Podocytes are responsible in part for maintaining the size and charge characteristics of the glomerular filter. The major sialoglycoprotein of the podocyte foot process glycocalyx is a 140-kDa sialoglycoprotein named podocalyxin. Monoclonal antibodies raised against isolated rabbit glomeruli that recognized a podocalyxin-like protein based upon size, Alcian blue staining, wheat germ agglutinin binding, and distribution in renal cortex were used to expression clone cDNAs from a rabbit glomerular library. On Northern blot the cDNAs hybridized to a 5.5-kilobase pair transcript predominantly present in glomerulus. The overlapping cDNAs spanned 5,313 base pairs that contained an open reading frame of 1,653 base pairs and were not homologous with a previously described sequence. The deduced 551-amino acid protein contained a putative 21-residue N-terminal signal peptide and a 26-amino acid transmembrane region. The mature protein has a calculated molecular mass of 55 kDa, an extracellular domain that contains putative O-linked glycosylation, and a potential glycosaminoglycan attachment site. The intracellular domain contains potential sites for phosphorylation. Processing of the full-length coding region in COS-7 cells resulted in a 140-kDa band, suggesting that the 55-kDa core protein undergoes extensive post-translational modification. The relationship between the cloned molecule and the monoclonal antibodies used for cloning was confirmed by making a fusion protein that inhibited binding of the monoclonal antibodies to renal cortical tissue sections and then raising polyclonal antibodies against the PCLP1 fusion protein that also recognized glomerular podocytes and endothelial cells in tissue sections in a similar distribution to the monoclonal antibodies. We conclude that we have cloned and sequenced a novel transmembrane core glycoprotein from rabbit glomerulus, which has many of the characteristics of podocalyxin. We have named this protein podocalyxin-like protein 1.

Podocalyxin is the major sialoglycoprotein of the glycocalyx lining the foot processes of glomerular epithelial cells (podocytes) where it is thought to maintain foot process structure and function in part by virtue of its negative charge (1). Podocalyxin was first identified by Kerjaschki, Sharkey, and Farquhar (1) as an Alcian blue staining 140-kDa sialoglycoprotein. Subsequent studies have shown that the negative charge is contributed by sulfate as well as by sialic acid (2) and that podocalyxin is present on the surface of endothelial cells as well as glomerular epithelial cells (3, 4).

The interdigitating foot processes of neighboring podocytes create the huge intercellular surface area for glomerular filtration. The importance of charge for maintenance of this structure has been demonstrated by experiments where charge neutralization with polycations or desialylation with neuraminidase is associated with loss of the interdigitating foot process structure of the podocyte (5–7). Similarly, the induction of podocyte foot process effacement in rats by injection of puromycin aminonucleoside is accompanied by leakage of the glomerular filter, podocyte foot process detachment, and a reduction in sialylation of podocalyxin (8, 9).

From these studies we have the concept of podocalyxin as an intensely negatively charged molecule present in large amounts on the podocyte foot processes and lining the surface of endothelial cells. At these sites podocalyxin may function in part by charge repulsion to maintain the distance between foot processes of neighboring cells and between circulating cells and the endothelium (“anti-adhesion molecule”). We report here the molecular structure of a transmembrane glycoprotein that we have cloned and sequenced as part of an effort to understand in molecular terms how the glomerular filter works and how it becomes dysfunctional in children and adults with the nephrotic syndrome. We have named this protein podocalyxin-like protein 1 (PCLP1).1

MATERIALS AND METHODS

Preparation of Monoclonal Antibodies—mAbs 5F7 and 4B3 were produced from BALB/C mice immunized with isolated rabbit glomeruli (10,000/immunization) by standard methods as described previously (10). The resulting hybridomas grown out in 96-well plates were selected and subcloned based on immunofluorescence pattern assayed on cryostat sections of rabbit renal cortex.

Glomerular Isolation, Protein Extraction, and Western Blots—Rabbit glomeruli were isolated from New Zealand White rabbits (2.0–2.5 kg)

1 The abbreviations used are: PCLP1, podocalyxin-like protein 1; kb, kilobase pair; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PAGE, polycrylamide gel electrophoresis; WGA, wheat germ agglutinin; GST, glutathione S-transferase; DMEM, Dulbecco’s modified Eagle’s medium.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) U35239.

