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Differential role of metallothionein on Zn, Cd and Cu accumulation in hepatic cytosol of rats

S. Saito* and Y. Kojima

Department of Environmental Medicine and Informatics, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060 (Japan), Fax +81 11 747 9780
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Abstract. To examine the role of metallothionein (MT) on heavy metal accumulation in hepatic cytosol of rats, this study was carried out to determine the relative Zn, Cd and Cu-binding capacities of MT in hepatic cytosol of Zn, Cd and Cu-injected rats, respectively. The heavy metal contents were detected in the liver and cytosol in the following proportions: 65.2–74.8% of the Zn content, 61.9–65.6% of the Cu content, and approx. 65% of the Cd content. Each rat was given a single intraperitoneal injection of saline, $ZnSO_4$ (1, 5, 10 and 20 mg Zn/kg b.w.), $CuSO_4$ (2, 4 and 6 mg Cu/kg b.w.) or $CdCl_2$ (1, 2 and 3 mg Cd/kg b.w.). The amounts of the increased Zn and Cu were attributable to MT and high molecular weight proteins, while most of the increased Cd was attributable to MT. There was a close relationship between heavy metal content of the cytosol and MT in all heavy metal-injected rats. Our data demonstrated that approx. 60% of the increased Zn or Cu in the hepatic cytosol of Zn or Cu-injected rats was bound to MT, while 83% of the increased Cd in hepatic cytosol of Cd-injected rats was bound to MT. Therefore the order of relative binding capacity in vivo of MT determined for several metals (Cd > Zn > Cu) did not correlate with the published in vitro order of affinity to MT, Cu > Cd > Zn. These results suggested that the role of MT in Zn or Cu accumulation in the liver of Zn or Cu-injected rats was different from that of MT in Cd accumulation in the liver of Cd-injected rats.
Key words. Metallothionein; zinc; cadmium; copper; rat liver.

Metallothionein (MT) (for reviews see [1, 2], and references therein) is characterized by a low molecular weight (6500–7000 Da), a high affinity for heavy metals such as zinc (Zn), cadmium (Cd) and copper (Cu), a high cysteine content and a lack of aromatic amino acids. MT occurs throughout the animal kingdom and is also found in higher plants, eukaryotic microorganisms, and some prokaryotes. A remarkable feature of all MT is its inducibility by several heavy metals, hormones, cytotoxic agents, various physiological conditions associated with physical or chemical stresses and X-ray irradiation. The physiological function of MT is not completely understood but appears to be associated mainly with detoxification of heavy-metal ions, e.g. Cd [3], and homeostasis of essential metals, e.g. Zn and Cu [4, 5]. Although the levels of MT and its inducibility following administration of heavy metals to rats have been demonstrated in adult rat liver [6–9], hepatic heavy metal metabolism has not been investigated in detail. In order to estimate the role of MT in heavy metal accumulation in hepatic cytosol of rats, this study was carried out to determine the relative Zn, Cd and Cu-binding capacities of MT (the ratio of heavy metal content associated with MT to increased heavy metal content in hepatic cytosol) in hepatic cytosol of Zn, Cd and Cu-injected rats, respectively.

* Corresponding author.

Materials and methods

Chemicals. Sephadex G-75 was obtained from Pharmacia LKB (Uppsala, Sweden). All other chemicals were of analytical grade commercially available. The glassware for acid oxidation was acid-washed and rinsed with deionized and distilled water.

Animal treatments. Thirty-three male Sprague-Dawley rats (Japan SLC, Inc., Japan), weighing 120–145 g were divided into 11 groups of three rats and housed at a constant temperature of 21.5 ± 1.5 °C on a 12 h light/12 h dark cycle for two weeks prior to starting the experiments. They were provided with a commercial diet (Clea Japan, Inc., Japan) and water ad libitum. $ZnSO_4$, $CuSO_4$ and $CdCl_2$ were dissolved in saline solution to obtain 1 (15 μmole), 5 (76 μmole), 10 (152 μmole) and 20 (305 μmole) mg Zn/kg b.w., 2 (31 μmole), 4 (62 μmole), and 6 (94 μmole) mg Cu/kg b.w. or 1 (8 μmole), 2 (17 μmole) and 3 (26 μmole) mg Cd/kg b.w., respectively. Zn (20 mg/kg b.w.), Cu (6 mg/kg b.w.) and Cd (3 mg/kg b.w.) were the maximum tolerated doses. The control rat group received only saline solution. Each rat was given a single intraperitoneal injection of heavy metal or saline solution, and killed 14 h after injection by anesthesia with diethyl ether. The livers were removed immediately and stored at −20 °C until use.

Determination of heavy metal content of liver. To analyse the heavy metal content of liver, 1 g of each liver (8.7–11.7 g) was digested with mixed acids (1 ml

Table 1. Distribution of Zn in the liver and cytosol of Zn-injected rats. All results are per rat liver.

