A Novel Human Serum Lectin with Collagen- and Fibrinogen-like Domains That Functions as an Opsonin*

(Received for publication, May 3, 1995, and in revised form, September 29, 1995)

Misao Matsushita, Yuichi Endo, Satoshi Taira, Yasuo Sato, Teizo Fujita, Narumi Ichikawa, Munehiro Nakata, and Tsuguo Mizuochi

From the Department of Biochemistry, Fukushima Medical College, 1-Hirakiga-oka, Fukushima 960-12 and the Laboratory of Biomedical Chemistry, Department of Applied Chemistry, Tokai University, Hiratsuka, Kanagawa 259-12, Japan

Collectins are C-type animal lectins with both collagenous and carbohydrate recognition domains and are involved in the first line host defense against pathogens. We report here a novel Ca²⁺-dependent and GlcNAc-binding lectin consisting of subunits of 35 kDa (P35) with a collagen-like sequence. When P35 is isolated from human serum, it forms a homopolymer by means of intermolecular disulfide bonding, as is the case with collectins. P35 cDNA was cloned from a human liver cDNA library, and the deduced amino acid sequence of 313 residues revealed that the mature form of P35 consists mainly of collagen- and fibrinogen-like domains. The latter contained two potential Ca²⁺-binding sites that may be involved in carbohydrate binding. The overall sequence of P35 was highly homologous to porcine ficollin and β. Northern blots of various human tissues showed that the major product of the 3.1-kilobase-long P35 transcript is expressed in liver. P35 enhanced phagocytosis of Salmonella typhimurium by neutrophils, suggesting an opsonic effect via the collagen region. P35 was found to bind to GlcNAc-conjugated bovine serum albumin, a neoglycoprotein, as well as to neoglycolipids containing complex-type oligosaccharides derived from glycoproteins, suggesting that P35 recognizes GlcNAc residues such as those found in microbial glycoconjugates and complex-type oligosaccharides. Therefore, P35 represents a new type of GlcNAc-binding lectin with structural and functional similarities to collectins involved in innate immunity.

Animal lectins are mainly involved in endocytosis, cell-cell interactions, and host defense. Collectins (1–4) are a group of collagenous and carbohydrate recognition domains and are involved in the first line host defense against pathogens. We report here a novel Ca²⁺-dependent and GlcNAc-binding lectin consisting of subunits of 35 kDa (P35) with a collagen-like sequence. When P35 is isolated from human serum, it forms a homopolymer by means of intermolecular disulfide bonding, as is the case with collectins. P35 cDNA was cloned from a human liver cDNA library, and the deduced amino acid sequence of 313 residues revealed that the mature form of P35 consists mainly of collagen- and fibrinogen-like domains. The latter contained two potential Ca²⁺-binding sites that may be involved in carbohydrate binding. The overall sequence of P35 was highly homologous to porcine ficollin and β. Northern blots of various human tissues showed that the major product of the 3.1-kilobase-long P35 transcript is expressed in liver. P35 enhanced phagocytosis of Salmonella typhimurium by neutrophils, suggesting an opsonic effect via the collagen region. P35 was found to bind to GlcNAc-conjugated bovine serum albumin, a neoglycoprotein, as well as to neoglycolipids containing complex-type oligosaccharides derived from glycoproteins, suggesting that P35 recognizes GlcNAc residues such as those found in microbial glycoconjugates and complex-type oligosaccharides. Therefore, P35 represents a new type of GlcNAc-binding lectin with structural and functional similarities to collectins involved in innate immunity.

Collectins possess a structural unit made up of three polypeptide chains, each containing at least three domains. The first domain consists of an NH₂-terminal sequence containing cysteine residues that make it possible for structural units to form oligomers via disulfide bonds. The second domain is a collagenous region consisting of Gly-Y-X triplet repeats (where X and Y represent any amino acid). The third domain contains a COOH-terminal sequence consisting of a carbohydrate recognition domain (CRD) (10) that is also involved in Ca²⁺ binding. It has 14 invariant and 18 highly conserved residues spread over ~120 amino acids and is found in many animal lectins (11, 12).

