Isolation of mannose-binding proteins from human and rat liver

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A binding assay for the detection of mannose-binding proteins was developed, which uses a ligand of mammalian origin, ^{125}I -labelled bovine pancreatic ribonuclease B. The binding assay was validated by using the recognized mannose-binding protein, concanavalin A. Microgram quantities of concanavalin A or mannose-binding proteins could be assayed. A mannose-binding protein was isolated from rat liver by affinity chromatography on mannose–Sephharose 6B. It has a M_r of approx. 900 000 under non-dissociating conditions and contains a subunit of approx. 34 000 M_r . When ribonuclease B–Sephharose was used as a ligand for affinity chromatography, the predominant mannose-binding material isolated from rat liver had a native M_r of 205 000–225 000 and consisted largely of a subunit of M_r 70 000, which yielded subunits of M_r 28 500 and 34 000 on reduction. It is suggested that different mannose-binding proteins are isolated by the two affinity-chromatography ligands. A mannose-binding protein was also purified from human liver by affinity chromatography on mannose–Sephharose 6B. It has a native M_r of over 1 000 000 and consists of subunits with M_r 28 000 and 30 500. Its isolation suggests that mannose-mediated endocytosis or intracellular transport of glycoproteins occurs in human liver.

Lectins or carbohydrate-binding proteins specific for galactose (Hudgin *et al.*, 1974), mannose/*N*-acetylglucosamine (Schlesinger *et al.*, 1978; Stahl *et al.*, 1978), fucose (Prieels *et al.*, 1978), phosphomannose (Kaplan *et al.*, 1977; Sando & Neufeld, 1977) and *N*-acetylglucosamine (Kawasaki & Ashwell, 1977) have been detected in eukaryotic cells. In contrast with these membrane-associated lectins, another class of soluble, low-molecular-weight, galactose-specific binding proteins has been found in vertebrate tissues (Teichberg *et al.*, 1975). Several of these lectins of both types have appropriate synthetic or natural glycoconjugate as a ligand. A mannose-binding protein that was not associated with enzymic activity was first detected during purification of human liver α -D-mannosidase by affinity chromatography (Robinson *et al.*, 1975). Subsequently, mannose-binding proteins have been isolated from rabbit liver (Kawasaki *et al.*, 1978), rabbit serum (Kozutsumi *et al.*, 1980), rat lymph nodes (Kawasaki *et al.*, 1980) and rat liver (Mizuno *et al.*, 1981; Maynard & Baenziger, 1982) by affinity chromatography on yeast mannan–Sephharose 4B. Townsend & Stahl (1981) have also isolated a mannose/*N*-acetylglucosamine/fucose-binding pro-

tein from rat liver, using yeast invertase–Sephharose 4B and mannose–Sephharose 4B as affinity-chromatography media. RNAase B is a glycoprotein, with a single ‘high-mannose’ glycan side chain (Liang *et al.*, 1980). It is known to be cleared from the circulation in the rat by the mannose-specific receptors of liver non-parenchymal cells (Baynes & Wold, 1976; Brown *et al.*, 1978; Schlesinger *et al.*, 1978) and to be endocytosed by isolated rat hepatic reticuloendothelial cells (Maynard & Baenziger, 1981). We have chosen to use RNAase B as a ligand in an assay for mannose-binding proteins and to investigate its use as a ligand for the isolation of mannose-binding proteins by affinity chromatography. In this paper, we demonstrate that apparently different mannose-binding proteins can be isolated from rat liver by affinity chromatography on mannose–Sephharose 4B and RNAase B–Sephharose 4B respectively, and also report the isolation of a mannose-binding protein from human liver.

Materials and methods

Purification of bovine pancreatic ribonuclease B

RNAase B was purified from commercial RNAase B [type IIIB or XIIB; Sigma (London) Chemical Co.

Abbreviations used: SDS, sodium dodecyl sulphate; RNAase, bovine pancreatic ribonuclease.

Ltd., Poole, Dorset, U.K.] by affinity chromatography on concanavalin A-Sepharose [Pharmacia (G.B.) Ltd., Hounslow, Middx., U.K.] as described by Baynes & Wold (1976). It was shown to be homogeneous by electrophoresis in polyacrylamide gel at pH 4.0 (McAllister *et al.*, 1963). Ribonuclease activity was assayed with the synthetic substrate cytidine 2':3'-cyclic phosphate (Crook *et al.*, 1960).