Dose (Zn mg/kg b.w.)	Liver μg of Zn	Cytosol	
		μg of Zn	% of liver
(n = 3, means ± S.D.)			
0	251 ± 18.5	182 ± 8.6	72.5
1	315 ± 15.2	218 ± 8.8	69.2
5	417 ± 22.0	312 ± 11.1	74.8
10	741 ± 11.5	483 ± 10.4	65.2
20	913 ± 120	610 ± 65.8	66.8

Table 2. Distribution of Cu in the liver and cytosol of Cu-injected rats. All results are per rat liver.

Dose (Cu mg/kg b.w.)	Liver μg of Cu	Cytosol	
		μg of Cu	% of liver
(n = 3, means ± S.D.)			
0	43.1 ± 5.9	28.3 ± 0.8	65.6
2	237 ± 31.4	153 ± 2.7	64.7
4	372 ± 45.1	244 ± 16.2	65.5
6	497 ± 42.2	307 ± 25.2	61.9

Table 3. Distribution of Cd in the liver and cytosol of Cd-injected rats. All results are per rat liver.

Dose (Cd mg/kg b.w.)	Liver μg of Cd	Cytosol	
		μg of Cd	% of liver
(n = 3, means ± S.D.)			
0	ND	ND	ND
1	53.5 ± 37.1	36.2 ± 24.9	67.6
2	180 ± 25.5	116 ± 12.1	64.6
3	186 ± 26.3	121 ± 17.1	65.0

H_2SO_4, 5 ml $HClO_4$ and 10 ml HNO_3). The Zn and Cu were measured with a Hitachi flame atomic absorption spectrophotometer, model 180-30, and the Cd was assayed using an inductively coupled argon plasma-atomic emission spectrometer (Hewlett 4500 Series, Packard, USA).

Determination of heavy metal content of metallothionein. Five grams of frozen liver were thawed at 4 °C, cut into pieces and homogenized (2:1 = v:w) in ice-cold 50 mM Tris/HCl, pH 8.1, with a polytron, model PT 10-35 (Kinematica GmbH, Switzerland) three times for 30 sec. The homogenate was centrifuged at 10,000 × g for 30 min at 4 °C with a Kubota centrifuge, model KR/200B. The supernatant was centrifuged at 110,000 × g for 60 min at 4 °C using a Hitachi ultracentrifuge, model 70P-72. The cytosol was applied to a Sephadex G-75 column (3.0 × 100 cm), equilibrated with 10 mM Tris/HCl, pH 8.1, and eluted with the same buffer at a flow rate of approx. 30 ml/h at 4 °C. The eluent was collected in 10 ml fractions and assayed for Zn, Cu and Cd concentrations with a Hitachi flame atomic absorption spectrophotometer, model 180-30.

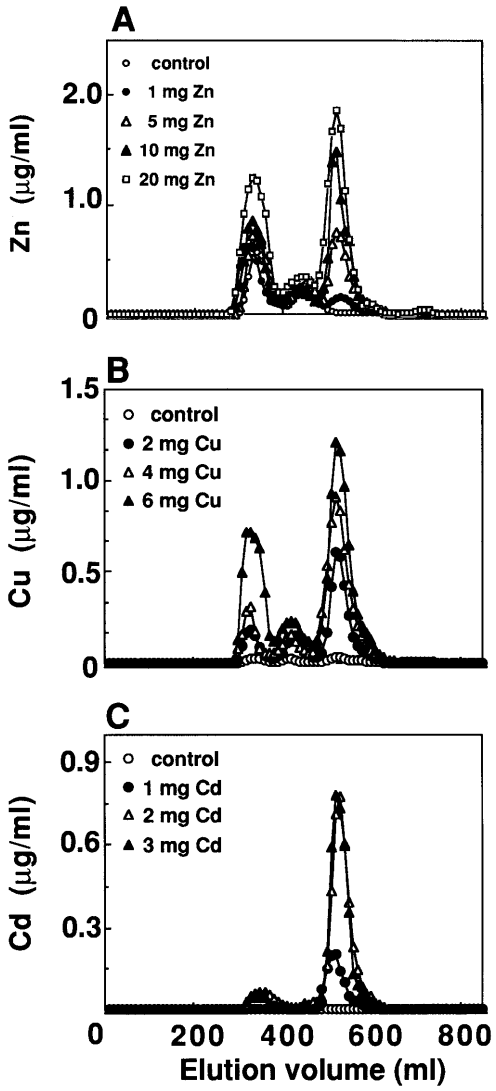

Figure 1. Elution patterns of gel filtration on Sephadex G-75 of the cytosol fractions obtained from the livers of Zn-injected (A), Cu-injected (B) and Cd-injected (C) rats respectively.

