

Isolation and Characterization of a Mannan-binding Protein from Rat Liver*

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A binding protein from liver which binds reversibly to yeast mannan depending on the presence or absence of calcium has been purified to near homogeneity by affinity chromatography. The binding of the isolated protein to ^{125}I -mannan is proportional to protein concentration and is apparently an unsaturable process. The Scatchard plot of the binding is a curvilinear, indicating the presence of a high affinity binding site with a dissociation constant of 1.62×10^{-8} M. Evidence is presented to show that the protein recognizes and binds mannose and *N*-acetylglucosamine residues almost indiscriminately at the same binding site. Physical and chemical studies suggest the intact binding protein with an approximate molecular weight of 194,000 to be composed of six identical subunits. The protein is characterized as a glycine-rich protein. The apparent ubiquitous distribution of mannan-binding protein in mammalian liver is consistent with the proposal that the binding protein is the cellular receptor mediating the hepatic uptake of glycoproteins terminated with mannose and/or *N*-acetylglucosamine residues.

MATERIALS AND METHODS

Saccharomyces cerevisiae mannan was prepared according to the procedure of Lee and Ballou (25) from a commercially available baker's yeast purchased from the Oriental Yeast Co., Tokyo, Japan, and was further purified by gel filtration on Sephadex G-100. The purified material comprised approximately 3% of protein and 140 nmol of organic phosphate/mg. Mannan free of phosphate isolated from *S. cerevisiae* (*mnn4*) was kindly supplied by Dr. G. Ashwell, National Institutes of Health, MD, to whom it was provided by Dr. C. E. Ballou, University of California, Berkeley, CA. Neoglycoproteins, prepared by the method of Lee *et al.* (26), were generously provided by Dr. Y. C. Lee, Johns Hopkins University, Baltimore, MD. *N*-Acetylglucosaminyl-bovine serum albumin and mannosyl-bovine serum albumin contained 32 mol and 38 mol, respectively, of the sugars per mol of bovine serum albumin. Ceruloplasmin (human) was supplied from Karolinska Institutet, Stockholm, Sweden, through the courtesy of Dr. B. Blombäck. Immunoglobulin (IgG) was isolated from rabbit serum by ammonium sulfate precipitation and subsequent DEAE-cellulose chromatography (4). Other proteins used in this study were obtained from Boehringer Mannheim, Miles Laboratories, Pharmacia, Sigma, and Worthington. Sugars and sugar phosphates were obtained from Nakarai Chemicals Ltd., Kyoto, Japan, and Sigma. Sepharose 4B, Sepharose CL-6B, Sephadex G-100, and Sephadex G-25 were purchased from Pharmacia.

Iodination of Glycoproteins— Na^{125}I , carrier free, was obtained from Amersham. Between 100 and 200 μg of mannan or neoglycoprotein was iodinated with 1 to 2 mCi of Na^{125}I by a modification of the procedure of Greenwood *et al.* (27). The iodinated glycoproteins, purified by passage through a column of Sephadex G-25, were recovered with specific activities ranging from 1 to 4 $\mu\text{Ci}/\mu\text{g}$. More than 95% of the radioactivity in the freshly labeled mannan was precipitated by antimannan antibodies specific for the $\text{Man}\alpha 1\text{-}3\text{Man}$ structure; precipitation was completely blocked by the addition of a mannan oligosaccharide, $\text{Man}\alpha 1\text{-}3\text{Man}\alpha 1\text{-}2\text{Man}\alpha 1\text{-}2\text{Man}$ (28), providing reasonable grounds for utilization of the ^{125}I -labeled material as a tracer of mannan. However, the label was stable only for a few weeks. To circumvent this problem, mannan was freshly labeled or labeled mannan was repurified before the experiments by affinity chromatography on a column of Sepharose 4B-antimannan antibodies (28).

Preparation of Sepharose 4B-Mannan—Sephadex 4B-mannan was prepared as described previously (22).

Binding Assay—The binding assay was carried out as described before (22) except that 0.1 to 1.5 μg of purified binding protein or equivalent amount of crude extract was incubated with 300 ng of ^{125}I -mannan or 100 ng of ^{125}I -neoglycoprotein. Blanks were prepared by incubation of labeled ligand without added binding protein or were determined by the addition of a 100-fold excess of unlabeled ligand. The value so obtained, less than 1% of the total radioactivity added for mannan and 4 to 5% for neoglycoproteins, was subtracted from the value obtained for each sample. A unit of activity was operationally defined as 1 ng of labeled glycoprotein bound under the conditions described above. Specific binding activity is expressed as units per μg of protein.

