Bull. Environ. Contam. Toxicol. (1988) 40:418–424

Environmental Contamination and Toxicology

Binding of Cadmium Chloride and Cd-metallothionein to Mucosal Brush Border Membrane of the Rat Small Intestinal Tract

Naoki Sugawara, Chieko Sugawara, and Hirotsugu Miyake

Department of Public Health, Sapporo Medical College, S-1, W-17, Central Ward, 060 Sapporo, Japan

Even though Cd is categorized as a metal with low absorption from the gastrointestinal tract,its total body burden predominantly results from its gastric absorption. Oral Cd also eventually triggers an endemic disease, Itai-Itai disease (Nogawa 1981), in certain areas of Japan. Cd absorption is influenced by several endogenous or exogenous factors such as aging (Engstrom and Nordberg 1979), sex (Kello et al 1979) and food components (Suzuki et al 1969).

To resolve the mechanism of Cd absorption from the gastrointesinal tract, metallothionein (MT) is one of the most interesting endogenous substances. Although the protein induced by Cd exists mainly in the mucosal cytosol fraction (Taguchi and Suzuki 1978) as well as in those of liver or kidneys, compared to the function of renal or hepatic MT, its function seems to be slightly different. So far, it is reasonable to assume that the protein is involved in the prevention of the transfer of Cd from the mucosa to the body (Foulkes and McMullen 1986; Sugawara and Sugawara 1987). However,the behavior of MT in/on the mucosal brush border membrane (BBM) is unclear. In order to gain a better understanding of this point, we estimated the Cd binding of $CdCl_2$ and Cd-MT to BBM isolated from control and Cd-exposed rats.

MATERIALS AND METHODS

Wistar male rats (5 weeks old) were maintained for 10 days with food and water ad libitum. After 10 days, they were divided into two groups, the control- and Cd-group. The Cd-group was given deionized water containing 100 ppm Cd ($CdCl_2$) for 10 days. A bottle of water (200 ml volume) having one drop of CH_3COOH was prepared every day. The control-group was given deionized water acidified with CH_3COOH. At 10 days, they were killed 12 hr after starvation. Small intestines (about 30 cm from the pylorus) were excised from each group.

The mucosa was scraped off with a glass slide on a chilled glass plate. To get mucosal brush border membrane (BBM), a method of adding $CaCl_2$ was used (Victery et al 1984). The mucosa was homogenized with Polytron in 20 volumes of 10 mM mannitol-5 mM Tris/HEPES buffer (pH 7.0). Calcium chloride solution (100 mM) of 1/50 volume was added to this homogenate at 4°C for 20 min with gentle stirring. This homogenate containing Ca ions was centrifuged at 3,000 g for 10 min. The layer phase was further centrifuged at 35,000 g for 20 min. The first precipitate (FP) was mainly composed of BBM. The FP was suspended into 10 volumes of 300 mM mannitol-5 mM Tris/HEPES buffer (pH 7.0).

To estimate binding of Cd to the BBM, $CdCl_2$ or Cd-MT(II) was added to the FP suspension in vitro. One incubation tube contained 5 μg Cd as $CdCl_2$ or Cd-MT, and 3.0-4.8 mg protein of BBM. Twenty min after incubation at 25°C, the mixture was centrifuged at 35,000 g for 20 min at 4°C. The same steps were carried out for a total of four times with a changing buffer volume. The final precipitate was suspended to 2.0 ml buffer solution and then sonicated (Branson Sonifier). The sonicated solution was used for measurement of Cd. The rest (1.7 ml) was applied to the Sephadex G-75 column (1.0X45 cm) eluted with 0.02 M Tris-HCl buffer (pH 8.5). Cadmium in column fractions was analyzed with a polarized Zeeman flameless or flame atomic absorption spectrophotometer.

