Identification and Characterization of Podocalyxin—the Major Sialoprotein of the Renal Glomerular Epithelial Cell

DONTSCHO KERJASCHKI, DAVID J. SHARKEY, and MARILYN GIST FARQUHAR
Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510

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ABSTRACT The glomerular epithelial polyanion is a specialized cell surface component found on renal glomerular epithelial cells (podocytes) that is rich in sialoprotein(s), as detected by staining with cationic dyes (colloidal iron, alcian blue) and wheat germ agglutinin (WGA). We have isolated rat glomeruli and analyzed their protein composition by SDS PAGE in 5–10% gradient gels. When the gels were stained with alcian blue or "Stains All," a single band with an apparent M_r of 140,000 was detected that also stained very prominently with silver, but not with Coomassie Blue. This band predominated in fluorograms of gels of isolated glomeruli that had been labeled in their sialic acid residues by periodate-[^{3}H]borohydride. In lectin overlays, the 140-kilodalton (kd) band was virtually the only one that bound [^{125}I]wheat germ agglutinin, and this binding could be prevented by predigestion with neuraminidase. [^{125}I]Peanut lectin bound exclusively to the 140-kd band after neuraminidase treatment. An antibody was prepared that specifically recognizes only the 140-kd band by immunoprecipitation and immuneoverlay. By immunoperoxidase and immunogold techniques, it was localized to the surface coat of the glomerular epithelium and, less extensively, to that of endothelial cells. When analyzed (after electroelution from preparative SDS gels), the 140-kd band was found to contain ~20% hexose and ~4.5% sialic acid.

These findings indicate that the 140-kd protein is the major sialoprotein of the glomerulus, and it is the only component of glomerular lysates with an affinity for cationic dyes and lectins identical to that defined histochemically for the epithelial polyanion in situ. Since this molecule is a major component of the cell coat or glycocalyx of the podocytes, we have called it "podocalyxin."

Glomerular epithelial cells or "podocytes" are endowed with an unusually highly negatively charged cell surface coat, called "epithelial polyanion," which was originally detected using various cationic histochemical stains such as colloidal iron (1–4), alcian blue (4) and ruthenium red (5), and was identified as sialic acid–rich based on the sensitivity of the staining to digestion with neuraminidase (1–4). More recently its sialic acid–rich nature was corroborated by the finding that fluorescent- or peroxidase-labeled wheat germ agglutinin (WGA)[1] binds to the visceral glomerular epithelium in several species (6–9), and this binding is sensitive to neuraminidase treatment. The presence of stainable epithelial polyanion is believed to be essential for maintaining the normal epithelial organization because when it is absent (e.g., in certain glomerular diseases [4, 10] and in differentiating glomeruli [11]), the usual foot process and filtration slit organization is distorted or absent, and because derangement of this organization and collapse of the slits can be induced by infusion of polycationic compounds into the kidney (12).

There is little information available on the specific cell surface components responsible for the staining. Nevins and Michael (13) identified three sialic acid–rich glycopeptides from tryptic digests of rat glomeruli, raised antibodies against two of these (a ~110-kilodalton [kd] band and one of undetermined M_r), and found that they stained the visceral epithelium. It was not clear, however, whether these proteolytic fragments were derived from one or several cell surface components.

In this report we have taken advantage of the known histochemical staining and lectin-binding properties of the epithelial polyanion in situ to identify and to isolate the molecular component responsible for the histochemical staining. We have identified a single sialoprotein with an apparent M_r (on 5–10% SDS gradient gels) of 140,000, and have

[1] *Abbreviations used in this paper:* Con A, concanavalin A; kd, kilodaltons; WGA, wheat germ agglutinin.

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obtained evidence that this protein constitutes the main component of epithelial polyanion.

MATERIALS AND METHODS

Materials: Alcian blue GX was obtained from Sigma Chemical Co. (St. Louis, MO); [^{3}H]borohydride from Research Products International Corp. (Mount Prospect, IL), and Fluoram from Roche (Nutley, NJ). The sources of other reagents were the same as given previously (14).

