Purification and characterization of the core-specific lectin from human serum and liver

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A lectin that displays specificity for the core region of asparagine-linked oligosaccharides (Man₃GlcNAc₂-Asn) was isolated from human serum and liver by affinity chromatography on mannan–Sephadex. The designation ‘core-specific lectin’ (CSL) is used to indicate its specificity. Selective elution of human CSL from mannan–Sephadex was accomplished with 50 mM-mannose. Two additional proteins that displayed Ca²⁺-dependent binding to mannan–Sephadex were eluted by mannose 6-phosphate or β -glycerophosphate but not by mannose. The latter proteins were identified as C-reactive protein and serum amyloid protein. Human CSL isolated from liver was indistinguishable from serum CSL in its physicochemical properties, immunological properties and specificity. The N-terminal sequence of human CSL is homologous to that reported for ‘mannan-binding protein C’ (MBP-C) [Drickamer, Dordal & Reynolds (1986) *J. Biol. Chem.* 261, 6878–6887]. The amino acid composition of human CSL is similar to that of rat MBP-C, including the presence of hydroxyproline and hydroxylysine residues. Collagen-like sequences with hydroxylated proline and lysine residues appear to be present in human CSL as well as in rat CSL. The collagen-like regions of human and rat CSL may play a role in assembly of CSL subunits into complexes consisting of nine subunits that display Ca²⁺-dependent carbohydrate-binding activity.

INTRODUCTION

Soluble lectins or carbohydrate-binding proteins are widespread in mammals. Their physiological function, however, is not known. One such lectin, the mannan-binding protein (MBP), was initially purified from Triton X-100 extracts of rabbit (Kawasaki *et al.*, 1978) and rat liver (Mizuno *et al.*, 1981; Townsend & Stahl, 1981). Mannan, N-acetylglucosaminylated bovine serum albumin (GlcNAc-BSA), fucosylated bovine serum albumin (Fuc-BSA) and mannosylated bovine serum albumin (Man-BSA) are bound by purified MBP and their binding is inhibited by monosaccharides such as mannose, fucose and N-acetylglucosamine (Mizuno *et al.*, 1981; Townsend & Stahl, 1981; Mori *et al.*, 1983). Although MBP was initially thought to be the macrophage receptor for structures containing mannose, fucose or N-acetylglucosamine, it was later established that MBP is a soluble protein synthesized by hepatocytes (Maynard & Baenziger, 1982; Mori *et al.*, 1983; Brownell *et al.*, 1984) and found in plasma (Kozutsumi *et al.*, 1980, 1981; Kawasaki *et al.*, 1983, 1985). MBP displays specificity for the ‘core’ region of asparagine-linked oligosaccharides (i.e. Man₃GlcNAc₂-Asn) rather than terminal monosaccharides, and we refer to it as the ‘core-specific’ lectin (CSL) to indicate this specificity (Maynard & Baenziger, 1982).

Drickamer *et al.* (1986) have described two distinct forms of rat MBP, MBP-A and MBP-C, which differ in amino acid sequence. A notable feature of MBP-A and MBP-C is the presence of a collagen-like domain with multiple repeats of the triplet Gly-Xaa-Yaa. We

have examined the synthesis, assembly and secretion of rat CSL [MBP-C of Drickamer *et al.* (1986)] using a cultured rat hepatoma cell line (Brownell *et al.*, 1984; Colley & Baenziger, 1987a,b,c). The kinetics of secretion are unusual, since rat CSL is released from the cell with a *t*_{1/2} of greater than 4 h (Brownell *et al.*, 1984; Colley & Baenziger, 1987a). Assembly of CSL subunits into a complex consisting of nine subunits occurs following arrival in the Golgi apparatus and is required for CSL to display binding activity for mannan (Colley & Baenziger, 1987a,c). Rat CSL contains hydroxylysine, hydroxyproline and glycosylated hydroxylysine (Glc-Gal-Hyl) residues (Colley & Baenziger, 1987b). Glycosylation of hydroxylysine residues occurs in the distal region of the Golgi apparatus; however, neither assembly of CSL subunits nor attainment of carbohydrate-binding activity requires this glycosylation step (Colley & Baenziger, 1987a,c). Secreted CSL also displays Ca²⁺-independent binding to hydrophobic matrices such as decyl-agarose (Colley & Baenziger, 1987c). Binding of CSL to hydrophobic matrices is dependent on hydroxylation and glycosylation of CSL, suggesting that the collagen-like domains of CSL may promote interaction with hydrophobic species but not with carbohydrates (Colley & Baenziger, 1987c). Although the functional significance of assembly and post-translational modification of rat CSL requires further definition, they significantly influence the properties of CSL.