Collectins play a role in the first line of host defense against microorganisms possessing certain oligosaccharides on their surface. Opsonization appears to be their common function, as is the case with MBP (13, 14) and conglutinin (15), and may occur through the interaction of collagenous regions with the C1q receptor on phagocytes (16, 17). MBP and conglutinin have been shown to bind to gp120 and gp160 of the envelope glycoproteins of human immunodeficiency virus type 1, respectively (18, 19), thereby inhibiting virus infection of cells (20) or the interaction between gp160 and CD4 (19). In addition, MBP has the unique property of being able to activate the complement system (21–24), probably by utilizing a C1s-like serine protease termed MBP-associated serine protease (25, 26), which is capable of cleaving C4 and C2, leading to the killing of bacteria or viruses by direct lysis or opsonization.

We describe here a novel type of human serum Ca²⁺-dependent and GlcNAc-binding lectin with opsonic activity. This lectin has a collagenous domain similar to that of collectins. Unlike the CRD in collectins, however, it possesses a fibrinogen-like domain that is probably involved in carbohydrate binding.

EXPERIMENTAL PROCEDURES

Reagents—Mannan from Saccharomyces cerevisiae, GlcNAc-agarose, ovalbumin, ribonuclease B, human IgG, fetuin, asialofetuin, human α₁-acid glycoprotein, and bovine serum albumin (BSA) conjugated with lactose (lactose-BSA) and cellulose (cellulose-BSA) were purchased from Sigma. Human fibrinogen was from Kabivitrum (Stockholm, Sweden), and neoglycoproteins of Man-BSA, Glc-BSA, GlcNAc-BSA, and Gal-BSA were from E. Y. Laboratories (San Mateo, CA). In each neoglycoprotein, ~19–42 mol of monosaccharide were conjugated to 1 mol of BSA. Mannose and GlcNAc were from Wako Pure Chemical Industries (Osaka, J. apan). CNBr-activated Sepharose 4B was from Pharmacia Fine Chemicals (Uppsala, Sweden). Coupling of mannase or asialofetuin to CNBr-activated Sepharose was performed according to the manufacturer’s instructions. Na⁺- and (32P)3-CTP were purchased from Amersham International (Buckinghamshire, United Kingdom). P35 was labeled using Na¹²⁵I and [O-¹⁴C]glucose from Pierce, and its specific activity was 0.24 MBq/μg.

Purification of P35—Human serum obtained by recalcification of outdated plasma was treated with polyethylene glycol 4000 at a final concentration of 7%. The precipitate was dissolved in 50 mM Tris, 1 mM NaCl, 50 mM CaCl₂, pH 7.8 (starting buffer), and applied to a mannana...
RESULTS

Preparation of P35 Binding to Glycoproteins and Neoglycoproteins—The six glycoproteins used were those whose oligosaccharides had been used for preparation of the neoglycolipids for the P35 overlay assay, and the six neoglycoproteins used were those in which BSA had been conjugated with mannos, glucose, GlcNAc, galactose, lactose, and cellobiose. These were dot-blotted on an Immobilon-P membrane (Millipore Co., Bedford, MA), which was then blocked and incubated with 125I-labeled P35. After the membranes were washed, binding of P35 to the glycoprotein and neoglycoprotein dots was assessed by autoradiography as reported previously (36, 38).

Preparation of Monoclonal Antibodies against P35.—Isolated P35 (10 μg) was injected into each female Balb/C mouse. After 3 weeks, the mice were boosted with 10 μg of P35 at intervals of 10 days. The mice were then bled from the tail vein, and the sera were then tested for the presence of anti-P35 antibodies by enzyme-linked immunosorbent assay. One positive mouse monoclonal antibody, F5, was purified from ascites fluid of mice bearing the hybridoma by precipitation with ammonium sulfate at 50% saturation followed by chromatography on DEAE-Toyopearl. F5 was of the IgG1 isotype.

cDNA Cloning—Based on analysis of the NH2-terminal amino acid sequence of the purified P35 protein (see Fig. 2), two oligonucleotides were synthesized as probes for the cloning of P35 cDNA: 5'-GA(A/G)CTNAA(A/G)GCGTNTGG-3' corresponding to amino acids 9–14 and 5'-GCT(N/G)C(T/G)T(G/A)TGTTNGG-3' corresponding to amino acids 12–22 of mature P35. By screening for a clone that was positive for both oligonucleotides, a P35 cDNA clone lacking a portion of the 5'-end was isolated from a human liver cDNA library. By further screening using the cDNA clone as a probe, 11 positive clones were obtained. These positive clones were subcloned into pBluescript II SK+ by in vivo excision according to the instructions provided by Stratagene (La Jolla, CA) and sequenced by the dyeoxy method with a Sequenase DNA sequencing kit (U. S. Biochemical Corp.). By overlapping the sequences of these clones, a stretch of P35 cDNA was constructed.