Isolation of Binding Protein—Frozen and thawed livers of male Wistar rats (100 g) were blended in a Waring Blendor with 400 ml of cold acetone for 1 min. The suspension was filtered under reduced pressure on Whatman No. 1 paper. The cake was allowed to air dry and to stand overnight at 4 °C. The acetone powder was suspended in 10 volumes of the extracting buffer, consisting of 0.02 M imidazole/HCl at pH 7.8, 0.4 M KCl, 0.5 mM EDTA, and 2% Triton X-100. After

Carbohydrate recognition systems for receptor-mediated endocytosis have been receiving considerable attention (1, 2). A binding protein associated with the rapid clearance of galactose-terminated glycoproteins from the circulation has been isolated from the liver and characterized intensively (3-11). While the presence of a second route of clearance in mammals for mannose- and/or *N*-acetylglucosamine-terminated glycoproteins has been suggested (12-21), no receptor associated with this process was identified until we described the isolation of a protein from rabbits which bound to yeast mannan (22, 23). The protein, designated as a mannan-binding protein, was thought from the specificity of binding to be the receptor which mediates hepatic uptake of glycoproteins with mannose and/or *N*-acetylglucosamine terminals. The present study was undertaken in an attempt to isolate and characterize a mannan-binding protein in rats, in which species most of the clearance studies on glycoproteins in the circulation have been carried out. Utilizing a modified procedure, we have succeeded in the purification to near homogeneity of a binding protein with dual specificity for mannose and *N*-acetylglucosamine from rat liver. Preliminary report of this work has been presented (24).

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stirring for 30 min at 4 °C, the crude extract was obtained by centrifugation at $12,000 \times g$ for 15 min. The extraction was repeated two more times with the same buffer. To the combined crude extracts, an aqueous suspension of the affinity gel (Sephacrose 4B-mannan, settled volume 100 ml) was added. The suspension was made to 0.02 M CaCl_2 by adding 1 M CaCl_2 . After stirring for 60 min at 4 °C, the affinity gel was separated by centrifugation at $200 \times g$ for 10 min, washed several times with the loading buffer, consisting of 0.02 M imidazole/HCl at pH 7.8, 1.25 M NaCl, 0.05 M CaCl_2 , and 0.5% Triton X-100, and poured into a column. Elution of the binding protein was accomplished with an eluting buffer consisting of 0.02 M imidazole/HCl at pH 7.8, 1.25 M NaCl, 0.002 M EDTA, and 0.5% Triton X-100. The major portion of the binding activity was recovered in the second and third bed volume of the eluting buffer. Upon addition of CaCl_2 to 0.02 M this material was adsorbed onto a second smaller affinity column containing about 10 ml of gel. The column was washed with the loading buffer and the binding protein was eluted with the eluting buffer. The affinity chromatography on a small column was repeated once more. The binding protein recovered in the eluting buffer could be stored at 4 °C over 3 months without loss of binding activity. Removal of Triton X-100 from the binding protein was accomplished by cold ethanol precipitation as described previously (4).

Polyacrylamide Gel Electrophoresis—Polyacrylamide gel electrophoresis was performed using System A (pH 9.45) as described by Rodbard and Chrambach (29). Ferguson plots (30) were obtained from gels of seven different total acrylamide concentrations ranging from 3.5 to 6.5%, but with a constant ratio of methylenebisacrylamide to acrylamide (3%). The following proteins were used as the reference standards (molecular weight given in parentheses): bovine serum albumin monomer (67,000), transferrin (90,000), bovine serum albumin dimer (134,000), ceruloplasmin (151,000), aldolase (160,000), catalase (232,000), and ferritin (443,000). Mobilities were determined relative to bromphenol blue.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed using a phosphate buffer system as described by Weber and Osborn (31). The following proteins were used as the reference standards (molecular weight given in parentheses): cytochrome *c* (12,400), lysozyme (14,500), IgG light chain (23,000), chymotrypsinogen (25,100), pepsin (35,000), aldolase (40,000), ovalbumin (43,500), IgG heavy chain (50,000), and catalase (57,000). The mobilities were determined relative to Pyronine Y.

