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Metallothionein–like Cadmium Binding Protein in Rat Testes Administered with Cadmium and Selenium

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It is well known that the testicular damage caused by acute cadmium toxicity: hemorrhagic inflammation, atrophy and necrosis, are protected by simultaneous selenium administration with cadmium, and that the cadmium concentration in the testis increases remarkably as compared with that of only cadmium administration (Parizek 1957, Kar et al. 1960, Chen et al. 1974, Gunn et al. 1968). The increased cadmium in the testis was found in the high molecular weight fraction containing selenium, and it has been thought that the shift of cadmium from the low molecular weight fraction (30000 dalton) to the high molecular weight fraction containing selenium is an important protection mechanism (Chen et al. 1974). However, the cadmium concentration in this high molecular weight fraction decreased with time, an re-shifted to the fraction of metallothionein, a low molecular weight protein having a protective effect against cadmium toxicity. The testicular damage mentioned above and the inhibition of enzyme activity were protected remarkably and also recovered to the normal level as a result of cadmium binding by induced metallothionein in the testis. Consequently, the secondary induction of metallothionein in testis is suggested as the important cause of this protection mechanism against the acute cadmium toxicity by simultaneous selenium administration (Omaye and Tappel 1975, Ohta 1985, Ohta and Imamiya 1986). While recently studying the cadmium binding protein, like metallothionein, in testes, it has been reported that the amino acid composition of cadmium binding protein in testis is not similar to that of the hepatic metallothionein (Deagen and Whanger 1985, Waalkes et al. 1984 a&b). The present study was undertaken to clarify the properties of the increased cadmium binding protein in the testis protected by simultaneous selenium administration with cadmium.

MATERIAL AND METHODS

Male Wistar strain rats, weighing 250-300 g, were used in this experiment. All rats were housed by providing food and water ad

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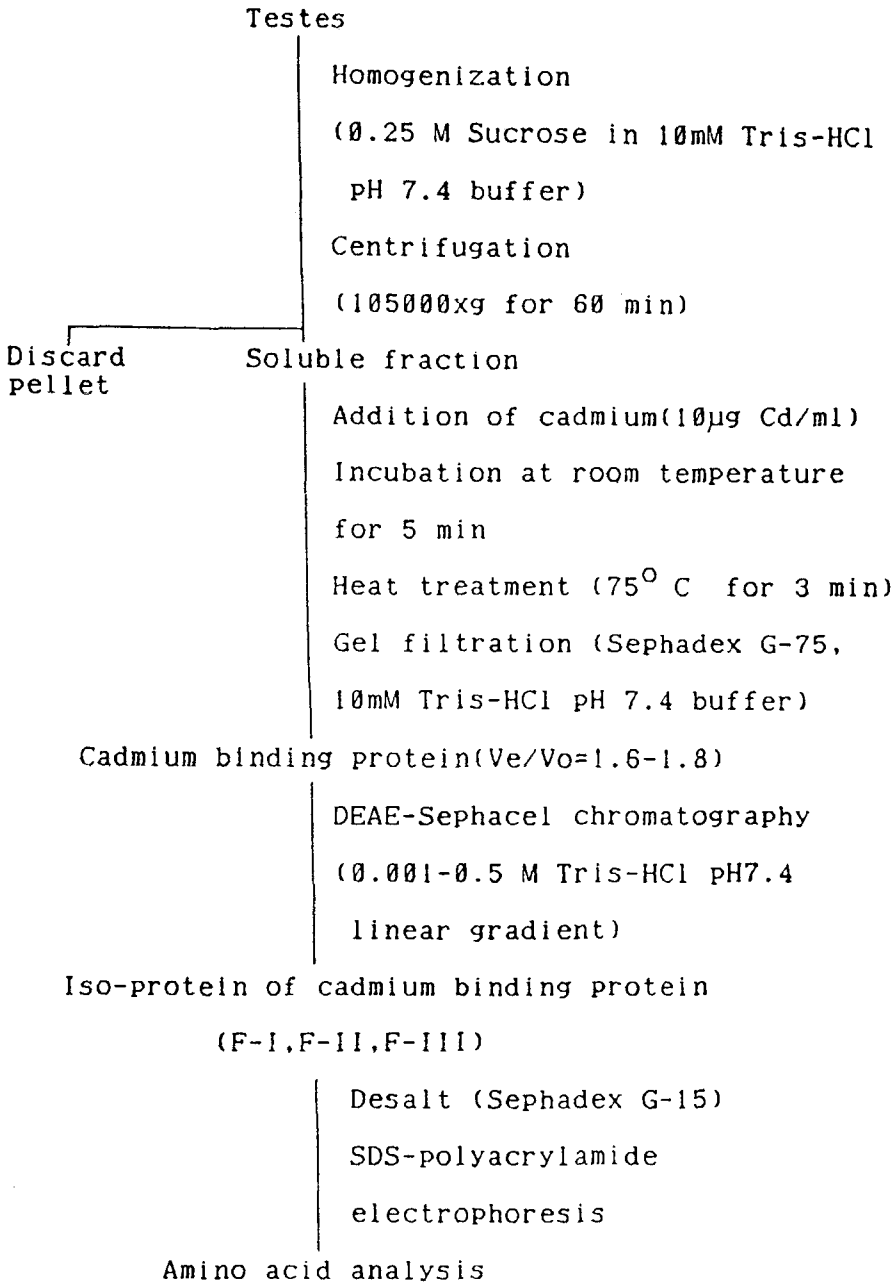