Isolation of Glomeruli: Rat kidneys were perfused with ice-cold Tris-buffered saline, containing protease inhibitors (1 μg/ml pepstatin A, 1 μg/ml antipain, 1 mM benzamidine, and 1 mM diisopropyl-fluorophosphate) via the abdominal aorta after which glomeruli were prepared by pressing cortical tissue through graded sieves (14). The purity and yield of the preparations was assessed by phase-contrast microscopy.

SDS PAGE: Freshly isolated glomeruli were pelleted in a microfuge, suspended in SDS PAGE sample buffer (3.65% SDS, 18 mM dithiothreitol, 4.5 mM EDTA, 40 mm Tris-phosphate buffer, pH 6.8, 10% glycerol) at a protein concentration of ~2 mg/ml, and heated for 5 min in a boiling water bath. With this protocol ~95% of the counts that had been incorporated into sialic acid residues by the periodate-[^{3}H]borohydride procedure (see below) were solubilized. The extract was cleared by spinning for 5 min in a microfuge, and aliquots were loaded on 5–10% gradient gels in a Maizel-buffer system (15). Gels were run at ~20 mA constant current for 4–6 h after which they were fixed in 25% isopropanol for 12 h at 20°C and stained with 0.1% Alcian blue GX in 3% acetic acid (16) or "Stains All" to detect sialoproteins (17). After destaining in distilled water the gels were photographed, the position of the blue band(s) was marked, and subsequently, they were restained with silver (18). Other gels were stained and fixed in 0.2% Coomassie Blue in 50% methanol-7% acetic acid.

Chemical Analysis: Preparative SDS gradient gels (5–10%, 3-mm thick) were run as described above, side lanes were stained with Stains All, and the corresponding region of the unstained gel was cut out and electroeluted. Protein was determined by the Fluoram method (19) using BSA as standard, total hexose by the phenol-sulfuric acid reaction (20) with glucose, mannose, fucose, and galactose as standards, and sialic acid by the thiobarbituric acid method (21).

Transferring to Nitrocellulose and Lectin Overlays: SDS gels were transferred onto nitrocellulose paper (22) for 12 h at 4°C. The transfer was stained with Ponceau S (23), and the gels were then stained with silver (18) to assess the completeness of the transfer (25). The stained paper strips were excised and incubated with radioiodinated (24) WGA, concanavalin A (Con A), or peanut lectin, and exposed for autoradiography. Some paper strips were digested with neuraminidase (0.05 U/ml, in 50 mM Na acetate buffer, pH 5.5, with 10 mM Ca-acetate) for 24 h at 37°C, before lectin overlay (26). As controls, the bound radioactive lectins were released by incubation of the paper strips in the appropriate competing sugar (200 mM) (26) after autoradiography.

Preparation of Antibodies: To prepare a partially purified fraction of glomerular sialoproteins for immunization of a rabbit, we took advantage of the known affinity of the proteins of interest for WGA and fractionated glomerular lysates on WGA-Sepharose columns. Isolated glomeruli were extracted in 0.2% Triton X-100 in PBS containing protease inhibitors (20 min at 20°C), and the insoluble material was removed by centrifugation (50,000 g for 20 min). The supernatant and the pellet were assayed for protein (27) and sialic acid (21) content, and 1% sodium deoxycholate was added. About 75% of the total glomerular sialic acid was solubilized by this procedure. The extract was then incubated with WGA-Sepharose 4B for 12 h at 4°C, and unbound material was removed by washing (four times) in PBS containing 0.1% Triton. The sialic acid–rich material that bound to the WGA column (28) was released with 120 mM *N*-acetyl-β-glucopyranoside in PBS with 0.1% Triton, precipitated with 0.2% phosphotungstic acid in 0.2 M HCl, and pelleted in a microfuge. About 85% of the sialic acid but only ~3.7% of the protein was recovered. The pellet was then extracted in 1 ml acetone for 20 min at 4°C, air dried, and analyzed by SDS PAGE. Two prominent bands (~125 and ~110 kd) were seen after staining with Stains All (see Fig. 2, lane *4*). In silver-stained gels, a number of other bands (~330 and 70 kd and several smaller bands) were detected as well (see Fig. *2*, lane *2*). Since attempts to further subfractionate the lysate were unsuccessful, the entire WGA-bound material was used as the antigen. For immunization, it was suspended in 0.5 ml PBS, and mixed with complete Freund's adjuvant.