Recently it was reported that the serum form of the rat MBP is able to activate complement through the classical pathway (Ikeda *et al.*, 1987). On the basis of the N-terminal amino acid sequence of serum MBP, it was

Abbreviations used: CSL, core-specific lectin; MBP, mannose- or mannan-binding protein; BSA, bovine serum albumin; CRP, C-reactive protein; SAP, serum amyloid protein; BBS, borate-buffered saline (0.15 M-NaCl/0.1 M-sodium borate buffer, pH 8.5); PBS, phosphate-buffered saline (0.15 M-NaCl/0.02 M-sodium phosphate buffer, pH 7.4); TBS, Tris-buffered saline (0.15 M-NaCl/0.02 M-Tris/HCl buffer, pH 7.5).

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determined that the serum form of rat MBP is identical with MBP-A reported by Drickamer *et al.* (1986). Since MBP and the complement component C1q both contain collagen-like domains, it was further suggested that MBP might in some fashion mimic C1q if it is bound to a cell surface.

Human serum and liver forms of MBPs have been described (Kawasaki *et al.*, 1983; Wild *et al.*, 1983; Summerfield & Taylor, 1986); however, their specificity for oligosaccharides has not been established, nor have their physical-chemical properties been compared with those of rat MBPs. Should it be possible for the human lectin to activate complement, as has been reported for the rat, its oligosaccharide-specificity is likely to determine which cells are targets for lysis. Since both assembly of subunits into a large complex and modification of hydroxylysine and hydroxyproline residues markedly affect the properties of rat CSL, it is of interest to determine if these features have been retained by the human equivalent.

MATERIALS

Sepharose CL-4B, Sephacryl S-300, yeast mannan, phosphocholine chloride, β -glycerophosphate and phosphorylated and sulphated monosaccharides were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Other monosaccharides were purchased from Pfanstiehl Laboratories, Waukegan, IL, U.S.A. Fractogel TSK HW-55(F) was obtained from EM Science, Gibbstown, NJ, U.S.A. Nitrocellulose for Western transfer-immunoblot analysis was from Schleicher and Schuell, Hanover, NH, U.S.A. Immunolon II Removawell Strips for the solid-phase radioimmunoassay and binding assays were obtained from Dynatech Laboratories, U.S.A. Fucosylated BSA was purchased from E-Y Laboratories, San Mateo, CA, U.S.A. Goat anti-[rabbit IgG F(ab')₂ fragment], sheep anti-(human CRP) and goat anti-(human SAP) sera were purchased from Cappel Laboratories, Downingtown, PA, U.S.A. Na¹²⁵I (100 mCi/ml) was obtained from Amersham-Searle, Arlington Heights, IL, U.S.A. [³⁵S]Methionine (> 1000 Ci/mmol) was purchased from New England Nuclear, Boston, MA, U.S.A.

METHODS

Affinity columns

Yeast mannan was coupled to CNBr-activated Sepharose CL-4B as described previously (Maynard & Baenziger, 1982) and yielded 1 mg of mannan/ml. Phosphocholine-Sepharose was prepared as previously described (deBeer & Pepys, 1982; Pepys *et al.*, 1977).

Iodination of proteins

The purified rat liver CSL and the goat anti-[rabbit F(ab')₂ fragment] antibody were iodinated by using Iodobeads (Pierce Chemical Co., Rockford, IL, U.S.A.) in 25 mM-Tris/HCl buffer, pH 7.8, containing 0.1 M-NaCl and stored in 25 mM-Tris/HCl buffer, pH 7.8, containing 0.1 M-NaCl and 0.5% BSA after separation from free [¹²⁵I]iodide by gel filtration on Sephadex G-25.

Binding assays

¹²⁵I-labelled agalacto-orosomucoid was prepared by digestion of ¹²⁵I-labelled asialo-orosomucoid with diplo-

coccal β -galactosidase as described previously (Maynard & Baenziger, 1981). Lectin binding activity was determined as previously described for the rat CSL (Maynard & Baenziger, 1982). Samples of the partially purified lectins were incubated at 25 °C for 30 min with 400 ng of ¹²⁵I-Fuc-BSA in 50 mM-Tris/HCl buffer, pH 7.8, containing 1 M-NaCl, 40 mM-CaCl₂ and 0.25% Triton X-100 in a final volume of 500 μ l. Samples were precipitated with saturated (NH₄)₂SO₄, pH 7.8 (pH adjusted with solid Trizma base). Precipitates were filtered on Gelman type GF/C glass-fibre filters, washed with 50%-saturated (NH₄)₂SO₄, pH 7.8, containing 25 mM-CaCl₂, and the radioactivity of the filters was counted. Non-specific binding accounted for less than 5% of the input radiolabel and was corrected for in all assays.