Protein bands were stained with Coomassie brilliant blue G-250 in 10% trichloroacetic acid and staining for carbohydrate was performed by the periodic acid-Schiff method of Zacharius *et al.* (32).

Protein Determination—Protein was determined by a microversion of the method of Lowry *et al.* (33) with minor modification to remove the interference by Triton X-100 (3); bovine serum albumin was the standard.

Phosphate Determination—The phosphate content of mannan was determined on 200 μg of ashed sample by the method of Chen *et al.* (34) according to the procedure of Ames and Dubin (35).

Amino Acid Analysis—Amino acid analysis was performed according to the method of Moore and Stein (36). Amino acids and hexosamines were determined with a Hitachi 835 amino acid analyzer after hydrolysis in 6 M HCl at 110 °C for 24 h. Tryptophan was determined according to the method of Matsubara and Sasaki (37).

RESULTS

Isolation of the Binding Protein—The results of a typical preparation of the binding protein from rat liver are summarized in Table I. Approximately 80% of the binding activity detectable in the acetone powder was recovered in the Triton X-100 extract. The unduly high yield of binding activity in the first affinity chromatography may be explained by the presence, in the column effluent, of a competitive inhibitor of ^{125}I -mannan binding to the purified binding protein, the K_i of which was 3.5 mg of protein/ml as estimated by a Lineweaver-Burk plot (38). Further characterization of the inhibitor(s) was not pursued.

Gel Filtration on Sepharose CL-6B—Gel filtration of the isolated binding protein resulted in the appearance of a single symmetrical major peak, in which approximately 90% of the protein and essentially all of the binding activity applied were recovered (Fig. 1). The specific activity was practically the same throughout the peak, wherein all values fell within the

TABLE I

Purification of binding protein

Approximately 100 g of frozen rat liver were used for the preparation of the Triton X-100 extract.

Fraction	Total protein	Total activity	Specific activity ^a	Yield	Purification
	mg	units ^b $\times 10^{-3}$		%	
Triton X-100 extract	10,800	32.8	0.003	100	1
Eluate, affinity column 1	99.2	50.8	5.0	154	1,660
Eluate, affinity column 2	1.08	41.3	38.3	126	12,800
Eluate, affinity column 3	0.32	21.8	68.2	66	22,700

^a Units per μg of protein.

^b A unit is defined as a nanogram of ^{125}I -mannan bound in the standard assay.

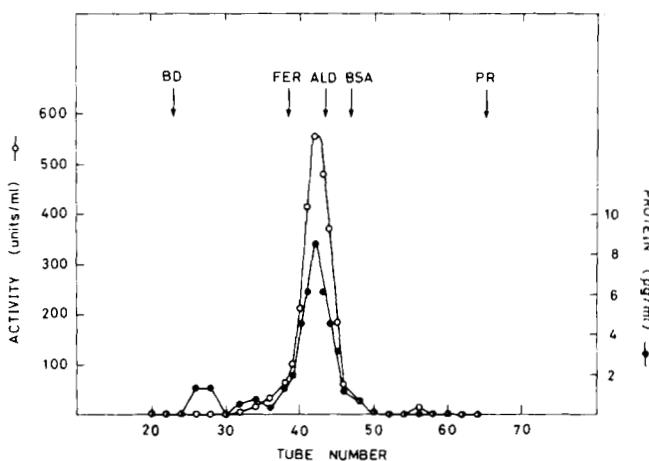


FIG. 1. Sepharose CL-6B chromatography of the purified binding protein in the presence of Triton X-100. Purified binding protein (70 μg) was loaded onto a column of Sepharose CL-6B (0.9 \times 100 cm) which had been equilibrated with the eluting buffer, consisting of 20 mM imidazole-HCl, pH 7.8, 0.5 M NaCl, 0.1% Triton X-100, and 1.0 mM EDTA. Fractions of 1.26 ml were collected at a flow rate of 4 ml/h and aliquots of each fraction were analyzed for binding activity and protein. BD, blue dextran (void volume); FER, ferritin; ALD, aldolase; BSA, bovine serum albumin; PR, phenol red.

range of 48 to 82 units/ μg of protein. The elution position of the binding protein corresponded to that of a globular protein of 200,000 daltons.