For assessment of BBM purity, disaccharidase activity was measured according to the method published by Dahlqvist (1968). Protein concentration was determined by the method of Lowry et al (1951). Cd-MT isolated from rat livers exposed to Cd was purified by the Sephadex G-75 and then Sephadex A-25 separations. The Cd-MT(II) used here was 5.9 (mol/mol) in the ratio of Cd/Zn, and 6.2 in the ratio of A_{254}/A_{280}. All chemicals were of analytical reagent grade.

RESULTS AND DISCUSSION

The BBM showed a 4.7 times higher activity of disaccharidase (μg glucose/g protein) than did the whole homogenate. When the control BBM was incubated with $CdCl_2$, 72% of the added Cd was yielded in the BBM fraction (Table 1). Furthermore, Cd binding to the BBM was recovered only in the HMW region (around fraction No. 16) on Sephadex G-75 (Fig. 1). When Cd-MT was added, its recovery was only 6% (Table 1). The Cd binding to BBM in the incubation was not of the MT-form (Fig. 1). In the case using deoxycholate (0.5%) as a solubilizer, the Cd binding to the BBM was not recovered in the MT-form (Fig. not shown).

Table 1. Cd binding to isolated brush border membranes

Brush Border Membrane/ Cd compound in vitro	Cd(μg/mg protein) in BBM fraction	Recovery (%)
Control / -	-	-
Control / +$CdCl_2$	0.720±0.031(6)	71.8±1.1
Control / +Cd-MT	0.069±0.043(4)	6.1±3.3
Exposed / -	0.292±0.051(4)	-
Exposed / +$CdCl_2$	0.936±0.093(4)	42.0±5.7
Exposed / +Cd-MT	0.260±0.107(4)	a-

The first precipitate (FP) was suspended in 300 mM mannitol-5 mM Tris/HEPES buffer (pH 7.0) solution. The solution was incubated with or without Cd compounds for 20 min at 25°C. After the incubation, the mixture was washed four times. The final precipitate was suspended in the mannitol buffer and sonicated to measure Cd and protein concentrations. Data expressed as the mean±SD. a: Calculation was not done.

The Cd released from the MT may be directly associated to the HMW substances. The HMW substances were positive to the Lowry protein assay (Lowry et al 1951). These data suggest that intact BBM isolated from the control group does not possess binding sites for MT.

Selenke and Foulkes reported previously that Cd-MT (hepatic 109Cd-MT containing two isomers) binds to isolated renal proximal tubular BBM, in vitro and in addition that there exists two classes of binding sites with different affinities for Cd-MT. The difference between our results and theirs may be due to the differences of some techniques and materials, binding assay of MT (determination of 109Cd activity but not 109Cd-MT), source of BBM and purity of MT.

Cd was detected in the BBM isolated from the $CdCl_2$-exposed rats (Table 1). The Cd existed in the two regions, the HMW-and MT-region, on the Sephadex G-75 column (Fig. 2). Even though the supernatant obtained from the first centrifugation contained overwhelmingly the induced Cd-MT (Fig. 3a), MT was not a large component for Cd in the BBM fraction (Fig. 2). The MT peak could not be detected in the supernatant (2.0 ml) obtained by the final centrifugation (Fig. 3b). Accordingly, the BBM MT peak was not a contaminant from the supernatant fraction. When $CdCl_2$ was added to the BBM fraction, 42% of its Cd was recovered in the BBM (Table 1). In addition, Cd was found in the HMW region on Sephadex G-75 (Fig. 4). On the other hand, when Cd-MT(II) was incubated with the BBM, the added Cd-MT

