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Cadmium Uptake and Induction of Cadmium-binding Protein in the Waterflea (*Moina macrocopa*)

Mitsuru Yamamura, Shigehisa Hatakeyama, and Kazuo T. Suzuki

National Institute for Environmental Studies, Yatabe, Tsukuba, Ibaraki 305, Japan

Cadmium is an accumulative pollutant and aquatic organisms take up the metal from food and water. WILLIAMS & GIESY (1978) have reported that there is no significant cadmium uptake from food when mosquitofish were fed with commercial fish food spiked with $CdCl_2$. However, HATAKEYAMA *et al.* have reported the effects of cadmium through food by using experimental food chain systems; guppy was less affected by cadmium when the fish was fed with the cadmium-accumulated zooplankton, *Moina macrocopa*. In contrast, hydra was more affected by the metal through food chain compared to the effects directly through water in *M. macrocopa*-hydra system (HATAKEYAMA *et al.* 1979, HATAKEYAMA & YASUNO 1982).

Zooplankton is one of the important foods for many aquatic organisms and seems to be an adequate food to study the effects of cadmium through food chain. However, the chemical forms of the metal in zooplankton are not known. The present study was intended to clarify the accumulation and chemical forms of cadmium in the zooplankton, *Moina macrocopa*.

MATERIALS AND METHODS

M. macrocopa collected from a pond at this institute was kept with food (Moina Px and Py, Japan Bioresearch Co., Noda, Japan) in aerated water tanks at 23°C. Cadmium was added to underground water (water hardness, 80 to 84 ppm as $CaCO_3$) in the tanks at a concentration of 20 μg Cd/ℓ and the cadmium-containing water was changed to fresh one every day. The animals exposed to cadmium were collected after 0, 24, 48, and 72 hr. The collected animals were washed with distilled water and then homogenized in two volumes of 0.1 M Tris-HCl buffer solution (pH 7.4, 0.25 M glucose) in an atmosphere of nitrogen under ice-water cooling. The homogenates were centrifuged at 170,000 g for 60 min at 4°C. Concentrations of cadmium, zinc, and copper in the homogenates and supernatants were determined on an atomic absorption spectrophotometer (Hitachi 170-50A) after digestion with $HClO_4$-HNO_3 (V/V=1/5).

A 10 mℓ portion of the supernatants was applied on a Sephadex G-75 column (2.6 X 90 cm) and the column was eluted with 10 mM Tris-HCl buffer solution (pH 8.6 at 25°C). Five mℓ fractions were collected. Concentrations of the metals and molecular absorbances at 254 and 280 nm in the fractions were determined on an atomic

absorption spectrophotometer and a spectrophotometer, respectively.

The cadmium-binding protein fraction from a Sephadex column was concentrated by ultrafiltration on a Diaflo UM-2 membrane. A 100 µℓ aliquot of the sample was applied on an SW column (TSK GEL SW3,000, 7.5 X 600 mm with a precolumn of 7.5 X 75 mm) and the column was eluted with 50 mM Tris-HCl buffer solution (pH 8.0 at 25°C, 0.1% NaN_3) at a flow rate of 1.0 mℓ/min. The outlet was directly connected to the nebulizer tube of an atomic absorption spectrophotometer as previously reported (SUZUKI 1980). Atomic absorbance of cadmium was monitored continuously.

RESULTS AND DISCUSSION

HATAKEYAMA & YASUNO (1981) have reported that LD_{50} value of cadmium to *M. macrocopa* is 28 µg Cd/ℓ at 72 hr. However, the distinct decrease in number of the animals was not observed during cadmium exposure at the present condition. Fig. 1 shows the accumlation of the metal in *M. macrocopa* during exposure to the metal. The concentration of the metal in the homogenates attained a maximum level in 72 hr. At this time, the concentrations of zinc and copper in the homogenates were not affected (the control values were 2.8 and 0.29 µg/mℓ, respectively). Approximately 60% of cadmium in the homogenates was extracted into the supernatant fraction and the metal concentration in the supernatants increased with accumulation of the metal in the animals.

Cadmium in the supernatant fraction was separated into the three fractions on a Sephadex G-75 column; the void volume, Ve/Vo

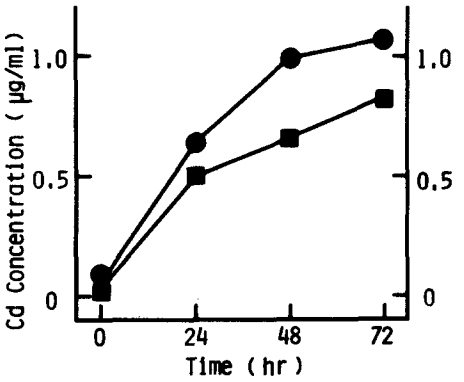

Fig. 1. Cadmium uptake into *M. macrocopa* during cadmium exposure. *M. macrocopa* exposed to cadmium at a concentration of 20 µg Cd/ℓ was collected after 0, 24, 48, and 72 hr. The animals were homogenized in two volumes of 0.1 M Tris-HCl buffer solution and centrifuged at 170,000 g for 60 min. —●— , homogenate; —■— , supernatant.

