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Purification and Characterization of Lead-Induced Zinc Thionein in the Liver of Rats

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It has been reported that certain heavy metals, such as cadmium, zinc and mercury, can induced a low molecular-weight protein, that is metallothionein, however it is known that lead among heavy metals did not induce this metallothionein (Cherian & Goyer 1978).

Metallothioneins are involved in homeostasis of essential metals, but its exact biological function remains obscure (Kagi & Nordberg 1979, Webb 1979, Foulkes 1982). Recently, Suzuki & Yoshikawa (1976) and Arizono et al.(1982) observed that the administration of lead increased zinc content in the low molecular-weight protein fraction in the rat hepatic 105000g soluble fraction by using Sephadex G-75 gel filtration, and that this fraction contained only zinc, not lead, as metal.

Therefore, we have investigated the effects of some drugs on this zinc contained low molecular-weight protein (zinc binding protein, Zn-BP), and further isolated and purified the Zn-BP from liver of rats treated with lead.

MATERIALS AND METHODS

Male Wistar rats were used in all experiments. Animals recieved normal chow and water *ad libitum*. Animals were usually given a single intraperitoneally injection of lead acetate (100mg/kg) or lead chloride (25mg/kg) 18 h prior to sacrifice. In case of treatment with drugs, phenobarbital (80mg/kg) intraperitoneally injected to animals once a day for 3 days prior to the injection of lead acetate, and CCl_4 (4ml/kg) which dissolved in olive oil (1:1) intraperitoneally treated to rats with simultaneous administration of lead acetate.

The rats were killed by decapitation, and the liver was perfused *in situ* with a cold 0.9% NaCl solution, and then homogenized with 4 volumes of 0.25M sucrose in a Potter-Elvehjem homogenizer with a teflon pestle. Preparation of 105000g soluble fraction was carried out by procedures described previously (Arizono et al. 1982).

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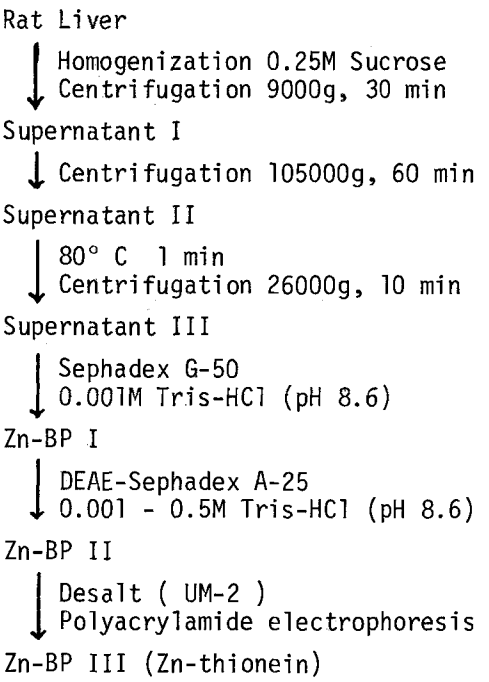
Five ml aliquots of 105000g soluble fraction were applied to a column of Sephadex G-75 (1.9x90cm), that has been equilibrated with 0.001M Tris-HCl, pH 8.6, and the column was eluted with the same buffer at flow rate of 20ml/h.

Isolation and purification of Zn-BP were carried out by procedures briefly described in Scheme 1. Lead and zinc analysis were performed using a atomic absorption spectrophotometer.



Scheme 1. Schematic Diagram of Zn-BP Purification Step

RESULTS AND DISCUSSION

In lead acetate or lead chloride treated rats, zinc content in low molecular-weight protein fraction increased markedly, and lead was detected only in the high molecular-weight protein fraction by using Sephadex G-75 gel filtration under this experimental condition as observed in the previous study (Arizono et al. 1982)(Figure 1 a,b). However, zinc in Zn-BP was substituted with lead added in vitro and this zinc shifted to high molecular-weight protein fraction the same as when cadmium was added (Figure 1 c,d).