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## References

1. *Podocalyxin-like Protein 1 from Rabbit as a Transmembrane Protein of Glomerular Podocytes and Vascular Endothelium*.

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by iron oxide magnetization as described previously (11). For glomerular extraction, 5 \times 10^4 glomeruli were suspended in 1 ml of PBS containing 1% Triton X-100, 0.1% SDS, 2 mM phenylmethylsulfonyl fluoride, 5 mM N-ethylmaleimide, 2 mM EDTA, and 8 M urea and sonicated in six short bursts of 10 s as described previously (10). Iron oxide and debris were removed by centrifugation. The supernatant was stored at -70°C. Glomerular extracts were analyzed by SDS-PAGE. For Alcian blue staining, gels were fixed in 25% isopropanol overnight, stained with 0.1% Alcian blue in 2% acetic acid, and destained with 1% acetic acid. Blots (model SBD-1000 polyblot, American Bionetics, Hayward, CA) were performed as described previously (12). After transfer, lanes were excised and nitrocellulose strips were incubated with 125I-WGA. After incubation an equal volume of PBS containing 1% bovine serum albumin (Sigma) was used for 125I-WGA. Western blots were incubated with the primary antibody 4B3, 5F7, or a control mAb (BB5) that does not recognize rabbit tissues. All mAbs were subclass IgG1. Alternatively blots were incubated with polyclonal rabbit anti-mouse antibody (JacksonImmunoresearch, Philadelphia, PA) and then gold conjugated polyclonal goat anti-rabbit antibody (JacksonImmunoresearch, Philadelphia, PA). Immunoperoxidase and Immunofluorescence Studies—Kidney sections (1 mm) were microwaved in 0.5% glutaraldehyde according to the protocol of Login et al. (15). Sections (2 mm) were cut on a cryostat for subsequent analysis using the immunoperoxidase methodology according to Vector Laboratories (Burlingame, CA). For immunofluorescence, cryostat sections of rabbit renal cortex were used. Immunoperoxidase and immunofluorescence reactions were performed using the primary antibodies described for Western blot.

cDNA Library, Screening, and Sequence Analysis—Total RNA was prepared from isolated glomeruli and renal cortex by modification of the CsCl/guanidine isocyanate method of Chirgwin et al. (16) as described previously (17). Polyadenylated RNA isolated from these preparations was then reverse transcribed into cDNA using random primers and a cDNA library was constructed in Uni-ZAP XR Lambda vectors through the custom library services of Stratagene, Inc. (La Jolla, CA). These libraries were screened using the 4B3 and 5F7 mAbs by the method of Young and Davis (18). The cDNA inserts were rescued in Bluescript SK- phagemid by the in vivo excision protocol of Stratagene or by excision and ligation into Bluescript SK- plasmid. Subsequent screens were performed using cDNA inserts as probes (19). Double-stranded DNA sequencing was done by the dieoxy chain termination method of Sanger et al. (20) using the Sequenase kit (U.S. Biochemical Corp.). For regions of high secondary structure dimethyl sulfoxide 10% (v/v) was added to the reactions (21). All clones shown were in both directions. Size and identity of cDNA ends were performed using 2 \times 10^3 cpm of glomerular RNA and a kit from Life Technologies, Inc. according to the manufacturer’s protocol. The PCR product was ligated into the pAMP vector (Life Technologies Inc.) and used to transform DH5α competent cells. Data base management and sequence analysis was done with version 8.0 of the Wisconsin Sequence Analysis Package (Genetics Computer Group, Madison, WI). Data base searches were performed using the Blast Network Service from the National Center for Biotechnology Information on the "nonredundant" data base from the Brookhaven Protein Data Bank, Genbank, EMBL, PIR, and SwissProt data bases (22).

Construction and Purification of Fusion Proteins—The fusion protein derived from the extracellular domain was prepared as follows: A region of the PCLP1 extracellular domain (bases 490-1002) was PCR-amplified using the primers TTTGATATCGGCGCTACGTCGAAGGCTT and TTTGATATCGGCGCTACGTCGAAGGCTT and TTTGATATCGGCGCTACGTCGAAGGCTT. The expression vector pGEX-KT and the PCR product were digested with EcoRI and BamHI, purified, and ligated. The ligation mixture was used to transform competent Escherichia coli TG1. The reading frame was confirmed by DNA sequencing. Fusion protein expression was performed as described by Smith and Johnson (23). Fusion protein purification was performed as described by Guan and Dixon (24).

