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Purification of Metallothionein-like Protein in Rat Placenta

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It has been reported that certain heavy metals, such as cadmium, mercury, copper and zinc, can induce a low molecular weight protein, which is known as metallothionein (KAGI & VALLEE 1960, CHERIAN & GOYER 1978). This metallothionein has been found in several diverse species and has been isolated from a variety of tissues (WEBB 1979). Metallothioneins are involved in homeostasis of essential metals and in protection from metal toxicity in living animals, but its exact biological function still remains unsolved.

LUCIS et al. (1972) and ARIYOSHI et al. (1978) reported the presence of metallothionein-like protein in rat placenta. Therefore, we attempted to isolate and purify this protein from rat placenta and further to clarify the biological function in placenta.

MATERIALS AND METHODS

Wistar rats weighing 250-300g were used in this experiments. All animals received normal rat chow and water ad libitum. Zero day of gestation was confirmed by the presence of sperm cells in vaginal smears as positive indication of pregnancy. Animals were subcutaneously injected with cadmium chloride at a dose of 1 mg/kg once daily for three days on day 15 to 17 of gestation, and were sacrificed on day 18 of gestation. The fetuses and placenta were removed by Caesarean section, and the placenta of littermates were pooled and saved for preparation of cytosol. Isolation and purification of cadmium (Cd)-binding protein in placenta were carried out by procedures briefly described in Fig. 1. Cd analysis was performed using a Hitachi model 208 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Fig. 2. shows a typical elution pattern from Sephadex G-50 gel filtration of the rat placenta homogenate supernatant II fraction. This fraction from numbers 58 to 72 contains about 80% of the Cd applied to the column. This Cd containing peak was pooled (Cd-binding protein fraction I) and was rechromatographed using Sephadex G-75 (Cd-binding protein fraction II), and further purified on a DEAE-cellulose column.

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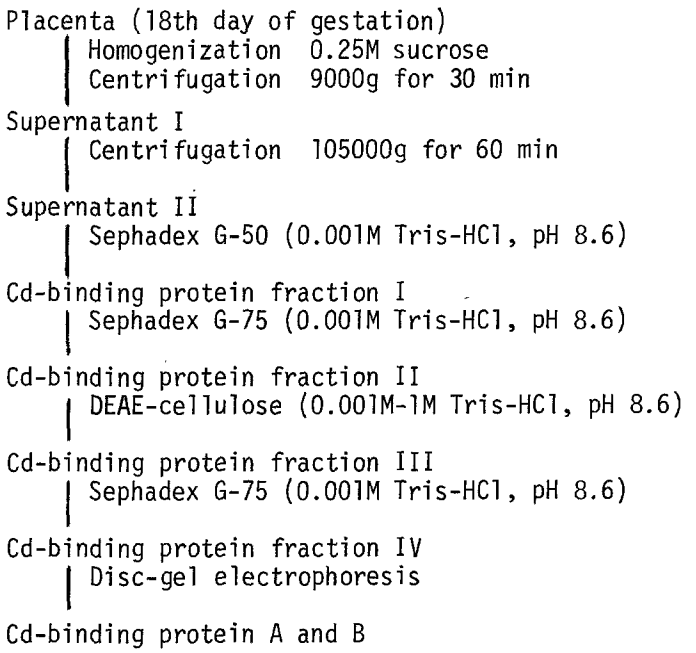

Fig. 1. Schematic diagram of the purification of Cd-binding protein from rat placenta.

The proteins were eluted with a stepwise gradient from 0.001M to 1M Tris-HCl, pH 8.6, as shown in Fig. 3. Two fractions which contained high levels of Cd were obtained. Each fraction, namely Cd-binding protein A (fraction numbers 71-92) and Cd-binding protein B (fraction numbers 178-187), was gel filtrated using Sephadex G-75. On disc-gel electrophoresis Cd-binding protein A and B each moved as a single band.

As shown in Fig. 4, the molecular weight of Cd-binding protein A and B were estimated to be about 6000 and 11000 daltons, respectively. This molecular weight of 6000 agreed with that of metallothionein obtained from other tissues (KOJIMA & KAGI 1978, WEBB 1979).

Table 1 shows the amino acid composition of Cd-binding protein A and B from rat placenta. Both Cd-binding protein A and B have a very low cysteine content and unusually aromatic amino acid residues. In addition, these proteins are marked variations in the proportions of many other amino acids. For example, Cd-binding protein A contains more aspartic acid, glutamic acid, glycine, isoleucine and leucine but less proline, alanine, valine and lysine than Cd-binding protein B. These results were markedly different from the amino acid composition of metallothionein which were reported previously (WEBB 1979). This difference might depend upon the tissue or organ difference. In addition, it was observed that these Cd-binding protein A and B contained 3.14g atom Cd/mole and o.86g atom Cd/mole, respectively.

