Heavy metals induced alterations in the acid phosphatase activity in the edible freshwater mussel Lamellidens marginalis (Lamarck)

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Citation

Abstract

Variations in the profile of biomarkers present in the inhabitant species have been widely used to assess environmental contamination and it is also known that environmental contaminants could challenge the lysosomal integrity and their functions in case of mussels exposed to them. Such xenobiotics induced lysosomal membrane destabilization and the associated alterations in the lysosomal enzymes are useful diagnostic tools for determining the health of ecosystems facing contamination threats. The present study pays attention to the variations in the profile of one such lysosomal enzyme viz., acid phosphatase (ACP) in the edible freshwater mussel Lamellidens marginalis (Lamarck) exposed to sub lethal concentrations of the heavy metal salts mercuric chloride (0.03 ppb) and cadmium chloride (0.13 ppb). The mussels were exposed to the heavy metals up to 15 days (d) and the variations in the activity of the biomarker ACP were analysed after the expiry of 1, 5, 10 and 15d of exposure in the gills and hepatopancreas of the exposed as well as control groups. Both the heavy metals were found to influence the activity of the lysosomal biomarker ACP to a great extent. However, the patterns of influences were different for both the metals. Further, the enzyme activity was found to be dependent not only on the nature of the toxicant but on the duration of exposure and the morpho-physiological role of the target organ also. Mercury was found to cause a higher degree of toxicity than cadmium.

INTRODUCTION

Aquatic ecosystems are progressively coming under permanent pressure of anthropogenic pollutants and heavy metal contamination of aquatic ecosystems is a worldwide problem posing health hazards not only to the inhabitant organisms but also to the non-target populations including human beings through food chains and food webs. Since the appearance of itai-itai disease and minamata disease, heavy metals have gained a prominent place in environmental research. Pollution by mercury is widespread in the aquatic environment with a clear tendency towards an increase in its intensity ([1,2,3]). Similarly, cadmium contamination also poses an important threat to human health because of its established harmful effects ([4]). These heavy metals are biologically non-essential, persistent and are having great bioaccumulation potential ([2,5]). In India, cadmium concentration in the range of 16 to 176 µg/g has been reported from marketed fishes ([6]). Further, bivalve molluscan forms are also reported to have great ability to bioconcentrate heavy metals in their body tissues ([7,8,9]).

In this regard, it is worth mentioning that human beings consume a number of molluscan species around the world including Indian subcontinent. While around 20 ethnic groups in Bangladesh consume snail meat ([10]), a number of bivalves and snails are utilized in Nepal ([11]). Different species of molluscs including the freshwater bivalve Lamellidens marginalis (Lamarck) are consumed by local people in many parts of India ([12,13,14]). At the same time, molluscs are considered as useful indicator species for biological assessment of water quality also ([15]). In general, even the food organisms growing in aquatic environments sub lethally contaminated with heavy metals could bioaccumulate them in their tissues leading to human health hazards. Therefore it is desirable to have potential biomarkers to understand the impact of heavy metal toxicities on food organisms such as fishes and shellfishes. Lysosomal enzyme release assay is generally considered as a biodindicator of the presence of pollutants in the ambient environment and organisms. Even though many such studies have been conducted in marine bivalves ([16,17,18,19]), reports on freshwater mussels are scanty ([20]). Acid phosphatase

(ACP) is a lysosomal biomarker, which is widely utilized as a potential indicator for assessing the impact of heavy metal pollutants on biological organisms because of the fact that lysosomal integrity and functions are generally altered to a great extend due to the actions of pollutants ([20,21]).

Many xenobiotics could act upon the tissues leading to lysosomal membrane damages. Such alterations usually make the membrane more permeable to enzymes and such membrane damage leads the expression of lysosomal enzymes. The property of lysosomes requiring membrane damage for enzyme expression is usually referred to as lability ([22]). ACP is a hydrolytic lysosomal enzyme and is released by the lysosomes during stress induced tissue/cell damage. It is also concerned with the process of transphosphorylation with an important role in the general energetics of the organism ([23]). In this context, the present study is an attempt to explore the possibility of exploiting the activity of ACP as a reliable biomarker for assessing the biological impact of the heavy metals mercury (Hg) and cadmium (Cd) in the edible freshwater mussel L. marginalis.

