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Note

Protein binding of cadmium, zinc and copper in environmentally insulted limpets *Patella vulgata*

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Despite the rapidly increasing store of information on the distribution of heavy metal levels in the environment, very little is known about the means by which these metals are stored in biological and botanical systems.

It is well known that cadmium is predominantly found in the kidneys and livers of mammals exposed to this metal[1]. The discovery of metallothionein, a low-molecular-weight protein, which is capable of selectively binding cadmium, zinc, copper and mercury, in horse kidney cortex by Margoshes and Vallee[2] has prompted the investigation of the storage methods of other mammals. Similar cadmium-binding proteins have since been found in the livers and kidneys of mice[3,4], rats[4–7] and humans[8–10]. It is believed that the same protein which binds cadmium is also important in the detoxification of mercury [11–13], but plays no part in the sequestration of the more toxic organic mercury compounds[14].

Butterworth *et al.*[15] have demonstrated that heavy metals, such as cadmium, zinc and lead, are concentrated by molluscs living in the littoral zones of the Severn Estuary. The accumulation of these metals implies some mechanism for their retention, such as the existance of one or more proteins capable of binding these metals.

We have investigated the distribution of cadmium, zinc and copper within the water-soluble protein extract from the common limpet *(Patella vulgata)* found in the Severn Estuary. These specimens were obtained from Brean Down (Somerset, Great Britain), where the estuarine species are exposed to severe industrial pollution and are therefore ideal for study.

EXPERIMENTAL AND RESULTS

Forty limpets were washed with doubly distilled water and then removed whole from their shells. These were homogenised with an equal volume of phosphate buffer (0.025 *M* in Na_2HPO_4, 0.025 *M* in KH_2PO_4, made up in doubly distilled water to give a pH of 7.0) and the homogenate was left for 12 h, with stirring. The homogenate was then centrifuged at 35,000 *g* for 3 h to remove particulate material and the homogenate was lyophillized. This extraction procedure was found to give higher metal recoveries than the published extraction procedures involving ethanol–trichloromethane[8] or rivanol ($C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$) precipitation stages[10]. Typical recoveries are given in Table I.

TABLE I

SAMPLE METAL CONTENT AND EXTRACTION EFFICIENCES

All concentrations are given in ppm (fresh weight).

Metal	*Sample concentration*	*Extraction (%)*
Cd	27.8	73.0
Zn	51.0	16.2
Cu	7.4	68.2

Gel filtration of 0.1-g samples of the extract was performed on 1.0 m × 2.6 cm columns of Sephadex G-75 (Pharmacia, Uppsala, Sweden). The protein samples were eluted with 0.001 *M* Tris–HCl buffer, pH 8.6, and 5-ml fractions of the eluate were collected. These were analysed for protein by ultraviolet spectrophotometry and for metal content by atomic absorption spectrophotometry.

Typical chromatograms are shown in Fig. 1, from which it can be seen that a predominant proportion of the cadmium present in the water-soluble extract is found bound to a protein of low molecular weight (approximately 10,000 daltons). This same protein also appears to be responsible for the binding of most of the copper which is present in the extract. The mechanism for the retention of zinc, however, is still unclear. Practically all of the zinc which is present in the extract (which is only 16% of that which is present in the original mollusc sample) is excluded by the gel filtration column, and is therefore bound to proteins of molecular weight greater than 60,000 daltons.

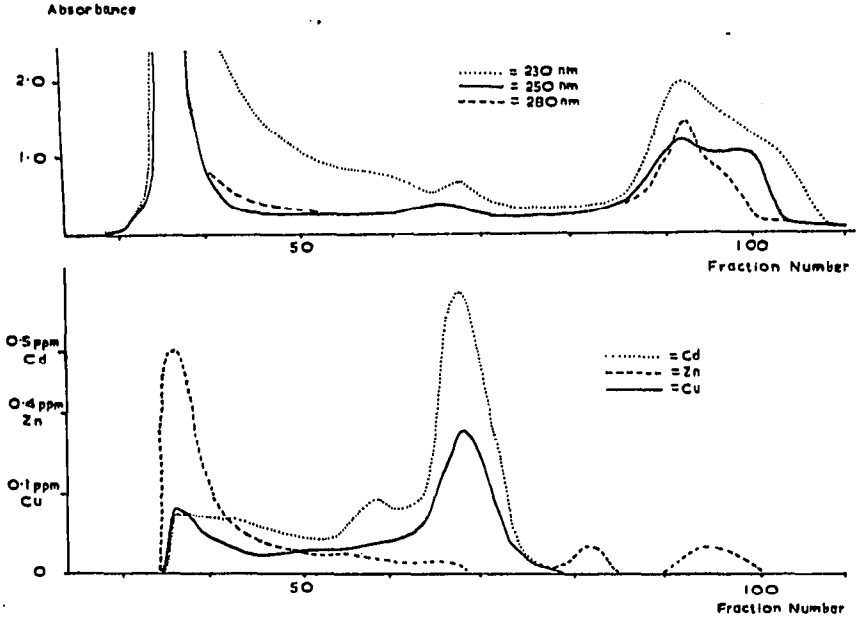

Fig. 1. Gel filtration of water-soluble limpet extract on Sephadex G-75. The sample was eluted with 0.001 *M* Tris–HCl buffer, pH 8.6, at a flow-rate of 10 ml/h.

These initial studies have indicated possible mechanisms for the retention of cadmium and copper by the common limpet and may account for the differences observed in the rate of uptake of zinc, cadmium and copper by dog whelks *(Nucella lapillus)*[16]. It is hoped that further investigation may reveal whether the high binding capacity of this protein may be due to high cysteine content, as has been postulated for mammalian metallothionein[17].

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