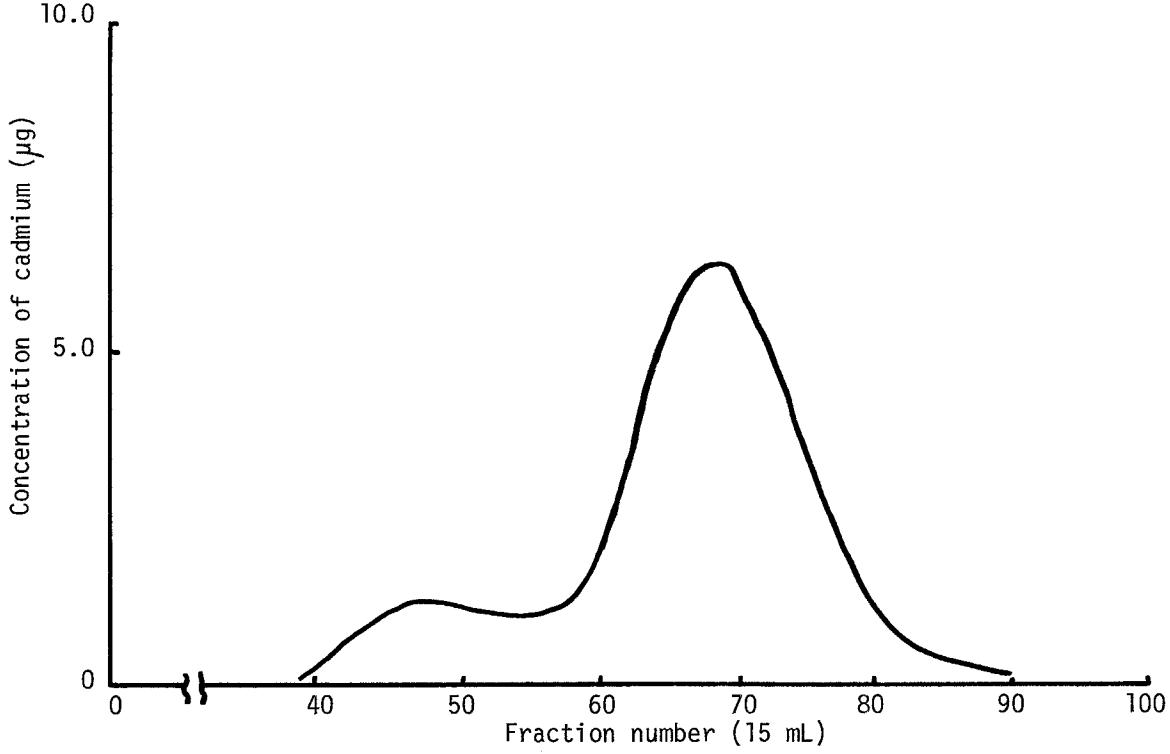

Fig. 2. Gel filtration of rat placenta supernatant II fraction on a Sephadex G-50 column.

The supernatant was applied to a Sephadex G-50 column (5.0x60cm), eluted with 0.001M Tris-HCl, pH 8.6, at a flow rate of 30 mL/h and collected (15 mL/tube).