Immunoadsorption Studies—mAbs 4B3 and 5F7 culture supernatants were assayed by dilution using immunofluorescence intensity as an end point. In the experiment shown, mAb 4B3 (1/25 dilution in PBS containing 1% bovine serum albumin) was incubated with the purified PCLP1-GST fusion protein (20 \mu g) or a fusion protein derived from the extracellular domain of GLEPP1 (20 \mu g) constructed in the same fusion protein system (12).

For transfection experiments COS-7 cells were plated at 2.7 \times 10^5 cells/60-mm dish overnight in DME (BioWhittaker, Walkersville, MD) with 10% newborn calf serum. Cells were washed once with serum-free DME before DME with Lipopectamine (Life Technologies, Inc.) 6 \mu M/1 ml and 2 \mu g of either the PCLP1 mammalian expression construct or pcDNA3 vector. After 6 h of incubation, one volume of DME with 10% fetal calf serum and 10% newborn calf serum was added. Cells were lysed and extracted at 24 h after transfection in 200 \mu M PBS containing 1% Triton X-100. 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 5 mM N-ethylmaleimide, 2 mM EDTA, and 8 M urea. Protein content of the extracts were analyzed by a modified Bradford technique (Bio-Rad, Richmond, CA).

Partial Purification of PCLP1 and Digestion with Glycosidases—Rabbit glomerular extract from 450,000 glomeruli was prepared in 9 ml of glomerular extraction buffer (PBS containing 1% Brij 35, 2 mM EDTA, leupeptin (2 \mu g/ml), and aprotinin (2 \mu g/ml)). The extract was clarified at 100,000 \times g for 1 h at 4°C. 1% Triton X-100, 0.1% SDS, 5 mM N-ethylmaleimide, 2 mM EDTA, and 8 M urea. Protein content of the extract was digested with a modified Bradford technique.

For transfection experiments COS-7 cells were plated at 2.7 \times 10^5 cells/60-mm dish overnight in DME (BioWhittaker, Walkersville, MD) with 10% newborn calf serum. Cells were washed once with serum-free DME before DME with Lipopectamine (Life Technologies, Inc.) 6 \mu M/1 ml and 2 \mu g of either the PCLP1 mammalian expression construct or pcDNA3 vector. After 6 h of incubation, one volume of DME with 10% fetal calf serum and 10% newborn calf serum was added. Cells were lysed and extracted at 24 h after transfection in 200 \mu M PBS containing 1% Triton X-100. 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 5 mM N-ethylmaleimide, 2 mM EDTA, and 8 M urea. Protein content of the extracts were analyzed by a modified Bradford technique.

The first three 5-mL fractions containing the PCLP1 were dialyzed against four changes of 10 mM Tris-HCl, pH 7.5, and finally against distilled water. These fractions were then lyophilized, and the dry powder was dissolved in a minimum volume of 20 mM Tris-HCl, pH 7.5.

The wheat germ agglutinin purified fraction of PCLP1 (8 \mu l containing 16 \mu g of total protein) was mixed with 1 \mu l of 2% SDS and 1 \mu l of 2-mercaptoethanol and kept in boiling water bath for 5 min. The mixture was diluted to 200 \mu l in 20 mM Tris-HCl, pH 7.5. 20 \mu l aliquots of the above preparation were digested with heparinases I and II, heparitinase mix (0.5 units each), chondroitinase ABC (0.5 units), N-glycosidase F (0.4 units), O-glycosidase (2 milliunits), and neuraminidase (0.1 unit) by incubating at room temperature overnight followed by an additional 3 h at 37°C (endo-β-galactosidase and N- and O-glycosidase were obtained from Boehringer Mannheim; the other enzymes were obtained from Sigma). Multiple enzyme digestions were done similarly by adding the enzymes sequentially to the aliquot of denatured PCLP1 buffer mix. For endo-β-galactosidase digestion, 8 \mu l of wheat germ agglutinin was boiled as described above but diluted with sterile distilled water. Endo-β-galactosidase digestion was performed in 50 mM sodium acetate buffer, pH 5.8, with 5 milliunits of the enzyme. Aliquots without the addition of any enzyme incubated under identical conditions served as controls. After incubation an equal volume of 2 \times 50 mM SDS loading buffer with 2-mercaptoethanol was added, and the reaction mixtures were kept in boiling water bath for 5 min, separated on SDS-PAGE, and immunoblotted with 483 and BB5 mAbs as described above.
mAbs prepared from splenocytes of mice immunized against whole rabbit glomeruli were selected based upon immunofluorescent patterns on cryostat sections of rabbit renal cortex. mAbs 5F7 and 4B3 showed the following properties.