Figure 1. Schematic diagram of the purification of cadmium binding protein in testis.

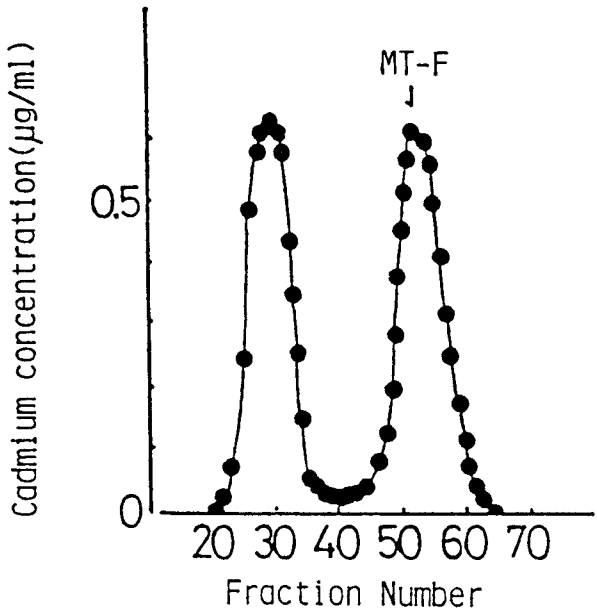

Figure 2. Cadmium distribution in testicular cytosol.

MT-F; Metallothionein fraction.

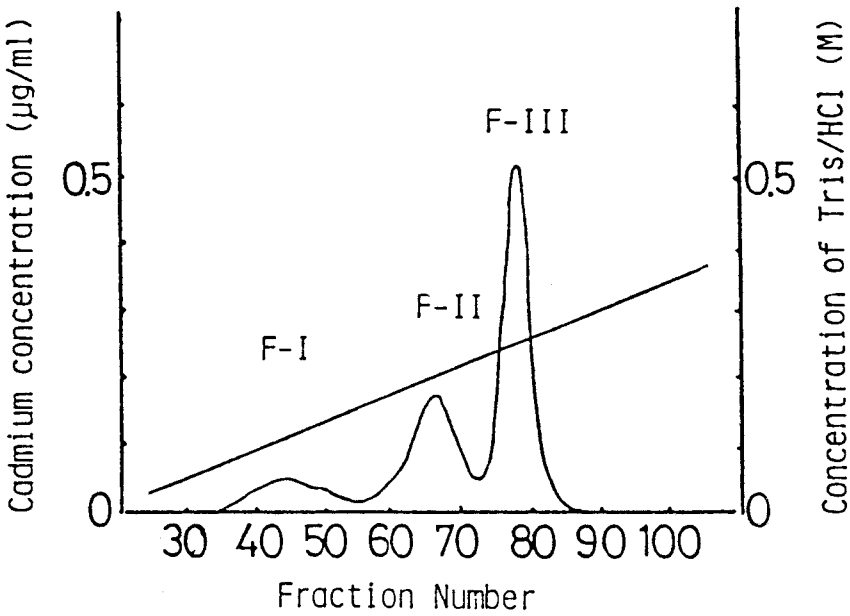

Figure 3. DEAE-Sephacel chromatography of the cadmium binding protein in metallothionein fraction.

