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Note

Hemolymph acid phosphatase activity in the green mussel (*Perna viridis,* Linnaeus, 1758) exposed to copper and mercury

S. R. Krupesha Sharma* and [1]Sujitha Thomas

Calicut Research Centre of Central Marine Fisheries Research Institute
*West Hill Post, Calicut-673 005, Kerala, India. *E-mail: krupeshsharma@gmail.com,*
[1]Mangalore Research Centre of CMFRI, Bolar, Mangalore -575 001, Karnataka, India

Abstract

The lysosomal system in bivalves is sensitive to changes in the intra and extra cellular environment. The activity pattern of hemolymph acid phosphatase, a marker enzyme of the lysosomal system, was studied in the green mussel (*Perna viridis*) exposed to sub lethal concentration of 25 µg/l of copper and mercury at 24 hr, 72 hr, 7th day and 14th day. The results of the study revealed that at the sub lethal level, copper can induce remarkably higher acid phosphatase activity (hypersynthesis) than mercury. Hypersynthesis of the enzyme occurs much earlier in copper treated mussels than observed in mercury exposed mussels, but on continued exposure, the acid phosphatase activity shows a considerable decline.

Because of their coastal and estuarine distribution, commercially exploited mussel beds are often subjected to sewage and industrial pollution and these bivalves accumulate and concentrate most of the potentially toxic heavy metals in their tissues to concentrations significantly above ambient level in the environment (Roberts, 1976). Many physiological processes including activity of many lysosomal hydrolytic enzymes are inhibited by heavy metals (Suresh and Mohandas, 1989) even though these metals may also activate certain enzymes (Have, 1969). The lysosomal system is very sensitive to changes in the intra and extra cellular environment directly or indirectly in controlling many physiological and pathological processes (Chandy and Patel, 1985).

Cheng (1983a) suggested that quantitative assessment of enzymes is a reliable indicator of stress imposed on the organism by environmental pollutants like heavy metals. Acid phosphatase activity pattern in the hemolymph of the copper stressed clam was investigated by Suresh and Mohandas (1989). However, no information is available on hemolymph acid phosphatase activity response in *Perna viridis* exposed to copper and mercury. Considering the wide spread distribution of the green mussel along the coastal areas, a study was carried out to determine the influence of sub lethal levels of copper and mercury on the activity of acid phosphatase in the hemolymph.

Materials and methods

Green mussels (65 to 85 mm shell length) were collected from a natural mussel bed (salinity: 35‰) off Calicut (74^0 40' N lat. 11^0 30'E long.) and immediately brought to the laboratory. After two days of acclimatization, the mussels were divided into three groups of 25 mussels with three replicates for each group and maintained in 40 litre plastic basins containing 30 litres of sand-filtered sea water (salinity: 35‰ ±0.5‰; pH: 7.5±0.1; temperature: 28±0.5 ^{O}C; dissolved oxygen: >85% saturation). The first group was exposed to copper as copper sulphate [($CuSO_4$).5H_2O, E. Merck, Mumbai] at concentration of 25 µg/l and the second group was exposed to mercury as mercuric chloride [($HgCl_2$), E. Merck, Mumbai] at 25 µg/l. The above experimental concentration was selected based on the acute toxicity (96 hr LC_{50} for copper, 86µg/l; mercury, 155µg/l) levels (Krishnakumar *et al*.,

1987). The third group served as control. Water was changed daily to replenish the metals.

Hemolymph was collected from five mussels of each group at zero hr, 24 hr, 72 hr, on 7th and 14th day from the anterior adductor muscle with the help of a three ml syringe fitted to a 23 gauze needle after making a notch on the shell using a triangular file (Santarem and Figueras, 1995). The hemolymph (approximately 0.5 ml) was transferred into a clean test tube for acid phosphatase activity and total protein assay.

Acid phosphatase activity in the hemolymph was determined following the method described by Andersch and Szcypinski (1947) with slight modification. Hemolymph sample of 0.1 ml was transferred into a test tube containing one ml of chilled 0.1M citrate buffer (pH: 3.6), kept in a water bath at 37 ^{O}C and 100µl of p-nitrophenyl phosphate disodium salt (20 mg/ml of citrate buffer) was added as substrate to start the reaction. After incubation for one hour, 2 ml of 0.25N NaOH was added to stop the reaction. The absorbance was measured at 410 nm in a Jenway 6300 spectrophotometer. Different concentrations of p-nitrophenol (HiMedia, Mumbai) in 0.25N NaOH were used to construct the standard curve. The velocity of the reaction was measured in terms of µmols of p- nitrophenol liberated/ hour. The enzyme activity was expressed as µmols of p- nitrophenol liberated/mg protein/hour.

The enzyme activity of experimental groups and that of the control group was analysed by employing paired sample t test using Systat software (Systat Software Inc, USA, Version: 10.2.01).