Immunostaining Pattern on Cryostat Sections of Renal Cortex—mAbs 5F7 and 4B3 produced an intense reaction product in glomeruli and also stained endothelium of arteries, veins, and peritubular capillaries (Fig. 1a).

Western Blotting—Blots of extracts from isolated glomeruli developed with mAbs 5F7 and 4B3 showed a major band at 140 kDa under reducing and nonreducing conditions (Fig. 1b).

Alcian Blue Binding—A major band at 140 kDa was also recognized by Alcian blue (Fig. 1b).

125I-WGA Binding—A major band at 140 kDa was also bound by 125I-WGA on a Western blot of rabbit renal glomerular extract.

Abolition of 125I-WGA Binding by Prior Neuraminidase Treatment—125I-WGA binding was abolished by prior neuraminidase treatment of the blot (Fig. 1b). In all reduced gels in this experiment, a second band at lower molecular mass was recognized by each detection system, thereby providing further data that the same target molecule was being recognized by the mAb, Alcian blue, and WGA.

Ultrastructural Localization of mAb Binding to Podocyte Foot Processes and Endothelium—Fig. 1c shows immunogold labeling of PCLP1 localized to the nonbasement membrane surface of the epithelial foot processes and to a lesser extent the endothelial plasma membrane. No immunogold staining was observed in the absence of primary antibodies or the presence of nonspecific anti-rabbit IgG. These characteristics are identical to those defined by Kerjaschki, Sharkey, and Farquhar (1) for rat podocin. We therefore elected to call the molecule recognized by these mAbs podocin-like protein 1.

Cloning and Sequencing of PCLP1 cDNAs

A rabbit glomerular cDNA library was constructed in Uni-Zap XR vector and screened with mAbs 5F7 and 4B3 in an expression system. Six positive cDNA clones were isolated with overlapping nucleotide sequence. 42 additional cDNAs were obtained by using these cDNAs as probes and by anchored PCR strategies. Fig. 2 (upper panel) shows in a diagrammatic form nine selected overlapping cDNAs used to construct the nucleotide sequence.

Northern blot analysis was performed to determine the approximate length of the transcript and to show the pattern of mRNA tissue expression. Northern blots performed using a 3.5-kb PCLP1 clone showed a major band at approximately 5.5 kb with minor bands at 7.1 and 4.4 kb (Fig. 3). Screening of different tissues shows the relatively much greater amount of mRNA in glomerulus as compared with other tissues (Fig. 3). This relative distribution of PCLP1 mRNA is similar to PCLP1 protein distribution as assessed by immunofluorescence using the 5F7 and 4B3 mAbs.