Antiserum

Rabbit antibodies directed against the human CSL were raised by popliteal-node immunization (Newbould, 1965) of New Zealand White rabbits with complete Freund's adjuvant containing 100 μ g of human CSL. Rabbits were boosted with incomplete Freund's adjuvant containing 100 μ g of CSL 3 and 6 weeks after the primary immunization. Serum was obtained commencing at 10 days after the final immunization.

Radio/immunoassay

A solid-phase radioimmunoassay was used to detect the human CSL in serum and in partially purified fractions. CSL (0.5 μ g) in BBS was bound to Immunolon II Removawell Strips overnight at 4 °C. After three washes with PBS containing 0.05% Triton X-100, the wells were incubated with PBS containing 0.05% Triton X-100 and 10 mg of BSA/ml for 2 h at 25 °C. For the inhibition assay, fractions containing various amounts of the human CSL were incubated with rabbit anti-(human CSL) serum for 30 min at 25 °C (300 μ l total volume). Then 200 μ l of this mixture was incubated in the wells for 90 min at 37 °C. After the wells had been washed, bound antibody was detected by incubation with ¹²⁵I-labelled goat anti-[rabbit IgG F(ab')₂ fragment] antibody (200 000 c.p.m./well) for 90 min at 37 °C. Wells were washed and the radioactivity was counted. Standard inhibition curves were constructed and used to quantify CSL in serum and partially purified fractions.

Western transfer-immunoblot analysis

Proteins were transferred from polyacrylamide gels to nitrocellulose by means of a Hoefer Scientific Instruments Transphor apparatus according to the method of Burnette (1981). Transfers were performed for 8–20 h at 10 °C. ¹⁴C-labelled protein standards were electrophoresed and transferred to the nitrocellulose with the samples. After electrophoretic transfer, the nitrocellulose was first incubated with blocking buffer (10 mM-Tris/HCl buffer, pH 7.8, containing 150 mM-NaCl, 2 mM-CaCl₂, 5% BSA and 0.2% Nonidet P-40) and then washed. It was then incubated with a 1:250 dilution of rabbit antiserum in blocking buffer and washed. Finally, the nitrocellulose was incubated with ¹²⁵I-labelled goat anti-[rabbit IgG F(ab')₂ fragment] antibody in blocking buffer (2 $\times$ 10⁷/100 ml) and washed. All incubations were performed at 25 °C for 1 h with agitation and were followed by two 20 min washes with 10 mM-Tris/HCl buffer, pH 7.8, containing 150 mM-NaCl, 2 mM-CaCl₂,

0.5% BSA, 0.2% Nonidet P-40 and 0.1% SDS. The nitrocellulose was then air-dried and exposed to Kodak XAR film for 24–48 h at -70°C .

Determinations of native M_r

Gel filtration of rat serum, cell media or purified proteins on columns of Sephacryl S-300 was used to determine the native M_r values of the CSL and CRP. A 2 cm $\times$ 75 cm column of Sephacryl S-300 equilibrated in 20 mM-Tris/HCl buffer, pH 7.8, containing 0.1 M-NaCl and 2 mM-EDTA was calibrated with the marker proteins ferritin (M_r 440 000), BSA (M_r 66 200) and agalactoorosomucoid (M_r 40 000). Blue Dextran was used to determine the void volume of the column (V_0), and glucose was used to determine the included volume of the column (V_s).

During gel filtration, fractions (1 ml) were collected and either directly counted for radioactivity (^{125}I), or immunoprecipitated from labelled cell media, or submitted to Western transfer-immunoblot analysis (rat serum) to detect the purified CSL or the lectin in rat serum or the media of cells.

RESULTS

Purification of the human serum CSL

A lectin that displays specificity for the 'core' region of certain asparagine-linked oligosaccharides was isolated from human serum by affinity chromatography on mannan-Sepharose. ^{125}I -Fuc-BSA was used to detect binding by the human serum CSL by using the $(\text{NH}_4)_2\text{SO}_4$ precipitation assay described previously (Maynard & Baenziger, 1982). Human serum (500–1000 ml) was adjusted to a final concentration of 25 mM- CaCl_2 and applied batchwise to a phosphocholine-Sepharose column (50 ml). Human serum CSL was not retained by phosphocholine-Sepharose. Material not bound by phosphocholine-Sepharose was applied batchwise to a column (100 ml) of mannan-Sepharose, which was extensively washed with TBS containing 25 mM- CaCl_2 on a sintered-glass funnel before being poured into a column. Material bound to the mannan-Sepharose was eluted with TBS containing 2 mM-EDTA. The eluate was re-adjusted to a final concentration of 25 mM- CaCl_2 and applied to a smaller mannan-Sepharose column (3–5 ml). After extensive washing with TBS containing 25 mM- CaCl_2 , human CSL was eluted with TBS containing 50 mM-mannose. Material still bound to the mannan-Sepharose after elution with mannose was eluted with TBS containing 2 mM-EDTA.

