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Lipid Peroxidation in the Gill and Hepatopancreas of *Oziotelphusa senex senex* Fabricius during Cadmium and Copper Exposure

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Environmental contamination by metals has increased in recent years due to the excessive use of metals in agriculture and industry. Due to their bioconcentration, immutable and non-degradable properties, these metals constitute a major source of pollutants. Among these metals cadmium, lead and mercury are non-essential, where as copper, iron, manganese, and zinc are essential elements. They are required in trace amounts by all forms of life but are toxic when present in excess.

Considerable information is available on the toxic effects of cadmium on biological mechanisms at all integration levels, such as molecular, biochemical, physiological and behavioural, in animals (Stoeppler and Piscator 1988). It is also well known that heavy metal contamination alters cellular physiology, particularly by affecting aspects such as transport across plasma membranes, mitochondrial functions, lysosomal stability etc. (Viarengo et al. 1980; Viarengo 1989). Eventhough it has been demonstrated that the *in vitro* addition of heavy metals stimulates membrane lipid peroxidation (Halliwell and Gutteridge 1984; Aruoma et al. 1989), the *in vivo* effects exerted by different cations on this process are still not clear (Stacey and Klaassen 1981). The present work reports the effect of exposure to sublethal concentrations of heavy metals such as Cu and Cd on lipid peroxidation in the tissues of the edible freshwater crab, *Oziotelphusa senex senex*.

MATERIALS AND METHODS

Adult *Oziotelphusa senex senex* (30-32 g) were used in the present study. The crabs were maintained in glass aquaria for 10 d under a natural photoperiod prior to use in experiments. They were fed frog muscle *ad libi-*

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tum on alternate days. The properties of the test medium (tap water) were: temperature, 30 ± 2°C; pH, 7.1; dissolved oxygen content, 5.88 ± 0.5 ml/l; hardness, 35 ± 3 ppm as $CaCO_3$; alkalinity, 9.7 ± 0.3 ppm as $CaCO_3$.

One hundred and fifty crabs were divided into three equal groups. The first group served as a control and the second and third groups were exposed to 100 µg/l of Cu^{2+} and 100 µg/l of Cd^{2+}, respectively. The dose was arrived at after determining the LC_{50} and then testing a number of sublethal concentrations. The metals were added daily to the medium as $CuCl_2$ or $CdCl_2$. The medium (1000 ml/crab) was changed daily and the animals were maintained for 7 days.

The animals were sacrificed at the same time of the day (10-11 AM) to avoid circadian variations. The gill and hepatopancreas were quickly isolated on ice and used immediately for biochemical analysis. The metal concentrations and biochemical estimations were done at 1,3 and 7 d of daily treatments.

The concentrations of Cu and Cd in the hepatopancreas and gill tissues were determined using an atomic absorption spectrophotometer (Perkin-Elmer Model 2380). Calibration curves were made on standard solutions and used for calculation of metal concentration.

Lipid peroxidation was evaluated by determining the levels of malondialdehyde (MDA) in the tissues. The concentrations of glutathione in the tissues were also estimated during heavy metal exposure. For malondialdehyde analysis, the tissues were homogenized in 30 mM Tris-HCl buffer, pH, 7.4. Aliquots of homogenates were added with an equal volume of acetonitril and centrifuged at 5000 g for 15 min at 0°C. The supernatants were utilized for evaluation of MDA content by high performance liquid chromatography (HPLC) using Lichrosorb NH_2 column (25 cm x 4.0 mm) and 30 mM Tris-HCl/acetonitril (9:1, V/V) as elution buffer. A standard solution of MDA was prepared (Esterbauer et al. 1984) and used to calibrate the HPLC assay.

Total glutathione content in the gill and hepatopancreas of crabs was estimated using GSH reductase enzymatic method (Akerboom and Sies 1981). Glutathione content is expressed as 'GSH equivalents' (GSH + ½ GSSG).

Statistical analysis was performed using Student's t-test (Pillai and Sinha 1968). Statistical significance was set at the $P < 0.05$ level.

Table 1. Concentration of Cd and Cu in hepatopancreas and gill of the freshwater crab Oziotelphusa senex senex exposed to 100 µg/l of either Cd or Cu.

Metal	Control	Exposure Time (days)		
		1	3	7
		Hepatopancreas		
Cd	2.73 ± 0.13	4.66* ± 0.26 (70.69)	9.66* ± 0.13 (253.84)	26.31* ± 0.20 (863.74)
Cu	0.93 ± 0.09	1.27* ± 0.11 (36.56)	1.85* ± 0.14 (98.92)	3.06* ± 0.29 (229.03)
		Gill		
Cd	1.32 ± 0.09	1.99* ± 0.23 (50.75)	4.65* ± 0.27 (252.27)	7.66* ± 0.26 (480.30)
Cu	0.70 ± 0.06	1.06* ± 0.19 (51.43)	1.69* ± 0.29 (141.43)	2.63* ± 0.35 (275.71)

Values expressed as µg/g dry wt. are mean ± S.D. of 8 individual crabs. Values in parentheses are % increase over control. Values are significant at * $P < 0.001$.

RESULTS AND DISCUSSION

Metal concentrations in the gill and hepatopancreas of crabs exposed for 7 d to Cu and Cd are presented in Table 1. Maximum uptake of both cadmium and copper ions is recorded in the tissues of crabs exposed for 7 days. Variations in lipid peroxidation (MDA content) in the tissues of crabs during Cu and Cd exposure are presented in Table 2. The MDA content increased significantly in the tissues of Cu-exposed crabs, while the Cd-exposure did not affect the level of MDA in either of the tissues examined. Glutathione concentration decreased significantly in gill and hepatopancreas following exposure to Cu, but was not affected by Cd exposure (Table 3).