A rabbit was immunized according to the schedule of Louvard et al. (29). 10 d after the last injection, the rabbit was bled, and IgG was purified on Protein A-Sepharose 4B (14). To remove contaminating gp330 cross-reactivity, the IgG was circulated over a gp330 column (14).

Characterization of IgG by Immunoprecipitation and Immuneoverlay: Freshly isolated glomeruli were labeled on their sialic acid residues with periodate-[^{3}H]borohydride (30), and lysed in RIPA buffer containing protease inhibitors. About 90% of the incorporated counts were solubilized. Immunoprecipitation and analysis of precipitates (5–10% gradient SDS gels) were performed as described previously (31). Gels were soaked in En^{3}Hance and exposed for fluorography.

Nitrocellulose transfers were incubated in hemoglobin buffer (0.1% hemoglobin, PBS) for 1 h at 20°C for quenching, and then in immune (anti-sgp140) IgG (20 μg/ml) in hemoglobin buffer for 1 h at 20°C. The overlays were washed, incubated with 3×10^6 cpm of ^{125}I-labeled goat anti-rabbit F(ab')$_2$ fragments, and then washed and exposed for autoradiography (25).

Immunocytochemistry: For immunoperoxidase, cryostat sections of fixed rat kidneys were incubated sequentially in immune (anti-sgp140) IgG, peroxidase-conjugated sheep anti-rabbit Fab, and diaminobenzidine medium (14, 32–34). For immunogold localizations, cryostat sections were incubated in anti-sgp140 IgG and Protein A-gold conjugate and processed as described previously (34).

RESULTS

Identification of Sialoproteins in Glomerular Lysates by Selective Staining of SDS Gels

When freshly isolated glomeruli were directly extracted in SDS sample buffer and their constituents were analyzed by SDS PAGE, multiple bands were seen after Coomassie Blue or silver staining (Fig. 1, lanes *1* and *2*). When gels were stained with alcian blue (not shown) or Stains All (Fig. 1, lane *3*), procedures that are known (16, 17) to detect mainly sialoproteins and other highly anionic glycoproteins and proteoglycans, a single band with an apparent M_r of 140,000 was seen. In heavily loaded gels, two additional, more lightly stained bands of greater mobility (~125 and 110 kd) were also detected. After silver staining, a diffuse band (probably corresponding to extracted proteoglycans) was also detected at the top of the gels (Fig. 1 *a*, lane *2*). When the 140-kd band was marked in Stains All–treated gels, and the gel was subsequently stained with silver, a prominent silver-positive band was observed in the 140-kd position (Fig. 2, lane *1*) which was not detected by Coomassie Blue staining (Fig. 1, lane 1). This is in keeping with the finding that other heavily sialylated glycoproteins such as glycophorin (35) do not stain with Coomassie Blue. Therefore, the 140 kd-band was by far the most prominent band after staining glomerular extracts with basic dyes.

Identification of Sialoproteins in Lectin Overlays

Since it has been shown by histochemical staining that the glomerular epithelium has a high affinity for WGA and peanut lectin (6–9) (the latter only after neuraminidase treatment), we tested the binding of these lectins to individual glomerular proteins (separated on SDS gels) in nitrocellulose transfers. In autoradiograms of nitrocellulose strips (containing the entire protein pattern of glomerular extracts) that had been incubated with [^{125}I]Con A, numerous bands were detected, including the 140-kd band (Fig. 1, lane *4*). By contrast, with [^{125}I]WGA, a single prominent 140-kd band was detected (Fig. 1, lane *5*). [^{125}I]WGA binding was completely abolished by pretreatment of the paper strips with neuraminidase (Fig. 1, lane *6*). Only after very long exposure of the transfers were a few other bands (~330, 125, 110, and 50 kd) detectable (not shown). With [^{125}I]peanut lectin, no binding was detected to any band (Fig. 1, lane *7*); however, after predigestion with neuraminidase the 140-kd band was labeled virtually exclusively (Fig. 1, lane *8*). Thus, the affinity of the 140-kd band