It was not possible to detect binding activity for Fuc-BSA or mannan in unfractionated serum. The purified lectin was used to raise a monospecific antiserum, and a solid-phase radioimmunoassay was established for quantification. On the basis of this radioimmunoassay it was estimated that 0.4–1.0 μg of CSL is present per ml of serum and that less than 5% remained in the serum after exposure to the initial mannan-Sepharose column. The yield of CSL by the radioimmunoassay was 25–30% and represented a 70 000-fold purification. Binding activity for ^{125}I -Fuc-BSA could first be detected after elution from the initial mannan-Sepharose column. The yield of lectin binding activity was considerably greater from the second mannan-Sepharose column (90%) than the yield of antigenic material (38%), resulting in a 2.4-fold increase in specific activity with respect to antigenic

Fig. 1. Purification and characterization of the human CSL

Human serum CSL purified as described in the text was analysed by SDS/polyacrylamide-gel electrophoresis and Coomassie Blue staining (a) and by Western transfer immunoblotting with antisera against human CSL (b), human CRP (c) and human SAP (d). Bound antibodies were detected by autoradiography following incubation with ^{125}I -labelled affinity-purified goat anti-[rabbit IgG F(ab')₂ fragment] antibody. Lane 1, human serum CSL eluted from mannan-Sepharose with 50 mM-mannose; lane 2, proteins eluted from phosphocholine-Sepharose with 2 mM-EDTA; lane 3, proteins eluted from mannan-Sepharose with 2 mM-EDTA after elution of CSL with mannose. Arrowheads indicate the migration position of a 30 000- M_r standard.

activity. The increase in specific activity with respect to total protein was 15.5-fold at this step. A 1 μg portion of purified CSL was able to bind 5.8 pmol of Fuc-BSA.

Selective elution of human CSL from mannan-Sepharose with mannose yielded a single protein of M_r 33 000 when examined by SDS polyacrylamide-gel electrophoresis (Fig. 1a, lane 1). In some instances (see Fig. 3) CSL was not quantitatively converted into a monomeric species during SDS/polyacrylamide gel electrophoresis, resulting in the appearance of small amounts of dimeric material of M_r 67 000. If serum was not first applied to the phosphocholine-Sepharose column and/or if CSL was eluted from mannan-Sepharose with EDTA rather than mannose, the preparations were more heterogeneous. Western transfer immunoblots were used to assess the purity of the protein fractions from phosphocholine-Sepharose and mannan-Sepharose. Human serum CSL was not bound by phospho-

Fig. 2. Comparison of human CSL isolated from serum and liver

Human CSL was purified from serum (lane 1) and liver (lanes 2 and 3) by mannan-Sepharose affinity chromatography as described in the text. Purified CSL was analysed by SDS/polyacrylamide-gel electrophoresis and silver staining (a) and Western transfer immunoblotting (b) with a monospecific rabbit antiserum raised against human serum CSL. Two separate preparations of liver CSL were examined (lanes 2 and 3). Arrowheads indicate the migration positions for standards of M_r 30000 and 43000.

choline-Sepharose (Fig. 1b, lane 2), and relatively small amounts of CSL remained bound to mannan-Sepharose after elution with 50 mM-mannose (Fig. 1b, lane 3). The protein species bound to phosphocholine-Sepharose and eluted by Ca^{2+} chelation had a M_r of 30000 and was identified as C-reactive protein (CRP) by the use of commercial antiserum (Figs. 1a and 1c, lanes 2). The protein species bound by mannan-Sepharose and released with 2 mM-EDTA after the elution of CSL with mannose also had an M_r of 30000 by SDS/polyacrylamide-gel electrophoresis and was identified as serum amyloid protein (SAP) by the use of commercial antiserum (Figs. 1a and 1d, lanes 3). Although no CRP was found in the EDTA eluate from the mannan-Sepharose shown in Fig. 1, CRP was found in the EDTA eluate from the mannan-Sepharose when the initial application of serum to a phosphocholine-Sepharose column was omitted (results not shown). We have found that both human CRP and rat CRP (K. J. Colley & J. U. Baenziger, unpublished work) are bound by mannan-Sepharose and can be eluted from this affinity column with TBS containing 2 mM-EDTA (results not shown). Neither CRP nor SAP displayed detectable binding

Fig. 3. Characterization of human serum CSL by gel filtration on Fractogel TSK HW-55 (F)

CSL that had been eluted from mannan-Sepharose with TBS containing 25 mM-mannose was applied to Fractogel TSK HW-55(F) equilibrated with PBS containing 2 mM-EDTA. Column fractions were assayed for protein (A_{280} , Δ), and the presence of human CSL by radioimmunoassay ($\bullet$), ^{125}I -Fuc-BSA binding ($\square$) and SDS/polyacrylamide-gel electrophoresis with Coomassie Blue staining (inset gel).

activity for Fuc-BSA or mannan by use of the $(\text{NH}_4)_2\text{SO}_4$ precipitation assay. Human serum CSL prepared as described accounted for the Ca^{2+} -dependent Fuc-BSA-binding activity present in serum and was free of both CRP and SAP.