MATERIALS AND METHODS
EXPERIMENTAL ORGANISMS AND THEIR MAINTENANCE

The freshwater pearl mussel L. marginalis having 23 to 25 g body weight and 5 to 6 cm shell length were collected from a pristine lake and were acclimated to the laboratory conditions for 20 days in plastic aquaria bearing well water. Feeding was done following Sreedevi et al. ([24]) and Sonawane ([25]) on everyday (d). The medium was renewed after every 24 hours (h).

TOXICANTS AND TEST CONCENTRATIONS

The heavy metal toxicants selected for exposure were mercuric chloride and cadmium chloride (sd fine Chemical Ltd., India). Prior to the commencement of the experiment, the 96 h LC_{50s} of mercuric chloride and cadmium chloride were estimated by following Finney's ([26]) method and were found to be 3 and 13 ppm respectively. For the present study, the sub lethal exposure concentrations of 0.03 ppb mercuric chloride and 0.13 ppb cadmium chloride were selected.

BIOASSAY

Toxicants exposures were done by following a 24 h renewal bioassay system. Four groups of 10 mussels each were exposed separately to 50 l of mercuric chloride solution (0.03 ppb) and cadmium chloride solution (0.13 ppb) respectively. The test solutions were prepared separately by mixing the required quantities of the respective heavy metals in well water having dissolved oxygen 5.8 ± 0.2 ppm, pH 7.3 ± 0.1, water hardness 82 ± 3.0 mg l^{-1} and a water temperature of 27 ± 2°C. Parallel control groups were also kept in separate aquaria bearing 50 l of well water without the addition of any heavy metals. The experimental and control aquaria were maintained up to 15d and the respective media were renewed after every 24 h. Feeding was allowed in the experimental as well as control groups everyday throughout the tenure of the experiment.

TISSUE PREPARATION

At the expiry of each of the stipulated exposure periods (1, 5, 10 and 15d of exposure), 5 mussels each from each group (control, Hg exposed, Cd exposed) were taken separately; shells were cleaned with distilled water and sacrificed separately to collect the gills and hepatopancreas. The respective tissues were rinsed immediately in ice-cold triple distilled water separately and were weighed accurately. The tissues were then homogenized separately in glass homogenizers over ice. Ten per cent homogenates were centrifuged in a refrigerated centrifuge and the supernatants were collected. The enzyme activity and protein concentration of each of the supernatants were determined within 2 h.

ENZYME PROFILING

ACP activity was determined by following King and Jagatheesan ([27]) and Varley ([28]) as described in the Span Diagnostics (India) test kit No. 25901 with slight modification. The enzyme activity was measured as the amount of p-nitrophenol formed in μmol per mg protein per hour. Protein concentrations in the homogenates were determined following the method of Lowry et al. ([29]).

STATISTICAL ANALYSIS

The data generated were analysed for level of significance using student's 't' test.

RESULTS

Alterations in the ACP profile of the mussels exposed to mercury and cadmium were not similar. ACP of gills and hepatopancreas responded differently to the two heavy metals at various exposure periods (Tables 1, 2 and Figs. 1, 2). Gill born ACP responded immediately to the heavy metals with significant increases in the enzymatic activity

(Table 1 and Fig. 1) whereas in the case of hepatopancreas, the response was delayed and only the mercury treated mussels exhibited increase in the enzymatic activity, that also after 5d only. In general, while cadmium was found to induce a slow but steady increase in the enzymatic profile, mercury caused drastic variations in hepatopancreas. In both the tissues of the mussels exposed to mercury, the ACP profile declined drastically during the later half of the experiment (Figs. 1, 2). On the other hand, in the case of cadmium exposed mussels; while the ACP titre decreased significantly in the gills during the later half of the experiment, it showed significant increases in the hepatopancreas during the later stages.

