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 (Man₉GlcNAc₂₋₋₋Asn) was isolated from human serum and liver by affinity chromatography on mannan–Sepharose. The designation ‘core-specific lectin’ (CSL) is used to indicate its specificity. Selective elution of human CSL from mannan–Sepharose was accomplished with 50 mM-mannose. Two additional proteins that displayed Ca²⁺-dependent binding to mannan–Sepharose 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-acetylgalactosaminylated bovine serum albumin (GlcNAc-BSA), fucosylated bovine serum albumin (Fuc-BSA) and mannosylated serum albumin bovine serum albumin (Man-BSA) are bound by purified MBP and their binding is inhibited by monosaccharides such as mannose, fucose and N-acetylgalactosamine (Mizuno et al., 1981; Townsend & Stahl, 1981; Mori et al., 1983). Although MBP was initially thought to be the macrophage receptor for structures containing mannan, fucose or N-acetylgalactosamine, 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. Man₉GlcNAc₂₋₋₋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,c). The kinetics of secretion are unusual, since rat CSL is released from the cell with a t½ 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
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 Pfannstiel 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. Immunonol 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, Downington, PA, U.S.A. Na\(^{125}\)I (100 mCi/ml) was obtained from Amersham-Searle, Arlington Heights, IL, U.S.A. \(^{35}\)S]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 \(^{125}\)Iiodide by gel filtration on Sephadex G-25.

Binding assays

\(^{125}\)I-labelled agalacto-orosomucoid was prepared by digestion of \(^{125}\)I-labelled asialo-orosomucoid with diplococcal 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 \(^{125}\)I-Fuc-BSA in 50 mM-Tris/HCl buffer, pH 7.8, containing 1 M-NaCl, 40 mM-CaCl\(_2\) and 0.25% Triton X-100 in a final volume of 500 μl. Samples were precipitated with saturated (NH\(_4\))\(_2\)SO\(_4\), pH 7.8 (pH adjusted with solid Trizma base). Precipitates were filtered on Gelman type GF/C glass-fibre filters, washed with 50% saturated (NH\(_4\))\(_2\)SO\(_4\), pH 7.8, containing 25 mM-CaCl\(_2\), 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 μg of human CSL. Rabbits were boosted with incomplete Freud’s adjuvant containing 100 μg 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 μg) in BBS was bound to Immunonol 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 μl total volume). Then 200 μl 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 \(^{125}\)I-labelled goat anti-[rabbit IgG F(ab′)2 fragment] antibody (200000 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 Hoeffer Scientific Instruments Transphor apparatus according to the method of Burnette (1981). Transfers were performed for 8–20 h at 10 °C. \(^{14}\)C-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-CaCl\(_2\), 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 \(^{125}\)I-labelled goat anti-[rabbit IgG F(ab′)2 fragment] antibody in blocking buffer (2 × 10\(^7\)/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-CaCl\(_2\), 5% BSA, 0.2% Nonidet P-40 (PBST). After the final wash, the nitrocellulose was air-dried for 30 min and air-dried for 30 min. The dried nitrocellulose was cut into 1 cm squares and counted in a gamma counter. The radioactivity was quantified as a percentage of the input radiolabel.
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 Sephacyr S-300 was used to determine the native $M_r$ values of the CSL and CRP. A 2 cm x 75 cm column of Sephacyr S-300 equilibrated in 20 mM-Tris/HCl buffer, pH 7.8, containing 0.1 mM-NaCl and 2 mM-EDTA was calibrated with the marker proteins ferritin ($M_r$ 440000), BSA ($M_r$ 66200) and agalacto- orosomucoid ($M_r$ 40000). 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 ($V_e$).

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 (NH$_4$)$_2$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 antibody, and a solid-phase radioimmunoassay was established for quantification. On the basis of this radioimmunoassay it was estimated that 0.4–1.0 mg 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 activity. The increase in specific activity with respect to total protein was 15.5-fold at this step. A 1 mg 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 $M_r$ 33000 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 $M_r$ 67000. 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. 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 antiserum 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 30000-$M_r$ standard.
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 analyzed 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 Mr 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-Sephrose 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 ($A_{280}$, △), and the presence of human CSL by radioimmunoassay (●), $^{125}$I-Fuc-BSA binding (□) and SDS/polyacrylamide-gel electrophoresis with Coomassie Blue staining (inset gel).
activity for Fuc-BSA or mannan by use of the (NH₄)₂SO₄ precipitation assay. Human serum CSL prepared as described accounted for the Ca²⁺-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-CaCl₂ 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 Mr 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 (A₂₈₀), 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 Mr of 33000 and small amounts of dimeric material migrating with an Mr of 67000. Gel filtration on a column of Sephacryl S-300 calibrated with proteins of known Mr yielded an estimated native Mr of 270000. Non-equilibrium sedimentation on a sucrose gradient calibrated with known standards yielded a native Mr 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

| 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 |
| 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.

Vol. 256
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 $\mu$l of 50 mM-Tris/HC1 buffer, pH 7.8, containing 1 mM-NaCl, 4 mM-CaCl$_2$ and 0.25% Triton X-100. The Fuc-BSA-lectin complex was precipitated with 50% saturated (NH$_4$)$_2$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).

Carbohydrate-specificity of human serum CSL.

With the use of the (NH$_4$)$_2$SO$_4$ precipitation assay Fuc-BSA displayed saturable binding to the human lectin (Fig. 5). The apparent association constant was $1.3 \times 10^{-18}$ 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 $\mu$M and 61 $\mu$M 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 inhibitory 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 ahexosamino-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 ahexosamino-orosomucoid were the most effective inhibitors of binding. Since digestion of the triantennary oligosaccharides on

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

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fetuin releases the \(\beta_1,2\)-linked N-acetylgalactosamine moieties but not the \(\beta_1,4\)-linked N-acetylgalactosamine, 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-acetylgalactosamine linked \(\beta_1,4\) to the \(\alpha_1,3\)-linked core mannose. The presence of the additional \(\beta_1,6\)-linked N-acetylgalactosamine that is found on tetra-antennary structures may decrease binding and account for the weaker activity of agalacto-orosomucoid and ahexosamino-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, 1987a,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, 1987b,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 26000 and 33000 respectively, when examined by SDS/polyacrylamide-gel electrophoresis. Their native \(M_r\) values determined by gel filtration are similar, 260000 for rat CSL and 270000–300000 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 150000–260000- \(M_r\) complexes to display carbohydrate-binding activity (Colley & Baenziger, 1987a). 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-acetylgalactosamine residues in the ‘core’ region of asparagine-linked oligosaccharides; however, the presence of an N-acetylgalactosamine residue linked \(\beta_1,4\) to the \(\alpha_1,3\)-linked mannose in the core appears to enhance binding by human CSL. In contrast, the rat reticuloendothelial-cell mannose/N-acetylgalactosamine/fucose-specific receptor requires either a mannose residue linked \(\alpha_1,6\) or an N-acetylgalactosamine residue linked \(\beta_1,6\) to the \(\alpha_1,6\)-linked mannose in the core of asparagine-linked oligosaccharides (Maynard & Baenziger, 1981).

Summerfield & Taylor (1986) have reported the isolation of a 30000-\(M_r\), Ca\(^{2+}\)-dependent mannan-binding protein from human serum. The mannan-binding protein isolated by these investigators appears to be identical with the 30000-\(M_r\) 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 \(\beta\)-glycerocephosphate 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 \( \mathrm{Ca}^{2+} \)-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 Mr values, amino acid compositions and N-terminal amino acid sequences, and demonstrate specificity for mannose and N-acetylgalcosamine 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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