Purification of CSL from human liver

The rat CSL was initially isolated from detergent extracts of liver (Mizuno *et al.*, 1981; Townsend & Stahl, 1981). Later it was recognized that this lectin is present in serum and is secreted by hepatocytes and a rat hepatoma maintained in culture (Maynard & Baenziger, 1982; Mori *et al.*, 1983; Brownell *et al.*, 1984). Human CSL can also be isolated from liver. Human liver (100 g) was homogenized with a Polytron homogenizer (P35K probe; 3 min at 4 °C) in 10 vol. (v/w) of TBS containing 0.05% Triton X-100. Non-soluble material was removed by sedimentation at 10000 g for 20 min, and the extract

was then adjusted to 25 mM- CaCl_2 and submitted to the same purification procedure as for human serum. The human CSL from both sources migrates on electrophoresis in SDS/polyacrylamide gels with an M_r of 33000 (Fig. 2a). In addition, the human liver CSL reacts upon Western transfer-immunoblot analysis with the antiserum raised against the human serum CSL (Fig. 2b). The liver and serum proteins are therefore closely related if not identical.

Physical-chemical properties of the human serum CSL

To remove minor contaminants and aggregated material, the human CSL was subjected to analysis by gel filtration on Fractogel TSK HW-55(F) in PBS containing 2 mM-EDTA (Fig. 3). The protein (A_{280}), ligand-binding activity (Fuc-BSA) and antigenic activity co-migrated and displayed constant proportions across the peak (Fig. 3). Electrophoretic separation on SDS/polyacrylamide gels followed by staining with Coomassie Blue demonstrated the presence of a peptide with an M_r of 33000 and small amounts of dimeric material migrating with an M_r of 67000. Gel filtration on a column of Sephacryl S-300 calibrated with proteins of known M_r yielded an estimated native M_r of 270000. Non-equilibrium sedimentation on a sucrose gradient calibrated with known standards yielded a native M_r of 300000 (results not shown). Thus, like rat CSL, human CSL is assembled into complexes consisting of eight or nine subunits.

The amino acid composition of human CSL is similar to that of rat CSL, which is believed to be identical with MBP-C (Drickamer *et al.*, 1986). Both lectins contain large amounts of aspartic acid/asparagine, glutamic acid/glutamine and glycine (Table 1) and the hydroxylated amino acids hydroxyproline and hydroxylysine. Human CSL therefore appears to have collagen-like domains similar to those described for rat CSL (Drickamer *et al.*, 1986). Amino sugars were not detected during amino acid analysis, and the lectin was not sensitive to digestion with *N*-glycanase, suggesting that it, like rat CSL, does not contain *N*-linked oligosaccharide.

The *N*-terminal sequence for two different preparations of human CSL was determined and yielded a single sequence, which is shown in Fig. 4. No identifiable product was obtained at position 5, as indicated by the Xaa. The *N*-terminal sequence of human CSL is closely related to the sequence determined for rat MBP-C (Drickamer *et al.*, 1986). Introduction of a gap of one residue in the rat protein at the position of the unidentified residue (Xaa) in human CSL results in six identities among the ten positions identified. Four of the ten residues present between positions 19 and 28 of MBP-C are not present in the closely related lectin MBP-A (Drickamer *et al.*, 1986). On the basis of this limited

Table 1. Amino acid compositions of human serum CSL and rat serum CSL

Amino acid	Human CSL		Rat CSL (mol %) [†]
	(mol %)	(residues/molecule)*	
Asx	11.8	35.3 (35)	12.4
Thr	6.6	19.8 (20)	4.9
Ser	7.5	22.4 (22)	8.6
Glx	13.6	40.7 (41)	12.6
Pro	5.7	17.0 (17)	5.0
Gly	13.8	41.4 (41)	13.9
Ala	7.9	23.8 (24)	6.7
Cys	1.4	4.3 (4)	N.D. [‡]
Val	4.4	13.3 (13)	5.8
Ile	2.9	8.7 (9)	3.6
Leu	7.9	23.6 (24)	8.8
Tyr	1.3	3.9 (4)	1.8
Phe	3.9	11.6 (12)	3.6
His	1.6	4.6 (5)	0.9
Lys	6.3	19.0 (19)	5.0
Arg	4.1	12.2 (12)	5.1
Met	0.6	1.7 (2)	1.3
Hyp	1.2	3.5 (4)	
Hyl	0.5	1.5 (2)	

* The M_r of the human CSL was estimated to be 33000 on the basis of relative mobility on SDS/polyacrylamide-gel electrophoresis.

