regoli et al. 1998).

The results of the present experiments indicate that hyperactivity of acid phosphatase occur only at the time of stress and when the stress gets dissipated, enzyme activity also get back to the normal level. In 150 and 300 ppb metal exposed mussels, as the metal concentrations were very high, hyper ACP activity might have occurred at a period earlier than 24 h and hence not detected. However, extended period of exposure caused a second spell of hyperactivity at 72 and 168 h. in 150 and 300 ppb exposed mussels. Unlike in the case of 75 ppb exposed mussels, this higher influx of metal into the mussel system might have triggered hyper enzyme activity at a period earlier than 24 h and hence not detected in mussels exposed to 150 and 300 ppb mercury. In 150 and 300 ppb mercury exposed mussels, metal concentration was many fold higher than that of mussels exposed to 75 ppb mercury. Acid phosphatase activity and metallothionein synthesis are so closely related that binding of metals to the lysosomal membrane causes increased loading of metal binding proteins within the lysosomal compartment (Regoli et al. 1998). Therefore, metal stress causes hyper activity of acid phosphatase. Mussels from the most heavily polluted stations exhibited reduced lysosomal membrane stability and relatively higher lysosomal heavy metal content compared

Table 1 Gill acid phosphatase activity (μmol/mg protein/h) in *Lamellidens corrianus* exposed to sublethal concentrations of mercury

Hours	24	72	168
Control: *N*	8	8	8
Mean value±	0.280	0.287	0.234
SD	0.055	0.076	0.052
Range	0. 202–0.355	0.200–0.395	0.178–0.322
75 ppb exposed: *N*	8	8	8
Mean value±	0.524***	0.426*	0.234
SD	0.065	0.062	0.027
Range	0.443–0.630	0.343–0.530	0.195–0.271
150 ppb exposed: *N*	8	8	8
Mean value±	0.305	0.772***	0.580***
SD	0.030	0.215	0.172
Range	0.259–0.357	0.398–1.062	0.356–0.879
300 ppb exposed: *N*	8	8	8
Mean value±	0.255	0.396*	0.334**
SD	0.035	0.074	0.045
Range	0.215–0.329	0.299–0.526	0.308–0.420

Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2 Hepatopancreas acid phosphatase activity (μmol/mg protein/h) in *Lamellidens corrianus* exposed to sublethal concentrations of mercury

Hours	24	72	168
Control: *N*	8	8	8
Mean value±	0.805	0.965	0.869
SD	0.263	0.106	0.289
Range	0.557–1.365	0.790–1.170	0.532–1.353
75 ppb exposed: *N*	8	8	8
Mean value±	0.1.793***	0.118	0.708
SD	0.204	0.308	0.107
Range	1.60–2.271	0.886–1.850	0.540–0.916
150 ppb exposed: *N*	8	8	8
Mean value±	0.709	0.354***	0.925***
SD	0.039	0.091	0.260
Range	0.635–0.761	0.246–0.511	0.678–1.510
300 ppb exposed: *N*	8	8	8
Mean value±	0.747	1.653**	0.984
SD	0.083	0.459	0.166
Range	0.585–0.837	1.027–2.183	0.785–1.298

Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

to mussels from less polluted areas (Domouhtsidou and Dimitriadis 2001). Duration and /or concentration of mercury could be an important criteria in determining the level of induction of metal binding protein. In 72 and 168 h exposures, as mussels remain exposed to mercury for extended period of time, metal accumulation in the tissues can be higher leading to higher rate of toxicity.

Metal detoxification by metallothionein is an inducible process which might be relatively economical to the cell because it may be turned on or off when required. (Carpene 1993).

Hepatopancreas enzyme activity in 75 ppb mercury exposed mussels revealed a pattern similar to that of gill tissue during the different time-periods. As in the case of gill, in the hepatopancreas also, hyper enzyme activity observed during the earlier period dropped to the level of control enzyme activity during the course of the experiment. Hyper enzyme activity in 75 ppb exposed mussels at 24 h time-period declined linearly towards 168 h indicating detoxification of the metal accumulated. But in 150 ppb exposed mussels, a near normal enzyme activity at 24 h was found to increase at 168 h after a significant drop at 72 h. In 300 ppb exposed mussels, a near normal activity at 24 h increased significantly at 72 h before falling to a near normal level at 168 h. (see Table 2). This pattern of activity may be related to the on and off mechanism of metallothionein synthesis as cited above (Carpene 1993). In bivalves, Metallothioneins are often cited to play an important role in controlling the kinetics of bioaccumulation of metals and their toxic effects (Baudrimont et al. 2003) A close and complex relationship exist between lysosomal responses and antioxidant responses to metals in mussels (Regoli et al. 1998). Moore and Viarengo (1987) indicated that destabilization of lysosomal membrane is associated with enhanced protein catabolism and failure of membrane to recover in the short to medium period. However, significantly lower enzyme activity at 72 h in 150 ppb exposed mussels remain unclear.

In nature, metals are bound differently in the gill and in the digestive gland of the mussels and metal detoxification in the former organ may be less effective than the latter (Bonneris et al. 2005). The intermittent high and low enzyme activity observed in both the tissues in mussels exposed to the different concentrations of metals shall also be attributed to valve closure, often reported in mussels exposed to higher concentration of metals (Tran et al. 2003). Erratic valve movement causes drastic fluctuation in gill irrigation resulting in variation in the effective concentration of metal to which the tissues are exposed to. Thus higher metal concentration and prolonged exposure might have led to intermittent valve closure and opening, leading to a variability in metal uptake and resultant enzyme activity. Exposure to metals considerably reduces the siphon activity in mussels and that the mussels possibly remain closed for extended period during the treatment which shows that they were not effectively exposed to the toxicants. This type of behavioral adaptation in turn might

have affected the biomarker responses measured (see Lehtonen and Leino 2003). The apparent sensitivity of *L. corrianus* to mercury in terms of Acid phosphatase activity suggests that this species may be used as an indicator organism in assessing the extent of metal pollution in freshwater bodies.

Difference in antioxidant profile and oxyradical metabolism for tissues with digestive or respiratory functions have been indicated in invertebrates and fishes (Regoli et al. 2002). Also, in nature, metals are bound differently in the gills and in the digestive gland of mussels and that metal detoxification in the former organ may be less effective than in the latter (Bonneris et al. 2005).

Thus differences in the antioxidant profile of the two tissues coupled with the differential binding of metals in the gills and the digestive gland might have resulted in the variation in the pattern of enzyme activity in the gill and the hepatopancreas of mercury exposed mussels. Oxidative stress is reported to be more in gills as inhibition of antioxidants is more pronounced in the gills (Cossu et al. 1997).

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