Northern Blot Hybridization—A membrane filter containing 2 μg of poly(A)+ mRNA from various human tissues (CLONTECH, Palo Alto, CA) was hybridized with 32P-labeled cDNA (EcoRI-XhoI fragment of 971 base pairs corresponding to nucleotides 110-1080) at 42°C over-night in 50% formamide, 5× SSPE (1× SSPE is 9 mM sodium phosphate, 150 mM NaCl, 1 mM EDTA, pH 7.4) 0.5% SDS, and 200 μg/ml salmon sperm DNA. After a final washing at 60°C for 30 min in 0.2× SSPE (15 mM sodium citrate, 150 mM NaCl, pH 7.0) containing 1.0% SDS, the filter was exposed to an autoradiogram imaging system, and the image was then read using a bioimaging analyzing system (BAS 1000, Fujifilm, Tokyo, J. anpan).

Amino Acid Sequence Analysis—The NH2-terminal amino acid sequence of P35 was determined with a gas-phase protein sequenzer (PSQ-1, Shimadzu Corp., Kyoto, Japan) for P35 Binding to Mannan—Microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with 100 μg of mannan (0.5 mg/ml 50 mM carbonate buffer, pH 9.6) by incubation at 4°C overnight. Wells were washed with Veronal buffer containing 0.1% Tween 20 and then blocked with Blockace (Dainippon Pharmaceutical Co., Ltd., Osaka, J. anpan) at 37°C for 2 h. After washing, wells were treated with 30 μl of P35 diluted in Veronal buffer containing either 2 μM CaCl2 or 10 μM EDTA at 37°C for 2 h. Wells were then washed with Veronal buffer/Tween 20 containing 2 μM CaCl2 or 10 μM EDTA and then with Veronal buffer/Tween 20 containing 2 μM CaCl2, followed by incubation with 30 μl of anti-P35 monoclonal antibody (2F5) at 37°C for 2 h. After washing, horseradish peroxidase-conjugated goat anti-mouse IgG (Vector, CA) was added, and the wells were washed, and incubation was carried out at 37°C for 2 h. The color was developed by addition of azinodi-(3-ethylbenzthiazoline)sulfonic acid (ABTS, Zymed Laboratories, Inc., South San Francisco, CA) in citrate buffer, pH 4.4, containing 0.025% hydrogen peroxide. The absorbance at 405 nm was determined using a Bio-Rad photometer (Model 450 Microplate Reader).

Preparation of Neoglycopolidids and the P35 Overlay Assay—Oligosaccharides were quantitatively released by hydrazinolysis, as described previously in detail (28, 29), from the following six glycoproteins: ovalbumin with high mannose-type and hybrid-type oligosaccharides (30); bovine pancreatic RNase B with only high mannose-type oligosaccharides (31); human IgG with di-, mono-, and non-galactosylated β-antennary complex-type oligosaccharides (32); human fibrinogen with digalactosylated biantennary complex-type oligosaccharides as the major oligosaccharides (33); fetuin with triantennary complex-type oligosaccharides as the major oligosaccharides (34); and human α1-acid glycoprotein with tetraantennary complex-type oligosaccharides as the major oligosaccharides (35). Oligosaccharide mixtures containing complex-type oligosaccharides were converted into neutral oligosaccharides by sialidase treatment. The neutral oligosaccharide mixtures obtained from the six glycoproteins were conjugated to dipalmitoylphosphatidylcholine previously described (26-30), 37). The reaction mixtures containing 2 μg of oligosaccharides were subjected to TLC using chloroform/methanol/water (105:100:28, by volume). Since all the neoglycolipids thus prepared contained a common lipid, they were separated according to their oligosaccharide structures using this TLC system.