[†] Based on an M_r of 26000 for the rat CSL (Maynard & Baenziger, 1982).

[‡] Not determined.

Human CSL	Glu-Thr-Val-Thr-Xaa-Glu-Asp-Ala-Gln-Lys
Rat MBP-C	Glu-Thr-Leu-Thr-----Glu-Gly-Ala-Gln-Ser-Cys 19
Rat MBP-A	Glu-Thr-Leu-Lys-----Thr-Cys

Fig. 4. *N*-Terminal amino acid sequences of rat CSL and human CSL

The data for rat MBP-A and MBP-C are from Drickamer *et al.* (1986). The *N*-terminal glutamic acid residue shown in MBP-C is at position 19.

Fig. 5. Binding of Fuc-BSA by human serum CSL

Increasing amounts of ^{125}I -Fuc-BSA were incubated with the equivalent of 1.52 pmol of the 33000- M_r subunit of human serum CSL in 500 μl of 50 mM-Tris/HCl buffer, pH 7.8, containing 1 M-NaCl, 4 mM- CaCl_2 and 0.25% Triton X-100. The Fuc-BSA-lectin complex was precipitated with 50%-saturated $(\text{NH}_4)_2\text{SO}_4$ and collected on GF/C glass-fibre filters. A Scatchard plot for the saturation curve is shown in the inset. B indicates the amount of ^{125}I -Fuc-BSA bound and F indicates that which remained free (total ^{125}I -Fuc-BSA minus that which was bound to CSL).

sequence information, human serum CSL is more closely related to MBP-C than to MBP-A.

Carbohydrate-specificity of human serum CSL.

With the use of the $(\text{NH}_4)_2\text{SO}_4$ precipitation assay Fuc-BSA displayed saturable binding to the human lectin (Fig. 5). The apparent association constant was 1.3×10^{-10} M, and 1 mol of Fuc-BSA was bound/7.6 mol of 33000- M_r subunit at saturation. This suggests that the native complex, consisting of eight or nine subunits, has a single binding site for molecules such as Fuc-BSA. Rat CSL does not display carbohydrate-binding activity until the subunits are assembled into high- M_r complexes similar in size to those of the human CSL (Colley & Baenziger, 1987a).

The ability of a variety of monosaccharides, oligosaccharides and glycoproteins to inhibit binding of Fuc-BSA was assessed to determine if the specificity of the human lectin was similar to that of the rat CSL. These studies are summarized in Table 2. Among the hexoses mannose and fucose inhibited binding by 50% at similar concentrations, 51 mM and 61 mM respectively. Glucose was 4–5-fold less potent, and galactose showed no inhibitory activity. *N*-Acetylglucosamine and *N*-acetylmannosamine showed similar inhibitory activity and were slightly more potent than mannose or fucose. *N*-Acetylgalactosamine was inactive. The pattern of monosaccharide inhibition was similar to that which we and others have observed for rat CSL (Mizuno *et al.*, 1981; Townsend & Stahl, 1981; Maynard & Baenziger, 1982; Mori *et al.*, 1983). The inhibitory effect of mannose and *N*-acetylglucosamine suggested that the human CSL, like the rat CSL, would display specificity for the core region of asparagine-linked oligosaccharides.

Among polysaccharides only mannan displayed in-

Table 2. Inhibitors of binding by human core-specific lectin

ID_{50} is the concentration of inhibitor that decreases the binding of ^{125}I -Fuc-BSA by 50%.

Inhibitor	ID_{50}
Monosaccharides:	
Mannose	51 mM
Fucose	61 mM
Glucose	190 mM
Galactose	> 200 mM
<i>N</i> -Acetylglucosamine	43 mM
<i>N</i> -Acetylmannosamine	48 mM
<i>N</i> -Acetylgalactosamine	> 200 mM
Polysaccharides:	
Mannan	0.21 $\mu\text{g/ml}$
Fucoidin	No inhibition
Chondroitin sulphate	No inhibition
Glycoproteins/glycopeptides/oligosaccharides:	
Ribonuclease B	> 10 μM
Ribonuclease B/R&A	> 0.6 μM
Ovalbumin GP-IV	> 1.1 μM
Ovalbumin GP-IIIa	> 1.1 μM
Ovalbumin GP-IIIC	> 1.0 μM
Asialo-transferrin	21 μM
Agalacto-transferrin	4.3 μM
Ahexosamino-transferrin	5.8 μM
Asialo-fetuin	> 0.18 μM
Agalacto-fetuin	0.034 μM
Ahexosamino-fetuin	0.027 μM
Orosomucoid	> 10 μM
Asialo-orosomucoid	5.2 μM
Agalacto-orosomucoid	0.19 μM
Ahexosamino-orosomucoid	0.47 μM

