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Effects of Metals on α-Amylase Activity in the Digestive Gland of the Green Mussel, *Perna viridis* L.

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A number of digestive enzymes *in* the green mussel, *Perna viridis* L., have been reported (Teo and Sabapathy 1990; Sabapathy and Teo 1992; Teo and Lim 1993), and α-amylase is believed to have a higher activity than the others (Teo and Sabapathy 1990; Sabapathy and Teo 1992). Small plankton, on which the green mussel feeds, may supply plenty of starch and glycogen (Teo and Sabapathy 1990). They may be an important source of nutrients for the green mussel and the ability of the latter to make good use of them depends mainly on the activities of amylase. The effect of heavy metals on amylase activity is also important as the ability of the mussel's digestive gland to accumulate these metals is well known (Tan and Lim 1984; Lakshmanan and Nambisan 1989). High concentrations of heavy metals, especially lead, have been observed in the water around Singapore (Sin et al. 1991).

The *in vitro* inhibition of some metals on the activities of digestive enzymes from the green mussel has been observed (Teo et al. 1990; Sabapathy and Teo 1992; Teo and Lim 1993), but kinetic properties of the inhibition and the *in vivo* inhibition of the heavy metals on digestive enzymes are little understood. In the present study, *in* vitro inhibition of four metals (Pb, Cd, Zn and Hg) on the activity of α-amylase from the digestive gland of the green mussel will be compared. Their effects on the K_M and V_{max} values of α-amylase will also be compared. Finally, lead is either added to the food or water, to see how it affects the activity of α-amylase and how this effect acts in combination with starvation.

MATERIALS AND METHODS

Specimens of the green mussel, *Perna viridis* L., for *in vitro* experiments were collected from Lim Chu Kang at the northwestern part of Singapore, and those for *in vivo* experiments were from Changi Village at the northeastern part of Singapore. The animals (7-8 cm length) were maintained in the laboratory (salinity:

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30‰, and temperature: 29°C) for three days, and were fed with a culture of algae, *Pavlova salina*.

The digestive glands were excised from 10 mussels and immediately pooled in ice-cold citrate-phosphate buffer (0.2 *M*, pH 5.8) at a ratio of one gland to 2.0 mL buffer. The gland tissues were then homogenized with an Ultra-Turrax homogenizer. The homogenate was centrifuged at 7000 x g for 5 min at 4°C. The supernatant was dialyzed for 24 hr against three changes of ice-cold distilled water (2 liters) at 4°C using Visking dialysis tubing. The dialyzed supernatant was immediately stored at 4°C and used for the amylase assay within 12 hr.

The amylase activity was determined as described by Sabapathy and Teo (1992), with a slight modification. The mixture, consisting of 0.15 mL enzyme extract, 0.25 mL citrate-phosphate buffer (0.2 *M*, pH 5.8) and 0.1 mL stock solution of the heavy metal, was pre-incubated at room temperature (25°C) for 10 min before 0.5 mL glycogen solution (1%) was added. The reaction mixture was incubated again at 37°C for 1 hr. Then, the reaction was stopped by adding 1.0 mL of 3,5-dinitrosalicylic acid agent to the reaction tube which was heated in boiling water for 5 min. Its absorbance was read at 546 nm on an LKB Ultrospec II spectrophotometer against a control blank. A maltose standard curve was prepared similarly.

The activity of amylase was also measured by varying the glycogen concentration from 0.1% to 1.6%, in the presence of Pb (17.1 m*M*), Cd (15.3 m*M*) or Zn (11.6 m*M*) at the LC_{50} of each compound. The apparent V_{max} and K_M values were calculated by the Lineweaver-Burk transformation.

The alga, *Pavlova salina*, was cultured in Conway medium. One mg lead nitrate per liter was added to the medium. The lead is believed to be tightly bound on or within the algae(Schulz-Baldes 1974). Before being fed to the mussels, the algae culture was centrifuged at low speed (400 x g) for 5 min to separate the algae from the medium, and then the algae were re-suspended in filtered seawater. The algae concentration was adjusted with a Coulter Counter to the same concentration as that before centrifuging.

Duplicate groups of 6 mussels each were exposed to clean or lead-contaminated filtered seawater (1 mg/L) and fed with clean or lead-contaminated algae (maintained at a concentration of 1.5x104 cells/ml in the tank) or without feeding (Table 1), for 8 days. After the 8-d exposure, all the mussels from each group were sampled and the amylase assay for individual mussel was done as described above but without dialysis and preincubation. Data were statistically analyzed with the two-tailed Student-t test.

Protein in the supernatant was determined by the Bradford method [Bradford 1976), using the Bio-rad protein assay kit, with bovine serum albumin as the standard.