Synthesis of affinity-chromatography media

RNAase B-Sepharose 4B was prepared by coupling purified RNAase B (25 mg) with CNBr-activated Sepharose 4B (Pharmacia) (2 g dry wt.) according to the manufacturer's instructions for conjugating proteins. Ethanolamine (1 M) was used to block any groups on the Sepharose 4B that had not reacted. Another batch of CNBr-activated Sepharose 4B was treated in the same manner, except that the RNAase B was omitted. This product was designated hydroxyethyl-Sepharose 4B.

D-Mannose (Sigma) was coupled to Sepharose 6B (Pharmacia) by reaction with divinyl sulphone (Aldrich, Gillingham, Dorset, U.K.) by the method of Fornstedt & Porath (1975). Typically, 20 g of mannose was made to react with 100 ml of suspended Sepharose 6B. Again, another batch of Sepharose 6B was treated in the same way, but in the absence of mannose to yield hydroxyethyl-Sepharose 6B.

To demonstrate that RNAase B and mannose had been coupled to Sepharose 4B and 6B respectively, and had retained the capacity to bind to a mannose-binding protein, concanavalin A (10 mg; Pharmacia) was passed through 1 ml-capacity columns of RNAase B-Sepharose 4B and mannose-Sepharose 6B. In both cases over 90% of the concanavalin A bound to the columns and was specifically eluted by methyl α -D-glucopyranoside (10%, w/v). In contrast, concanavalin A did not bind to hydroxyethyl-Sepharose 4B or 6B. Chromatography on concanavalin A-Sepharose and chromatography of concanavalin A on the affinity media were carried out in 10 mM-sodium phosphate buffer, pH 6.8, containing 0.1 mM- CaCl_2 , 0.1 mM- MnCl_2 , 0.1 mM- MgCl_2 and 0.5 M- NaCl .

Isolation of mannose-binding proteins from human and rat liver

Post mortem human liver (50 g), which had been stored at -20°C until required, or rat liver (50 g), which was frozen immediately on removal and stored at -20°C , was homogenized in 100 ml of ice-cold acetone in an MSE Ato-Mix homogenizer. The slurry was filtered on Whatman no. 1 filter paper under reduced pressure and allowed to dry in air. Proteins were extracted from the acetone-dried powder in a buffer consisting of 10 mM-Tris/HCl,

pH 7.8, 0.4 M- KCl and 2% (v/v) Triton X-100 as described by Kawasaki *et al.* (1978). After addition of solid CaCl_2 to give a final concentration of 20 mM, the crude extract (1.5–2.0 litres) was applied to a column (80 ml capacity) of mannose-Sepharose 6B which had been equilibrated with a loading buffer consisting of 10 mM-Tris/HCl, pH 7.8, 20 mM- CaCl_2 , 1.25 M- NaCl and 0.5% (v/v) Triton X-100. The eluate was made 20 mM in CaCl_2 and rechromatographed on an analytical column of mannose-Sepharose 6B (5 ml). Material eluted with EDTA as described above was pooled, dialysed against 10 mM-Tris/HCl, pH 7.5, containing 20 mM- CaCl_2 and stored frozen at -20°C . For affinity chromatography of the rat liver extract (600 ml) on RNAase B-Sepharose 4B (11 ml capacity column), the same procedure was followed.

Electrophoretic analysis

Electrophoresis of proteins under non-denaturing conditions was performed in vertical pre-cast gels containing a gradient (2.5–28%, w/v) of polyacrylamide (Universal Scientific, Ltd., London E.12, U.K.). The buffer system, pH 8.4, contained 0.09 M-Tris, 0.08 M-boric acid and 2.5 mM-EDTA. Protein samples (10 μl) reached their pore limits after electrophoresis for 24 h at 125 V at 4°C . Molecular weights were estimated as described by Manwell (1977).

Electrophoresis of proteins under denaturing conditions was performed at pH 8.4 in vertical gels (80 mm $\times$ 80 mm $\times$ 1.5 mm) of polyacrylamide (7.5%, w/v) as described by Laemmli (1970). Disulphide bonds in the polypeptides were reduced and alkylated with iodoacetamide in some experiments (Roberts & Lord, 1981).