hibitory activity. Fucoidin and chondroitin sulphate were inactive. Inhibition by glycoproteins, glycopeptides and oligosaccharides did not fall into a simple pattern. Glycopeptides and glycoproteins bearing high-mannose-type oligosaccharides were not inhibitory at the concentrations tested. Transferrin, which bears predominantly biantennary complex oligosaccharides, was inhibitory after removal of sialic acid and was roughly 50-fold more potent after removal of galactose. Subsequent removal of *N*-acetylglucosamine did not significantly alter the inhibitory potency of transferrin. Fetuin was the most potent inhibitor of binding among the glycoproteins tested. Agalacto- and ahexasamino-fetuin were roughly 100-fold more potent inhibitors of binding than agalacto-transferrin. Since binding was not inhibited by the monosaccharides galactose and *N*-acetylgalactosamine, it is not likely that the *O*-glycosidically linked oligosaccharides present on fetuin contributed to this inhibition. Notably, as much as 25% of transferrin bears a triantennary complex oligosaccharide at one of its two glycosylation sites, and it may be the triantennary oligosaccharides present a fraction of the transferrin that account for its inhibitory activity rather than the biantennary structures. Orosomucoid, which bears a heterogeneous array of complex oligosaccharides, was not as potent an inhibitor of binding as fetuin. As with fetuin, agalacto-orosomucoid and ahexasamino-orosomucoid were the most effective inhibitors of binding. Since digestion of the triantennary oligosaccharides on

fetuin releases the β 1,2-linked *N*-acetylglucosamine moieties but not the β 1,4-linked *N*-acetylglucosamine, it may be that human CSL, like the rat CSL, recognizes primarily features of the 'core' region of complex oligosaccharides and that this recognition is significantly enhanced by the presence of the *N*-acetylglucosamine linked β 1,4 to the α 1,3-linked core mannose. The presence of the additional β 1,6-linked *N*-acetylglucosamine that is found on tetra-antennary structures may decrease binding and account for the weaker activity of agalacto-orosomucoid and ahexasamino-orosomucoid. More detailed studies with individual glycopeptides will be required to resolve such issues. We have, however, used the term human 'core-specific' lectin to indicate that, like rat CSL, human CSL recognizes features of the core region of asparagine-linked oligosaccharides.

DISCUSSION

We have isolated a lectin from human serum and liver by affinity chromatography on mannan-Sepharose. The human lectin closely resembles rat CSL in its physical-chemical characteristics and carbohydrate-binding specificity, and we refer to it as the human 'core-specific' lectin. Drickamer *et al.* (1986) determined the amino acid sequences of two closely related mannan-binding proteins, MBP-A and MBP-C, from their respective cDNA sequences. MBP-C is the major form obtained from rat liver and appears to be identical with the CSL that we have previously characterized (Maynard & Baenziger, 1982). Ikeda *et al.* (1987) reported that rat serum MBP has an *N*-terminal sequence identical with that of MBP-A, suggesting that MBP-C is retained by the liver whereas MBP-A is released into the circulation. The *N*-terminal sequence of human serum CSL shows greater homology to MBP-C than to MBP-A (Fig. 4). The sequence of a human lectin (human MBP) has recently been determined from its cDNA sequence (Ezekowitz *et al.*, 1988). The human MBP shows 51 % homology with rat MBP-C and 48 % homology with rat MBP-A. Removal of a hydrophobic signal sequence from human MBP would produce a protein with an *N*-terminal sequence identical with that we have found for human serum CSL. On the basis of immunoreactivity, physical-chemical properties and oligosaccharide-specificity we cannot distinguish between the liver and serum forms of human CSL. Thus, unlike the rat, there may be a single form of the human CSL.

