Effect of lead on Na\(^+\), K\(^+\)-ATPase activity in *Penaeus indicus* postlarvae

C Satyavathi & Y Prabhakara Rao*

Department of Zoology, Andhra University, Visakhapatnam 530 003, India.

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In vivo effect of lead on Na\(^+\), K\(^+\)-ATPase was studied in plasma membrane/mitochondrial fraction of *P. indicus* postlarvae (PL), exposed to 30 days to a sublethal concentration (1.44 ppm) of lead. A significant (P < 0.05) decrease in the enzyme activity was observed for exposed PL with respect to their controls at different intervals except 24 hr. Further the substrate (ATP) and ion (Na\(^+\) and K\(^+\))-dependent kinetics of Na\(^+\), K\(^+\)-ATPase was studied with the plasma membrane/mitochondrial fractions of control and 30 days exposed PL. The apparent K_m and V_max values were calculated to determine the nature of inhibition. Both the control and exposed PL showed almost the same apparent K_m values in the presence of different substrate or ion concentrations indicating that lead interacts with the enzyme at a different binding site.

The post-larval (PL) stage of *Penaeus indicus* is very critical as its environment changes from estuarine to marine condition in the later stages necessitating an alteration in its physiology. The ionic concentrations of haemolymph in crustaceans are maintained by active absorption of sodium chloride from the surrounding medium. Enzymes like Na\(^+\)-ATPase, K\(^+\)-ATPase, Na\(^+\), K\(^+\)-ATPase, Mg\(^{2+}\)-ATPase, Ca\(^{2+}\)-ATPase which are responsible for the ionic regulation of haemolymph, have been found in the gills of crustaceans. Recently, the presence of sodium (Na\(^+\)) or potassium (K\(^+\)) or sodium potassium (Na\(^+\), K\(^+\))-stimulated ATPase has been reported in the PL of *P. indicus*. These ATPases are associated with not only osmoregulation but also neurotransmitter release, cardiac contractility and other cellular functions such as cell volume regulation, calcium concentration and membrane potential. As lead is one of the most common heavy metals reported in the east coast of India, an attempt has been made to examine the effect of lead on Na\(^+\), K\(^+\)-ATPase activity in PL of *P. indicus*. In the present investigation, the enzyme activity and its kinetics were studied in the PL on exposure to lead.

*Penaeus indicus* PL were obtained from Gosthani estuary (Lat. 18°19' and Long. 82°57') of Bheemunipatnam, east coast of India. PL of almost the same size (10-12 mm) were selected and acclimatized to laboratory conditions at ambient salinity (20 ppt) and temperature (29\(^o\) ± 1\(^o\))C for 48 hr. Care was taken that there was no contamination of lead either in the feed or in the ambient water.

Plasma membrane/mitochondrial fraction of PL was prepared by following the method of Proverbio et al. and Ho & Chan. About 100 PL were used to prepare the above fraction and the protein content in the fraction was determined as per Lowry et al. The ATPase activity was estimated by the hydrolysis of ATP in presence of the enzyme according to the method of Proverbio et al. and the liberated inorganic phosphate (Pi) was determined. The enzyme activity was calculated based on the difference between the assays carried out in presence and absence of ouabain (1 mM) which is a specific inhibitor of Na\(^+\), K\(^+\)-ATPase. The activity of the enzyme was expressed as nmols of Pi liberated/mg protein/min.

Bioassays were carried out earlier with different concentrations of lead and an LC_{50} (96 hr) value of 7.22 ppm was reported for these PL. In the present investigation, the PL were exposed to a sublethal concentration of lead for 30 days. The sublethal concentration of lead was calculated as 1/5th of LC_{50} (96 hr) value i.e., 1.44 ppm. Appropriate amount of lead acetate stock solution was added to the medium to get the final desired concentration and the stock solution was prepared by dissolving lead acetate (GR) in double distilled water. Parallel control was maintained along with the experiment without the lead toxicant. Both control and exposed PL were fed with commercial feed (Lux Water Base, Nellore, India; 44% protein) twice a day based on 20% of body weight and the medium was renewed daily. The PL were sacrificed...
and the membrane fraction was prepared as described earlier for the enzyme assay at intervals of 24, 48, 96 hr, 10 days and 30 days. The enzyme activity of 10 K pellet obtained from the control and exposed PL (30 days) was studied with different concentrations of ATP (0.25, 0.5, 1, 2 and 4 mM) or Na⁺ (5, 10, 25, 50 and 100 mM) or K⁺ (5, 10, 25, 50 and 100 mM) in the reaction mixture. ATP, NaCl and KCl were dissolved individually in double distilled water and appropriate amounts were added to the reaction mixture to obtain the above desired final concentrations. The other contents in the reaction mixture were kept constant.