Flow Cytometric Analysis—Binding of P35 to S. typhimurium was analyzed by flow cytometry. S. typhimurium cells (TV119 or LT2; 1 × 107 colony-forming units/ml) in 100 μl of GHBSS containing 2 μM CaCl2 were incubated with 200 μl of pepsinized Veronal buffer containing 2 μM CaCl2 at 37°C for 30 min. In inhibition experiments, P35 was preincubated for 30 min with 100 μg GlcNAc or mannan (50 mg/ml) before it was added to the bacteria. Bacteria were then incubated with the antibody 2F5 (100 μg/ml) at 37°C for 40 min and stained with 20 μl of 125I-labeled goat antihuman-IgG conjugated rabbit anti-mouse immunoglobulins (Dako J anpan Co., Ltd., Tokyo, J. anpan) at 4°C for 30 min. The bacteria were washed twice between each reaction with gelatin/Veronal buffer containing 2 μM CaCl2. Reactivities were evaluated on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and compared with a control consisting of bacteria treated with 2F5 and fluorescent isothiocyanate-conjugated rabbit anti-mouse immunoglobulins.

Phagocytosis of S. typhimurium by PMN—S. typhimurium cells (TV119 or LT2; 2 × 107 colony-forming units) were added to human neutrophils (PMN) (1 × 107/ml) followed by 200 μl of GHBSS, followed by incubation at 37°C for 30 min. The mixture containing bacterial cells and PMN was added to a 0.4% agarose plate. After the cells were washed with buffer, they were stained with May-Grins, and phagocytosis of bacteria by PMN was observed by light microscopy. The number of bacteria ingested per 100 PMN was defined as the phagocytic index. Significance was tested using Student’s t test.

RESULTS

Purification of P35—In the purification scheme of MBP, the 7% polyethylene glycol precipitate of human serum was first subjected to affinity chromatography on a yeast mannan-Sepharose column, and MBP was eluted with mannos in the presence of calcium ions as described previously (25). In the present study, we then carried out elution with GlcNAc to examine whether lectins such as conglutinin-like protein were
retained on mannan-Sepharose after MBP had been eluted. As a result, a protein with a molecular mass of ~35 kDa under reducing conditions was found in the eluate from mannan-Sepharose. We tentatively named this lectin P35. Further purification of P35 was achieved by ion-exchange chromatography using a Mono Q column since the P35 preparation from mannan-Sepharose was contaminated with IgG and IgM.

As shown in Fig. 1, P35 showed a single band that was slightly larger than the MBP subunit under reducing conditions. Under nonreducing conditions, it appeared as a 320-kDa moiety. These results suggest that P35 is composed of subunits linked to form a homopolymer via disulfide bonds, as is the case with MBP. The NH₂-terminal 43-amino acid sequence of P35 determined using a protein sequencer shows glycine residues at intervals of two amino acids, suggesting a collagenous type of sequence (Fig. 2).

cDNA Cloning of P35—Based on the NH₂-terminal amino acid sequence of the P35 subunit, two oligonucleotides were synthesized and used to screen for cDNA clones of P35 in a human liver λZAP cDNA library. We eventually obtained 11 positive clones that we sequenced. cDNA sequence analysis showed that P35 contains a 339-base pair open reading frame encoding 313 amino acids preceded by a leader peptide of 25 amino acids (Fig. 3). The predicted molecular size of mature P35 was calculated to be 31.4 kDa, which nearly matched that of the P35 subunit. There are two potential N-glycosylation sites at residues 240 and 300 and two potential calcium-binding sites in the COOH-terminal half. P35 consists of three major domains: an NH₂-terminal noncollagenous region of 1–13 amino acids; a collagen-like sequence starting at amino acid 14 with two Gly-X-Y triplets followed by a gap of six amino acid residues and 15 Gly-X-Y repeats; and a COOH-terminus of 218 amino acids, which in P35 has fibrinogen and fibrinogen-like characteristics.

By conducting a homology search, we found that P35 shows 77.4 and 75.4% homology to ficolins α and β, respectively, at the...
amino acid level (Fig. 4). Ficolin was first identified in porcine uterus membranes and characterized as a transforming growth factor-β-binding protein by Ichijo et al. (39), although its physiological functions remain unknown. These investigators isolated two closely related cDNA clones encoding two proteins designated ficolins α and β, which have 83% homology and which consist of collagen- and fibrinogen-like domains (40), as seen in P35.