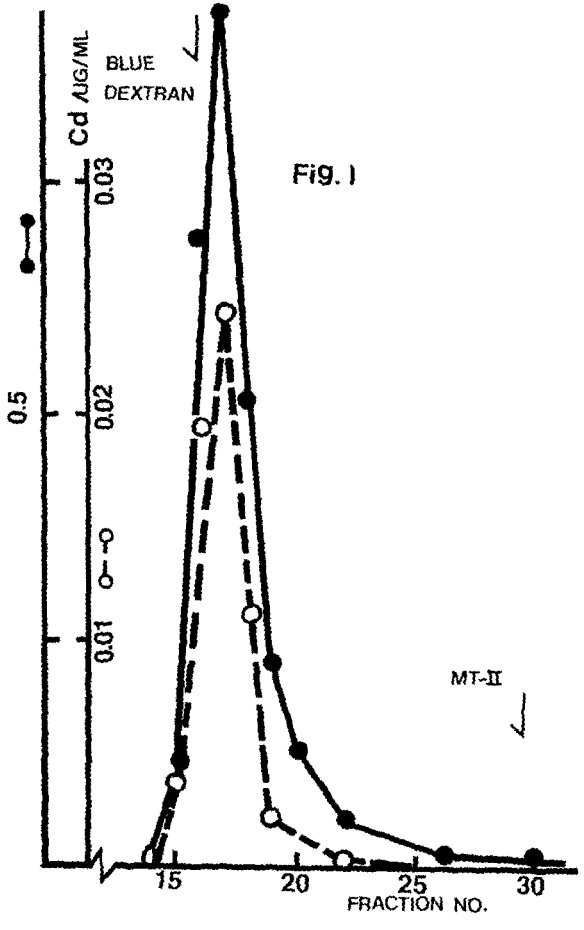

Cd AUG/ML
BLUE
DEXTRAN
0.5
0.03
0.02
0.01
MT-II
15
20
25
30
FRACTION NO.

Fig. 1

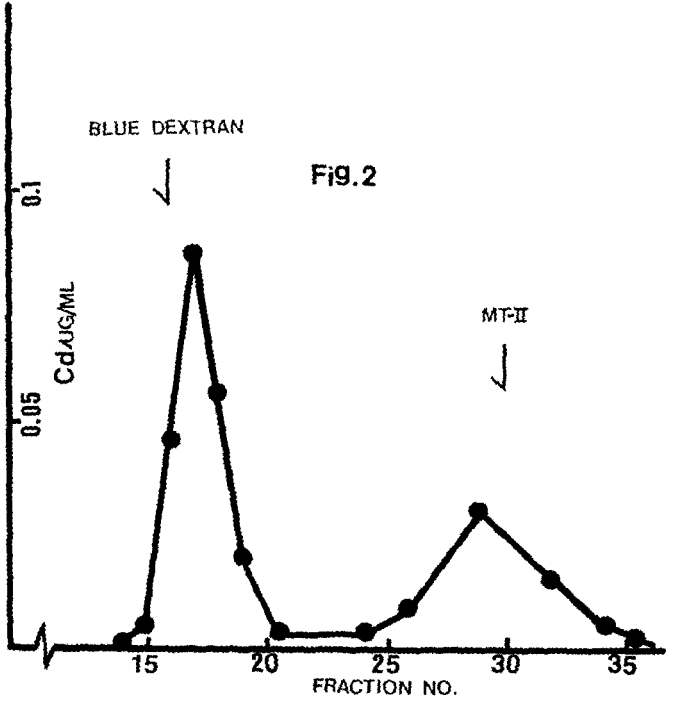
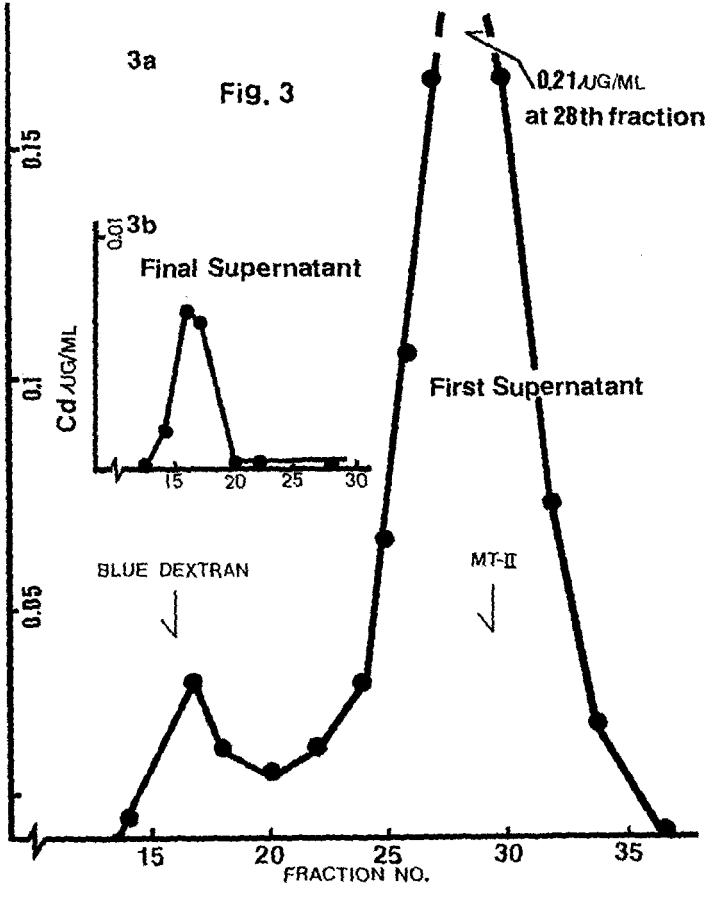