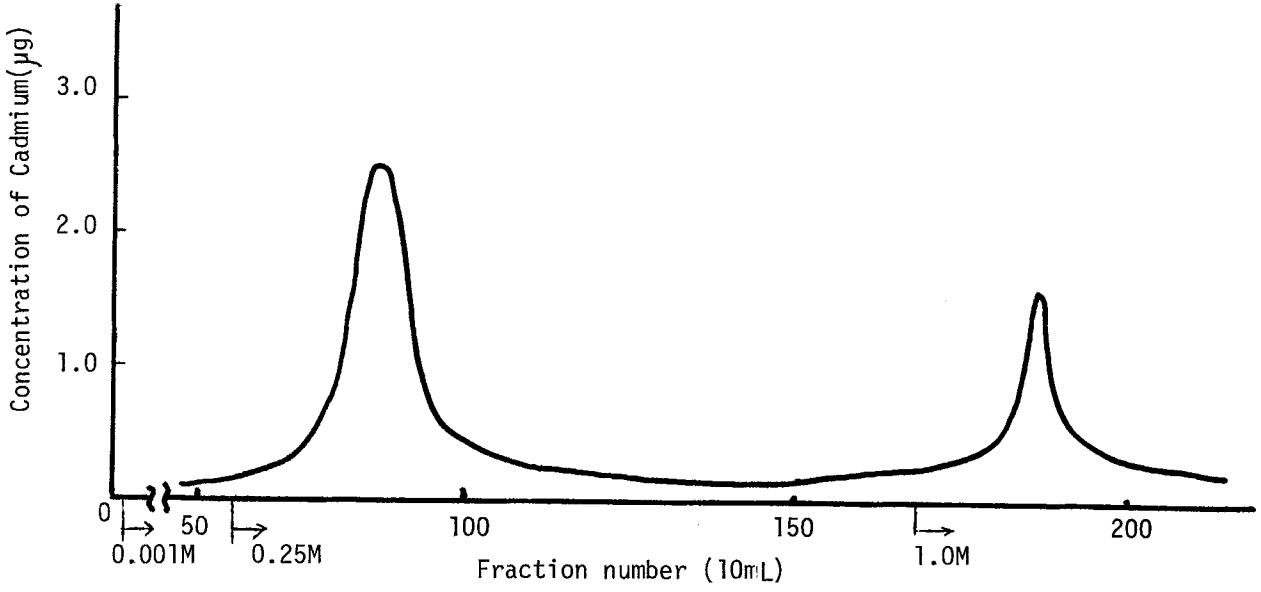

Fig. 3. Chromatography of the Cd-binding protein fraction on a DEAE-cellulose column.

Cd containing fraction were pooled from Sephadex G-75 gel-filtration and were applied to a DEAE-cellulose column(2.5x18cm) equilibrated with 0.001M Tris-HCl pH 8.6. Elution was carried out by a stepwise salt gradient(limiting buffer 1.0M Tris-HCl, pH 8.6) at flow rate of 30mL/h . Fractions(10mL) were collected.

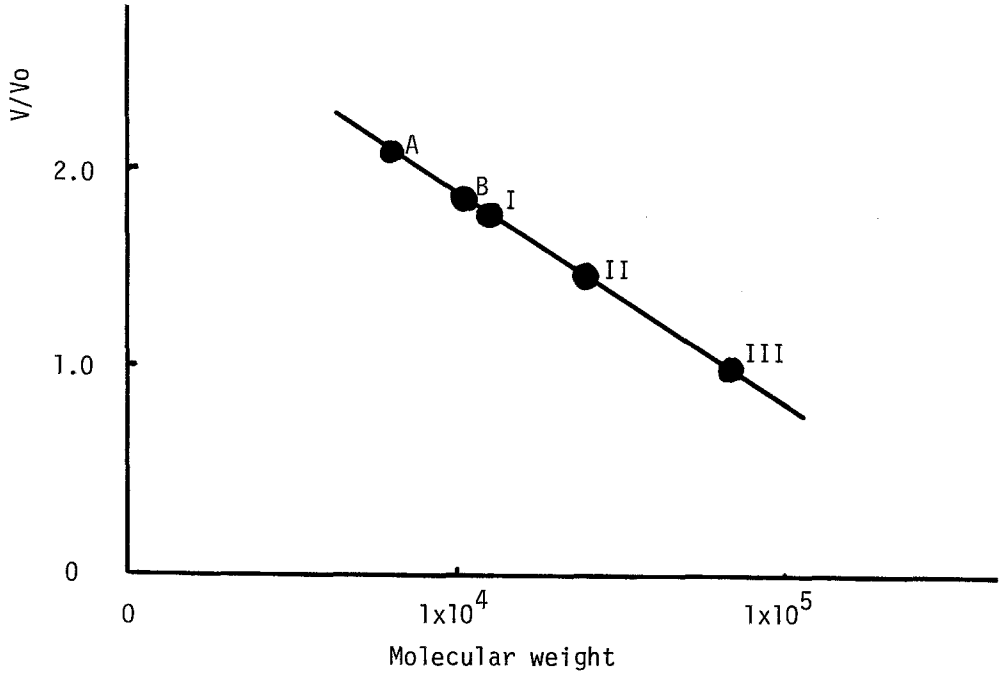

Fig. 4. Determination of molecular weight of the Cd-binding protein A and B from rat placenta by Sephadex G-75 gel filtration.
The marker proteins used are the following--I:cytochrome c, II:chymotrypsin, III:bovine serum albumin.

TABLE 1. Amino acid composition of the Cd-binding protein A and B from rat placenta.

Amino acid	Cd-binding protein A	Cd-binding protein B
	(% of total residues)	
Aspartic acid	15.5	9.5
Threonine	4.9	4.8
Serine	4.9	3.6
Glutamic acid	8.7	1.2
Proline	6.8	15.5
Glycine	8.7	3.6
Alanine	7.8	13.1
1/2 Cysteine*	3.9	6.0
Valine	3.9	6.0
Methionine	1.0	1.2
Isoleucine	7.8	3.6
Leucine	8.7	3.6
Tyrosine	1.9	2.4
Phenylalanine	4.9	4.8
Lysine	6.8	14.3
Histidine	1.0	3.6
Arginine	2.9	3.6

* Determined from cysteic acid content.

It is not known whether the Cd-binding protein A and B obtained from rat placenta are considerably specific metal-binding protein. OSE et al. (1977) reported that there was metal-binding protein in human placenta and its molecular weight estimated about 10000 daltons, but this study did not investigate the amino acid composition.

It would be interesting to determine if the treatment to pregnant animals with other toxic heavy metals such as mercury cause the induction of metal-binding protein in placenta and further such proteins have any fundamental significance for feto-placental unit. Further studies will be needed to clarify this relation.

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