MECHANISM FOR CADMIUM AND ZINC ANTAGONISM OF COPPER METABOLISM

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SUMMARY

Gel filtration analysis of the soluble fraction from both bovine duodenum and liver demonstrate that the major fraction of copper is bound to a protein similar to metallothionein. Cadmium, zinc and p-hydroxymercuribenzoate significantly decrease copper binding by partially purified metallothionein. These results indicate that cadmium, zinc and other mercaptide forming agents antagonize copper metabolism by displacing copper from sulfhydryl binding sites on metallothionein.

Several investigations have demonstrated that cadmium and zinc alter copper metabolism (1-4). However, the mechanism of interaction among these elements has not been demonstrated. Starcher first identified a protein of 10,000 molecular weight in chick intestine and suggested that cadmium and zinc antagonize copper absorption by displacing copper from the duodenal protein (4). Recently, we examined the metabolism of copper and zinc at the molecular level and demonstrated that the two elements are bound to a single 10,000 molecular weight protein in the duodenum, liver and kidney from rat (5).

The metal-binding characteristics and molecular weight of the protein from both the chick duodenum and rat organs suggests that the metal-binding protein is similar to metallothionein isolated from equine and human kidney (6-8). Metallothionein has a molecular weight of 10,000 and binds cadmium, zinc and copper. Therefore, to further explain the antagonism among these elements, we have

examined the effect of cadmium and zinc on copper binding by metallothionein from bovine duodenum and liver.

METHODS

To identify the major copper-binding proteins in the soluble fraction of duodenum and liver, homogenates were prepared from 50 g of tissue. The tissue was homogenized in 1 volume of 0.05 M K_2HPO_4 pH 7.0 using a Potter-Elvehjem homogenizer. The homogenates were centrifuged for 60 min at 105,000 g. The pellet was discarded and the supernatant (soluble fraction) was taken for gel filtration analysis. Prior to gel filtration, the supernatant was concentrated by ultrafiltration. Following ultrafiltration, 5 ml of the concentrated soluble fraction was applied to a Sephadex G-75 column 2.5 x 40 cm equilibrated with 0.001 M Tris and 0.05 M KCl at pH 8.6. The material was eluted from the column using the same buffer. Gel filtration was carried out at 22 C with a flow rate of 0.5 ml/min and 3.0 ml fractions were collected for analysis. Copper content of the eluted fractions was analyzed by atomic absorption spectrophotometry. In addition, ultraviolet absorption at 250 mμ and 280 mμ was measured in each fraction. Molecular weights were estimated by the method of Andrews (9), using horse heart cytochrome c, and bovine hemoglobin.

Metallothionein was partially purified from both bovine duodenum and liver by the method of Pulido et al (8). The final purification procedure utilizing Porath column electrophoresis was omitted. Protein sulfhydryl groups were determined at pH 4.6 by the colorimetric method of Boyer (10).

To analyze copper binding by metallothionein, 4.9×10^{-7}M $^{64}Cu(NO_3)_2$ (spec. act. = 11.5 mCi/mg) and 7.7×10^{-6}M $ZnCl_2$ or 9.0×10^{-7}M $CdCl_2$ or 10^{-4}M p-hydroxymercuribenzoate (PMB) were mixed with 1.58×10^{-6}M metallothionein in 0.001 M tris pH 8.6. The

solutions were stirred continuously at 4 C for 2 hours after which the contents were dialyzed for 24 hours against two changes of distilled water. The dialyzed fractions were counted for radioactivity in a gamma well scintillation counter and protein concentration was determined using biuret reagent (11).

RESULTS AND DISCUSSION

As shown in Figure 1, protein-bound copper from the soluble fraction of both duodenum and liver was eluted in two fractions. One copper fraction was eluted at 120 ml which corresponds with