The full length of the nucleotides sequenced from the overlapping cDNA clones spans 5313 base pairs (Figs. 2 and 4). An initiator methionine (base pairs 304–306) was identified by the following criteria, (a) The amino acid sequence was consistent with Kozak’s consensus sequence (first methionine in the open reading frame, purine in position –3 (25); (b) this methionine was followed by a 21-amino acid putative signal peptide containing 12 consecutive hydrophobic amino acids (ALA-LAALLLL (Fig. 4) (26); (c) the presence of numerous CpG-rich “islands” is compatible with this region being 5′-untranslated sequence (underlined in Fig. 4) (27). A stop codon was found at base pairs 1957–1959, indicating the end of the open reading frame (Fig. 4). This would correspond to an open reading frame of 1653 base pairs or a total of 551 amino acids. If the putative signal peptide (21 amino acids) is cleaved off in post-translational processing, then the mature protein will be 530 amino acids long, and the N-terminal amino
region were analyzed for the presence of potential
sequence. The remaining 429 amino acids of the extracellular
PCLP1 open reading frame are typical of a signal peptide
Computer Group sequence software package indicates there
amino acid sequence using the Motifs program of the Genetics
N
putative transmembrane domain must be extracellular.
Thus the region of the molecule N-terminal to the
these two mAbs also bind to nonpermeabilized isolated rabbit
thecloneJo3isolatedusingthemAbscodesfortheregionofthe
transmembrane region (Figs. 2 and 4). Immediately C-terminal
to the putative transmembrane region are positively charged
amino acids (HQRSLHRK) as is typically found at the cyto-
plasmic side of a transmembrane region (28). This orientation
relative to the cell membrane is also supported by the fact that
the clone Jo3 isolated using the mAbs codes for the region of
the molecule N-terminal to the transmembrane region and that
these two mAbs also bind to nonpermeabilized isolated rabbit
glomeruli as assessed by immunofluorescence (data not
shown). Thus the region of the molecule N-terminal to the
putative transmembrane domain must be extracellular.

Analysis of the Extracellular Domain

As indicated above, the N-terminal 21 amino acids of the
PCLP1 open reading frame are typical of a signal peptide
sequence. The remaining 429 amino acids of the extracellular
region were analyzed for the presence of potential O- and
N-linked glycosylation sites. Analysis of the putative PCLP1
amino acid sequence using the Motifs program of the Genetics
Computer Group sequence software package indicates there

are three potential sites for N-linked glycosylation (Fig. 4). One
of these potential N-linked sites (amino acid 333) is flanked
by leucines as has been described in chick lumican (29). The
putative PCLP1 amino acid sequence has five serine-threonine
clusters providing potential acceptor sites for O-linked oligo-
saccharides (Fig. 4). Rat podocalyxin has been suggested to
contain at least two N-linked oligosaccharide groups (8) and
O-linked oligosaccharide groups (1, 2). There is one potential
glycosaminoglycan attachment site (amino acids 215–218) that
contains the consensus sequence (SGXG) found in small pro-
teoglycans (30). There are four other glycine-serine or serine-
glycine groups that could also potentially serve as attachment
sites for glycosaminoglycan chains, although they lack associ-
ated acidic residues that are frequently but not always found
adjacent to glycosaminoglycan attachment sites (31, 32).

There are several other features of the extracellular domain
that appear to be noteworthy. A 24-amino acid span (amino
acids 245–268) is very rich in serine and proline (21 of 24 amino
acids) and lies in the middle of the extracellular domain (Figs.
2 and 4). There are 4 cysteines available in the extracellular
domain for potential disulfide linkage to form two loop struc-
tures (Figs. 2 and 4). One clone (GN2) from the glomerular
cDNA library contained an additional 60-nucleotide insert (1324–
1384) coding for 20 additional amino acids (Figs. 2 and 4). This
putative alternately spliced region contains an unusual series
of 3 alternating glutamines followed by 3 alternating glutamic
acids (QRQSQGEGETE). The extracellular domain has 5 dou-
blets of acidic amino acids (281–282, 366–367, 430–431, 445–
446, and 448–449) similar to clusters of acidic amino acids
thought to mediate calcium binding in other proteins (33, 34).

Analysis of the Intracellular Domain

The intracellular domain contains 75 amino acids (amino
acids 477–551). Overall this region is highly acidic (pl = 4.3).
There are two potential casein kinase II phosphorylation sites
(amino acids 511 and 539) and one potential protein kinase C
phosphorylation site (amino acid 481). At the C-terminal end

