Fate and Comparative Toxicity of Metallothioneins with Differing Cd/Zn Ratios in Rat Kidney

Kazuo T. Suzuki, Shinji Takenaka, and Kentaro Kubota
National Institute for Environmental Studies, P. O. Yatabe, Ibaraki 300-21, Japan

Abstract. Metallothioneins with differing Cd/Zn ratios were prepared in vitro from rat liver zinc-thionein by replacing zinc with cadmium and were injected intraperitoneally to female rats. The distribution of cadmium, zinc, and copper in the kidney supernatant fraction was determined using a Sephadex G-75 column. The distribution pattern of cadmium and zinc changed dramatically within 24 hr after the injection. The changes were explained by the degradation and re-synthesis of metallothionein in the kidneys. The necrotic changes of renal tubular lining cells were correlated to the amount of cadmium in the metallothionein but not to the amount of metallothionein (protein).

The selective toxicity of injected metallothionein to the kidneys (Cherian et al. 1976, Nordberg et al. 1975, Webb and Etienne 1977) and the assumed protective role of the protein (Kägi and Vallee 1960, Piscator 1964, Pulido et al. 1966, Winge and Rajagopalan 1972) are of interest from the viewpoint of the relationships between the adverse effects and the chemical forms of cadmium in living tissues (Suzuki et al. 1977). The present work is intended to clarify whether the renal lesion induced by the injection of metallothionein is caused by metallothionein itself or by the inorganic ion liberated from the protein. One approach to the problem is to trace the metabolic fate of the injected metallothionein in the kidneys. Another approach is to compare the relative toxicity of the metallothionein with differing Cd/Zn ratios. For those purposes, metallothioneins which contained cadmium and zinc in differing ratios were prepared in vitro from rat liver zinc-thionein by replacing zinc in the protein with inorganic cadmium. The comparative toxicity of the metallothioneins and the fate of the injected cadmium-thionein in rat kidneys were determined.

Methods

The rats were maintained on a standard laboratory chow (product of Clea Japan, Tokyo) and distilled water ad libitum. All glassware was rinsed three times with double distilled water. Metal contents
were determined by means of a Hitachi 508 Atomic Absorption Spectrophotometer. Absorbances at 254 and 280 nm were recorded on a Hitachi 191E Spectrophotometer.

**Preparation of Metallothioneins with Differing Cd/Zn Ratios**

Zinc-thionein was induced in the livers of 10-weeks-old female rats of Wistar strain by the ip injection of \( \text{ZnCl}_2 \) in the amount of 18.7 mg Zn\( ^{2+} \)/kg body weight. The animals were sacrificed 18 hr after the injection by removing blood under light ether anesthesia. The livers were homogenized in four times the volume of Tris buffer solution (0.1 M, pH 7.4) containing glucose (0.25 M) with a teflon homogenizer under a nitrogen atmosphere and cooled with ice-water. The homogenate was centrifuged at 105000 \( \times g \) for 75 min at 2\( ^{\circ} \) to 4\( ^{\circ} \)C. The supernatant contained zinc in the metallothionein fractions in the amount of about 10 \( \mu g \) Zn/ml of the supernatant. Metallothioneins, which contained cadmium and zinc in differing ratios, were prepared by adding different amounts of \( \text{CdCl}_2 \) to the homogenate or to the supernatant. Cadmium-thionein (A group in Table 1) was derived by adding \( \text{CdCl}_2 \) (1.12 mg as Cd\( ^{2+} \)) to the supernatant (60 ml). The solution was mixed and allowed to stand for 15 min at room temperature. The solution was then applied to a Sephadex G-75 column (5 \( \times \) 80 cm) and eluted with Tris buffer solution (10 mM, pH 8.6). The metallothionein fractions (monitored by atomic absorption analysis of cadmium and/or zinc) were combined and the solution was concentrated by ultrafiltration on a Diaflo UM-10 membrane. Zinc-thionein (E group in Table 1) was isolated as above, but without adding cadmium. Metallothioneins of B, C, and D groups in Table 1 were prepared as follows: The livers were pre-homogenized in twice the volume of Tris buffer solution for extraction as above. The homogenate (40 ml) was mixed with the same volume of the buffer solution which contained \( \text{CdCl}_2 \) (672, 448, and 224 \( \mu g \) as Cd\( ^{2+} \) in 40 ml for B, C, and D groups in Table 1, respectively) and further homogenized. The homogenate was ultracentrifuged and the supernatant was applied to a Sephadex G-75 column as above. Then, the metallothionein solution was concentrated by ultrafiltration on a Diaflo UM-10 membrane. The concentration of metallothionein solution for injection was adjusted with Tris buffer solution (10 mM, pH 8.6).

**Co-chromatography of Zinc-thionein and Cadmium, Zinc-thionein on a DEAE Sephadex A-25 Column**

The isolated zinc-thionein (E group in Table 1) was mixed with metallothionein solution (Cd,Zn-thionein) isolated from cadmium-exposed rat liver. The mixed solution contained zinc (2.56 \( \times \) 10\(^{-7}\) mole; 56\% from zinc-thionein, and 44\% from Cd,Zn-thionein) and cadmium (2.16 \( \times \) 10\(^{-7}\) mole) as metallothionein. The sample was applied to a DEAE Sephadex A-25 column (1.5 \( \times \) 28 cm) which was pre-equilibrated with Tris buffer solution (1 mM, pH 8.6), washed with the same buffer solution (25 ml), and eluted with Tris buffer solution of concentration gradient between 1 mM (pH 8.6, 100 ml) and 300 mM (pH 8.6, 300 ml), flow rate 19 ml/hr. Cadmium and zinc were analyzed in each eluate (2.7 ml/tube).

**Metabolic Fate of Cadmium-thionein in the Kidneys**

Metallothionein solution was injected ip into 6-weeks-old female rats (body weight, 92.5 \( \pm \) 4.1 g) of Wistar strain (SLC, Hamamatsu, Japan). The animals (6 rats/group) were sacrificed 6 hr, 1, 2, 4, and 7 days after the injection by removing blood under light ether anesthesia for metal analysis. The kidneys of three rats were combined and homogenized in four times the volume of Tris buffer solution (0.1 M, pH 7.4) containing glucose (0.25 M) using a teflon homogenizer. The homogenate was centrifuged at 105000 \( g \) for 75 min at 2\( ^{\circ} \) to 4\( ^{\circ} \)C. The metal contents in the tissue homogenate and in the supernatant were determined after digesting with mixed acids of \( \text{HClO}_4 \), 0.2 ml, and \( \text{HNO}_3 \), 0.5 ml \( \times \) 2 for 0.5 ml sample (acids for heavy metal analyses, Wako, Japan) and diluting to 5 ml with double distilled water. The distribution patterns of the metals, Cd, Zn, and Cu, in the supernatant fractions of