BLUE DEXTRAN
0.1
0.05
CdAUG/ML
MT-II
15
20
25
30
35
FRACTION NO.

Fig.2


3a
Fig. 3
0.21 μG/ML
at 28th fraction
0.15
0.1
0.05
3b
0.01
Final Supernatant
Cd μG/ML
15
20
25
30
First Supernatant
BLUE DEXTRAN
MT-II
15
20
25
30
35
FRACTION NO.

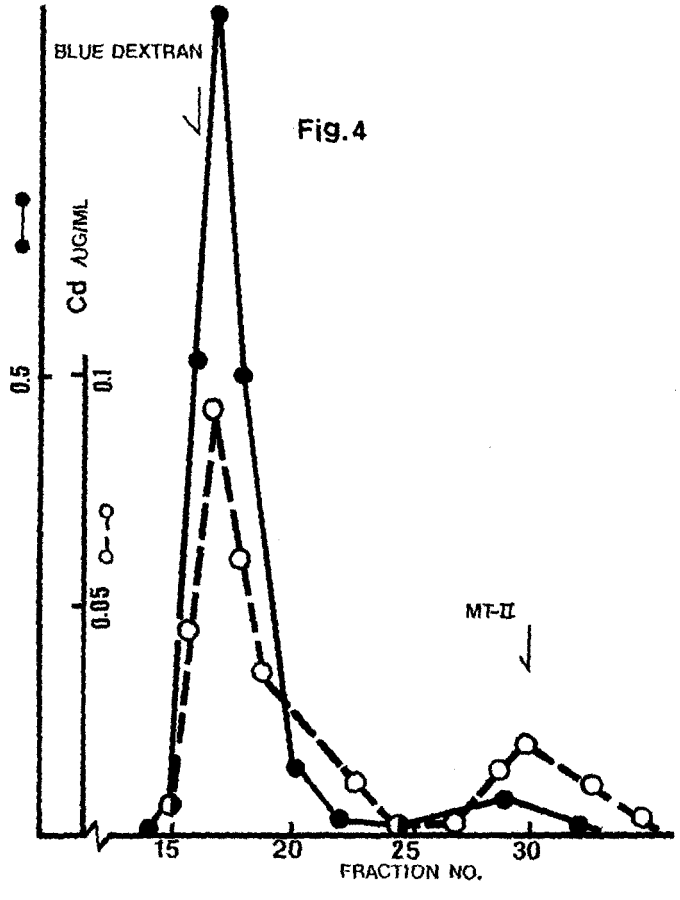

BLUE DEXTRAN
Fig. 4
0.5
Cd μG/ML
0.1
0.05
MT-II
15
20
25
30
FRACTION NO.

Figure 1 Cd Distribution in BBM from Intact Intestinal Mucosa. The mucosal BBMs were incubated with $CdCl_2$ (●—●) or Cd-MT (O--O) solution at 25°C for 20 min. The further procedures, centrifugations, sonication and application were seen in MATERIALS AND METHODS.