Figure 1

Table 1: Alterations in the activity of acid phosphatase (ACP) in the gills of exposed to sub lethal concentrations of mercuric chloride and cadmium chloride in comparison to the control groups

Parameter		ACP activity (µmol/mg protein/h)			
		1d	5d	10d	15d
Control	$\overline{X}$	0.6258	0.6149	0.6018	0.6107
	± SD	± 0.022	± 0.015	± 0.075	± 0.030
	Range	0.5986-0.6472	0.5991-0.6364	0.5091-0.6896	0.5686-0.6519
Hg exposed	$\overline{X}$	0.6227[NS]	0.7216***	0.5092*	0.3909***
	± SD	± 0.019	± 0.026	± 0.025	± 0.052
	Range	0.6061-0.6536	0.7039-0.7481	0.4846-0.5501	0.3281-0.4590
Cd exposed	$\overline{X}$	0.6218[NS]	0.6260[NS]	0.6980*	0.7540**
	± SD	± 0.024	± 0.032	± 0.019	± 0.043
	Range	0.6013-0.6573	0.5836-0.6601	0.6742-0.7236	0.7018-0.8001

Note: Based on student's 't' test, * = p < 0.05; ** = p < 0.01; *** = p < 0.001; NS = not significant.
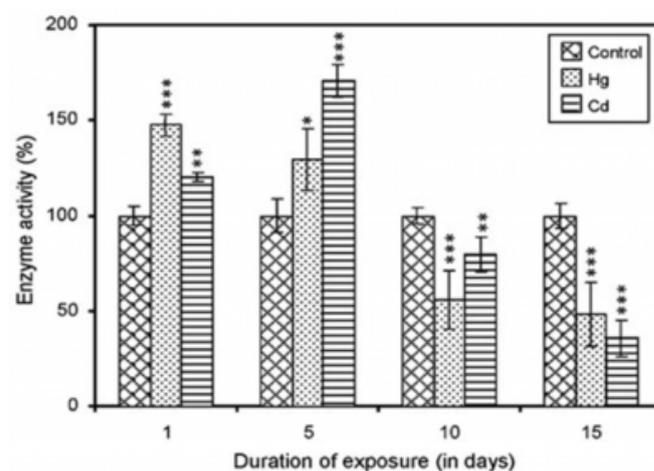
Figure 2

Table 2: Alterations in the activity of acid phosphatase (ACP) in the hepatopancreas of exposed to sub lethal concentrations of mercuric chloride and cadmium chloride in comparison to the control groups

Parameter		ACP activity (µmol/mg protein/h)			
		1d	5d	10d	15d
Control	$\overline{X}$	0.3158	0.3185	0.3157	0.3160
	± SD	± 0.016	± 0.027	± 0.013	± 0.020
	Range	0.2892-0.3314	0.2962-0.3391	0.2976-0.3341	0.2987-0.3396
Hg exposed	$\overline{X}$	0.4669***	0.4128*	0.1762***	0.1529***
	± SD	± 0.027	± 0.066	± 0.027	± 0.026
	Range	0.4291-0.4973	0.3314-0.4936	0.1402-0.2014	0.1246-0.1892
Cd exposed	$\overline{X}$	0.3813**	0.5436***	0.2518**	0.1134***
	± SD	± 0.009	± 0.046	± 0.023	± 0.011
	Range	0.3672-0.3901	0.4839-0.5866	0.2266-0.2839	0.1036-0.1300

Note: Based on student's 't' test, * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Figure 3

Figure 1: Variations in the percentages of acid phosphatase (ACP) activities in the gills of exposed to sub lethal concentrations of mercuric chloride and cadmium chloride

Note: Based on student's 't' test, control values are taken as 100%; $\overline{X}$ ± SD in %; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Figure 4

Figure 2: Variations in the percentages of acid phosphatase (ACP) activities in the hepatopancreas of exposed to sub lethal concentrations of mercuric chloride and cadmium chloride
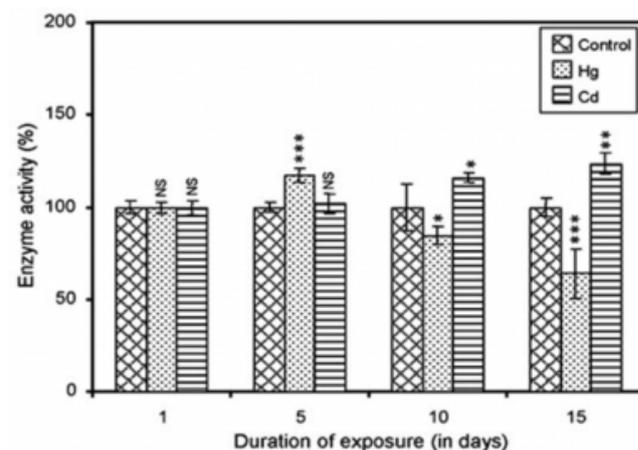
Note: Based on student's 't' test, control values are taken as 100%; $\overline{X}$ ± SD in %; * = p < 0.05; ** = p < 0.01; *** = p < 0.001; NS = not significant.