Fig. 2. Diagrammatic illustration of PCLP1 cDNA and derived
protein structure. Top, diagrammatic representation of the cDNAs
used to derive the PCLP1 nucleotide structure. Clone Jo3 is one of the
cDNAs cloned with the mAbs 5F7 and 4B3. Clone RACE 10 was cloned
by PCR techniques. The remainder of the cDNAs shown were obtained
using labeled cDNAs as probes. All clones were sequenced in both
directions. Clone GN2 contains a 60-nucleotide amino acid putative
alternative splice region in the coding region (see below). Bottom, dia-
agrammatic representation of PCLP1 protein structure derived from the
nucleotide sequence and aligned with a Kyle-Doolittle amino acid hy-
Fig. 3. Northern blot developed using a 3.5-kb PCLP1 cDNA as
a probe. Glomerular RNA was loaded in lanes A (30 μg) and B (5 μg).
Lanes C–H were each loaded with 30 μg of RNA from renal cortex (lane
C), liver (lane D), lung (lane E), intestine (lane F), spleen (lane G), and
muscle (lane H). The major transcript is at 5.5 kb, with minor bands at
4.4 and 7.1 kb. The intensity of the signal is comparable between lane
B (5 μg of glomerular RNA) and lane C (30 μg of renal cortical RNA),
confirming that the amount of transcript detected is highest in the
glomerular RNA sample. The positions of the 28S and 18S ribosomal
RNA bands are shown (arrowheads). The blot was stained with meth-
ylene blue prior to transfer to confirm comparable loading of RNA onto
lanes A and C–H with 6-fold less RNA loaded onto lane B.
there is a highly acidic 10-amino acid region containing 4 aspartic acid and 3 glutamic acid residues (pI 3.5).

Fusion Protein and Immunoadsorption Studies
To demonstrate that the cDNA sequence obtained codes for a protein recognized by the mAbs, a PCLP1 fusion protein was prepared. The region of the cDNA corresponding to amino acids 63–233 (Fig. 4) was amplified by PCR, ligated into the expression vector pGEX-KT, and expressed in frame with GST in E. coli TG1. After purification by glutathione affinity chromatography, the fusion protein was used to demonstrate the capacity to immunoadsorb the binding of the mAbs 5F7 and 4B3 to kidney tissue (Fig. 5A). The fusion protein of another podocyte protein, GLEPP1 (12), did not immunoadsorb the binding of mAbs 5F7 and 4B3. Thus, this region of the cDNA (bases 490–1002) codes for an epitope recognized by mAbs 5F7 and 4B3.

FIG. 4. PCLP1 nucleotide and derived amino acid sequences obtained from cDNA sequencing. The initiation methionine was identified as the first ATG in the open reading frame and obeys Kozak’s consensus. The underlined nucleotides in the putative 5′-untranslated region represent probable CpG regions. The underlined N-terminal 21 hydrophobic amino acids represent a putative signal peptide. The double underlined 26 hydrophobic amino acids represent a putative transmembrane region. Potential sites of N-linked glycosylation (black triangles), O-linked glycosylation (dotted underlines), glycosaminoglycan attachment (dashed underlines), and disulfide linkage (black circles) are shown. The 60-nucleic acid sequence number 1324–1383 (lowercase) was found only in clone GN2.
The PCLP1-GST fusion protein was also used to immunize guinea pigs to raise polyclonal antibodies. On Western blot both the polyclonal antiserum and mAb 4B3 recognized a major band at approximately 140 kDa under both reducing and nonreducing conditions (Fig. 5B). The fusion protein was able to immunoadsorb the binding of both polyclonal and monoclonal antibodies to PCLP1 as assessed by immunofluorescence and Western blot (Fig. 5A and B). Furthermore both the polyclonal anti-PCLP1 fusion protein serum and mAb 4B3 showed identical staining on immunofluorescence of the glomerulus and peritubular capillaries to that seen with the monoclonal antibodies (Fig. 5C). We conclude that the cDNAs cloned code for a molecule with the same size and distribution as that recognized by the monoclonal antibodies.