Northern Blotting—Northern blot analysis of mRNA from various human tissues showed that the major transcript of P35 is expressed in liver and is 1.3 kb in length (Fig. 5). Liver also contains three additional transcripts of 3.0, 3.2, and 4.0 kb. Lung and placenta expressed cross-hybridized messages of 1.4 kb in length, which is slightly longer than the major transcript in liver.

Requirement for Calcium Ions for P35 Binding to Mannan—To examine whether P35 is Ca\(^{2+}\)-dependent, P35 was incubated with mannan coated on enzyme-linked immunosorbent assay plates in the presence of calcium ions or EDTA, and P35 binding to mannan was then determined using monoclonal anti-P35 antibody. The results show that P35 bound to mannan in the presence of calcium ions (A\(_{405}\) = 0.22), whereas EDTA prevented this binding (A\(_{405}\) = 0.06). Furthermore, purified P35 bound to mannan-Sepharose in the presence of Ca\(^{2+}\) and was later eluted with EDTA (data not shown). Therefore, it is likely that Ca\(^{2+}\) is required for P35 binding to mannan.

Binding Specificity of P35 for Glycoconjugates—P35 specificity for various glycoproteins and neoglycoproteins was evaluated by a binding assay. As shown in Fig. 6, P35 bound to GlcNAc-BSA, one of the neoglycoproteins, but did not bind to the others (Man-BSA, Glc-BSA, lactose-BSA, and celllobiose-BSA). It also did not bind to any of the glycoproteins tested, i.e. ovalbumin (OVA) with high mannose-type and hybrid-type oligosaccharides (30); bovine pancreatic RNase B (RN) with only high mannose-type oligosaccharides (31); human IgG with di-, mono-, and non-galactosylated biantennary complex-type oligosaccharides (32); human fibrinogen (Fib) with digalactosylated biantennary complex-type oligosaccharides as the major oligosaccharides (33); fetuin (Fet) with triantennary complex-type oligosaccharides as the major oligosaccharides (34); and human α\(_1\)-acid glycoprotein (AGP) with tetraantennary complex-type oligosaccharides as the major oligosaccharides (35). From these results, it appears likely that P35 recognizes GlcNAc. The reason why P35 bound to GlcNAc-BSA but not to human IgG with non-galactosylated (GlcNAc-exposed) oligosaccharides may have been due to steric hindrance by the polypeptide moiety. Another possibility may involve a lack of residue clusters because GlcNAc-BSA, to which P35 bound, contains clustered GlcNAc residues (29 mol of GlcNAc/mol of BSA), whereas the six glycoproteins tested, to which P35 did not bind, contain only one to five N-linked oligosaccharide chains with GlcNAc residues.

We next performed a similar binding assay using neoglycolipids containing neutral oligosaccharides derived from the above six glycoproteins. Neoglycolipids were prepared by conjugating neutral oligosaccharides derived from the glycopro-
Proteins to dipalmitoyl phosphatidylethanolamine. These conjugates were chromatographed on a TLC plate, which was then overlaid with \(^{125}\)I-P35. As shown in Fig. 7, radiolabeled P35 gates were chromatographed on a TLC plate, which was then stained with orcinol reagent for oligosaccharide detection of the neoglycolipids (lower panel). Arrows in lane c indicate a neoglycolipid lacking terminal galactose derived from IgG.

**Fig. 6.** Binding of P35 to neoglycoproteins and natural glycoproteins. Neoglycoproteins and natural glycoproteins were dot-blotted on an Immobilon-P membrane. The membrane was incubated with \(^{125}\)I-labeled P35 and then subjected to autoradiography for 24 h for detection of P35 binding. Details are described under "Experimental Procedures" and "Results." OVA, ovalbumin; RN, RNase B; Fib, fibrinogen; Fet, fetuin; AGP, \(\alpha_1\)-acid glycoprotein; Lac, lactose; Cel, cellobiose.