Protein bands were detected by staining with Coomassie Blue R-250 (Sigma) [0.02% (w/v) in 10% (v/v) acetic acid]. Glycoproteins that bind to concanavalin A were detected as described by Parish *et al.* (1977). Horseradish peroxidase and 3,3'-diaminobenzidine hydrochloride were obtained from Sigma.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Preparation of ^{125}I -labelled RNAase B

Purified RNAase B (0.5 or 1.0 mg) was iodinated with Na^{125}I (Amersham International, Amersham, Bucks., U.K.) by the chloramine-T method of Greenwood *et al.* (1963). Excess reagent and low-molecular-weight material were separated from the ^{125}I -labelled RNAase B by gel filtration on Sephadex G-50 (Pharmacia). To ascertain whether the iodination had significantly altered the carbo-

hydrate moiety of the RNAase B, a sample (100 μ l) of the labelled protein was analysed on a column of concanavalin A–Sepharose (5 ml capacity). In all preparations over 90% of the radioactivity bound to the column was specifically eluted by methyl α -D-glucopyranoside (10%, w/v). The specific radioactivity of 125 I-labelled RNAase B prepared in this way was in the range 300–500 μ Ci/mg.

Binding assay

The binding of concanavalin A or mammalian mannose-binding proteins to 125 I-labelled RNAase B was assayed essentially by a modification of 'Assay A' of Hudgin *et al.* (1974). 125 I-labelled RNAase B (1 μ g) was mixed with concanavalin A (0–100 μ g) or appropriate weight of mannose-binding protein in 0.5 ml of a binding buffer, which consisted of 10 mM-Tris/HCl, pH 7.8, 0.25 M-NaCl, 40 mM-CaCl₂, 0.1% (w/v) Triton X-100 and 0.6% (w/v) bovine serum albumin. After incubation for 10 min at room temperature, the complex was precipitated by addition of 0.5 ml of ice-cold saturated (NH₄)₂SO₄, which had been adjusted to pH 7.8 with solid Tris and also contained 10 mM-CaCl₂. The mixture was left on ice for 10 min before collection of the precipitate by filtration on a Whatman GF/C filter. After washing with cold (NH₄)₂SO₄ (50% saturation) containing 10 mM-CaCl₂ and bovine serum albumin (0.3%, w/v), the filter was placed in a vial and the radioactivity counted in a Packard Autogamma counter (Packard Instruments Ltd., Caversham, Berks., U.K.). Assays were performed in triplicate for each sample, and the non-specific precipitation, which was measured by adding the 125 I-labelled RNAase B just before filtration, was subtracted from each value. Preparations of mammalian mannose-binding proteins were usually concentrated 2–10-fold by freeze-drying and redissolving before assay.

Results

Validation of binding assay

The conditions for the standard assay procedure were selected after preliminary experiments in which concanavalin A was used as a recognized mannose-binding protein and in which the amount of 125 I-labelled RNAase B, the percentage saturation of (NH₄)₂SO₄ and the incubation time were varied. Variation of the amount of concanavalin A in the standard assay showed that the radioactivity precipitated was essentially directly proportional to the amount of concanavalin A over the range 0–10 μ g and that saturation occurred at about 80–100 μ g (Fig. 1). Analysis of the results for non-saturating amounts of concanavalin A (0–50 μ g) by the method of Scatchard (1949) gave a linear plot with an apparent association constant of approx. 10^6 M⁻¹.

Fig. 1. Assay for the binding of 125 I-labelled RNAase B to concanavalin A

125 I-labelled RNAase B (1 μ g) was incubated with different amounts of concanavalin A (0–100 μ g) under the conditions described in the Materials and methods section. The radioactive material precipitated by (NH₄)₂SO₄ (50% saturation) was counted. (a) Data plotted as c.p.m. versus μ g of concanavalin in assay, with inset for lower values obtained in different experiment from that giving higher values; (b) data analysed by method of Scatchard (1949).

By assuming that there was one binding site per subunit of concanavalin A of M_r 25000, it was calculated from the Scatchard plot that there was 0.7, i.e. 1, binding site on RNAase B. Thus the binding assay could be used reliably to measure amounts of concanavalin A in the range 0.5–50 μ g.

As pure mammalian mannose-binding proteins are not available, the mannose-binding activity of the proteins isolated from rat and human liver was expressed in terms of the equivalent amount of concanavalin A that would precipitate the same amount of ^{125}I -labelled RNAase B, obtained from a standard curve.