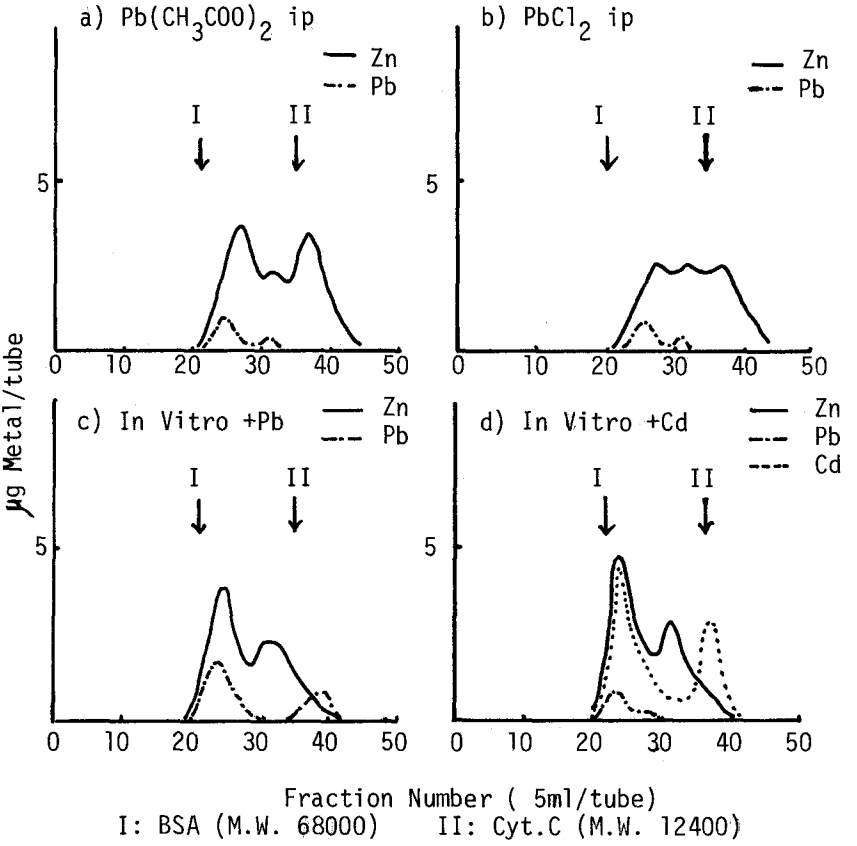


Figure 1. Sephadex G-75 Elution Profile of Liver Cytosol in Rat

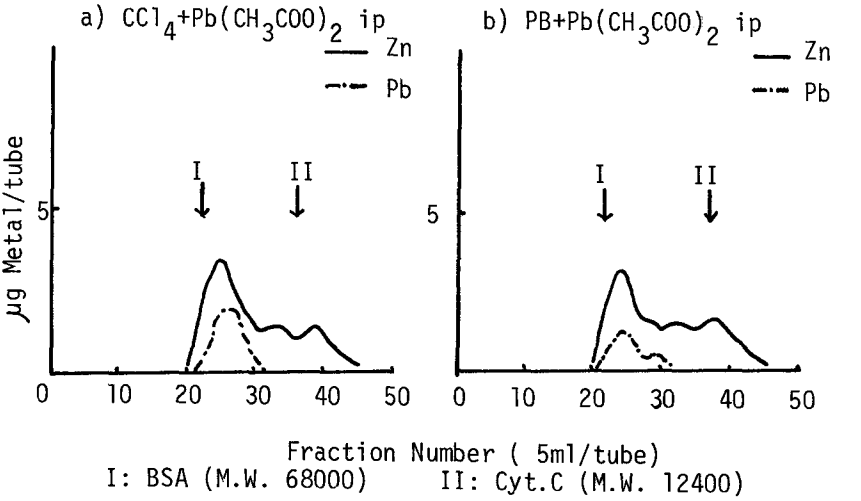


Figure 2. Gel Filtration Profile of Rat Liver Cytosol on Sephadex G-75

The formation of Zn-BP induced by lead acetate was slightly decreased by the pretreatment with phenobarbital or simultaneous treatment with CCl_4 when compared to lead treatment alone (Figure 2). On the other hand, Oh et al. (1978) and Cagen & Klaassen (1979) reported that CCl_4 induced the zinc thionein in rat liver.

Arizono et al. (1982) have already reported that the pretreatment with actinomycin D (0.8mg/kg, 4 h before lead injection) inhibit the formation of Zn-BP induced by lead acetate, but that the treatment with cycloheximide (0.5mg/kg, 4 h before and 6 h after lead acetate injection) could not inhibit Zn-BP biosynthesis by lead acetate. These findings suggest that induction mechanism of Zn-BP in rat liver is regulated by transcription level.

Oh et al.(1978) reported that some environmental stress induced Zn-MT in the liver of rats, however, we recently observed the Zn-BP in the liver of rats treated with lead acetate after adrenalectomy (Arizono et al. 1984). This assumed that induction mechanism of lead-induced Zn-BP is distinct from that of adrenal related induced zinc thionein.

The Zn-BP was purified from heat treated (80°C, 1min) hepatic soluble fraction of rats after the administration of lead. Figure 3a) shows Sephadex G-50 elution profile of heat stable fraction (Supernatant III). This Zn-BP from fraction numbers 48 to 55 was pooled (Zn-BP I) and further purified on a DEAE Sephadex A-25 column. As shown in Figure 3 b), the Zn-BP I was eluted with a linear gradient from 0.001M to 0.5M Tris-HCl, pH 8.6. The separation between Zn-BP fraction (fraction numbers 40-43) and zinc-free material was achieved by this step.