The results indicate conspicuous and noteworthy differences in lipid peroxidation in the tissues of crabs during Cu and Cd exposure. Copper stimulated lipid peroxidation in crabs, whereas Cd did not. In view of the stimulation by copper, it is reasonable to expect changes in glutathione levels, since glutathione

Table 2. Malondialdehyde content in the hepatopancreas and gill of the freshwater crab Oziotelphusa senex senex exposed to 100 µg/l of either Cd or Cu.

Metal	Control	Exposure Time (days) 1	3	7
		Hepatopancreas		
Cd	69.78 ± 7.81	72.58[NS] ± 7.88 (4.01)	69.89[NS] ± 6.43 (0.16)	71.80[NS] ± 7.92 (2.89)
Cu	71.92 ± 8.87	94.71* ± 9.87 (31.69)	124.86* ± 10.94 (73.61)	147.83* ± 11.65 (105.54)
		Gill		
Cd	45.41 ± 7.82	47.44[NS] ± 5.69 (4.47)	47.91[NS] ± 8.09 (5.55)	43.55[NS] ± 5.69 (-4.09)
Cu	43.78 ± 8.09	54.84** ± 7.02 (25.26)	75.09* ± 6.57 (71.52)	82.15* ± 7.08 (87.64)

Values expressed as nmol/g wet wt. are mean ± S.D of 8 individual crabs. Values in parentheses are % change over control. Values are significant at * $P < 0.001$; ** $P < 0.05$. NS = Not significant.

is usually considered the most powerful soluble antioxidant compound present in the cell and it is involved in the protection against oxidative damage (Meister and Anderson 1983). The concentration of glutathione decreased significantly only in the tissues of Cu-exposed crabs, thus indicating that Cu impairs one of the most important defense mechanisms of the cell against peroxidative stress.

Many in vivo and in vitro studies indicate that transition metals like iron and copper are involved in redox reactions which result in the formation of oxy-radicals (Wills 1969; Cheeseman et al. 1988)

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + HO^{-}$$
$$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + HO^{\bullet} + HO^{-}$$

Among these two, cuprous ions react with H_2O_2 with a much greater rate constant than do ferrous ions, giving rise to extremely reactive hydroxyl radicals in the Fenton reaction (Halliwell and Gutteridge 1984). With

Table 3. Glutathione content in the hepatopancreas and gill of the freshwater crab Oziotelphusa senex senex exposed to 100 μg/l of either Cd or Cu.

Metal	Control	Exposure Time (days) 1	3	7
		Hepatopancreas		
Cd	490.18 ± 47.51	494.67^{NS} ± 42.20 (0.92)	509.62^{NS} ± 31.02 (3.97)	496.09^{NS} ± 27.73 (1.21)
Cu	504.28 ± 45.09	450.09** ± 29.47 (-10.75)	378.66* ± 31.43 (-27.04)	318.62* ± 27.32 (-36.82)
		Gill		
Cd	135.95 ± 12.35	137.49^{NS} ± 11.61 (1.13)	139.56^{NS} ± 17.35 (2.66)	132.07^{Ns} ± 15.14 (-2.85)
Cu	137.19 ± 11.51	112.09* ± 11.14 (-18.30)	93.45* ± 9.87 (-31.88)	75.91* ± 8.14 (-44.67)

Values expressed as nmol/g wet wt. are mean ± S.D. of 8 individual crabs. Values in parentheses are % change over control. Values are significant at * $P < 0.001$; ** $P < 0.05$. NS = Not significant.

organic hydroperoxides (ROOH), homologous reaction is thought to occur leading to the formation of the peroxy ($ROO^{\bullet}$) and alkoxy ($RO^{\bullet}$) radicals

$$ROOH + Cu^{2+} \longrightarrow ROO^{\bullet} + Cu^{+} + H^{+}$$
$$ROOH + Cu^{+} \longrightarrow RO^{\bullet} + Cu^{2+} + OH^{-}$$

Therefore, copper ions may participate both in the initiation and the propagation of lipid peroxidation, thus stimulating the degradation of membrane lipids. The undegradable end products of lipid peroxidation processes tend to accumulate in the tertiary lysosomes in the form of an insoluble polymer containing oxidized lipids and proteins, usually named lipofuscin. Viarengo et al. (1990) reported that after exposure to Cu, lipofuscin granules accumulate in the lysosomes of digestive gland cells of mussels. These insoluble lipoprotein pigments are able to bind the Cu in a stable form, thus representing the main detoxification mechanism of Cu in cells (Viarengo 1989). Whereas Cd does not undergo redox cycling it was unable to stimulate lipid peroxidation process in the tissues of crab.

Excess heavy metals in the cells may also stimulate the synthesis of metallothioneins in crustaceans (Lerch et al. 1982; Otvos et al. 1982). These are soluble, heat stable, low molecular weight, SH-rich proteins having high affinity for metal ions and binds to metals in a non-toxic form, thus reducing their deleterious effects. These metallothioneins accumulate in the lysosomes in an insoluble form after the oxidation of SH residues by the formation of intra disulphide bridges. By considering the alterations in the lipid peroxidation in the tissues of crab, it is clear that Cu bound both to lipid peroxidation end products and to Cu-thioneins and was subsequently trapped into lysosomes, and further eliminated from cells by exocytosis. On the contrary, these routes of metal detoxification do not seem to be active in the case of Cd. This would explain the different biological half-lifes of these metals, which is short for Cu (9 days) and longer for Cd (7 months) (Viarengo et al. 1987).

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