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Effect of Cadmium on Antioxidant Enzyme Activities and Lipid Peroxidation in a Freshwater Field Crab, *Barytelphusa guerini*

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Oxidative stress is experienced potentially by all aerobic life when antioxidant defenses are overcome by prooxidant forces. Evidence indicates that the health of aquatic organisms might also be linked to oxidative stress (Di Giulio et al. 1989). Lipid peroxidation (LPO) is considered to be an important feature in cellular injury. Lipid peroxidation largely results from free radical reactions in membranes, which are rich in polyunsaturated fatty acids. The reactive oxygen forms ($^1O_2$, $O_2^{\cdot}$, H_2O_2, $^{\cdot}OH$) which are produced during oxidative stresses potentially damage cells and tissues (Machlin and Bendich 1987). The literature reports the oxyradical generation, antioxidant enzyme and scavenger responses in marine molluscs (Wenning and Di Giulio 1988; Livingstone et al.1989). Of particular colicern are processes by which environmental contaminants may enhance the oxidative stress in aquatic organisms. Moreover the epidemiology of highly elevated rates of ideopathic lesions and neoplasia among some aquatic animals is related increasingly to oxidative stress associated with environmental pollution (Malins et al. 1988).

In this work we studied the effectiveness of the antioxidant defense systems in relation to the cadmium-induced lipid peroxidation process in the freshwater crab Barytelphusa guerini. This edible crab is an important component of the paddy field ecosystem. The potential for pollution-enhanced oxyradical generation and the operation of antioxidant cellular defenses in crabs has not been addressed and, therefore, offers possibility for detecting the biological impact of pollution. Also the activity of xanthine oxidase which is indicative of superoxide radical generation (McCord 1985) was measured.

MATERIALS AND METHODS

Healthy male crabs Barytelphusa guerini weighing 24±0.5gm collected from an unpolluted river around Hyderabad were held in 50 L plastic containers at room temperature 27±0.50°C for 15 d. Crabs were ted fish *ad libitum* A density of 10 crabs per 8 L of tapwater was used with 10 individuals in each test container. The physico-chemical characteristics of the test water were as follows : pH 7.2-7.4; dissolved oxygen 7.8-8.0 mg/L; salinity 0.19%; chlorinity 0.110 gm/L; alkalinity 102 mg/L; hardness of water 112 mg/L (as $CaCO_3$) and CO_2 2.08 mg/L. A stock solution of cadmium chloride was prepared in deionized water and mixed

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in water to yield the required dilutions. Crabs were exposed to six serial concentrations of cadmium to determine the LC50 value. Each concentration was repeated six times with parallel controls, arid mortality was noted in each concentration at the end of 96 hr. The mortality in each concentration was taken to determine LC50 by plotting a graph taking log concentration on X-axis and % mortality on y-axis. The dead crabs, if any, were removed immediately. The LC50 value of 1.82 mg/L for 96 hr was calculated by using the probit curve according to Finney (1964).

Crabs were divided into two groups of 24 each. Group I served as a control and group II was exposed to a sublethal concentration (0.62 mg/L) of cadmium (as $CdCl_2$) for 30 d. Crabs were starved a day prior to biochemical analysis to deplete food reserves, if any, due to differential feeding. During treatment with cadmium, the crabs were fed every 24 hr before renewing the toxicant water. Both control and experimental crabs, six each, were sacrificed on the 1st, 7th, 15th and 30th day of exposure and the hepatopancreas and gills were isolated and immediately transferred to a freezer at -10°C. All biochemical estimations were conducted in triplicate samples from six animals each of the control and cadmium-treated groups.

Lipid peroxidation was estimated by the formation of thiobarbituric acid reactive substances and quantified in terms of malondialdehyde (MDA) as described by Livingstone et al. (1990). Liver and gill tissues were homogenized in cold KCl buffer (1.15%) and the crude homogenate was mixed with an equal amount of 20% TCA (trichloroacetic acid) and boiled for 10 min at 70°C. The solution then was centrifuged at 12,000 x g in cold centrifuge for 20 min. Then 1 mL of the supernatant was collected and treated with 0.5 mL of TBA (thiobarbituric acid) and boiled for 10 min at 70°C. The chromogens formed were measured at 532 nm in a spectrophotometer against a reagent blank. Tetraethoxy propane was used as an external standard. Lipid peroxidation was expressed as nanomoles of MDA.

