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Ligands Binding Cadmium, Zinc, and Copper in a Species of New Zealand Oyster (*Ostrea lutaria*)

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Long-term exposure to cadmium may cause damage in the renal proximal tubules (FRIBERG et al. 1974). Critical concentrations for such a damage may vary from 10-200 µg Cd/g kidney cortex depending upon the administered form of cadmium (CHERIAN et al. 1982). In a recent study (McKENZIE et al. 1982) it was shown that a species of oyster, *Ostrea lutaria*, which contained a high concentration of cadmium (on an average 5 µg/g wet tissue) was consumed widely in New Zealand. It was also shown that a sub-population group (oyster fishermen) who consumed a large number of the oysters per week, had as high cadmium intakes as the populations in Japan with known chronic manifestations of cadmium toxicity. But the blood and urine Cd levels in the oyster consumers were lower than expected in relation to the high intake (as much as 500 µg Cd/day). It was suggested that the Cd from this species of oyster was either poorly absorbed or was absorbed to a greater extent than indicated by the blood levels but was metabolised differently to Cd from other foods.

In light of the above data, it was therefore important to investigate the form(s) of Cd in the oyster, *Ostrea lutaria*. The results of preliminary studies indicate that the data may be of significant importance, first in the interpretation of the epidemiological data obtained earlier (McKENZIE et al. 1982), and secondly in evaluating the risk of high Cd intakes via food and subsequent health effects in humans. The results of the preliminary studies are therefore described in this report.

MATERIALS AND METHODS

Oyster Samples: *Ostrea lutaria*, a species of dredge oyster, was obtained from Foveaux Strait, South Island, New Zealand. The oysters were homogenised in groups of four with 0.25M sucrose/8mM Tris-HCl solution containing 0.1mM PMSF (Phenylmethylflurosulphonate) (Sigma Chemical Co.) a protease inhibitor. Three separate homogenisations were carried out to obtain the data in

triplicate. The homogenisation and the subsequent separation steps were carried out at 0-4°C.

Ultracentrifugation and Chromatography: The homogenates were centrifuged initially at 16,000 g (av.) for 20 min. and then at 100,000 g (av.) for 1 hr. Portions (2 ml) of the final supernatants (the cytosol) were chromatographed on Sephadex G-75 columns equilibrated and eluted with 0.1M ammonium formate/8mM Tris-HCl at pH 8.0 (SHARMA AND McQUEEN 1980). Eluate fractions of 5 ml were collected and analysed for Cd, Zn and Cu. The sedimented particulate fractions obtained after the centrifugation of the homogenates, were also analysed for Cd, Zn and Cu.

Characerisation of Cd-binding Ligands: The Sephadex columns were calibrated for molecular weight estimations with blue dextran (MW: 2,000,000), bovine serum albumin (MW: 67,000), horse heart muscle cytochrome C (MW: 12,400) rat liver Cd-thionein (MW: about 10,000) and potassium dichromate (MW: 249) as described before (SHARMA 1982). The Cd-binding ligands in the oyster cytosol were pooled according to their molecular weights into three groups, high molecular weight (HMW: > 20,000); metallothionein (MT) fraction (MW: 6000-12,000); and low molecular weight fraction (LMW: < 2000). The pooled MT fractions were tested for heat stability at 75°C for 5 min. Heat treated and untreated MT fractions were centrifuged at 1000 g (av.) for 5 min. and the $E_{250/280}$ ratios of the supernatants were determined. The heat treated MT fractions were chromatographed again on the Sephadex G-75 column and the concentration of Cd in the MT fractions was determined. Hemolysate test; this is an adaptation of the method described by Onosaka and Cherian, 1981. Rat blood erythrocytes were hemolised with deionised distilled water and the hemolysate (0.5 ml) was added to 5 ml of fresly chromatographed MT fraction of oyster cytosol. The mixture was heated to 75°C for 2 min. and then centrifuged at 1000 g (av.) for 5 min. The supernatant was chromatographed on the Sephadex G-75 column and the concentration of Cd determined in the fractions corresponding to MT.

Metal Analysis: Samples of the homogenate, cellular particulate fraction and cytosol were digested in concentrated ultrex grade nitric acid (J.T. Baker Chemical Co.) at 75°C overnight. The digests were appropriately diluted with deionised distilled water and analysed for Cd, Zn and Cu. The chromatographed eluate fractions were analysed without any pretreatment. All Cd analysis were carried out by a graphite furnace atomic absorption method (SHARMA et al. 1982). The Zn and Cu levels were determined by an air-acetylene flame atomic absorption method. Concentrations of Cd, Zn and Cu

were calculated using standard addition calibration curves for each matrix.

RESULTS

Analysis of Cd, Zn and Cu in the New Zealand oyster, *Ostrea lutaria*, showed that on an average it contained 5.8 µg Cd, 67 µg Zn and 14.1 µg Cu per g wet weight (Table 1).

Table 1. Concentrations of Cd, Zn and Cu in whole oyster, cellular particulate fraction and the cytosol (µg/g tissue ± s.d.)

Metal Analysed	Whole Tissue	Particulate Fraction	Cytosol
Cd	5.8 ± 1.9	2.8 ± 0.7	2.9 ± 1.0
Zn	67.0 ± 24.0	15.4 ± 4.6	48.2 ± 12.5
Cu	14.1 ± 3.5	2.6 ± 0.8	11.3 ± 2.8

Within the oyster tissue, the amount of Cd was equally distributed between the particulate and soluble (cytosol) fractions. The distribution of Zn and Cu was different, about 70% and 80% respectively being present in the cytosol. The concentrations of the metals found in the whole oyster are in agreement with those reported elsewhere (NIELSEN 1975).