DISCUSSION

Numerous studies have utilized biochemical measures to quantify the deleterious effects of environmental pollutants on aquatic organisms including mussels ([30,31,32]). Profiling of phosphatases activities is one such commonly used diagnostic tool to assess toxicity stress of chemicals in living organisms ([33]). Lysosomes are sub cellular membrane

bound organelles active in the catabolism of cellular and extra cellular material. Lysosomal enzymes including ACP, in bivalve molluscs are reportedly involved in a host of defense and cell degradative mechanisms ([34,35]). It is an inducible enzyme because its activity goes up when there is a toxic impact and the enzyme begins to counteract the toxic effect ([36,37]). Subsequently the enzyme activity may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage. Further, a number of stress sources including toxic substances such as heavy metals could induce alterations in cellular physiology leading to changes in the function of lysosome. In this context the role of intact lysosomal membrane in the regulation of lysosomal function is worth mentioning. The intact lysosomal membrane prevents the expression of lysosomal enzymes including ACP and thereby do not allow indiscriminate autophagy. This mechanism in fact lends the property of latency to lysosomal enzymes ([38]). However, alterations in cellular energy or metabolic requirements and exposure to toxicants could reportedly bring in changes in the size, quantity and membrane lability of lysosomes ([36,39]). According to Lowe et al. ([40]), alterations in the membrane permeability can have severe consequences such as leakage of hydrolytic enzymes including ACP, which could have detrimental effect on the cell.

The two heavy metals (mercury and cadmium) used in this study have gained their own notoriority as biologically non-essential and persistent type of pollutants causing widespread metal toxicosis in various organisms. While cadmium has significant effect on the lability of lysosomal membrane ([38,41]), mercury causes even greater lability ([42,43]). The results of the present study clearly shows that the effects of heavy metal toxicants on the lysosomal enzyme ACP depends at least on three factors viz., toxic nature of individual heavy metal, duration of exposure and the morpho-physiological state of the concerned organ/tissue.

As far as the nature of the toxicant is concerned; it is evident from the LC_{50s} that mercury is having greater toxicity than cadmium. The early setting in of high intensity alterations in the profile of ACP of mercury-exposed mussels also reflects this (Fig. 1-1d exposure and Fig. 2-5d exposure) in comparison to the cadmium exposed ones. Even at a lower exposure concentration (0.03 ppb), the intensity of interference with ACP by mercury is much higher when compared to that of cadmium (0.13 ppb). This is true for both the tissues investigated.

With regard to the duration of exposure, even though the exposure concentration of cadmium is higher, the intensity of alterations in the activity of ACP is low in the earlier stages of exposure when compared to that of mercury (Figs. 1, 2). This is more evident in the case of hepatopancreas (Fig. 2). In other words cadmium requires more duration of exposure to elicit toxicity than mercury. Therefore, the duration of exposure is also an important factor that modulates the toxic impact of heavy metals on the activity of ACP.

The third factor, which influences the ACP activity, is the target organ/tissue. There occurs rapid onset of toxicity in the case of gills than hepatopancreas (Figs. 1, 2) for both the heavy metals tested. The earlier commencement of variations in the enzymatic profile of gills (Table 1 and Fig. 1) may be attributed to its proximity to the ambient toxicant as well as to its morpho-physiological significance. According to Jayakumar and Paul ([5]), in the case of fish, crustaceans and molluscs, gills are one of the target organs to suffer instantaneously from ambient toxicants and being primarily a filter feeder; gills play pivotal roles in the physiology of mussels ([44]). The highly branched thin gills with the increased surface area when meet with the large volume of water and suspended particles passing through their surface, a highly conducive environment is created for active metal binding and subsequent interactions. This makes gills more susceptible to water borne toxicants leading to highly altered expression of ACP.