Polyacrylamide Gel Electrophoresis—Upon polyacrylamide gel electrophoresis in a conventional system at pH 9.45, the binding protein migrated as a single band as shown in Fig. 2A. The location of the binding activity, measured after extraction of gel segments, was consistent with that of the protein band (data not shown). An estimation of the molecular weight of the band was sought by means of a Ferguson plot (30) as described under "Materials and Methods." The slope of the line in the plot, the retardation coefficient (K_R), was related to the molecular weight according to the suggestion of Rodbard and Chrambach (39). Analyses of a series of standard proteins yielded a linear regression line of $(K_R)^{1/2}$ versus (molecular weight) $^{1/3}$ with a correlation coefficient of 0.974, based on which the molecular weight of the binding protein was estimated to be 194,000 (see Fig. 3).

Upon polyacrylamide gel electrophoresis in sodium dodecyl sulfate, the binding protein, treated with β -mercaptoethanol, revealed a single major component as well as a few minor components of lower mobility (Fig. 2C). The molecular weight of the major component, estimated by comparison with standard proteins, was 32,000 in either 8% or 10% acrylamide gels.

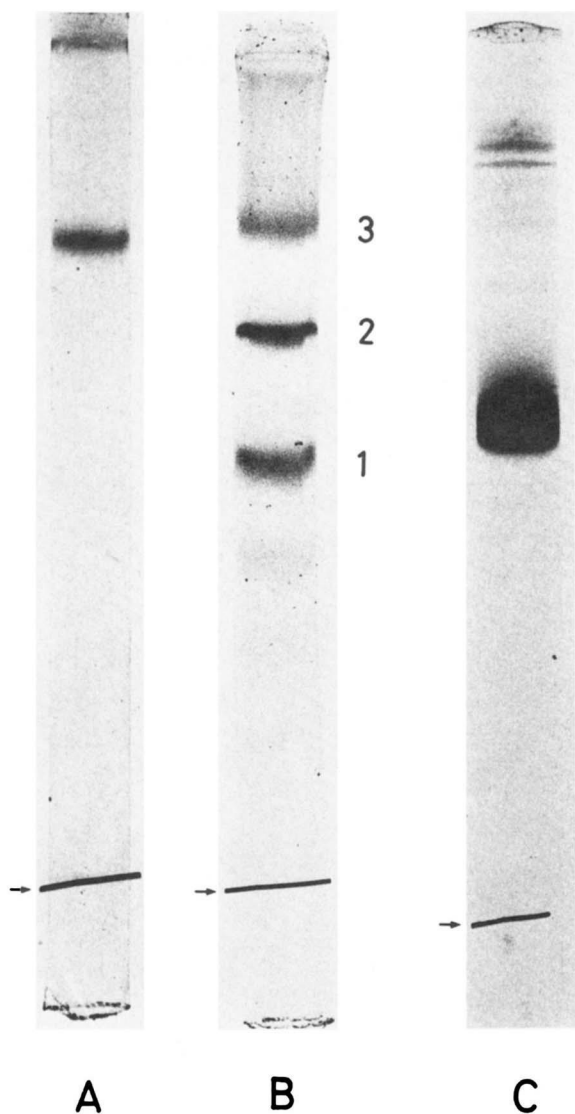


FIG. 2. Polyacrylamide gel electrophoresis of the binding protein and its subunits. *A*, the purified binding protein (8 μ g) in the eluting buffer was dialyzed against 50 mM Tris/glycine buffer, pH 9.0, and applied to a 5.5% acrylamide gel. Electrophoresis was carried out using a multiphasic buffer system "System A" (pH 9.45) for 2.5 h at 4 °C. Protein bands were stained with Coomassie brilliant blue G-250 in 10% trichloroacetic acid. *B*, a crude binding protein (7 μ g) (see "Discussion") was applied to a 5.5% acrylamide gel. Electrophoresis and staining were carried out as described in *A*. *C*, an aqueous solution of the purified binding protein (13 μ g) was made 10 mM in NaOH containing 1% sodium dodecyl sulfate. After a few minutes at room temperature, the solution was made 10% and 0.02 M with respect to β -mercaptoethanol and sodium phosphate buffer, pH 7.2, respectively. The sample was applied to a 10% acrylamide gel containing 0.1% sodium dodecyl sulfate. Electrophoresis was carried out at room temperature for 4 h according to the method of Weber and Osborn (31). The gel was soaked overnight in 7% acetic acid in 40% ethanol, prior to staining with Coomassie brilliant blue G-250 in 10% trichloroacetic acid. The arrows denote the migration of marker dyes.