**Fig. 7.** Binding of P35 to neoglycolipids derived from glycoproteins. Neoglycolipids prepared from neutral oligosaccharides of the six glycoproteins, as described under "Experimental Procedures," were chromatographed on a TLC plate. Lane a, ovalbumin; lane b, RNase B; lane c, IgG; lane d, fibrinogen; lane e, fetuin; lane f, \(\alpha_1\)-acid glycoprotein. The plate was overlaid with \(^{125}\)I-labeled P35, subjected to autoradiography for 24 h for detection of P35 binding (upper panel), and then stained with orcinol reagent for oligosaccharide detection of the neoglycolipids (lower panel). Arrows in lane c indicate a neoglycolipid lacking terminal galactose derived from IgG.

**DISCUSSION**

We isolated and characterized a human serum lectin tentatively named P35 that is capable of binding to yeast mannan-Sepharose and elutes with GlcNAc. Binding studies with neoglycoproteins and neoglycolipids indicate that P35 does not
bind to mannose-BSA or to high mannose-type or hybrid-type oligosaccharides, although it binds to GlcNac in GlcNac-BSA and complex-type oligosaccharide chains with GlcNac residues linked to the trimannosyl core. In experiments with mannan-Sepharose, P35 may not have bound to the mannose residue but to GlcNac-containing oligosaccharides present as contaminants in the yeast mannan used since yeast mannan itself does not contain this type of oligosaccharide (41). The binding specificity of P35 for GlcNac was also confirmed by affinity chromatography using GlcNAc-agarose and asialofetuin-Sepharose. Purified P35 was able to bind to both columns and eluted with GlcNac (Fig. 8).


P35, MBP, and conglutinin can be classified as serum lectins specific for GlcNAc. Based on binding studies with neoglycolipids, it has been shown that MBP (42, 43), conglutinin (36), and P35 recognize a GlcNAc next to a terminal galactose in oligosaccharides derived from IgG when this terminal galactose is missing. Unlike P35, MBP and conglutinin are unable to bind to GlcNac when an adjacent terminal galactose is present. MBP and conglutinin bind to mannose in oligosaccharides derived from glycoproteins such as RNase B and ovalbumin. In contrast, P35 is unable to bind to high mannose-type or hybrid-type oligosaccharides or to mannose-BSA. The lack of binding to hybrid-type oligosaccharides bearing a GlcNAc \(^{1}\text{-Man}\) group may be due to steric hindrance by the outer chain on the Man\(^{3}\text{-6Man}\) side. It can be concluded that P35 shows binding specificity toward GlcNAc and that, unlike MBP and conglutinin, it does not recognize mannose.

The precise mechanism by which P35 is able to bind to neoglycolipids is unknown, as is the reason why it does not bind to glycoproteins even though both contain the same oligosaccharide structure. As described under "Results," however, P35 may prefer clustered GlcNAc residues and clustered complex-type oligosaccharide chains with GlcNAc residues linked to the trimannosyl core. This preference is in accord with the binding of P35 to microorganisms that have a large number of nonreducing terminal GlcNAc residues exposed on their surface, as is the case with bacteria of S. typhimurium strain TV119. On the other hand, P35 was unable to bind to LT2, a smooth-type strain of S. typhimurium that possesses additional O-polysaccharides. These extra O-polysaccharides might render GlcNAc inaccessible to P35 due to steric hindrance. Therefore, we could not exclude the possibility that the GlcNAc residues of the glycoprotein oligosaccharide chains may be hidden by the polypeptide portion or by sialic acid residues that are linked to galactose residues on these oligosaccharides, resulting in the prevention of a P35 interaction.

By searching for sequence homology in a protein data base, we found that the NH\(_2\)-terminal six-amino acid sequence of P35 completely coincides with that of a 36-kDa protein that was identified as one of three soluble class I human leukocyte antigen proteins in human plasma by immunoprecipitation and immunoblotting using anti-human leukocyte antigen
monoclonal antibodies (44). Of the three proteins, the NH2-terminal amino acid sequences of two species were identical to that of cellular human leukocyte antigen, whereas no homology was found for the other 36-kDa protein. It is possible that the 36-kDa protein is identical to P35 and was obtained because of its ability to bind to IgG through complex-type oligosaccharides.