Results and discussion

The acid phosphatase activity pattern of the mussels exposed to copper and mercury at zero hour, 24 hr, 72 hr, 7th and 14th day are shown in Fig.1 & 2 respectively.

Mussels exposed to copper showed significantly (P<0.01) higher enzyme activity than the control groups at 24 hr, 72 hr and 7th day. The highest activity was observed at 24 hr of exposure to copper

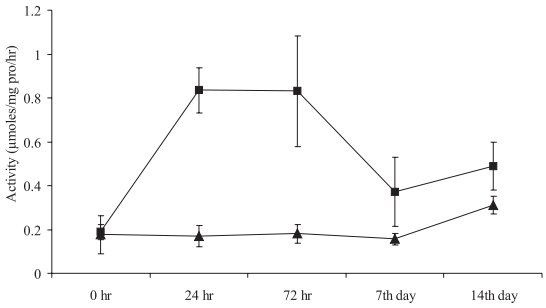

Fig. 1. Acid phosphatase activity pattern in the mussel (*Perna viridis*) exposed to copper (■ copper; ▲ control; vertical bars indicate standard deviation; n=15)

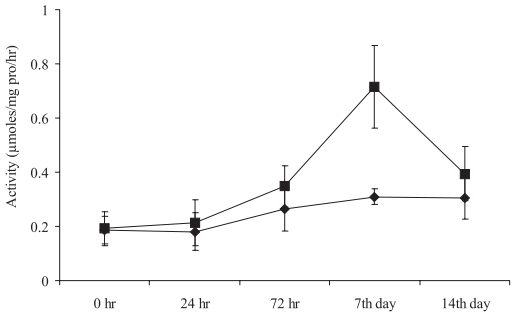

Fig. 2. Acid phosphatase activity pattern in the mussel (*Perna viridis*) exposed to mercury (■ mercury; ◆ control; vertical bars indicate standard deviation; n=15)

which was sustained up to 72 hr after which there was a decline in the activity till seventh day. On 14th day the activity in the copper treated groups was not significantly different from that of the mussels in the control groups.

In the case of mercury treated groups, the enzyme activity was significantly (P<0.01) higher than the control groups on the third and seventh days. The highest activity was observed on seventh day followed by a sharp decline in the enzyme activity and on 14th day, the enzyme activity in the mercury treated groups was not different from that of the control groups.

The toxicity of metals affects the integrity of the lysosomal membrane and causes its destabilization followed by release of stored

lysosomal hydrolases into the hemolymph component, thereby increasing the activity of the enzyme in hemolymph (Moore, 1976; Baccino, 1978). However, the increased activity in the present study may not be due to increase in the number of hemocytes as these metals do not stimulate hemopoesis in the early hours of exposure to copper and mercury (Cheng, 1987).

The difference in acid phosphatase activity pattern between mussels exposed to copper and mercury could be attributed to the nature of pathogenesis of the cellular changes caused by the two metals. The onset of destabilization leading to release of the enzymes into the cytosol could also be responsible for the difference in the activity pattern induced by the metals.The higher activity may be due to the hypersynthesis of acid phosphatase. Hypersynthesised enzymes play a protective role in the removal of inflammation-provoking agents (Cheng, 1983a). The hypersynthesis of the enzyme in hemocytes underline the involvement of the enzyme in defence reaction, so as to destroy the susceptible biotic and abiotic agents (Cheng, 1983ab).

The decline in acid phosphatase activity after a certain period in the case of both the metals may be either due to destabilization of the lysosomes or inhibition of hypersynthesis by the same metal responsible for the early enhanced activity. Besides, response of the enzyme activity to copper was more conspicuous than mercury in terms of onset and sustenance. Krishnakumar *et al*. (1990) in their study on physiological and cellular responses to copper and mercury in green mussel observed that copper was more toxic than mercury in inducing lysosomal changes in the digestive gland. Copper causes lysosomal membrane damage by prompting lipid oxidation (Baccino, 1978). This results in destabilization of the lysosomal membrane and release of hydrolytic enzymes into the hemolymph. This activity was delayed in the case of exposure to mercury when compared to copper.

The peak enzyme activity in the copper treated groups was at 24 hr whereas it was on 7th day in mercury treated mussels suggesting that the release of enzyme into the hemolymph occurred well before 24 hr in the case of copper exposed mussels, while it occurred after 24 hr in mercury exposed ones.

Exposure to heavy metals can cause decrease in the total hemocyte count in the later stages (Cheng, 1987) and continued exposure can lead to inhibition of hypersynthesis of the hydrolytic enzymes. Copper inhibited the acid phosphatase activity in the tropical blood clam *Anadara granosa* at sub lethal levels (Anthony and Patel, 2000). Based on the findings of the study, it can be concluded that at sub lethal level, copper can induce remarkably higher acid phosphatase activity than mercury and after a period of exposure, both metals can cause a decline in the enzyme activity probably due to inactivation of hypersynthesis.

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