Table 1. Comparison of the amino acid composition of testicular cadmium binding iso-proteins (Cd-BP) and hepatic metallothionein (MT). (residues mol/100 mol)

	Cd-BP in testis			MT in Liver*	
Amino acid	F-I	F-II	F-III	I	II
Asp	9.20	8.91	9.55	6.8	6.8
Thr	6.18	5.93	6.24	6.7	2.8
Ser	7.67	9.16	8.15	15.2	18.5
Glu	9.98	8.83	14.70	2.1	4.6
Pro	8.83	5.40	6.27	3.2	3.9
Gly	9.72	9.44	7.69	9.1	6.3
Ala	6.66	7.51	7.48	5.1	7.6
Val	7.75	5.03	4.79	3.3	1.6
½Cys**	4.02	13.22	6.02	34.5	32.0
Met	0.88	2.02	1.31	1.4	1.7
Ile	4.07	3.29	3.38	0.2	1.4
Leu	7.41	4.42	6.02	0.3	0.4
Tyr	trace	0.44	1.09	---	0.3
Phe	4.24	1.90	2.66	---	---
His	2.72	1.22	1.20	0.1	0.2
Lys	7.21	10.80	11.24	12.0	11.9
Arg	3.46	2.48	2.23	---	---

* Whanger & Deagen (1982)
** Determined from cysteic acid content.
Results are means of 2 or 4 determination.

libitum. Rats were given simultaneouly i.p. injection of cadmium with selenium (Cd as cadmium chloride 2.0 mg/kg, Se as sodium selenite 0.6 mg/kg). At the 7th day after the injection, rats were sacrificed, and the testes were removed after perfusion with 0.25 M Sucrose in 10 mM Tris-HCl (pH 7.4)) buffer, pooled and saved for the preparation of cytosol. The isolation and purification of the cadmium binding protein in testes were carried out by the procedure briefly described in Fig. 1 (Onosaka et al. 1978, Deagen and Whanger 1985, Waalkes et al. 1984). Amino acid analysis of samples was performed after hydrolysis with or without performic acid oxidation according to the standard method (Moore and Stein 1963). Cadmium analysis was performed using atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

The elution profile of testicular cytosol from Sephadex G-75 gel filtration is shown in Fig. 2. After the heat treatment of Onosaka et al. (1978), the cadmium in the cytosol fraction was found in the equivalent fraction to metallothionein and the void volume fraction. Furthermore, this cadmium binding protein in the metallothionein fraction (fraction numbers 49-61) was purified on a DEAE-Sephacel (Fig. 3), and fractionated into three species (Fraction I, II and III). Each of these iso-proteins showed a single band on SDS polyacrylamide gel electrophoresis, (unpublished data). From Table 1, the iso-proteins of the testicular cadmium binding protein which are heat stable like metallothionein, had a different amino acid composition compared to hepatic metallothionein (Whanger and Deagen 1982). Namely, these iso-proteins had a low cysteine content (4.02, 13.22 and 6.02 % of total residues, for F-I, F-II and F-III respectively), and also they contained aromatic amino acids. These results concerning the amino acid composition of cadmium binding proteins were similar to those results reported by Deagen and Whager (1985) and by Waalkes et al. (1984) for rat testes. However, it was strongly suggested that this cadmium binding protein in testis plays a protective role in acute cadmium toxicity analogous to metallothionein in liver and kidney (Nordberg 1971, Ohta and Imamiya 1986). On the other hand, it has been reported that the cadmium binding protein in testis is metallothionein on the basis of immunochemical analysis (Danielson et al., 1982). Moreover, many cadmium binding proteins, like metallothionein, have been reported in various species (Stone and Overnell 1985). It would be interesting to clarify the role of those proteins, and their relationship to metallothionein. Further studies will be needed on these problems.

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