Without being treated with β -mercaptoethanol, the binding protein migrated as multiple bands, the molecular size of which roughly corresponded to that of monomer, dimer, and trimer of the subunit of 32,000 daltons (data not shown). None of these bands gave a positive periodic acid-Schiff reaction for carbohydrate staining.

All of the above data may be rationalized as indicating that the intact binding protein of 194,000 daltons is composed of six identical subunits of 32,000 daltons.

Kinetics and Stoichiometry of Binding—The binding reaction was completed within 5 min at 25 °C and was essentially independent on the amount of ligand added. Dissociation also proceeded rapidly. After incubation of the binding protein with 125 I-mannan, a 200-fold excess of nonradioactive ligand was added; aliquots were removed at various times and assayed for bound radioactivity. Within 10 min, more than 95% of the original radioactivity disappeared. Fig. 4 demonstrates that binding was proportional to the amount of the binding protein added and was dependent markedly upon the amount of ligand present. A Scatchard plot (40) of the binding data suggested the presence of more than one binding site with different affinities (Fig. 5). The dissociation constant of a higher affinity site, estimated from the slope of the line, was 1.62×10^{-8} M, as the average of two separate experiments with different lots of the binding protein and 125 I-mannan. The maximum capacity of the binding with higher affinity was 254 units/ μ g of the binding protein. On the assumption that the molecular weights of the binding protein and mannan are

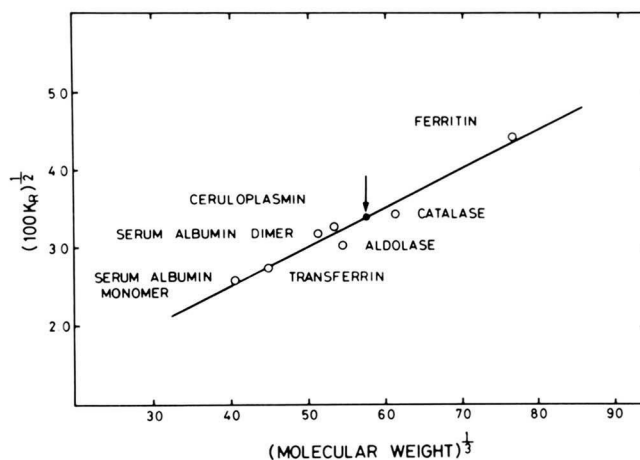


FIG. 3. Estimation of the molecular weight of the binding protein. The retardation coefficient (K_R) of each protein was calculated from Ferguson plot (not shown). The regression line was drawn by the method of least squares. The binding protein is indicated by the closed circle (●).

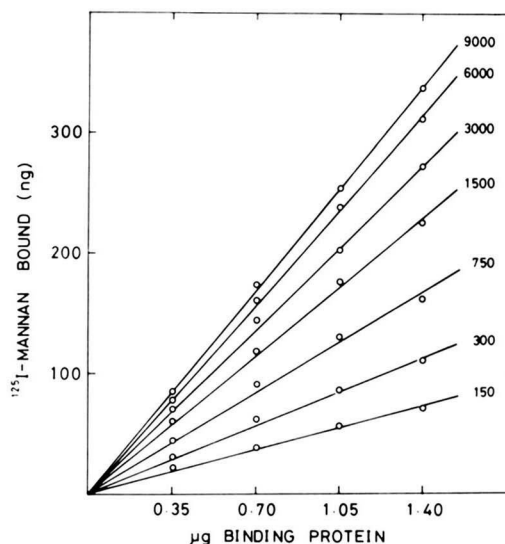


FIG. 4. Linearity of binding. Incubations were carried out in the presence of increasing amounts of 125 I-mannan. At each level, the binding protein was varied from 0.35 to 1.40 μ g/incubation. The numbers next to the curves indicate the nanograms of 125 I-mannan added.

