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ISOLATION OF A MANGANESE BINDING PROTEIN FROM MOUSE LIVER

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ABSTRACT

A manganese-binding protein with mol. wt. of approx. 71.000 has been identified in the membranous fractions of mouse liver. This protein seems to have characteristics similar to transferrin.

INTRODUCTION

It has been shown that absorbed manganese is transported to the liver and other tissues by a beta 1 globulin (10). Intraperitoneally injected Mn^{56} is rapidly distributed in association with the membranous fractions, concentrating primarily in organs rich in mitochondria (8). Our work with Mn^{54} showed that altough hepatic mitochondrial and nuclear fractions demonstrated the highest uptake, the values for the microsomal fraction were highly significative (Bonilla E., unpublished results). It is assumed that some of the manganese that reaches the hepatic cells is incorporated into certain proteins, at least temporarily (3). However, the relationship of liver proteins to the turnover of manganese is poorly understood.

The current study deals with the separation and characterization of a manganese binding protein from the membranous fractions of mouse liver.

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MATERIAL AND METHOD

Swiss male albino mice (18-25 gr) were used throughout. All animals were given Purina laboratory chow (32 μg Mn/g) and distilled demineralized water, ad libitum. One hour after the intraperitoneal injection with 10 μCi of $Mn^{54}Cl_2$ (Amersham-Searle) they were killed by cervical dislocation and the livers removed, excised and rinsed with 0.9% NaCl. A 10% homogenate in 0.25 M sucrose (pH 7.4) was prepared using a Potter-Elvehjem homogenizer and a teflon pestle. Five ml aliquots were centrifuged at 105.000 g for 60 min. The supernatants were discarded and the resulting precipitates homogenized with 2 ml of 0.9% NaCl and centrifuged at 105.000 g for 120 min. The latter supernatants were dialyzed for 18 hours with 200 vol. of 0.1M buffer tris-HCl pH 8.0 in 1M NaCl. After dialysis, 2.5 ml (20 mg protein) were poured onto a column of Sephadex G-200 (2.6 X 70 cm) which had been previously equilibrated with the same buffer. Fractions of 5 ml were collected and their absorbances at 280 nm monitored for determining relative protein concentrations in column effluents.

In the studies with Fe^{59}, 10 μCi of $Fe^{59}Cl_3$ (New England Nuclear) in 0.1 M citrate buffer, pH 7.4 were injected by the intraperitoneal route every 12 hours. One hour after the fourth injection the animals were killed and the NaCl extracts were obtained and treated as described for the Mn^{54} experiments. Fractions of 4.3 ml were collected from the column.

The molecular weight was determined following the gel-filtration method of Andrews (1). The peak proteins isolated from the columns were concentrated by ultrafiltration (Amicon UM-05). After 16 hours-dialysis with 200 vol. of buffer 0.05 M tris-HCl pH 7.5 containing KCl (0.1M), 2 ml of the concentrate (2 mg protein) were applied to a column of Sephadex G-200 (2.5 X 50 cm) and eluted with the same buffer. Chymotrypsinogen A, ovalbumin, bovine serum albumin, gammaglobulin and apoferritin were chosen as reference proteins and their elution volumes measured for each of them. In addition, the void volume was determined in experiments with blue dextran plus reference proteins.

Protein was estimated by the method of Lowry et al (7) with bovine serum albumin as the standard. Radioactivity was measured in a Packard well-type scintillation counter.

RESULTS AND DISCUSSION

The G-200 elution profile of the NaCl extract is characterized by two Mn^{54}-containing peaks (Fig. 1). Peak A centred at an elution volume of