The antioxidant enzyme superoxide dismutase (SOD E.C. 1.15.1.1) was estimated according to Marklund and Marklund (1974) indirectly by the ability of the enzyme to inhibit O_2-dependent autooxidation of pyrogallol. The rate of autooxidation was measured by noting the change in absorbance at 420 nm. The reaction mixture (2 mL) contained 1.5 mL of Tris-Hcl buffer (pH 8.2) 200 µL of DETPA (diethylene triamine penta acetic acid). 200 µL of pyrogallol and 100 µL of erlzyrrie. The reaction was initiated by the addition of pyrogallol and the increase in absorbance at 420 nm was noted over 3 min against a reagent blank which contained pyrogallol without the enzyme. Superoxide dismutase activity is expressed as one unit of enzyme that inhibits the rate of autooxidation of pyragallol by 50%.

The antioxidant enzyme, NADP-Glucose-6-phosphate dehydrogenase (G-6-PDH EC 1.1.1.49) was measured according to Lohr (1974). The reaction mixture in 2 mL contained 0.4 mL of phosphate-buffer (pH 7.8), 0.4 mL of lodo-nitro phenol-tetrazolium salt solution, 0.5 mL of glucose-6-phosphate. 0.1 mL of NADP, 0.1 mL of TEA (triethanolamine) and 0.5mL of enzyme. G-6-PDH activity was assayed by measuring the formation of Formazan at 495 nm in spekol against a toluene blank.

Xanthine Oxidase (XOD) activity was measured according to Govindappa and Swami (1965). The reaction mixture contained xanthine as a substrate and NAD as a coenzyme. The protein content of the enzyme source was estimated by the method of Lowry et al. (1951) using bovine serum albumen (Sigma

Chemical Co., St Louis, USA) as a standard. Data are presented as means ± S.D. of six animals per group. The Data were normally distributed and student's paired 't' test was used to compare the dilferences between control and experimental groups. A value of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The results of the present investigation demonstrated clear susceptibility of tissues to cadmium-induced oxidative stress (Tables 1-4). Oxidative damage is seen as an increase in lipid peroxide formation in the tissues of crab. A significant increase in lipid peroxidation was found in both liver and gill tissues of crab (Table 1). Thomas et al. (1982) observed that cadmium elevated lipid peroxidation in liver homogenates and microsomes of mullet (Mugil cephalus). Cadmium was reported to enhance lipid peroxidation in mussel (Mytilus edulis) tissues (Viarengo et al. 1988). A significant increase in lipid peroxidation was found in brain, liver and muscle tissues of the freshwater catfish Heteropneustes fossilis following exposure to mercuric chloride (Bano and Hasan 1989). Since peroxidation of membrane lipids is primarily an outcome of the generation of free radicals, it is possible that cadmium initiates free radical formation. Free radicals are involved at the early stages of Cd intoxication (Vincent et al. 1989). However, the mechanisms responsible for their formation are not well understood. Cadmium is a poor electron acceptor and donor under standard conditions prevailing in biological systems and, as such, direct induction of LPO reaction may be excluded. Consequently, free radicals should result from interactions between Cd and critical cellular sites. Xanthine oxidase (XOD), a flavoprotein, catalyzes the formation of superoxide radicals that can initiate lipid peroxidation (McCord 1985). The activity patterns of XOD measured in the present study indicated an enhancement in the enzyme activity on all days of exposure (Table 2). Increased generation of free radicals could lead to lipid peroxidation. In the present study the rate of lipid peroxidation, measured in the form of Malondialdehyde (MDA) content, significantly increased on all days of exposure in both hepatopancras and gill. In addition, a number of studies have demonstrated oxyradical generation in several bivalve species exposed to environmental pollutants (Jewell and Winston 1989, Livingstone et al. 1990).

As a result of the relative instability of free radicals and their potential to damage cells and tissues, free radical scavengers with antioxidant properties are present for protection. The antioxidant enzymes measured in the present study were: superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G-6-PDH) (Tables 3 & 4). Activities of SOD and G-6-PDH indicated an enhancement on all days of exposure, suggesting the responsiveness of these enzymes to the oxyradicals generated by cadmium in crab. Radi and Matkovies (1988) found variable responses of SOD in the tissues of common carp exposed to Cu or Zn and observed an elevation in liver SOD activity of the carp. The above study supports the present observations of elevation of SOD activity in the hepatopancreas of the crab. Roberts et al. (1987) observed generally higher activities of SOD in hepatic postmitochondrial supernatant in spot (Leiostomus xanthurus) from PAH - polluted sites. Vig and Nemosok (1989) investigated the single and interactive effects of paraquat on liver and gill activities of SOD and observed an elevation in enzyme activity. These observations substantially support our results. Recently Klaverkamp and Palace (1993) observed an increase in SOD activity in the liver of three species of fish exposed to cadmium. Glucose-6-phosphate dehydrogenase (G-6-PDH), another antioxidant enzyme and a major component of the pentose shunt pathway, was increased in our study. Oxidative stress depletes intracellular glutathione which will be restored by the enzyme

Table 1. Lipid peroxidation in the tissues of the freshwater crab Barytelphusa guerini exposed to a sublethal concentration of cadium.