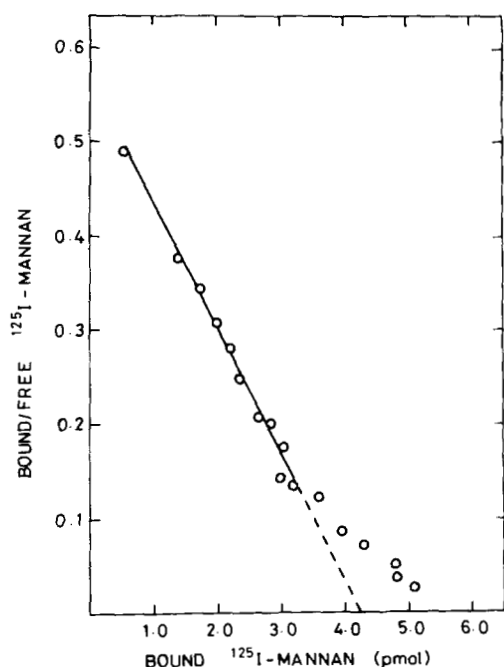


FIG. 5. Scatchard plot of the binding. The purified binding protein (0.7 μ g) was incubated with various amounts of 125 I-mannan from 150 to 9000 ng. The regression line was drawn by the method of least squares.

194,000 and 40,000 (41), respectively, it can be estimated that 1.3 mol of mannan were bound/mol of the binding protein.

Specificity of Binding—The relative affinities of the binding protein for a number of sugars were estimated from measurements of the ability of each substance to inhibit the binding of 125 I-mannan. As shown in Table II, *N*-acetylmannosamine was the most effective inhibitor tested, and mannose and *N*-acetylglucosamine were next. On the other hand, mannosamine, glucosamine, galactose, and *N*-acetylgalactosamine are very weak inhibitors. These results imply a dual specificity of the binding protein for mannose and *N*-acetylglucosamine. However, because of the branched structure of mannan, it was conceivable that substituted sugars generated artificial determinants that led us to an erroneous interpretation of the inhibition data. To meet this problem, neoglycoproteins were tested as ligands. These neoglycoproteins bound to the binding protein approximately one-half as much by weight as 125 I-mannan under the standard assay condition. The profiles of the inhibition were almost indistinguishable from each other whichever neoglycoprotein was used and were much simpler than but basically the same as with 125 I-mannan. More convincing evidence for the identification of a single binding site common to mannose and *N*-acetylglucosamine was provided by competition experiments between mannosyl-bovine serum albumin and *N*-acetylglucosaminyl-bovine serum albumin, as shown in Fig. 6. These two ligands gave rise to almost identical binding curves when assayed individually. However, in the presence of unlabeled mannosyl-bovine serum albumin practically sufficient to saturate the binding protein (6 μ g), the binding of 125 I-*N*-acetylglucosaminyl-bovine serum albumin to the binding protein was reduced close to the hypothetical levels expected as the consequence of the competition at the same binding site between two ligands with equivalent affinity.

Mannose 6-phosphate, which has been shown to be a recognition marker on lysosomal enzymes for uptake into human fibroblasts, was inactive at a concentration of 2 mM, where pinocytosis into human fibroblasts was inhibited almost completely (42, 43). At a concentration of 10 mM, mannose 6-

phosphate inhibited the binding significantly but less than one-third as with mannose. Additional evidence against the direct involvement of phosphate in the mannan binding was the comparable inhibitory potency of a mutant of mannan lacking phosphorylated mannose residues (*mnn4*). Thus, the amount of mannan required to give 50% inhibition under the standard assay condition was 375 ng and 330 ng for wild type and *mnn4* mannans, respectively.

Stability of the Ligand-binding Protein Complex—After

TABLE II

Specificity of binding as measured by inhibition assay

The assay was carried out as described under "Materials and Methods" in the presence of 0.5 to 1.0 μ g of the purified binding protein and the inhibitors listed in the table. Values are the mean of the two or three experiments, in each of which duplicate determinations were done. Control binding was measured under the same conditions in the absence of added inhibitors. Sugars are of the *D* configuration unless otherwise stated.