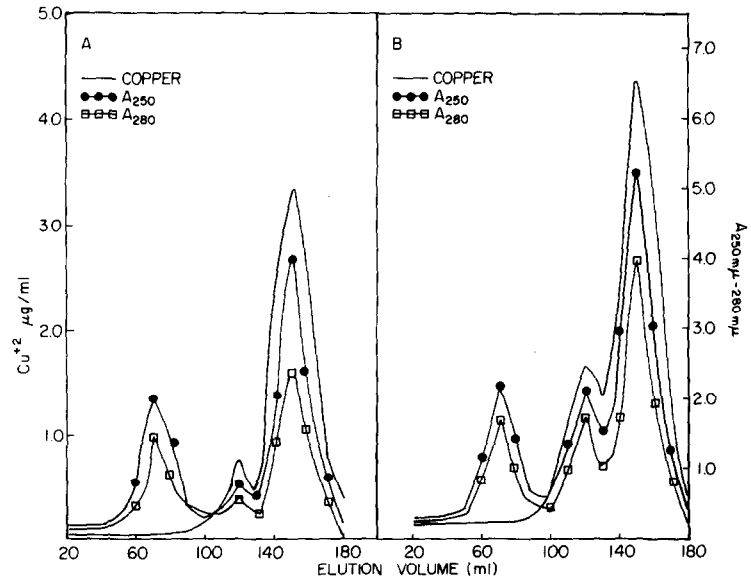

Figure 1. Elution of protein-bound copper from Sephadex G-75 column. (A) Bovine duodenum. (B) Bovine liver. For details see text.

protein of 33,000 molecular weight (Figure 2). The second and largest copper-protein fraction was eluted at 152 ml which corresponds with protein of 10,000 molecular weight.

Purified metallothionein from both equine and human kidney contains cadmium, zinc and copper (6-8). The protein has a molecular weight of approximately 10,000 and has an ultraviolet absorption

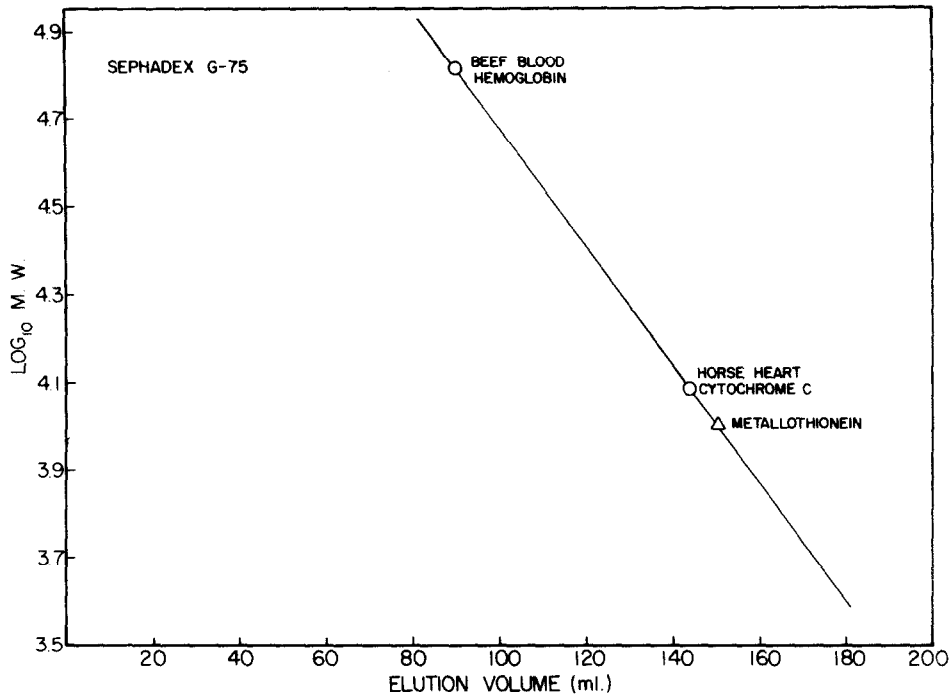

Figure 2. Elution volume of proteins of known molecular weight from Sephadex G-75 column.

maximum at 250 mμ. As described above, the major fraction of protein-bound copper from the crude soluble fraction of both duodenum and liver was eluted with a protein of 10,000 molecular weight. In addition, the copper-protein fraction had a high ultraviolet absorption at 250 mμ. These results indicate that the major fraction of soluble copper from both duodenum and liver is associated with a protein similar to metallothionein. In subsequent experiments, we have found that the second fraction of soluble copper is associated with a protein similar to cytocuprein which has been described by Carrico and Deutsch (12,13).