Results and discussion

This investigation studied the role of metallothionein on Zn, Cu and Cd accumulation in rat liver following these heavy metal injections. Table 1 shows quantitative data of the Zn content in the liver and cytosol of Zn-injected rats. The Zn content of the cytosol and the liver increased following the injection of Zn. After Zn injection, 65.2–74.8% of the Zn content of liver was detected in the cytosol. The levels in rat liver in our study were higher than those of the previous study (Bremner et al. [10]). Quantitative data of the Cu content of the liver and the cytosol of Cu-injected rats are shown in table 2. The Cu content of cytosol and liver increased following the injection of Cu. After Cu injection, 61.9–65.6% of the Cu content of the liver was detected in the cytosol. The Cd content of cytosol and liver increased in the range 0–2 mg/kg b.w., and declined thereafter (table 3).

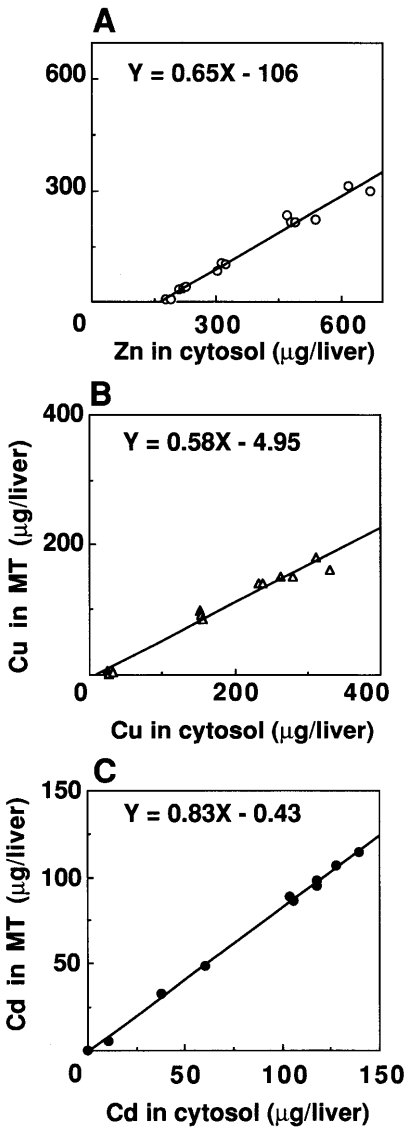

Figure 2. Relationships between Zn, Cu and Cd contents in the cytosol and the MT in rats injected with Zn (*A*), Cu (*B*) and Cd (*C*), respectively. The slope of the regression equation is shown in the figure.

Approx. 65% of the Cd content of the liver of Cd-injected rats was detected in the cytosol. The levels in the rat liver in our study were lower than those of the previous study by Sabbioni and Marafante [11].

These results indicated that in all three rats the proportions of the heavy metal contents in the livers of heavy metal-injected rats were very similar. The distribution profiles of the hepatic cytosol of Zn, Cu and Cd-injected rats on a Sephadex G-75 column are shown in figure 1A, 1B and 1C, respectively. Peak I comprised high molecular weight proteins, peak II corresponded to superoxide dismutase and peak III, with an elution volume of 470–570 ml ($Kd = 0.53$), was identified as MT [12]. The increase in Zn was attributable to MT and high molecular weight proteins (fig. 1A). The pattern was similar to the distribution profiles of Cu-injected rats (fig. 1B), while most of the increase in Cd was associated with MT (fig. 1C). The relationships between the Zn, Cu and Cd contents of the cytosol and the MT were examined in the liver (fig. 2A, 2B and 2C). There was a close relationship between heavy metal content of the cytosol and the MT in all heavy metal-injected rats. Positive correlations were observed between the Zn ($r = 0.991$, $p < 0.001$), Cu ($r = 0.985$, $p < 0.001$) and Cd ($r = 0.999$, $p < 0.001$) contents of the cytosol and the MT. The slopes of regression lines were: Zn 0.65, Cu 0.58 and Cd 0.83. These data demonstrate that approx. 60% of the increased Zn or Cu in the hepatic cytosol of Zn or Cu-injected rats is bound to MT. This value is similar to that reported for the renal cytosol of Zn-injected rats [13]. On the other hand, 83% of the increased Cd in the hepatic cytosol of Cd-injected rats is bound to MT. Many Zn or Cu-binding enzymes such as alcohol dehydrogenase, alkaline phosphatase and superoxide dismutase require Zn or Cu in vivo, while heavy metal-binding proteins in vivo do not need Cd. These responses may result in differences between the essential and the toxic, non-essential heavy metals. The order of relative binding capacity of MT in vivo determined for several metals ($Cd > Zn > Cu$) did not correlate with published in vitro affinities of $Cu > Cd > Zn$ to MT [14, 15]. This investigation will therefore lead to a better understanding of the role of MT in heavy metal metabolism in liver.

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