Rat CSL and human CSL have similar amino acid compositions, including the presence of hydroxyproline and hydroxylysine, high contents of aspartic acid/asparagine, glutamic acid/glutamine and glycine, and nearly identical molar percentage compositions for the remaining amino acids. The elevated contents of glycine and the presence of hydroxylated amino acids in human CSL suggest that, like rat CSL, it contains collagen-like sequences with the repeating triplet Gly-Xaa-Yaa. Such collagen-like sequences with the repeating triplet Gly-Xaa-Yaa have been found in the human lectin (Ezekowitz *et al.*, 1988). The presence of hydroxylated proline and lysine in human CSL indicates that, like rat CSL (Drickamer *et al.*, 1986; Colley & Baenziger, 1987*a,b,c*), human CSL undergoes hydroxylation and glycosylation within these collagen-like regions. Collagen-like regions with hydroxylated amino acids are also present in the

major pulmonary surfactant protein, which displays Ca^{2+} -dependent binding to immobilized mannose, fucose, galactose and glucose (Haagsman *et al.*, 1987). Although the post-translational modification of the collagen-like domain of rat CSL (MBP-C) is not essential for assembly and expression of binding activity (Colley & Baenziger, 1987*b,c*), it is likely that the collagen-like regions may provide the mechanism for assembly into an active complex for rat CSL and for surfactant protein SP 28–36 (Haagsman *et al.*, 1987).

Rat CSL and human CSL have similar subunit M_r values, of 26 000 and 33 000 respectively, when examined by SDS/polyacrylamide-gel electrophoresis. Their native M_r values determined by gel filtration are similar, 260 000 for rat CSL and 270 000–300 000 for human CSL. One mol of Fuc-BSA is bound/7.6 mol of human CSL subunit, suggesting that the native complex of nine subunits produces the functional equivalent of a single binding site for Fuc-BSA. Whether this is also true for oligosaccharides remains to be established; however, it is notable that we have found that newly synthesized rat CSL must be assembled into 150 000–260 000- M_r complexes to display carbohydrate-binding activity (Colley & Baenziger, 1987*a*). Binding of Fuc-BSA is Ca^{2+} -dependent, with maximal binding being attained at 1 mM- Ca^{2+} (K. J. Colley & J. U. Baenziger, unpublished work).

The carbohydrate-binding specificity of human CSL is similar to that of rat CSL. Both rat CSL and human CSL recognize mannose and *N*-acetylglucosamine residues in the 'core' region of asparagine-linked oligosaccharides; however, the presence of an *N*-acetylglucosamine residue linked β 1,4 to the α 1,3-linked mannose in the core appears to enhance binding by human CSL. In contrast, the rat reticuloendothelial-cell mannose/*N*-acetylglucosamine/fucose-specific receptor requires either a mannose residue linked α 1,6 or an *N*-acetylglucosamine residue linked β 1,6 to the α 1,6-linked mannose in the core of asparagine-linked oligosaccharides (Maynard & Baenziger, 1981).

Summerfield & Taylor (1986) have reported the isolation of a 30 000- M_r Ca^{2+} -dependent mannan-binding protein from human serum. The mannan-binding protein isolated by these investigators appears to be identical with the 33 000- M_r human lectin that we have isolated from both human liver extracts and serum by mannan-Sepharose affinity chromatography. Both have monosaccharide-binding specificities similar to those of the rat CSL, and both can be identified in liver and serum by immunoblot analysis. Summerfield & Taylor (1986) report that unsubstituted Sepharose could be used to remove all the SAP from human liver extracts before application on to the carbohydrate affinity matrices. In contrast, we have found that mannan-Sepharose has a greater capacity for SAP and CRP than does unsubstituted Sepharose. Even if material is applied to a phosphocholine-Sepharose column, which will remove CRP and SAP, we have found that separation of CSL and remaining CRP and SAP requires differential elution from mannan-Sepharose with β -glycerophosphate and mannose. Kawasaki *et al.* (1983) have also prepared a mannan-binding lectin from human serum that has features similar to those of the human CSL that we have described. CSL prepared in the manner that we have described is free of antibodies that bind to mannan. Mannan-specific antibodies are probably not eluted

from the initial mannan affinity column, since their binding is not Ca^{2+} -dependent.

Ikeda *et al.* (1987) have reported that rat serum MBP (MBP-A) but not rat liver MBP (MBP-C) is able to activate complement through the classical pathway. They also found that human serum MBP was able to activate complement. This suggests that human CSL (MBP) is functionally equivalent to rat MBP-A even though it shows greater sequence homology to rat MBP-C. Activation of complement by the classical pathway by rat CSL and human CSL raises the question of how activation is prevented under normal physiological conditions.

In summary, the human CSL can be isolated from both serum and liver extracts by mannan-Sepharose affinity chromatography. The human CSL and rat CSL have similar subunit and native M_r values, amino acid compositions and *N*-terminal amino acid sequences, and demonstrate specificity for mannose and *N*-acetylglucosamine residues in the core region of asparagine-linked oligosaccharides. A collagen-like domain with hydroxylysine and hydroxyproline residues is present in both rat CSL and human CSL and may play an important biological role.

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