Chromatography of an extract of rat liver on mannose-Sephacrose 6B

The extract of rat liver was passed through a preparative column of mannose-Sephacrose in a buffer containing 20 mM- CaCl_2 . When no further protein was eluted from the column under these conditions, the eluting buffer was changed to one containing 2 mM-EDTA and no Ca^{2+} . The material eluted from the mannose-Sephacrose by EDTA was pooled and made 20 mM with respect to CaCl_2 and applied to a smaller column of mannose-Sephacrose 6B, equilibrated in the Ca^{2+} -containing buffer. The same elution procedure was used and the material specifically eluted by the EDTA was pooled and dialysed extensively against 10 mM-Tris buffer, adjusted to pH 7.5 with HCl and containing 20 mM- CaCl_2 . The yields and properties of the proteins purified in this manner are summarized in Table 1. When the rat liver extract was applied to a column of hydroxyethyl-Sephacrose 6B under identical conditions, no protein was specifically eluted by EDTA.

Mannose-binding activity of material purified from rat liver by affinity chromatography on mannose-Sephacrose

The mannose-binding activity of the preparations purified by affinity chromatography on mannose-Sephacrose 6B was measured by using the binding assay with ^{125}I -labelled RNAase B as the ligand. All the preparations were found to have comparable binding activity, which was expressed as the equivalent weight of concanavalin A required to precipitate the same amount of ^{125}I -labelled RNAase B under the same conditions (Table 1). The binding activity was directly proportional to the amount of purified material in the binding assay (Fig. 2).

Electrophoretic analysis of rat liver mannose-binding protein

Material purified by affinity chromatography was electrophoresed under non-denaturing conditions in a gradient of polyacrylamide, showing that it consisted predominantly of a single protein with a M_r of at least 900 000. The apparent size of the native protein varied from 900 000 to an estimated several million. Electrophoresis of the material in the presence of SDS showed that it was mainly composed of a subunit of apparent M_r 34 000, together with smaller amounts of polypeptides corresponding to multiples of this size. This suggests

Table 1. Comparison of yields and properties of mannose-binding proteins purified from human and rat liver by affinity chromatography

Source (no. of preparations)	Affinity support	Yield (mg/kg of liver) [average (and range)]	Binding activity (equivalent μg of concanavalin A/mg of protein) (average $\pm$ s.d.) 23.6 ± 9.5	Native M_r > 900 000	M_r of subunits found in dissociating conditions without reduction of disulphide bonds 34 000 and (34 000) $_n$	M_r of polypeptide chains found in dis- sociating conditions after reduction and alkylation 63 000
Rat liver (5)	Mannose-Sephacrose	10 (2-24)	21	200 000 and 1 700 000 approx.	70 000	28 500, 34 000 and 70 000
Rat liver (2)	RNAase B-Sephacrose	140	10.6	> 1 000 000	30 000	28 000, 30 500 and 31 500
Human liver (1)	Mannose-Sephacrose	26.7

Fig. 2. Assay of mannose-binding activity of material purified by affinity chromatography on mannose-Sepharose 6B from rat liver

^{125}I -labelled RNAase B ($1\mu\text{g}$) was incubated with different volumes (0–500 μl) of concentrated binding protein in the binding assay buffer in a total volume of 500 μl .

that aggregation of the main subunit occurs. A positive reaction with the concanavalin A-peroxidase system showed that the main subunit was a glycoprotein. To investigate whether aggregation was responsible for the components with a higher molecular weight, the binding protein was reduced and alkylated with iodoacetamide before electrophoresis (Fig. 3a). Under these conditions the binding protein yielded predominantly a component of 63 000 M_r . However, when a larger amount of material was applied to a gel (Fig. 3b) a polypeptide of M_r 32 000 was also observed. The proportion of the larger component increased on storage and with the length of time of boiling in SDS.

Isolation of mannose-binding proteins from rat liver by affinity chromatography on ribonuclease B-Sepharose 4B

The single mannose-rich glycan side chain of RNAase B provides an alternative ligand for affinity chromatography of mannose-binding proteins. Therefore a crude extract of liver was passed through a column of RNAase B-Sepharose 4B, equilibrated in the buffer containing 20 mM- CaCl_2 . Subsequent elution of the column with a buffer lacking Ca^{2+} but containing 2 mM-EDTA resulted in the elution of protein material. After dialysis against 10 mM-Tris/HCl buffer, pH 7.8, containing 20 mM-

Fig. 3. Comparison of rat and human liver mannose-binding proteins by electrophoresis under denaturing conditions