Compound	Inhibition of binding (%)		
	125 I-Mannan	125 I-GlcNAc-BSA ^a	125 I-Man-BSA ^b
30 mM			
<i>N</i> -Acetylmannosamine	87	81	80
<i>N</i> -Acetylglucosamine	56	53	44
Mannose	61	44	52
L-Fucose	47	17	11
2-Deoxyglucose	36	2	1
Glucose	38	0	3
Glucosamine	24	0	0
Mannosamine	28	0	0
Galactose	20	0	0
Lactose	21	6	0
Galactosamine	2	5	0
<i>N</i> -Acetylgalactosamine	2	0	2
2 mM			
Mannose 6-phosphate	0	0	0
Glucose 6-phosphate	0	0	0
12 mM			
<i>N</i> -Acetylneuraminic acid	1	0	0

^a 125 I-*N*-acetylglucosaminyl-bovine serum albumin.

^b 125 I-mannosyl-bovine serum albumin.

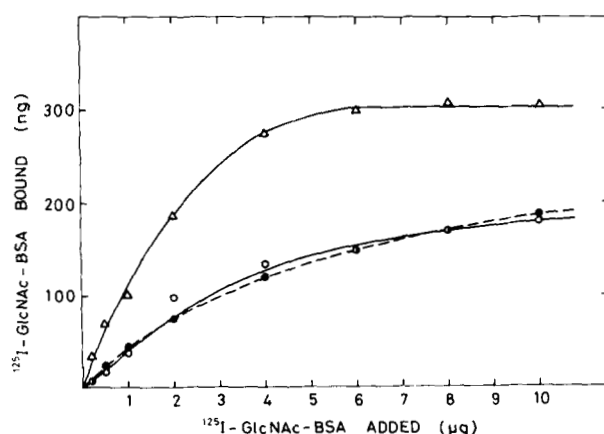


FIG. 6. Competitive binding between *N*-acetylglucosaminyl-bovine serum albumin and mannosyl-bovine serum albumin. The assay was carried out as described under "Materials and Methods." The binding protein (0.9 μ g) was incubated with increasing amounts of 125 I-labeled *N*-acetylglucosaminyl-bovine serum albumin (125 I-GlcNAc-BSA) in the presence (○) or the absence (Δ) of 6 μ g of unlabeled mannosyl-bovine serum albumin. The hypothetical binding curve calculated based on the assumption that these two neoglycoproteins compete for the identical binding site with equivalent affinity is shown for comparison (●).

incubation for 30 min under alkaline conditions up to pH 10, no loss of stability of the binding complex was observed as shown in Fig. 7. At acidic pH, on the other hand, the complex became progressively more unstable and at pH 5, dissociation

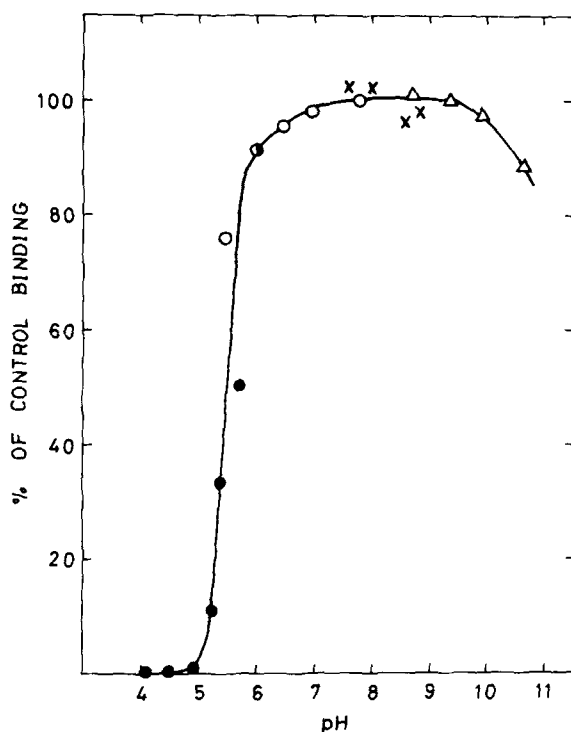


FIG. 7. Effect of pH on the stability of the complex. The reaction mixture, containing 0.7 μ g of the purified binding protein and 300 ng of 125 I-mannan/0.3 ml of solution, was held for 15 min at 25 °C as described under "Materials and Methods." Then the pH of the mixture was adjusted to the values shown on the curve by the addition of 0.2 ml of 0.5 M buffer solution, and the incubation continued for an additional 30 min prior to precipitation and filtration. ●, Sodium acetate; ○, 2-(N-morpholino)ethanesulfonic acid; ×, Tris-HCl; △, glycine/NaOH.