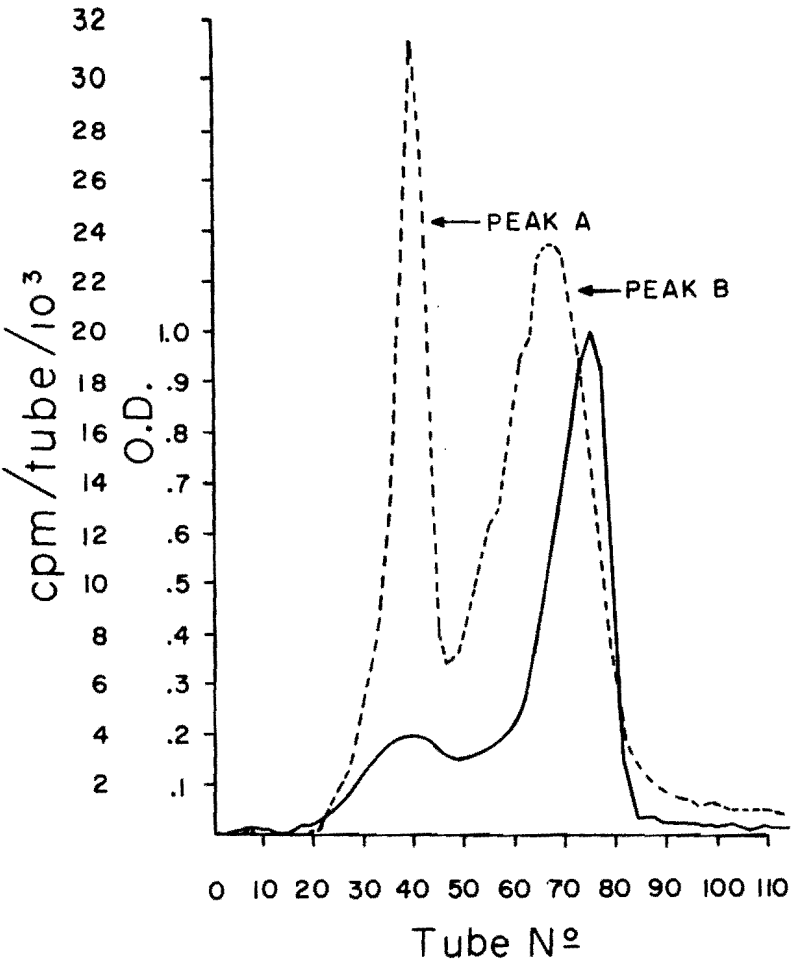

Fig. 1.– Sephadex G-200 elution profile of the NaCl extract from liver membranous fraction of mice administered with Mn^{54} Cl_2. The colum (2.6 X 70 cm) was eluted with 0.1 M tris-HCl buffer, pH 8.0, in 1M NaCl. Fractions of 5 ml were collected and monitored for absorbance at 280 nm (———) and for Mn^{54} (– – – – –).

195 ml and is associated to a small peak of proteins of large molecular weight. Peak B centred at an elution volume of 330 ml and precedes a peak of proteins of lower molecular weights. For the purpose of this study we only determine some characteristics of the proteins from peak A. Those of the lower molecular weight proteins (peak B) will not be considered.

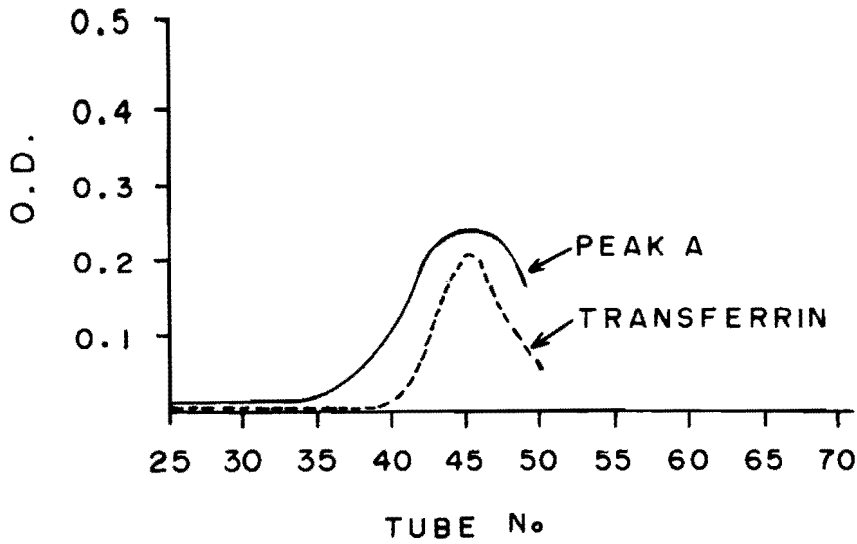

Fig. 2.– Sephadex G-200 elution profile of transferrin as compared with the protein of peak A. The column (2.6 X 70 cm) was eluted with 0.1 M tris-HCl buffer, pH 8.0, containing 1M NaCl. Fractions of 4.3 ml were collected.

Human transferrin (AB KABI, Sweden) when passed through a column of Sephadex G-200 is eluted in a way identical to the protein of peak A (Fig. 2). Panic (9) and Keefer et al (6) have demonstrated that manganese binds to transferrin, a beta 1 globulin synthesized chiefly by the hepatic parenchymal cells (11). This protein has two separate iron-binding sites, each capable of binding one atom of ferric ion (2). The two sites seem to behave differently (5). One site, called "A", release its iron preferentially to erythroblasts; the "B" site release its iron to other tissues such as liver which appears to have receptor sites for transferrin. In fact, of the non-erythrocyte iron pathways, the exchange with the hepatic parenchymal cells is one of the largest (12).