Samples of the human and the rat binding proteins were reduced and alkylated before denaturation and electrophoresis in the presence of SDS. The gels were stained with Coomassie Blue. (a) Lane 1, rat liver binding protein purified on mannose-Sepharose 6B (approx. 10 μg applied); lane 2, protein standards (M_r in parentheses): pyruvate kinase (60 000), 3-phosphoglycerate kinase (45 000), glyceraldehyde 3-phosphate dehydrogenase (36 000), carbonic anhydrase (29 000) and myoglobin (17 000). (b) Lane 2, rat liver protein purified on mannose-Sepharose 6B (approx. 50 μg applied); lane 3, human liver binding protein purified on mannose-Sepharose 6B; lane 4, rat liver protein purified on RNAase B-Sepharose 4B; lanes 1 and 5, protein standards. Values beside the gels are M_r ($\times 10^{-3}$).

CaCl_2 , the mannose-binding activity of this preparation was measured by the binding assay. The specific binding activity was similar to that for the protein isolated from rat liver by affinity chromatography on mannose-Sepharose (Table 1). Addition of 10 μg of ribonuclease A, which is unglycosylated, to the binding assay mixture did not alter the amount of ^{125}I -labelled RNAase B precipitated by a sample of the binding protein. This shows that recognition is dependent on the oligomannoside moiety and not the polypeptide chain of RNAase B.

Electrophoretic analysis of material isolated by affinity chromatography on RNAase B-Sepharose

The material binding to RNAase B-Sepharose 4B was found to contain two protein components when analysed by electrophoresis in a gradient of polyacrylamide. The majority of the material had a M_r of 205 000–225 000, but there was a small amount with

Fig. 4. Electrophoresis under denaturing conditions of mannose-binding protein isolated from human liver by affinity chromatography on mannose-Sepharose 6B Samples of the binding protein (lanes 1 and 3) and a mixture of protein standards (lanes 2 and 4) were analysed by electrophoresis under denaturing conditions. The proteins were not reduced and alkylated. The gel was cut in half, and one half (a) stained with Coomassie Blue and the other (b) for glycoproteins with concanavalin A and peroxidase.

a very high apparent M_r , of greater than 1 000 000. Analysis of the material by electrophoresis in the presence of SDS showed that it consisted predominantly of a glycoprotein subunit of M_r 70 000. This component was also observed when the binding protein was reduced and alkylated before electrophoresis in the presence of SDS (Fig. 3b), but polypeptides of M_r 34 000 and 28 500 accounted for most of the material.

Isolation of a mannose-binding protein from human liver by affinity chromatography on mannose-Sepharose

An extract of human liver was prepared in the same way as for rat liver. Material that bound to the mannose-Sepharose 6B in the presence of Ca^{2+} and could be eluted from the column specifically by EDTA was obtained by two chromatographic steps as described previously for rat liver. Its binding activity for RNAase B was comparable with that of the material isolated from rat liver (Table 1). The native putative human mannose-binding protein also had a high M_r of greater than 1 000 000 and appeared to be homogenous when analysed by electrophoresis under non-denaturing conditions. Electrophoresis in the presence of SDS showed that it consisted of a glycoprotein subunit of 30 000 M_r

(Fig. 4). However, when the material was reduced and alkylated before electrophoresis it was seen to consist of two, or possibly three, different subunits, of M_r 28 000, 30 500 and 31 500, together with a small amount of material of M_r 70 000 (Fig. 3b).

Discussion

^{125}I -labelled RNAase B has been shown to be a suitable ligand for the assay of mammalian and plant mannose-binding proteins. By using concanavalin A as a standard, the binding assay was shown to be valid over a range of concentrations and to be sensitive enough to measure microgram quantities of concanavalin A. The association constant for the binding of ^{125}I -labelled RNAase B to concanavalin A was found to be approx. $1 \times 10^6 \text{ M}^{-1}$ from a Scatchard plot. This value is comparable with the range of values of K_a (4.5×10^6 – $25 \times 10^6 \text{ M}^{-1}$) determined for the binding of glycopeptides to concanavalin A under similar conditions (Baenziger & Fiete, 1979). The intact polypeptide structure may account for the slightly lower value obtained for ^{125}I -labelled RNAase B. The apparent dissociation constant for the binding of ^{125}I -labelled RNAase B to isolated rat hepatic reticuloendothelial cells at 4°C is $6 \times 10^{-7} \text{ M}$ (Maynard & Baenziger, 1981). The similarity in values of the association constants for the binding of RNAase B to concanavalin A and to a cellular mannose-binding receptor further validates the use of this binding assay for mammalian binding proteins.

