Isolation of Cadmium-Binding Proteins

The existence of cadmium binding protein (Cd-BP) in various mammalian tissues and cells have been reported by us and a number of other workers[1–8]. The isolation procedure described for 'metallothionein' by KÄGI and VALLEE[2,9] and later modified by PULIDO et al.[4] was initially employed in our laboratory to investigate the rat liver cadmium binding components labeled with ^{109}Cd. Owing to the considerable loss of radioactivity and hence poor recovery of the proteins at the initial ethanol-chloroform precipitation step, an alternate procedure was devised. Using this method it was possible to isolate two distinct species of Cd-BP accounting for 60–70% of the total ^{109}Cd.

Materials and methods. Rats of Wistar strain were injected in the interscapular region with $CdCl_2$ and $^{109}CdCl_2$. Liver was cut into small pieces and homogenized (20% W/V) in sucrose solution using a glass homogenizer with teflon pestle. The homogenate was centrifuged in an ultracentrifuge (International Equipment Company, Model B-35), to obtain the soluble fraction[10]. *Tris* buffers, 0.001 *M* and 0.25 *M* with respect to tris (hydroxymethyl) aminomethane were prepared in glass distilled water and adjusted to pH 8.6 with HCl. Sodium azide (0.02%) was used as preservative. Sephadex gels and ion exchanger were prepared as recommended by the manufacturer (Pharmacia Fine Chemicals, Swe-

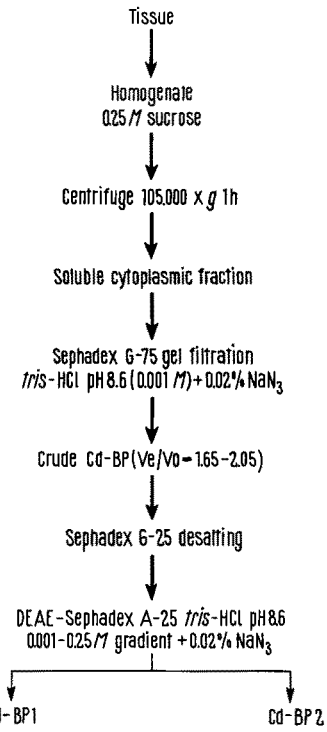

Fig. 1. Scheme for the isolation of Cd-binding proteins (Cd-BP).

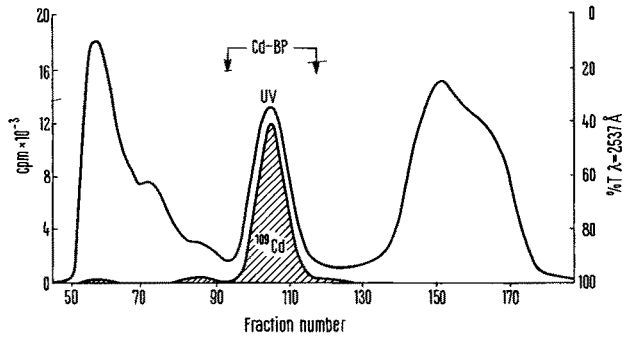

Fig. 2. Gel filtration of soluble fraction on Sephadex G-75 column (2.5 × 90 cm). Flow rate 18.6 ml/h. *Tris*-HCl pH 8.6, 0.001 *M* buffer. The rat was treated with 0.01 m*M* $CdCl_2$/kg daily for 12 days. A single s.c. injection of carrier-free $^{109}CdCl_2$ was given 24 h before sacrifice. Fresh liver was used and 14 ml of the soluble fraction was applied on the column.

