LETTERS TO THE EDITOR

A FURTHER PURIFICATION OF THE LOW MOLECULAR-WEIGHT CADMIUM-, COPPER- AND ZINC-BINDING PROTEINS IN THE BLOOD AND URINE OF CADMIUM POISONED RATS

Cadmium in the plasma and erythrocytes from the blood of mice¹⁾ and rats²⁾ given daily cadmium injection has been found to bind to a protein with low molecular-weight corresponding to metallothionein, cadmium- and zinc-binding protein³⁾. Cadmium in the urine of those animals was also found to bind to a metallothionein-like protein after the tubular lesions occurred by cadmium intoxication^{4) 5)}. The present study deals with a further purification of the low molecular-weight proteins in blood and urine of rats under a long-term cadmium administration.

Fifteen Sprague-Dawley male rats (8 weeks in age) were given daily subcutaneous injection of 0.5 mg Cd/kg, 6 days per week for 26 weeks. Blood was obtained from five of the animals at 4, 8 and 26 weeks of cadmium administration. Plasma and erythrocytes were separated by centrifugation for 10 min at 3,000 r.p.m. and pooled. Erythrocytes were hemolyzed by homogenizing them in an equivalent volume of 0.01 M Tris-HCl buffer, pH 7.4. Clear hemolysate was obtained by centrifuging the homogenate for 10 min at 15,000 r.p.m. The plasma and hemolysate were gel filtrated on a Sephadex G-75 column with 0.01 M Tris-HCl buffer, pH 8.6 containing 0.02% sodium azide. Cadmium copper and zinc were analysed on each fraction of the effluent.

The main cadmium-containing fractions were collected and then concentrated (UM-2, Amicon) at 4°C. The concentrated solution was supplied on a column of DEAE Sephadex A-25 (1.5×15 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 8.6. The solution was filtrated with 75 ml of the same buffer and then with 400 ml linear Tris-HCl gradient from 0.05 M to 0.4 M, pH 8.6 at a flow rate of 20 ml/hr. The effluent was collected in 5 ml fractions.

At 14 weeks of cadmium administration, urine was collected for 3-4 hr and pooled. The urine (30-40 ml) was immediately concentrated and gel filtrated by the above-mentioned methods.

On each fraction from the plasma, hemolysate and urine, absorbance of UV-ray at a wavelength of 250 nm was measured, then cadmium, copper and zinc analyses were carried out by atomic absorption spectrophotometry.

Cadmium concentrations in the whole blood at 4, 8 and 26 weeks were 0.81, 1.3 and 1.7 µg/ml (mean for five animals), respectively. Cadmium content of the plasma accounted for 2-3% of total cadmium in the whole blood until 8 weeks and was about 7% at 26 weeks. Sephadex G-75 gel filtration of the plasma revealed that at 4 weeks a small amount of cadmium and an easily detectable amount of copper bound to a protein

having the same molecular-weight as metallothionein has. At 8 and 26 weeks cadmium bound to the low molecular-weight protein was 31% and 58% of total cadmium in the plasma. Copper also bound to the protein but zinc was scarcely detected. In the hemolysate a large part of cadmium (80–90%) bound to the low molecular-weight protein together with a small amount of zinc.

As shown in Fig. 1, four clear cadmium peaks were isolated by DEAE Sephadex A-25 gel filtration of the low molecular-weight proteins from the plasma and hemolysate at 26 weeks. An absorption peak of UV-ray at a wavelength of 250 nm was observed at each cadmium peak of the hemolysate. But, the UV-absorption peaks of the plasma effluent were not entirely clear, probably because the protein content of the sample was too small. Of the profiles shown in Fig. 1, F-1 and F-2 were eluted with buffer concentration at the start. F-3 and F-4 were isolated at approximately the same buffer concentrations at which the two forms⁽⁶⁾⁷⁾ (Cd-Bp1 and Cd-Bp2) of metallothionein from liver and kidney of the rats were isolated under the same condition of gel filtration. Total cadmium contents of F-3 and F-4 were 65% and 87% of cadmium in the plasma and hemolysate, respectively. Besides cadmium, only copper in the four fractions from the plasma and only zinc in those from the hemolysate was detected.

At 14 weeks mean urinary excretion of cadmium increased up to 86 $\mu\text{g}/\text{day}$. Urinary excretion of copper and zinc also increased up to about four and ten times the normal

Fig. 1. Metal distributions by DEAE Sephadex A-25 gel filtration of the low molecular-weight cadmium-, copper- and zinc-binding proteins in the plasma and hemolysate from rats given repeated subcutaneous cadmium injection (0.5 mg Cd/kg, 6 days/week) for 26 weeks.

Cd, Cu, Zn-BINDING PROTEINS IN BLOOD AND URINE OF RATS

level, respectively. Sephadex G-75 gel filtration of the urine showed that most of cadmium (about 90% or more) bound to a low molecular-weight protein together with copper, but zinc was not found to bind to that protein. Fig. 2 shows a typical profiles of metal distributions by DEAE Sephadex A-25 gel filtration of the protein. Four peaks of cadmium and copper were isolated at almost the same concentrations of Tris-HCl buffer as in the case of the plasma and hemolysate. UV-absorption peaks were observed in accordance with the cadmium and copper peaks. Cadmium contents of F-1, F-2, F-3 and F-4 were 9%, 25%, 36% and 14% of total cadmium, respectively. F-3 and F-4 might represent the two forms (Cd-Bp1 and Cd-Bp2) of metallothionein⁽⁶⁾⁷⁾, while it remained obscure what F-1 and F-2 were. They may be only artifacts⁷⁾ under the purification of the urine. However, since the purification was carried out with as much care as possible, the consideration that they may be catabolites of metallothionein or some cadmium-binding components should not be simply denied.

Fig. 2. Metal distributions by DEAE Sephadex A-25 gel filtration of the low molecular-weight cadmium- and copper-binding protein in the urine of rats given repeated subcutaneous cadmium injection (0.5 mg Cd/kg, 6 days/week) for 14 weeks.

From the present results on the analysis of metals and UV-absorption study, it seems reasonable to think that the main components of the low molecular-weight cadmium-binding proteins in the blood and urine may be metallothionein. However, the low molecular-weight proteins of the plasma and urine scarcely bind zinc, which is different from metallothionein⁽⁸⁾. This suggests that these proteins may have excessively strong affinity to copper as well as cadmium compared with zinc in the plasma and urine.

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