On the contrary, the hepatopancreas born ACP largely remains at the control level (Table 2 and Fig. 2) especially in the earlier stages of exposure. Further, the alterations in the expression of ACP in the hepatopancreas are also at a low pace when compared to that of the gill. Two reasons could be attributed to this observation. Firstly, hepatopancreas is not a prime site for the contact effects of toxicants. The metal intake in the hepatopancreas is mainly from the oral route or from the metal transported from other parts of the body by the haemolymph. In case of the former route, metals first come in contact with the gills and associated structures while filtering the suspended particles and only those portions of the heavy metals, which still remained in the food particles, are ingested. Similarly, transport of metals by haemolymph to hepatopancreas is also a time consuming process. Therefore in the case of hepatopancreas one can naturally expect a delayed/reduced outcome of toxicosis as observed in the present investigation. Secondly,

hepatopancreas is a preferred site of detoxification and it is even referred to as the sink for toxicants ([20]). As the detoxification mechanisms are more active here, the ACP produced in the earlier stages of exposure might have been utilized immediately for this purpose thereby keeping the ACP titre more or less at the control level. The role of the metal binding protein metallothionein mediated detoxification ([45]) also cannot be ruled out, as hepatopancreas is a known site for the induction of metallothionein. The production of metallothionein could lead to sequestering of the metal present in the hepatopancreas and thereby reducing metal toxicity. This could be one of the reasons for the maintenance of ACP profile at the control level in the cadmium exposed mussels up to 5d (Table 2 and Fig. 2) because cadmium is well known to cause the induction of metallothionein ([46,47,48,49]).

Various workers have observed significant increases in phagocytic ability of haemocytes along with lysosomal ACP activity in the haemocytes of mussels exposed to cadmium and other metals ([35,50,51,52]). This observation is significant in view of the fact that phagocytosis by haemocytes is an integral part of immune defense in mussels. While studying the effect of cadmium exposure on Channa punctatus, Dubale and Shah ([41]) have also observed an increase in liver ACP activities. In the light of all these reports, the increase in the ACP activity in the gill tissue especially in the earlier stages of exposure and in the later stages of cadmium exposed hepatopancreas may be considered as a part of the physiological process to cope with the stress induced by the heavy metals mercury and cadmium. Further, the significant decreases in the ACP profiles of the gills (Hg and Cd exposed) and hepatopancreas (Hg exposed) during the later stages of the experiment could be due to the seepage of the enzyme into the haemolymph as a result of the lysosomal membrane damage caused by the sustained exposure to the heavy metals. The early commencement of the decreasing trend of ACP activity in the mercury treated mussels indicates the higher toxic potential of mercury in comparison to cadmium.

Free metals ions such as Cd^{2+} are known to cross the plasma membrane through ion channels ([53]) and Arillo et al. ([54]) have proposed that exogenous cations such as cadmium compete for anionic sites in the intralysosomal matrix and thereby displace the lysosomal enzymes from the matrix. This would make it easy for the enzymes to pass through the lysosomal membranes. This is in addition to the development of osmotic gradients in the lysosome due to the accumulation of toxicants. Such osmotic variations would result in the swelling of lysosome leading to increased lysosomal lability. It is also proposed that endogenous hormones elicited by the general stress response could increase lysosomal membrane lability ([38]). Whatever be the mode of action, lysosomal membrane lability could result in the leakage of ACP into the haemolymph leading to a decreased enzyme titre in the tissues of the pollutant-exposed mussels in comparison to the control ones.

It may be concluded that the heavy metals mercury and cadmium cause the differential expression of lysosomal ACP in the freshwater mussel L. marginalis at sub lethal exposure concentrations. The lysosomal destabilization in gills and hepatopancreas do not follow the same pattern. While ACP in the gills expressed early symptoms of commencement of toxicosis, hepatopancreas born ACP showed more latent periods before the onset of fluctuations. Similarly, the effects of mercury and cadmium on the ACP of gill and hepatopancreas vary considerably at different periods of exposure. It may be inferred that the ACP profile in the freshwater mussel L. marginalis when used in comparison with pristine control, could be used as a biomarker of sub lethal toxicity induced by the heavy metals mercury and cadmium. ACP profiling may be utilized as a reliable technique along with other criteria for evaluating the contamination status of mussel meat before human consumption.

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