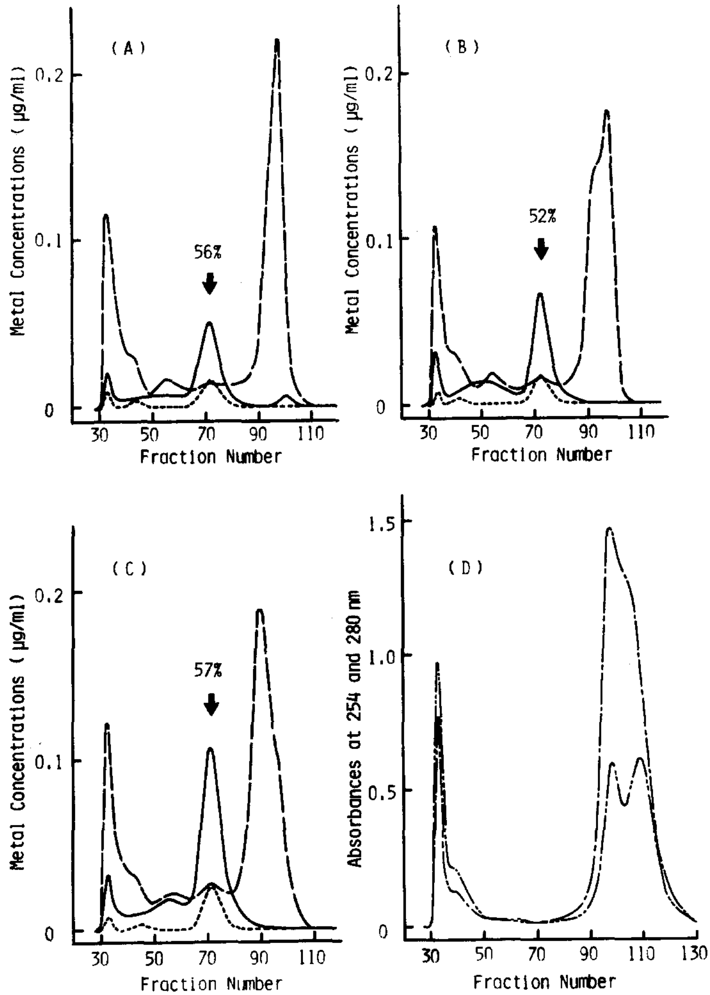

Fig. 2. Sephadex G-75 elution profiles of supernatants from *M. macrocopa* exposed to cadmium. A 10 mℓ portion of the supernatants obtained as shown in the legend to Fig. 1 was applied on a Sephadex G-75 column (2.6 X 90 cm). The column was eluted with 10 mM Tris-HCl buffer solution (pH 8.6) and 5 mℓ fractions were collected. Chromatograms (A), (B), and (C) show elution profiles of metals after 24, 48, and 72 hr, respectively. Chromatogram (D) shows UV absorbances of the supernatant corresponding to chromatogram (C). The arrows and figures indicate the cadmium -binding protein fraction and percent of cadmium in the fraction, respectively. ———— , Cd; — — — —, Zn; ----------, Cu; ——-—— , absorbance at 254 nm; ——--—— , absorbance at 280 nm.

=1.4-1.8, and the low molecular weight protein fraction (Fig. 2). More than 50% of the metal in the supernatants was eluted at the low molecular weight protein fraction (Ve/Vo=2.0-2.4) and the peak intensity increased with accumulation of the metal in *M. macrocopa*. Most of zinc in the supernatants showed a similar distribution to the UV profile; the high molecular weight protein fraction and the salts fraction. The low molecular weight cadmium -binding protein (Ve/Vo=2.0-2.4) contained only small amounts of zinc and copper, and the amounts did not increase with the increase of cadmium.

Metallothioneins induced in malacostracans are reported to have an apparent molecular weight of 10,000 daltons as that of mammalian metallothioneins (OLAFSON *et al.* 1979, OVERNELL & TREWHELLA 1979, RAY & WHITE 1981). However, the cadmium-binding protein induced in *M. macrocopa* was smaller than mammalian metallothioneins, the protein being eluted slower than rat metallothioneins (Ve/Vo=1.8-2.2) at the same condition.

The cadmium-binding protein induced in *M. macrocopa* was further separated into two isoproteins on an SW3,000 column which was shown to have a cation exchange chromatographic property when eluted with alkaline buffer solution (SUZUKI 1980) as shown in Fig. 3. At the same condition, rat metallothionein-I and -II were eluted at retention times of 20.4 and 19.5 min, respectively. These results suggest that cadmium accumulated in *M. macrocopa* is mainly bound to the low molecular weight cadmium-binding protein whose molecular weight is smaller than that of mammalian

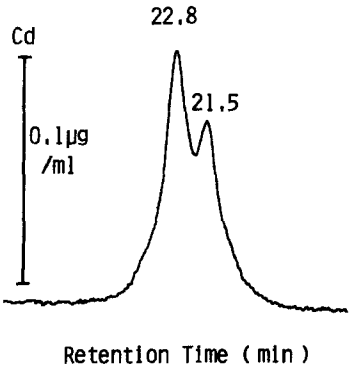

Fig. 3. Gel permeation-cadmium atomic absorption chromatogram of the cadmium-binding protein from *M. macrocopa* on an SW3,000 column. The cadmium-binding protein concentrated after isolation with a Sephadex column was applied on an SW column and the column was eluted with 50 mM Tris-HCl buffer solution (pH 8.0) at a flow rate of 1.0 mℓ/min. The detector level of an atomic absorption spectrophotometer was set as indicated by the vertical bar.

metallothioneins and that the protein is a mixture of two isoproteins as in the case of malacostracan metallothioneins (OLAFSON *et al.* 1979, OVERNELL & TREWHELLA 1979).

In short, the zooplankton, *M. macrocopa*, exposed to cadmium (20 µg Cd/ℓ) took up the metal and attained a maximum level in 72 hr. At this time, the concentrations of zinc and copper in *M. macrocopa* was not affected. Most of cadmium accumulated in the animals was bound to the low molecular weight cadmium-binding protein. The protein had an apparent molecular weight smaller than that of rat metallothioneins and was a mixture of two isoproteins.

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