Mannose-binding proteins have been isolated from extracts of rat liver by affinity chromatography on both mannose-Sepharose 6B and RNAase B-Sepharose 4B. The material purified on mannose-Sepharose 6B has a M_r under non-denaturing conditions of over 900 000. It appears to be composed of a subunit of M_r approx. 32 000–34 000, which undergoes aggregation readily, particularly to a dimer. The proportion of dimer present increased on storage and on prolonged boiling in SDS. The rat hepatocyte asialoglycoprotein receptor also associates readily to a dimer when boiled in SDS (Schwartz *et al.*, 1981). In contrast, the predominant native form of the mannose-binding protein isolated by chromatography on RNAase B-Sepharose had a M_r of 205 000–225 000, but some material with a very high M_r was also observed. It consisted largely of a subunit of M_r 70 000, but polypeptides of M_r 34 000 and 28 500 were observed after reduction. It is suggested that the native form is a trimer of the subunit of M_r 70 000, which may in turn contain two different smaller subunits. Thus the rat liver mannose-binding proteins isolated by the two ligands differ in their predominant native size and subunit composition.

Mannose-binding proteins have been isolated from rat liver by three other groups. Townsend & Stahl (1981) isolated a protein with a subunit of M_r 30000 by chromatography on mannose-Sepharose or invertase-Sepharose. It is antigenically distinct from the rat alveolar macrophage mannan-binding protein. An homogenous protein with a native M_r of 194000, consisting of six identical subunits of M_r 32000, has been isolated by affinity chromatography on mannose-Sepharose 4B by Mizuno *et al.* (1981). This protein did not give a positive reaction with the insensitive periodic acid-Schiff reaction for carbohydrate, whereas all the subunits detected by us reacted positively with the more sensitive concanavalin A-peroxidase stain for carbohydrate. More recently, three distinct mannose-binding proteins have been isolated by using the same affinity medium by specific sequential elution with mannose, mannose 6-phosphate and EDTA (Maynard & Baenziger, 1982). EDTA alone eluted a mixture of the proteins. The main component, which was a glycoprotein, was eluted specifically by mannose. It occurs predominantly in the microsomal fraction of hepatocytes and has a distinct oligosaccharide specificity from that of the receptor, which recognizes mannose and *N*-acetylglucosamine and mediates endocytosis by rat hepatic reticuloendothelial cells. It is immunologically identical with the binding protein isolated by Townsend & Stahl (1981), and both preparations are identical when analysed by the same electrophoretic system. The protein isolated by Mizuno *et al.* (1981) is probably also identical.

It is suggested that the material isolated by us by chromatography on mannose-Sepharose 6B under the same conditions as used by Townsend & Stahl (1981) is predominantly the hepatocyte binding protein. As elution was with EDTA, the heterogeneity observed with high-resolution electrophoresis would be due to the minor components observed by Maynard & Baenziger (1982). Although RNAase B binds to the hepatocyte lectin, it is endocytosed very readily by isolated rat liver reticuloendothelial cells (Maynard & Baenziger, 1981) and is removed from the circulation by non-parenchymal cells. Therefore it is tempting to speculate that the material obtained by affinity chromatography with RNAase B largely contains the reticuloendothelial-cell mannose-specific receptor. Elution by EDTA might again explain the observed heterogeneity.

The rat liver mannose-binding proteins have similar properties, e.g. Ca^{2+} -dependence and subunits of approx. M_r 30000 which form aggregates, to the mannose-binding proteins isolated from rat lymph nodes (Kawasaki *et al.*, 1980), rabbit liver (Kawasaki *et al.*, 1978) and rabbit serum (Kozutsumi *et al.*, 1980). These properties are also shared

by the human liver mannose-binding protein, which we have isolated for the first time. Its tissue and subcellular distribution, specificity and composition should be investigated to clarify its function. However, its presence does suggest that mannose-mediated endocytosis or intracellular transport of glycoproteins occurs in the human liver. This may be relevant to reports that recognition markers other than mannose 6-phosphate may be involved in the delivery of enzymes to lysosomes in human liver (Waheed *et al.*, 1982; Owada & Neufeld, 1982).

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