Studies on enzyme assays were carried out with five different sets of samples and each enzyme assay was done in triplicate. The mean values together with standard deviation were calculated for comparison. Student’s t test was used to compare the enzyme activity of exposed with that of control at different intervals. The enzyme kinetics was determined following Proverbio et al.²

The results indicate that there was a gradual fall in Na⁺, K⁺-ATPase activity in the exposed PL with increasing exposure period. However, the percent decrease in enzyme activity with respect to their controls was 2.2, 19.3, 46.5, 64.3 and 81.7 at 24, 48, 96 hr, 10 days and 30 days respectively. The inhibition was significant (P < 0.05) from their respective controls at all intervals except for 24 hr.

The ATPase activity was also measured at different ATP, Na⁺ and K⁺ concentrations with the membranes obtained from control and 30 days exposed PL of P. indicus and the results are presented in Fig. 1 (a-c). The enzyme activity showed an increase with increasing substrate (ATP) concentrations in both control and exposed PL but this increase was more in control than exposed PL (inset of Fig. 1a). However, the enzyme activity was almost saturated from 2 mM ATP onwards in both control and exposed PL. When substrate (ATP) concentrations were plotted against substrate/enzyme activity (S/V) (Fig. 1a), the apparent K_M value was found to be 1.95 mM for control and 1.87 mM for exposed PL. However, these values were almost the same for both control and exposed PL indicating that lead was not competing with the substrate for the active site on the enzyme. The V_max value of control (118.7 n mol Pi liberated/mg protein/min) was higher than exposed (27.14 n mol Pi liberated/mg protein/min) PL (Fig. 1a).

The enzyme activity showed a similar increase with increasing concentrations of Na⁺ or K⁺ in both control and exposed PL (inset of Figs 1b and c).

![Fig. 1—Hanes-Woolf plot for Na⁺, K⁺-ATPase activity in control and exposed PL with different ATP(a), Na⁺(b) and K⁺(c) concentrations in the reaction mixture. Inset: enzyme activity in control and exposed PL at different ATP(a), Na⁺(b) and K⁺(c) concentrations. However, it is also clear from the insets of Figs 1b and c that a saturation in the enzyme activity was obtained at 50 mM Na⁺ and 20 mM K⁺ concentrations. The plots of Na⁺ or K⁺ concentrations against their respective S/V values are presented in Figs 1b and c.](image-url)
There was not much difference between the apparent KM value of control (38.6 mM) and exposed (37.8 mM) PL with different concentrations of Na⁺ in the assay (Fig. 1b). A similar trend of almost the same apparent KM values was observed in control (12.37 mM) and exposed (11.98 mM) PL in the presence of different K⁺ concentrations in the reactions mixture (Fig. 1c). The above data from both the figures (1b and c) suggest that lead be not in competition with either Na⁺ or K⁺ ions. The Vmax values of control (140.6 and 107.8 n mol Pi liberated/mg protein/min) were higher than in exposed (22.73 and 20.18 n mol Pi liberated/mg protein/min) PL with respect to different N⁺ and K⁺ concentrations (Figs. 1b and c).

In vivo exposure of lead inhibited the Na⁺, K⁺-ATPase activity in P. indicus PL. This inhibition in ATPase activity may disturb the movement of Na⁺ or K⁺ ions across the membrane in these PL. Na/K exchange in crustacean gills is known to occur through Na, K pumps10,11. It has also been proposed that Na⁺, K⁺-ATPase was located predominantly in the basolateral plasma membrane of the thick epithelial cells from gill lamella12.

Similar inhibition of ATPase activity was reported by Proverbio et al.7 in the gills of the Macrobrachium amazonicu during exposure to metals such as Rh, Li and Cs. Torreblanca et al.13 observed that heavy metal pollution can be detected by gill ATPase activity in Procambarus clarkii. Dhavale et al.14 noticed cadmium-induced inhibition of Na⁺, K⁺-ATPase activity in the tissues of Scylla serrata. The results of the present investigation also demonstrate an increase in the activity of enzyme with increasing days of development in control PL as this enzyme was one of the ATPases that show a characteristic pattern during development4. Accumulation of lead was reported to increase with exposure period in PL of P. indicus15 and a concentration of lead at 48 hr (342 μg/g dry weight) may be sufficient in the tissues for the significant inhibition of enzyme activity.

It is evident from the data that there was a gradual inhibition of enzyme activity on exposure to lead and this is in correlation with in vitro results. The kinetic studies of the enzyme revealed that the apparent KM values did not change much in both control and exposed PL in contrast with Vmax values either in presence of different concentrations of substrate (ATP) or ions (Na⁺ and K⁺) suggesting that lead inhibits the enzyme activity in a non-competitive manner. The results of the present investigation are in correlation with in vitro observations on P. indicus PL and also further substantiate the nature of lead inhibition. Inhibition of enzyme activity may be due to interaction of lead with sulphydryl (-SH) groups of the enzyme molecule as has been suggested by Viarengo et al.16. Further investigations are needed to demonstrate the binding of lead with -SH groups of the enzyme.

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References