Table 1. Scheme of exposure of six experimental groups of green mussels to lead.

Groups	Food condition	Seawater condition
1	no	clean
2	no	Pb-contaminated
3	clean	clean
4	clean	Pb-contaminated
5	Pb-contaminated	clean
6	Pb-contaminated	Pb-contaminated

Table 2. Estimated concentration for 50% inactivation of amylase by each metal compound.

Metals	IC_{50} value(m*M*)
$HgCl_2$	0.9
$ZnCl_2$	11.6
$CdCl_2$	15.3
$Pb(NO_3)_2$	17.1

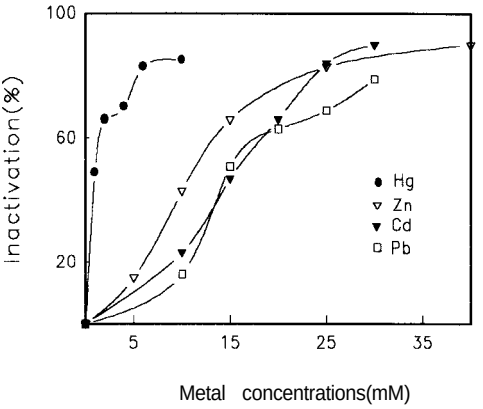

Figure 1. Reduction(% control) in amylase activity caused by heavy metals.

RESULTS AND DISCUSSION

The results showed that the amylase activity was significantly inhibited by the heavy metals studied in a concentration-dependent manner (Fig.1). The data obtained were analyzed statistically by the probit method (Finney 1952) and the IC_{50} value for each metal tested was estimated (Table 2). Of the heavy metals tested in this experiment, Hg was the most effective inhibitor, requiring only 0.9 m*M* for 50% inhibition. This value was even lower than 1.87 m*M* reported by Sabapathy and Teo (1992). The IC_{50} value of Cd on amylase by this study was also lower than 27.81 m*M* reported before (Sabapathy and Teo 1992). These differences resulted probably from the slight modification of the enzyme assay method used here, The preincubation of heavy metals with the supernanant of the digestive gland in the absence of substrate resulted in a greater inhibitory effect of the heavy metals. This might indicate that the amylase was less susceptible to inhibition in the presence of glycogen. No data on the inhibition of lead and zinc on amylase activity were available for comparison. The concentrations for 50% inactivation of sucrase from the green mussels were 7.9 μ*M* and 13.3 μ*M* for lead chloride and zinc chloride, respectively (Teo et al. 1990). A Hg concentration causing 80% inhibition of the trehalase from the digestive gland of green mussel was reported as 1 m*M*, but even at the concentration of 300 m*M*, the inhibitory effect of Zn on trehalase was less than 50% (Teo and Lim 1993). Thus, amylase is

more resistant to inhibitory effects of Pb and Zn than sucrase, but less resistant to Zn than trehalase.

Both the apparent V_{max} and K_M values were significantly decreased in the presence of metals (Table 3). As an example, the Lineweaver-Burk plot shows the inhibition of lead on the amylase activity (Fig.2).

After the green mussels were kept without food for 8 days, their amylase activity (Group 1, Fig.3) was 28% lower ($p<0.01$) than that of the control (Group 3) which were kept in clean seawater and fed with clean algae. Earlier work also showed that starvation would result in the decrease of enzyme activity and this is most likely due to reduced production of digestive enzymes (Reid 1968; Teo et al. 1990). The actual activity of a bivalve's enzyme is influenced not only by innate enzyme properties but also by the rate of enzyme production and level of enzyme concentration under the given ration levels (Brock and Brock 1989). When the green mussels were exposed to lead added to the seawater without food for 8 days (Group 2), the amylase activity in the homogenates of these mussels showed even lower activity. It was 44% lower ($p<0.01$) than that of the control (Group 3) and 22% lower ($p<0.01$) than those which were only starved for 8 days (Group 1). Compared to those which were exposed to lead in seawater, but not starved for the same period (Group 4), the activity was 32% lower ($p<0.01$). No data were available in the literature for any comparison.