The metallothionein preparation used in our experiments contained 14 titratable sulfhydryl groups per mole of protein

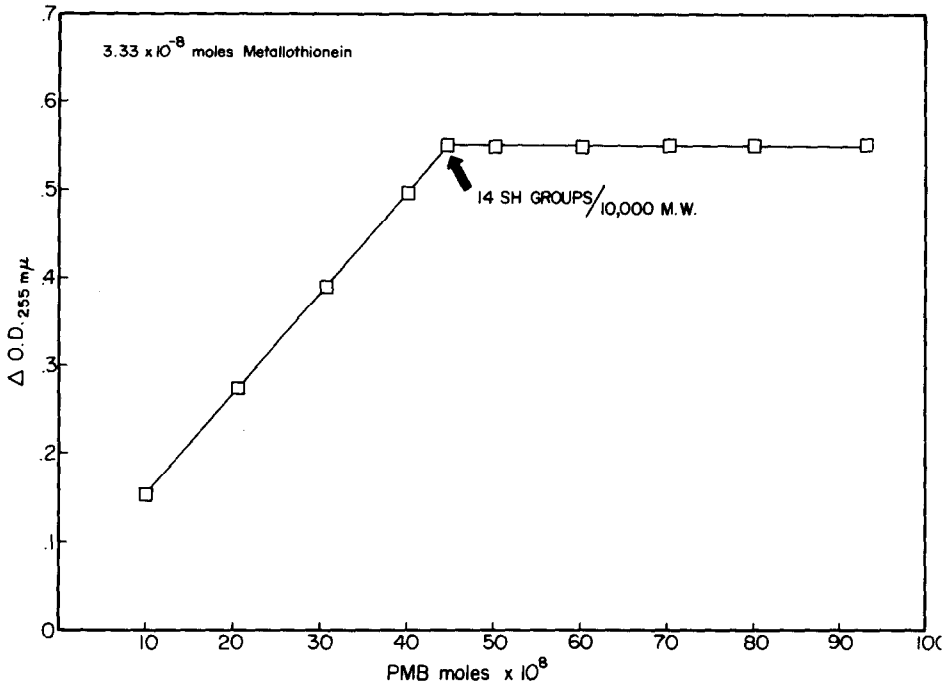

Figure 3. Sulfhydryl analysis of partially purified metallothionein from bovine liver. Metallothionein (1.33×10^{-5}M) in 0.05 M sodium acetate pH 4.6 was titrated with 1.0×10^{-3}M p-hydroxymercuribenzoate (PMB).

(Figure 3). When ^{64}Cu and PMB were added to metallothionein, ^{64}Cu binding by the protein was significantly decreased (Table 1). In addition, both cadmium and zinc significantly decreased ^{64}Cu binding by metallothionein.

Kagi and Vallee demonstrated that the metal ions of metallothionein are bound to mercapto groups (6-8). Highly purified metallothionein contains 26 sulfhydryl groups/mole, which is slightly higher than the number found in the crude metallothionein described in our experiments. Kagi and Vallee also demonstrated that cadmium and zinc compete for binding sites on metallothionein (6-8). As described in our experiments, cadmium, zinc and p-hydroxymercuribenzoate significantly decreased copper binding by

Table 1. Effect of p-hydroxymercuribenzoate, cadmium, and zinc on ^{64}Cu binding by duodenum and liver metallothionein.

Additions	Duodenum metallothionein CPM/mg	Liver metallothionein CPM/mg
^{64}Cu	226,896±14,938	194,962±12,422
^{64}Cu + PMB	83,860± 8,680	39,443± 6,889
$^{64}Cu + Cd^{+2}$	145,348±10,420	143,341± 9,683
$^{64}Cu + Zn^{+2}$	149,626±11,692	140,993±10,522

Values shown are mean ± S.E. of three experiments.
PMB = p-hydroxymercuribenzoate.

metallothionein. Cadmium, zinc and p-hydroxymercuribenzoate are known to form stable mercaptides (6,7), which indicates that these substances displace copper from the sulfhydryl binding sites on metallothionein. Since metallothionein is the major copper-binding protein in both duodenum and liver, these results explain the previously observed alterations in copper metabolism produced by cadmium and zinc.

Silver and mercury also produce alterations in copper metabolism (2,3,14). Since both of these elements form mercaptides, the effect on copper metabolism by silver and mercury probably results from competition for sulfhydryl binding sites on metallothionein.

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