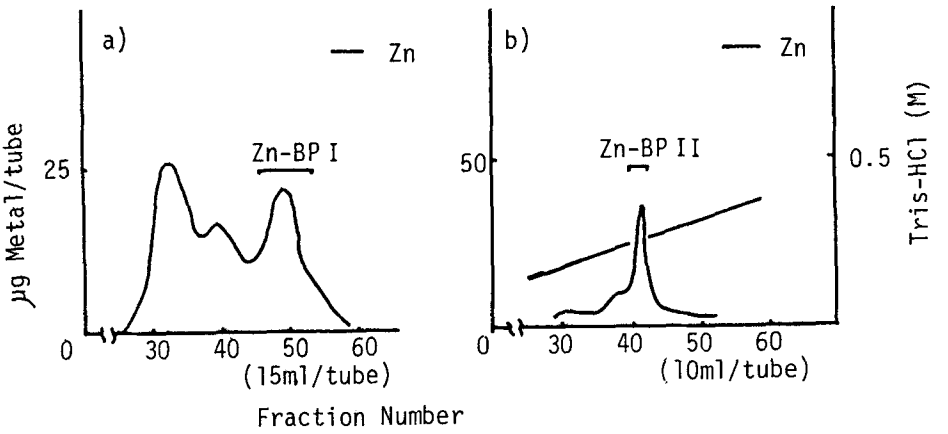


Figure 3. Gel Filtration Profile of Rat Liver Cytosol on Sephadex G-50 (a) and DEAE Sephadex A-25 Column Chromatogram of Zn-BP (b)

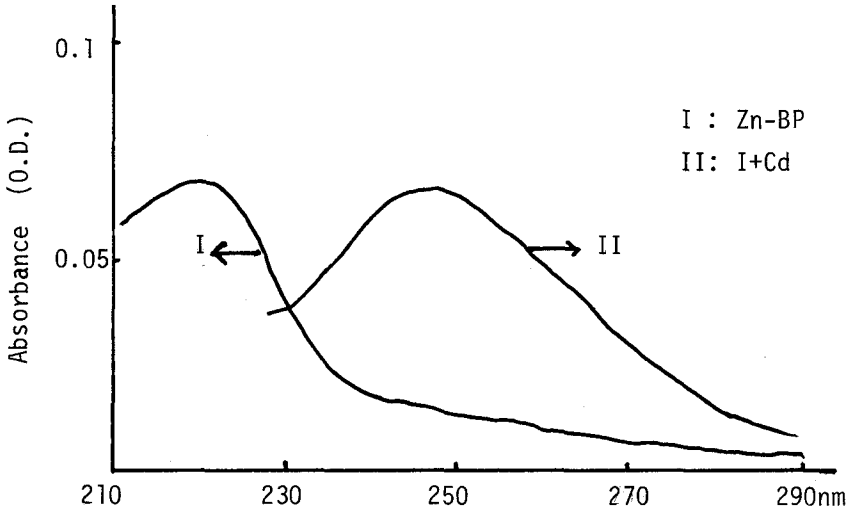


Figure 4. Difference Spectra of Zn-BP

Table 1. Amino acid compositions (% of total residues)

Amino acid	Cd-BP*	Zn-BP
Asp	7.6	8.2
Thr	6.4	3.9
Ser	13.0	7.3
Glu	5.6	6.8
Pro	2.8	3.1
Gly	9.6	10.4
Ala	6.4	4.9
1/2 Cys**	29.8	26.2
Val	4.1	3.9
Met	1.6	1.9
Ile	1.5	2.5
Leu	1.4	3.6
Lys	14.2	14.5
Arg	0.3	1.4
His	-	-
Phe	-	-

* Sokolowski & Weser (1975)
** Determined from cysteic acid content

After desalting and concentration of the obtained fraction, purified Zn-BP was obtained by using SDS polyacrylamide gel electrophoresis. This Zn-BP showed a single band and molecular weight was estimate to be about 6000 daltons. This molecular weight agreed with that of metallothionein obtained from other tissues (Webb 1979). As shown in the difference spectra, Zn-BP and cadmium substituted Zn-BP exhibited an absorption maximum at about 220nm and 250nm, respectively (Figure 4).

The amino acid composition of this Zn-BP is given in Table 1. This Zn-BP has a high cysteine content (26.2% of total residues) and a low aromatic amino acids and histidine content, which are common properties of metallothionein. However, serine content in the Zn-BP content was 7.3% of total residues. This is different from that of other known metallothionein (about 14% of total residues). This purified Zn-BP contents 7.9g atom zinc per mole.

These results suggest that this Zn-BP is thought to be zinc thionein(Zn-MT) and lead has an inducing potency for Zn-MT in the liver of rats.

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