Tissues		Exposure period in days			
		1	7	15	30
Hepato -	Control	11.66±1.21	10.91±1.01	11.55±1.66	10.58±1.04
pancreas	Experimental	13.32±1.42*	16.60±1.22***	18.92±1.32***	15.05±0.92***
	% variation	+ 14.2	+ 52.2	+ 63.8	+ 42.2
	Control	5.41±0.62	5.02±0.59	5.83±0.48	5.09±0.62
Gill	Experimental	6.25±0.81*	6.66±0.69**	7.08±0.82***	7.75±0.92***
	% variation	+ 15.5	+ 32.7	+ 21.4	+ 52.3

Values expressed as nanomoles of MDA (Malondialdehyde)/gm tissue. Values are Mean ± S.D. of six individual observations. Experimental values differ significantly from controls at the level *p<0.1; **p<0.01 and ***p<0.001 respectively.

Table 2. Xanthine oxidase (XOD) activity in tissues of the freshwater crab Barytelphusa guerini exposed to a sublethal concentration of cadmium.

Tissues		Exposure period in days			
		1	7	15	30
Hepato-	Control	1.07±0.23	1.05±0.31	1.06±0.29	1.07±0.28
pancreas	Experimental	1.23±0.24	1.35±1.26*	1.57±0.35**	1.52±0.21***
	% variation	+ 15.0	+ 28.6	+ 48.0	+ 42.2
	Control	0.52±0.05	0.51±0.04	0.49±0.03	0.51±0.05
Gill	Experimental	0.57± 0.04	0.67±0.03***	0.59±0.03***	0.74±0.08***
	% variation	+ 10.8	+ 32.2	+ 20.0	+ 44.5

Values expressed as μ moles of Formanzan/mg protein/hr. Values are Mean ± S.D. of six individual observations. Experimental values differ significantly from controls at the level *p<0.1; **p<0.05 and ***p<0.001 respectively.

Table 3. *Glucose*-6-phosphate dehydrogenase (G-6-PDH) activity in the tissues of the freshwater crab Barytelphusa guerini exposed to a sublethal concentration of cadmium.

Tissues		Exposure period in days			
		1	7	15	30
Hepato -	Control	0.28±0.02	0.29±0.03	0.30±0.02	0.30±0.3
pancreas	Experimental	0.39±0.02***	0.51±0.02***	0.49±0.03***	0.47±0.02***
	% variation	+ 39.6	+ 73.9	+ 62.9	+ 57.0
	Control	0.21± 0.03	0.23±0.03	0.21±0.02	0.21±0.03
Gill	Experimental	0.25±0.02*	0.27±0.03*	0.27±0.03**	0.29±0.03***
	% variation	+ 16.6	+ 20.4	+ 26.2	+ 34.6

Values expressed as μ moles of Formazan/mg protein/hr. Values are Mean± SD. of six individual observations. Experimental values differ significantly from controls at the level *p<0.05 **p<0.01 and ***p<0.001 respectively.

Table 4. Superoxide dismutase (SOD) activity in the tissues of the freshwater crab Barytelphusa guerini exposed to a sublethal concentration of cadmium.

Tissues		Exposure period in days			
		1	7	15	30
Hepato-	Control	2.33±0.22	1.64±0.18	2.30±0.39	2.02±0.30
pancreas	Experimental	3.38±0.22***	2.81±0.20***	3.50±0.39***	3.27±0.38***
	% variation	+ 45.6	+ 71.8	+ 52.1	+ 62.1
	Control	1.18±0.11	1.32±0.11	2.30±0.39	2.02±0.30
Gill	Experimental	1.43±0.10**	1.93±0.11***	1.69±0.12***	2.66±0.14***
	% variation	+ 20.9	+ 46.1	+ 31.2	+ 80.0

Values expressed as units/mg protein. Values are Mean ± S.D. of six individual observations. Experimental values differ significantly from controls at the level **p<0.01 and ***p<0.001 respectively.

glutathione reductase which utilize NADP furnished by G-6-PDH. The activity levels of G-6-PDH were significantly elevated, indicating antioxidant defense of this enzyme in view of its responsiveness to the cadmium-induced lipid peroxidation in crab.

The oxyradical generation (XOD activity) occurring in crabs and the presence of free-radical scavengers and specific antioxidant enzymes to counter cadmium-induced oxidative stress, i.e., lipid peroxidation, provide compelling evidence for the operation of an antioxidant defense system in the field crab. Further studies to assess the temporal evolution of LPO and elaboration of the mechanisms of action and the interrrelationships between free radical scavengers are under progress.

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