The amylase activity in the homogenate of the digestive gland from green mussels in Group 4 (Fig.3) was 18% lower ($p<0.01$) than that of the control (Group 3). Thus, the lead taken up by the gills and mantle might have accumulated in the digestive gland which might have acquired some lead by diffusion through the foot. On the other hand, the amylase activity in the homogenates of the digestive gland of the green mussels (Group 5, Fig.3), which were kept in clean seawater but fed with lead-contaminated algae, was 14% lower ($p<0.05$) than that of the control. The digestive gland could also take up lead from the food. The green mussels which were exposed to lead in both food and seawater (Group 6) showed 36% reduction ($p<0.01$) in amylase activity. Evidence indicates that lead taken up from food and from seawater had an equal inhibitory effect on the amylase activity present in the homogenates of the digestive gland. This indicates that about half of the amount of lead accumulated in the digestive gland was acquired from the food and the other half from the seawater. Both the seawater and the algae contained the same concentration of lead. Schulz-Baldes(1974) found that the lead accumulated by *Mytilus edulis* in its whole soft tissues after 35 days of lead exposure was 29% of the total amount of lead in the medium. In another series of experiments, the lead was added to the food, and the amount taken up was 23.5% of the amount present in the food, but the seawater only contained 0.01 ppm lead while the algal-culture medium contained 1.0 ppm lead. The concentrations of lead and the total amount of lead were highly variable in different tissues. That accumulated in the digestive gland and intestine of mussels given lead-contaminated food was about 50% higher than that kept in lead-contaminated seawater. In terms of total lead content, the difference was 45%, with those given

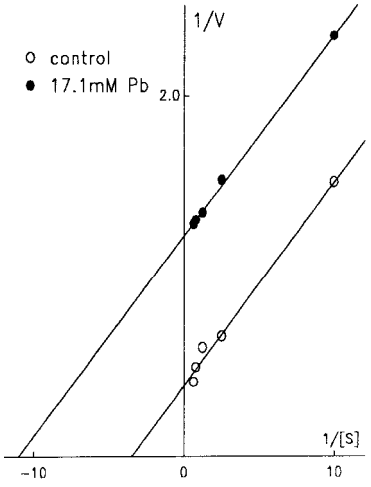

Figure 2. Lineweaver-Burk plot showing inhibition of lead on amylase activity in me green mussel.

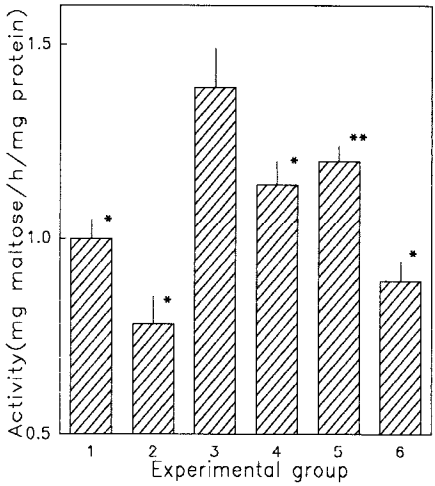

Figure 3. Effects of lead exposure and starvation on amylase activity in me green mussel (*: $p<0.01$; and **: $p<0.05$).

Table 3. Inhibition of heavy metals to amylase activity in the green mussel.

		Lineweaver-Burk regression	K_M*	V_{max}**
Pb	con.	Y=0.4084+0.1023X (r=0.92)	0.25	2.45
	expt.	Y=1.1765+0.1412X (r=0.94)	0.12	0.85
Cd	con.	Y=0.4084+0.1023X (r=0.92)	0.25	2.45
	expt.	Y=0.7039+0.1027X (r=0.98)	0.15	1.42
Zn	con.	Y=0.4084+0.1023X (r=0.92)	0.25	2.45
	expt.	Y=0.7692+0.1231X (r=0.93)	0.16	1.30

* Unit: %; and ** Unit: mg maltose/hr/mg protein

lead-contaminated food having a higher lead content. This was apparently due to the higher lead content of the food than the seawater. The work of Schulz-Baldes (1974) clearly shows that even with a very low concentration of lead in seawater (0.01 ppm), appreciable amount of lead can still accumulate in the digestive gland of these bivalves. This has great implications on the digestive capability of the mussels by affecting their growth.

Amiard et al. (1989) exposed the oyster *Crassostrea gigas* to either lead or copper by adding either one of these to the food or to the seawater and then determined

the quantities of these two metals accumulated in the soft tissues of the whole oyster. They concluded that the dietary uptake was almost negligible as compared to the direct uptake from the polluted seawater. Unfortunately, they did not determine the amount of lead accumulated in different tissues.

Our data show that mercury is the most effective inhibitor of amylase, followed by zinc, cadmium and lead, in that order. All of them caused significant alteration of K_M and V_{max} values. Our data also show that both lead exposure and starvation may affect the amylase activity. Starvation has a larger effect than lead exposure on the amylase activity. However, the bioaccumulation of heavy metals in the digestive gland and intestine is of special significance to mariculture of these bivalves. Since they cause *in vivo* inhibition of digestive enzymes, this will lead to lower growth rate of green mussels.

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