**The Effect of Processing on the Apparent Size of PCLP1 Protein**

The calculated molecular mass of the PCLP1 protein core from the derived amino acid sequence is about 55 kDa. In vitro transcription/translation of PCLP1 cDNA (bases 1–2943) in a T7 coupled reticulocyte lysate system shows the cDNA to encode a protein with an apparent molecular mass of 70 kDa (Fig. 6, left panel). Thus the unprocessed PCLP1 molecule migrates more slowly on SDS-PAGE than expected from its calculated size. Similar differences in calculated and apparent molecular mass have been reported for chromogranin A, an acidic protein similar to PCLP1 (35), and for the core proteins of proteoglycans such as syndecan (36) and neurocan (37). After processing by canine pancreatic microsomal membranes, the apparent molecular mass increases to about 80 kDa, representing the core proteins of proteoglycans such as syndecan (36) and neurocan (37). After processing by canine pancreatic microsomal membranes, the apparent molecular mass increases to about 80 kDa, representing the core proteins of proteoglycans such as syndecan (36) and neurocan (37). Processing by canine pancreatic microsomal membranes increases the apparent molecular mass of the PCLP1 core protein to approximately 80 kDa. The presence of microsomal membranes results in an increase in the apparent molecular mass to approximately 80 kDa. Right, expression of the PCLP1 protein in COS-7 cells. Western blotting with mAb 4B3 of extracts of COS-7 cells transfected with PCLP1 cDNA (base pairs 1–2943 in pcDNA3 vector) (lane G) shows the presence of a 140-kDa band that runs slightly higher than the band seen from a glomerular extract from isolated rabbit glomeruli (lane H). COS-7 cells alone (lane E) or COS-7 cells transfected with vector alone showed no band (lane F). Western blot of the same extract as that run in lane G but developed with a control monoclonal antibody (BB5) showed no band (lane I). These data clearly show that COS-7 cells transfected with PCLP1 cDNA produce a 140-kDa protein.
from in vitro enzymatic digestion with molecular mass of 140 kDa. Cells and from glomerular extract have the same apparent mass as described for podocalyxin (2). No shift was seen on digestion with O-glycosidase (Fig. 7, lane G).

That PCLP1 binds Alcian blue and has potential glycosaminoglycan attachment sites (Figs. 1B and 4) suggests it may be a proteoglycan. However, digestion with heparanases (Fig. 7, lane B), chondroitinase ABC (Fig. 7, lane C), heparanase and chondroitinase ABC (Fig. 7, lane D), and endo-β-galactosidase (Fig. 7, lane L) yielded no significant change in apparent molecular mass. Similar results were obtained for digests performed in the absence of SDS or 2-mercaptoethanol (data not shown). As a positive control blots of the same digests were probed with mAb 4C3, which we have recently reported and used to clone the podocyte membrane protein tyrosine phosphatase GLEPP1 (I2). These experiments clearly showed changes in molecular mass of GLEPP1 on incubation with chondroitinase ABC, N-glycosidase F, O-glycosidase, and neuraminidase (data not shown). Thus the conditions used allowed digestion in the same incubation mix of another glomerular epithelial glycoprotein. This result suggests that the glycosidic linkages of PCLP1 are not accessible to the glycosidic enzymes, that PCLP1 might be an unusual glycoconjugate not susceptible to the enzymes used, or that the post-translational increase in apparent molecular mass is not due to glycosylation.

Conclusions About the Glycosylation of PCLP1

The difference in the apparent molecular mass of the PCLP1 from in vitro transcription/translation system (70 kDa) and transfected cells (140 kDa) reflects a high degree of post-translational modification. However, as noted above, commonly used glycan linkages were not detected by digestion experiments described above. Farquhar and colleagues have determined that podocalyxin is highly glycosylated with 20% hexose, 4.5% sialic acid (1), and additional N-acetylglucosamine (2). Endothelial podocalyxin has been suggested to contain mixed glycosylated or an unusual oligosaccharide structure (38). PCLP1, with only about 50% of its apparent molecular mass due to its peptide core, also appears to be heavily glycosylated, is likely sulfated based on its Alcian blue binding, and may contain an unusual oligosaccharide/glycosaminoglycan structure based on its resistance to glycosidase digestion.

The Relationship of PCLP1 to Podocalyxin

We cannot conclude from these studies that PCLP1 (characterized in rabbit) is identical to podocalyxin (characterized in rat) because anti-podocalyxin (rat) IgG is reported not to react across species (39). The antibodies we have identified and developed also do not react across species. However, PCLP1 does appear to be similar to podocalyxin in all respects examined including molecular mass, sialylation, Alcian blue binding, and tissue distribution. Definitive confirmation will be available once rat podocalyxin is cloned.

The identification of the nucleotide sequence for this major protein distributed on glomerular epithelial cell and vascular endothelial cell surfaces is an important step toward understanding the role this molecule may play in glomerular and endothelial cell biology and pathology.

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Note Added in Proof—During the review of this manuscript, the following short human sequence submissions to GenBank® were found to be homologous to the 3’-untranslated region of PCLP1: T87928, R99975, R99976, H52052, T87719, and H64714.
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