Figure 2 Cd Distribution in BBM from Intestinal Mucosa Exposed to $CdCl_2$ for 10 days. The mucosal BBM was incubated with Cd-free buffer.

Figure 3 Cd Distribution in First and Final Supernatant Fractions from Intestinal Mucosa Exposed to $CdCl_2$ for 10 days. The first and final (2 ml) supernarant fractions were obtained from the first and final centrifugation (35,000 g, 20 min), respectively. The two fractions were applied to Sephadex G-75 column, respectively.

Figure 4 Cd Distribution in BBM from Intestinal Mucosa Exposed to $CdCl_2$ for 10 days. The mucosal BBMs were incubated with $CdCl_2$ (●—●) or Cd-MT (O--O) solution.

---

did not yield as MT in the BBM (Fig. 4). In addition, the recovery of added Cd could not be calculated. The result suggests that the BBM's sites for binding to MT are not stimulated by the exposure of Cd.

When the mucosa was exposed to Cd ($CdCl_2$), Cd-MT is induced in the mucosa cells. In its cytosol, Cd existed in the form of MT. The MT stimulates the uptake of Cd into the mucosal cells (Foulkes and McMullen 1986; Sugawara and Sugawara 1987), but by no means, increases the transfer of Cd into the body (Foulkes and McMullen 1986; Sugawara and Sugawara 1987). When Cd-MT is given orally, the protein exists also in the mucosal cytosol fraction (Cherian et al 1978). Its distribution of Cd from the HMW to MT regions was not different from that exposed to $CdCl_2$ (Cherian et al 1978). Furthermore,its absorption as Cd is nearly the same as that of $CdCl_2$ (Cherian et al 1978).

Our data showing the low affinity of Cd-MT to BBM *in vivo* and the lack of its binding to BBM *in vitro* may be the reason why cadmium chloride or Cd-MT is absorbed only slightly.

REFERENCES

Cherian MG, Goyer RA, Valber LS (1978) Gastrointestinal absorption and organ distribution of oral cadmium chloride and cadmium-metallothionein in mice. J Toxicol Environ Health 4: 861-868.

Dahlqvist A (1968) Assay of intestinal disaccharidases. Anal Biochem 22: 99-107.
Engstrom B, Nordberg GF (1979) Factors influencing absorption and retention of oral 109 Cd in mice; age, pretreatment and subsequent treatment with nonradioactive cadmium. Acta Pharmacol Toxicol 45: 313-324.
Foulkes EC, McMullen DM (1986) Endogenous metallothionein as determinant of intestinal cadmium absorption: A reevaluation. Toxicol 38: 285-291
Kello D, Dekanic D, Kostial K (1979) Influence of sex and dietary calcium on intestinal cadmium absorption in rats. Arch Environ Health 34: 30-33.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275.
Nogawa K (1981) Itai-itai disease and follow-up studies. In: Nriagu JO (ed) Cadmium in the Environment, part 2. John Wiley & Sons,Inc. p 2-54.
Selenke W, Foulkes EC (1981) The binding of cadmium metallothionein to isolated renal brush border membranes. Proc Soc Exp Biol Med 167: 40-44.
Sugawara N, Sugawara C (1987) Role of mucosal metallothionein preinduced by oral Cd or Zn on the intestinal absorption of a subsequent Cd dose. Bull Environ Contam Toxicol 38: 295-299
Suzuki S, Taguchi T, Yokohashi G (1969) Dietary factors influencing upon the retention rate of orally administered 115m $CdCl_2$ in mice. Ind Health 7: 155-159.
Taguchi T, Suzuki T (1978) Cadmium binding components in the supernatant fraction of the small intestinal mucosa of rats administered cadmium. Jap J Hyg 33: 467-473.
Victery W, Miller CR, Fowler BA (1984) Lead accumulation by rat renal brush border membrane vesicles. J Pharmacol Exp Therap 231: 589-596.

Received August 25, 1987; accepted October 15, 1987.