cDNA analysis revealed that P35 and porcine ficolins are highly homologous and share collagen- and fibrinogen-like domains. Two types of ficolin with sequence homology, ficolins α and β, have been identified from a porcine uterus cDNA library. The distribution of ficolin in various human tissues has been studied using porcine ficolin probes and mRNAs from these tissues; and ficolin β was shown to be present in lung and placenta, and ficolin β in skeletal muscle (40). With Northern blot analysis, we showed that the major P35 transcript of 1.3 kb is expressed in liver. This transcript seems to correspond to P35. Three larger signals that were also detected in liver probably correspond to products of alternative splicing because they were found in the cDNA sequences, and Northern blotting with the intron sequences as a probe showed that the probes were reacted with these three bands, but not with the 1.3-kb band. Lung and placenta expressed cross-hybridized messages 1.4 kb in length, which is slightly longer than that in liver, suggesting that these two tissues produce P35-related proteins that may differ in amino acid sequence from the one expressed in liver. It is possible that P35 and P35-related protein might be human analogues of the two types of porcine ficolin.

P35 and collectins share structural and functional similarities. They are both Ca2+-dependent lectins as well as hybrid proteins that have collagenous domains with either a fibrinogen-like domain (in P35) or a CRD (in collectins). Amino acid sequences in the CRD responsible for calcium and carbohydrate binding have been thoroughly investigated (11, 12). Although there is no sequence homology between the CRD and the fibrinogen-like domain, P35 has potential calcium-binding sequences in the CRD responsible for calcium and carbohydrate binding sites within the latter. It is therefore reasonable to expect that carbohydrate-binding sites also reside in this domain. It has been reported that fibrinogen-like domains occur in certain proteins such as tenascin and cytotactin (45, 46) and the scabrous protein of Drosophila melanogaster (47); however, their functions in these proteins are not yet fully understood. This is the first report demonstrating that one possible function of the fibrinogen-like domain might be to bind to carbohydrates in a calcium-dependent manner.

P35 has been demonstrated to bind to TV119, an Ar chemoattractant of S. typhimurium bearing rough core polysaccharides containing a terminal GlcNac. Binding of P35 to TV119 was carbohydrate-specific and mediated enhancement of phagocytosis by PMN. These activities were not directed to LT2, a smooth-type strain of S. typhimurium. O-Polysaccharides in LT2 might render P35 inaccessible to GlcNac due to steric hindrance. Collagen domains in collectins are crucial for exhibiting opsonic activities. Upon binding to their ligands, collectins interact with the C1q receptor on phagocytes, resulting in enhancement of phagocytosis. Since P35 is also a lectin containing a collagen-like domain structure, it is possible that in P35, this domain binds to the C1q receptor on PMN.

In conclusion, P35 is a human serum Ca2+-dependent lectin specific for GlcNac. It exhibits a high structural similarity to porcine ficolin and possesses both collagen- and fibrinogen-like domains. P35 also resembles collectins in that the collagen-like domains in both are presumably involved in opsonic activities towards microorganisms. Instead of the CRD found in collectins, however, P35 has a fibrinogen-like domain that could be responsible for carbohydrate binding. P35 is therefore a novel type of carbohydrate-specific and GlcNac-binding lectin with hybrid domains that are probably involved in innate immunity.

Acknowledgments—We thank Dr. William Campbell for English editing and Mihoko Mogi for technical assistance.

REFERENCES

1. Malhotra, R., Haurum, J., Thiel, S., and Sim, R. B. (1992) Eur. J. Immunol. 22, 1437–1445
2. Sastry, K., and Ezekowitz, R. A. (1993) Curr. Opin. Immunol. 5, 59–66
3. Hidramkov, U., Malhotra, R., Sim, R. B., and ensenijs, J. C. (1994) Immunol. Today 15, 67–74
4. Hoppe, H. J., and Reid, K. B. (1994) Protein Sci. 3, 1143–1158
5. Drickamer, K. (1988) Biochem. Biophys. Res. Commun. 151, 959–966
6. Thiel, S. (1992) Immunopharmacology 24, 91–99
7. Turner, M. (1994) Biochem. Soc. Trans. 22, 88–94
8. Ensenijs, J. C., Lausers, S. B., Zheng, Y., and Holmskog, U. (1994) Biochem. Soc. Trans. 22, 95–100
9. Y. Endo, S. Taira, Y. Sato, M. Matsushita, and T. Fujita, manuscript in preparation.