Molecular weights of 83.000 and 65.000-70 have been reported for human (2) and bovine transferrin (4), respectively. However, its gel filtration behavior is more consistent with a molecular weight of approximately 70.000 than with one of 83.000 (1). As calculated from fig. 3 the M.W. of the protein obtained from peak A was 71.000 ± 1.700 (mean ± s.e.m. from three experiments). This M.W. is similar to that reported by Andrews (1) for bovine transferrin.

After the injection of Fe^{59} two radioactive peaks are obtained from the NaCl extract. One peak is centred at an elution volume of 195 ml and

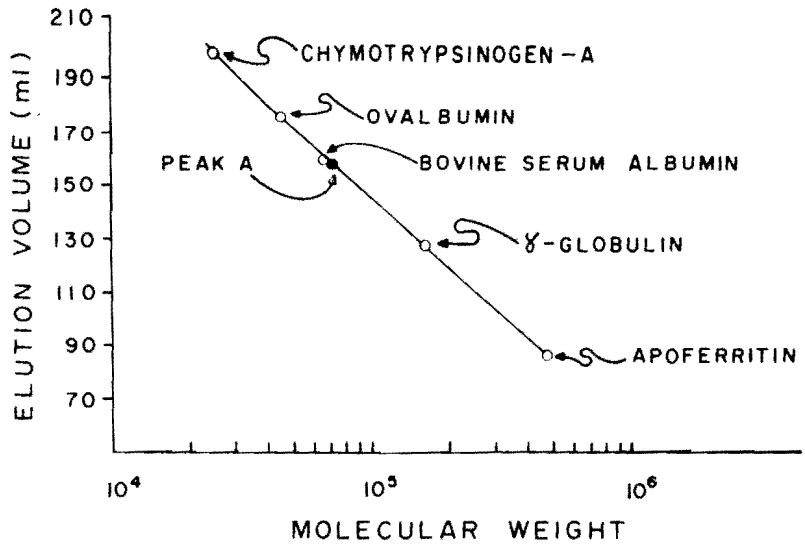

Fig. 3.– Plot of elution volume against log (mol. wt.) for proteins on Sephadex G-200 column (2.5 X 50 cm) eluted with buffer 0.05 M tris-HCl, pH 7.5, containing KCl (0.1M). Details are given in the text.

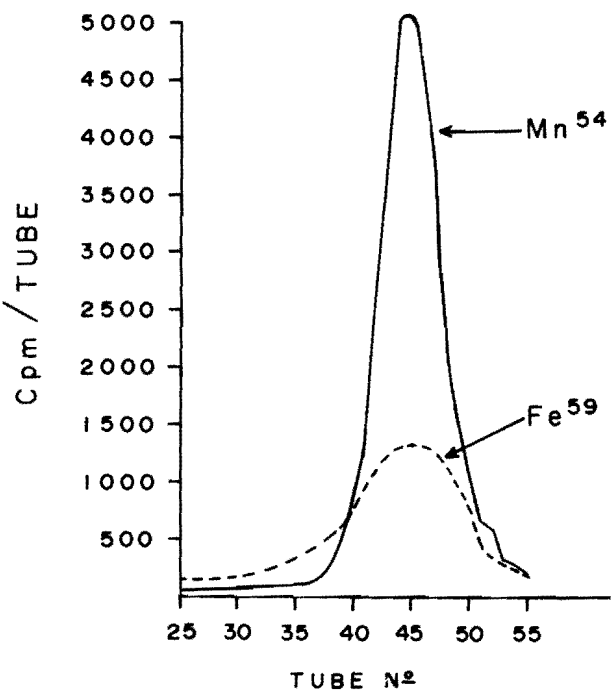

Fig. 4.– Sephadex G-200 elution profile of the NaCl extracts obtained from liver of mice administered with $Fe^{59}Cl_3$ or $Mn^{54}Cl_2$. Further details are given in the text.

the other at 279 ml. The former elution volume is similar to the one we found for the first Mn^{54} peak (peak A) (Fig. 4) and for human transferrin.

In conclusion, the manganese-binding protein isolated from the membranous fractions of mouse liver appears to be a protein with characteristics similar to transferrin. This finding rises the question about the importance of this glycoprotein in the liver uptake and metabolism of manganese.

RESUMEN

Aislamiento de una proteína fijadora de manganeso a partir de hígado de ratón. *Bonilla E. (Instituto de Investigación Clínica, Apartado 1151, Maracaibo, Venezuela).* **Invest Clín** *18(4): 197-203, 1977.*– Se identificó una proteína fijadora de manganeso en la fracción membranosa de hígado de ratón. Su peso molecular fue aproximadamente de 71.000 dalton. Esta proteína tiene características similares a la transferrina.

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