[1] M. MARGOSHES and B. L. VALLEE, J. Am. chem. Soc. *79*, 4813 (1957).
[2] J. H. R. KÄGI and B. L. VALLEE, J. biol. Chem. *235*, 3460 (1960).
[3] M. PISCATOR, Nord. hyg. Tidskr. *45*, 76 (1964).
[4] P. PULIDO, J. H. R. KÄGI and B. L. VALLEE, Biochemistry *5*, 1768 (1966).
[5] Z. A. SHAIKH and O. J. LUCIS, Fedn. Proc. *29*, 298 Abs. (1970).
[6] J. H. R. KÄGI, Abstracts, 8th International Congress of Biochemistry, Lucerne, Switzerland (Ed. J. GREGORY; Staples Printers, Ltd., Rochester, England 1970), p. 130.
[7] Z. A. SHAIKH and O. J. LUCIS, Fedn. Proc. *30*, 238 Abs. (1971).
[8] O. J. LUCIS, Z. A. SHAIKH and J. A. EMBIL JR., Experientia *26*, 1109 (1970).
[9] J. H. R. KÄGI and B. L. VALLEE, J. biol. Chem. *236*, 2435 (1961).
[10] W. C. SCHNEIDER, in *Manometric Techniques* (Eds. W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER; Burgess Publishing Company, Minneapolis 1964), p. 183.

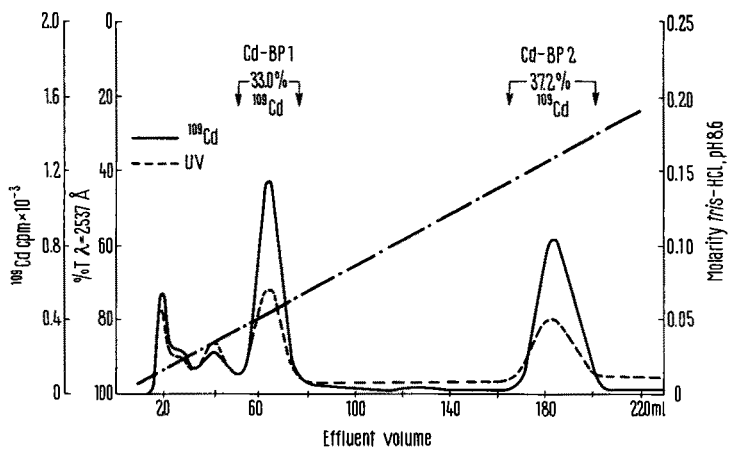

Fig. 3. Purification of Cd-BP on DEAE-Sephadex A-25 column (1.5 × 27 cm). The column was equilibrated with *Tris*-HCl pH 8.6, 0.001 *M* buffer for 3 h (2 void volumes). Flow rate 12 ml/h. The molarity of *Tris*-HCl represents the influent gradient.

den). The samples were applied on the columns under a layer of elution buffer. The effluent from the column was monitored at 2537 Å by LKB Uvicord and 10 min fractions were collected by LKB RadiRac fraction collector directly into the γ-counting vials. All operations were carried out at 4 °C. The column fractions were assayed for ^{109}Cd by a dual channel Nuclear Chicago Spectrometer with an efficiency of 51%. Cd-BP containing fractions were pooled and freeze-dried using VirTis freeze-drier. Polyacrylamide gels were prepared according to the manufacturer's instruction manual (Canalco, USA). Proteins were quantitated by LOWRY's method[11]. Radioactive cadmium-109 was purchased as $^{109}CdCl_2$ carrier free, with radiochemical purity better than 99% from NEN Corporation, Boston, Massachusetts.