TABLE III

Amino acid composition of the binding protein

Binding protein hydrolysis and amino acid analysis were performed as described under "Materials and Methods."

Amino acid	Residues/100 residues	Residues/mol ^a
Lysine	5.0	15
Histidine	1.8	5
Arginine	5.5	16
Aspartic acid	11.6	34
Threonine	5.7	17
Serine	8.1	24
Glutamic acid	11.2	33
Proline	3.7	11
Glycine	11.7	35
Alanine	6.8	20
Half-cystine	3.6	11
Valine	6.5	19
Methionine	1.2	3
Isoleucine	2.8	8
Leucine	7.9	24
Tyrosine	2.2	7
Phenylalanine	3.6	11
Tryptophan	1.1	3
Total	100	296

$M_r = 31,900$

^a The number of amino acid residues per mol of subunit was based on an estimated molecular weight of the subunit of 32,000.

was complete. At pH 7.8, the reaction product was stable for at least 1 h at 25 °C.

Effects of Cations—Calcium is required for binding; in its absence with 2 mM EDTA added, no binding was observed. The K_m for calcium was calculated to be 0.2 mM as determined by a Lineweaver-Burk plot (38). Upon comparison at a concentration of 2 mM, the substitution of calcium by strontium resulted in 20% of the control value, whereas other metals, zinc, cobalt, copper, manganese, magnesium, and barium, were all inert.

Amino Acid Composition—The amino acid composition of the mannan-binding protein is shown in Table III. A high content of glycine, which is in fact the most predominant amino acid, is a characteristic of this binding protein. Although a small amount of glucosamine (0.9 mol/mol of subunit) was detected on analysis, the significance of this finding is currently unclear.

DISCUSSION

Demonstrating a new route of clearance of glycoproteins from rat plasma, a binding protein specific for mannose and/or *N*-acetylglucosamine residues has been isolated from rat liver. This protein is analogous to the binding protein specific for asialoglycoproteins, which mediates the original route of clearance, in its predominant localization in the liver and requirement of calcium for binding. However, these two proteins are clearly distinguishable from each other in their specific properties. Besides binding specificity toward sugars, differences were extended to antigenicity,¹ to size of the subunits (32,000 versus 47,000 daltons), to reactivity to periodic acid-Schiff stain, and to yield of the proteins (9). The yield of mannan-binding protein shown in Table I is approximately one-fifth of the value reported for asialoglycoprotein-binding protein (9). However, taking into account the lower recovery of the latter binding protein, the quantities of the respective binding proteins in rat liver are even more disparate.

Despite its simplicity, the isolation procedure deserves comments. Unless the procedure described under "Materials and Methods" was followed strictly, three protein bands rather than one are obtained in the conventional polyacrylamide gel electrophoresis (see Fig. 2B). The additional two bands (band 1 and band 2) migrating ahead of the binding protein (band 3) had no binding activity. Washing of the acetone powder with salt solutions containing EDTA prior to extraction with Triton X-100 as described previously (22) proved harmful and standing the acetone powder overnight in a cold room appeared necessary.

The mannan-binding protein isolated in this study shares many properties with rabbit liver mannan-binding protein (22), such as reversible binding to yeast mannan in response to the presence or the absence of calcium, binding specificity for mannose and *N*-acetylglucosamine residues, and subunit structure consisting of a single component with similar molecular weight. Further resemblance of these two proteins with respect to the following criteria has been established by our recent studies on rabbit liver mannan-binding protein: reversibility and apparent unsaturability of the binding to mannan, curvilinear Scatchard plots of the binding to mannan, elution position of binding proteins on gel filtration in the presence of Triton X-100 and EDTA, dependence of the stability of the binding complex upon pH, and glycine-rich amino acid composition.²

The study presented here supports the idea that hepatic

¹ Y. Mizuno, Y. Kozutsumi, T. Kawasaki, and I. Yamashina, unpublished experiments.

² Y. Kozutsumi, T. Kawasaki, and I. Yamashina, manuscript in preparation.

mannan-binding protein is a unique mammalian lectin, which recognizes and binds mannose and *N*-acetylglucosamine residues, and is a reasonable candidate for the cellular receptor mediating the hepatic uptake of glycoproteins terminated with these sugars.

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