Separation of the Cd-binding ligands in the oyster cytosol on Sephadex G-75 column showed that about 60% of the cytosolic Cd was bound to HMW proteins eluted near the void volume (Table 2). Similarly about 40% of Zn and Cu was also present in the HMW proteins. A significant proportion (32%) of the cytosolic Cd was present in low molecular weight protein fractions eluting with relative elution volumes similar to that of rat liver metallothionein. The protein was estimated to have a molecular weight in the range 6000 to 12,000 daltons and the protein fraction was defined as the metallothionein fraction. Only a very small amount (< 1%) of Zn was associated with the MT fraction, giving a Cd: Zn ratio of about 15:1. In contrast, a high concentration of Cu was measured in the MT fraction (2.23 µg Cu/g wet tissue), with a Cd:Cu ratio of 1:2.4. The LMW fraction of the oyster tissue cytosol had less than 1% of cytosolic Cd, but

Table 2. Concentrations of Cd, Zn and Cu in the Cd-binding ligands in the tissue cytosol of Ostrea lutaria.

Metal Analysed (µg Metal/g tissue s.d.	HMW Fraction (MW: > 20000)	MT Fraction (MW:6000-12000)	LMW Fraction (MW: < 2000)
Cd	1.68 ± 0.52	0.92 ± 0.40	0.11 ± 0.06
Zn	18.6 ± 3.2	0.13 ± 0.06	27.7 ± 4.2
Cu	4.57 ± 1.83	2.23 ± 0.84	3.59± 1.18

it contained a large proportion of Zn (about 60%) and Cu (about 32%).

Data on the partial characterisation of the low molecular weight protein corresponding to metallothionein fractions is given in Table 3.

Table 3. Amount of Cd in treated and untreated MT fraction of oyster tissue cytosol.

Parameter	Untreated	Heat Treated	Hemolysate Treated
Cd, ng	467 ± 38	446 ± 36	425 ± 43
Recovery	100%	95%	91%

The heat and hemolysate treated MT fractions, both eluted with relative elution volumes corresponding to the untreated MT fractions showed over 90% of the metal was still bound to the MT-like protein after heat and hemolysate treatment. The heat treated MT fraction had a $E_{250/280}$ ratio of 6.6.

DISCUSSION

The results clearly show that most of the Cd (about 75%) in the oyster, *Ostrea lutaria*, was present in the particulate fraction of the tissue and bound to the HMW proteins in the cytosol. A smaller but significant amount (about 25%) was also bound to a low molecular weight protein fraction which had a similar elution profile on Sephadex G-75 column as rat liver metallothionein. The protein was heat stable and did not release the bound Cd when mixed with rat erythrocyte hemolysate. The $E_{250/280}$ ratio was low (6.6) compared to that for pure Cd-MT (12-14). But this discrepancy is usually associated with the impurities in partially purified Cd-MT (KAGI and NORDBERG 1979). These results indicate that the low molecular weight Cd-binding protein in *Ostrea lutaria* may be Cd-MT. The almost complete absence of Zn in this protein suggests that it is different to the mammalian MT but similar to the metalloprotein isolated from other invertebrates (OTVOS et al. 1982; WIEDOW et al 1982).

Following exposure to inorganic Cd, most of the absorbed metal is initially accumulated in the liver mainly bound to MT. In the long term there is a gradual transfer of Cd to the kidneys leading to a high accumulation in the renal cortex. As in the liver, MT is the major Cd-binding protein in the kidney cells. In people exposed to high levels of Cd, renal tubular damage may occur at Cd concentrations of about 200 µg/g renal cortex (FRIBERG et al. 1974). However data based onanimal studies indicate that the renal damage may occur at kidney Cd concentrations well below this value, and was dependent on the administered form of the metal. For instance, when Cd was administered either as an injection or orally as Cd-MT, Cd was absorbed directly and preferentially into the kidneys causing proximal tubular damage at kidney Cd concentrations as low as 10 µg/g renal cortex (NORDBERG et al. 1975, CHERIAN et al. 1976; CHERIAN 1979). Furthermore, based on LD_{50} values the MT bound Cd is seven times more toxic than inorganic Cd (WEBB and ETIENE 1977). In this context, if the Cd in the oyster, *Ostrea lutaria*, is indeed present as Cd-MT, it would be quickly transported to the kidney and possibly contribute less to blood Cd levels than non-metallothionein bound Cd and hence explain to some extent the lower blood Cd concentrations found in people who consume large numbers of these oysters.

Within the cell MT plays a protective role against the toxicity of Cd (NORDBERG 1971, RUGSTAD and NORSETH 1975). Extracellularly, the Cd-MT complex appears to be several times more toxic than the Cd^{2+} ion.

VALBERG et al. 1977 found that when the intestinal mucosal cells were exposed to Cd-MT, extensive necrosis of absorptive cells occurred. Under the same conditions, cadmium chloride caused only minor abnormalities. Based on these studies which show the cellular toxicity of Cd-MT, and the results of present study which shows a high concentration of Cd and metallothionein-like protein in *Ostrea lutaria*, it is important to elucidate the various forms of Cd in the oyster (and perhaps other food), their gastrointestinal absorption, tissue distribution and toxicity. The significance of the different Cd-binding ligands and the competitive interactions of other metals such as Zn and Cu in the oyster is not clear. However further continuing studies may provide some clues to the significance of these interactions and the apparent paradoxical role of MT in the toxicity and detoxification of Cd.

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