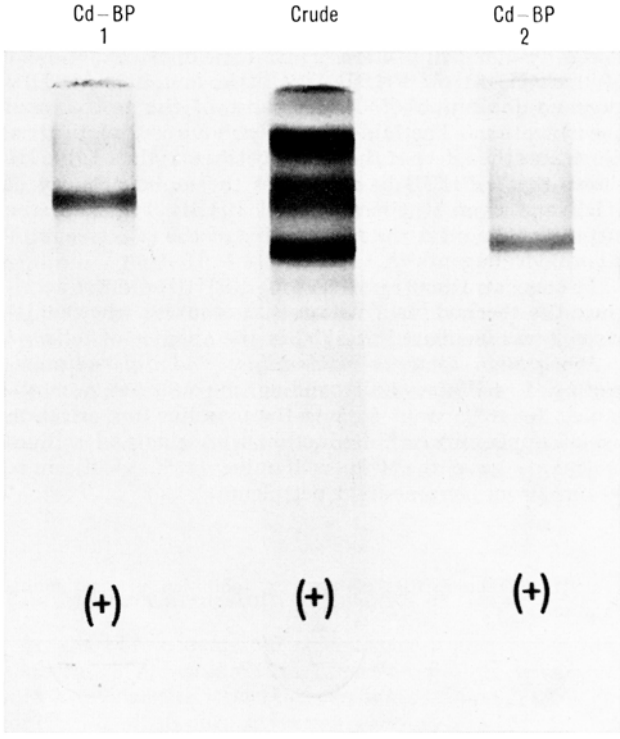

Fig. 4. Disc electrophoresis of Cd-BP (rat liver). Electrophoresis was carried out at room temperature in 7% polyacrylamide gel, using pH 9.5 *Tris*-glycine buffer. The applied current was 5 mA/column. Proteins were fixed in 12% TCA and stained with Coomassie blue.

Results and discussion. The Cd-BP were first induced in rat by s.c. injection of $CdCl_2$[5]. Liver tissue was homogenized and fractionated as described in Figure 1. Figure 2 shows the elution pattern of the soluble fraction on Sephadex G-75 column. Almost all ^{109}Cd was associated with a low molecular weight UV-light absorbing protein peak. The total recovery of ^{109}Cd from the column was better than 98%. Crude Cd-BP containing fractions were pooled and freeze-dried. Salts were removed by gel filtration on a Sephadex G-25 column (2.5 × 50 cm) and eluted with distilled water. Over 95% of the applied protein and ^{109}Cd were recovered in the void volume of the column. The desalted protein fractions were freeze-dried and kept in the dessicated state until further use. About 5 mg of crude protein was dissolved in 1 ml of 0.001 *M Tris* buffer and applied on DEAE-Sephadex column. The proteins were eluted by *Tris*-HCl concentration gradient formed by mixing 0.25 *M Tris* buffer (12 ml/h) with 100 ml of the starting buffer. As shown in Figure 3 two major UV-light absorbing protein peaks containing ^{109}Cd and numbered as Cd-BP 1 and Cd-BP 2 were isolated. Homogeneity of these proteins was verified by disc electrophoresis. As shown in Figure 4, Cd-BP 1 and Cd-BP 2 migrated as single bands having similar mobilities to those separated from the crude protein. To locate the position of ^{109}Cd, unstained gels were sliced into small discs 1 mm in thickness and counted in the γ-spectrometer. Radioactivity was present in the protein staining region.

The procedure described above is useful for analytical purposes, but it can be easily adopted for the preparative scale by appropriate adjustments in the column dimensions. The treatments given to the macromolecules are relatively mild and the principles involved in the isolation procedure can be utilized for the isolation of other metal binding proteins.

Résumé. Un procédé pour l'isolation des protéines combinées au Cd radioactif (^{109}Cd) a été mis au point. La méthode ne détruit pas les protéines. D'autres protéines ayant des affinités pour les métaux lourds pourront ainsi être isolées.

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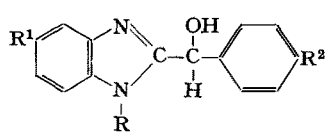
[11] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

The High Activity of Fluorohydroxybenzylbenzimidazoles Against Some Small RNA-Viruses

O'SULLIVAN, LUDLOW, PANTIC and WALLIS[1] reported that the introduction of a 5-fluoro substituent into 2-(α-hydroxybenzyl)benzimidazole (HBB; Ia) produced a notable increase in protective activity towards tissue cultures infected with poliovirus. Information is now given on this fluoro derivative (5-FHBB; Ib) and on two new highly active fluoro compounds, 2-(α-hydroxy-*p*-fluorobenzyl)benzimidazole (HFBB; Ic) and 5-fluoro-2-(α-hydroxybenzyl)-1-propylbenzimidazole (FPHBB; Id). The latter is by far the most active of all the antipicornaviral benzimidazoles hitherto investigated.



Ia; R = R^1 = R^2 = H
Ib; R = R^2 = H, R^1 = F
Ic; R = R^1 = H, R^2 = F
Id; R = Pr, R^1 = F, R^2 = H
Ie; R = Pr, R^1 = R^2 = H

The compounds were tested with polioviruses 1, 2 and 3 and coxsackievirus A21 in ERK (human) cell line monolayers and with coxsackievirus A9 in secondary MK monolayers. The ERK cells, on subculturing, were grown in slowly revolving tubes at 37 °C for 3 days in