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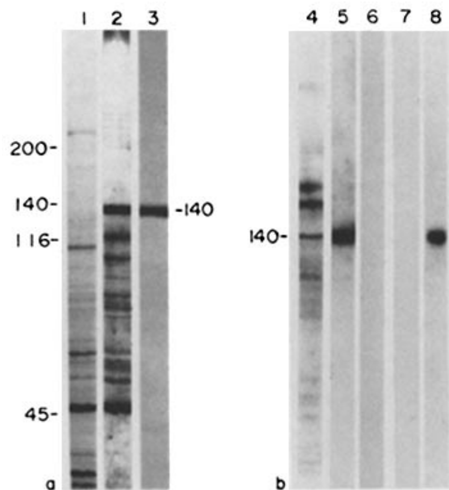

FIGURE 1 (*a*) Extracts of isolated glomeruli solubilized in SDS sample buffer and separated on a 5–10% gradient SDS gel. Lane 1, Coomassie Blue staining; lane 2, silver staining; lane 3, "Stains All" pattern. A prominent band with an apparent M_r of 140,000 is visible in the Stains All and silver-stained preparations that is not visible after Coomassie Blue staining. (*b*) Lectin overlays on glomerular extracts separated on 5–10% SDS gradient gels and transferred to nitrocellulose. Individual paper strips were incubated with [^{125}I]Con A (lane *4*); [^{125}I]WGA (lane *5*); neuraminidase, followed by [^{125}I]-WGA (lane *6*); [^{125}I]peanut lectin (lane *7*); or neuraminidase followed by [^{125}I]peanut lectin (lane *8*). Con A binds to a number of bands including the 140-kd band, but WGA and peanut lectin bind selectively to the 140-kd band (the latter only after neuraminidase treatment).

for WGA and peanut lectin is identical to that of the glomerular epithelium in histochemically stained preparations (6–9).

Identification of Sialoproteins by Radiolabeling of Sialic Acid Residues In Situ

When isolated glomeruli were radiolabeled with periodate-[^{3}H]borohydride to label sialic acid residues exposed on the cell surfaces (30) and the lysate was examined by SDS PAGE fluorography, a relatively simple pattern of sialoproteins was obtained (Fig. 2, lane *5*). Again, a 140-kd band predominated. In addition, however, several other bands (~330, 125, 100, and >50 kd) were also visible.

Thus, the 140-kd band is the predominant sialoprotein present in glomerular lysates as detected by three different approaches (cationic stains, WGA-binding, and periodate-[^{3}H]borohydride radiolabeling), and it has staining and lectin binding characteristics closely resembling those defined by histochemistry for glomerular epithelial polyanion in situ.

Characterization of the Antibodies Raised Against WGA-Sepharose Eluates By Immunoprecipitation and Immuneoverlay

To assist in the identification and characterization of glomerular sialoproteins, an antibody was raised against those components of Triton extracts of isolated glomeruli that bound to WGA-Sepharose. When periodate-[^{3}H]borohydride–labeled glomerular extracts (Fig. 2, lane *5*) were immunoprecipitated with IgG raised against the WGA eluate, the major protein precipitated was a 140-kd band. Traces of a ~330-kd band, and two bands with lower mobility (~125 and ~110 kd) were visible after long exposures (Fig. 2, lane *6*). When the IgG was deprived of its cross-reactivity to gp330 (31) by passing it over an affinity column (14) before use, no 330-kd band was precipitated.

When glomeruli were dissolved directly in SDS sample buffer, separated by SDS PAGE, transferred to nitrocellulose, and overlayed with gp330-depleted IgG, only the 140-kd band was seen (Fig. 2*b*, lane *7*). However, when transfers of Triton X-100 glomerular extract or the WGA-Sepharose binding material were similarly overlayed with the same IgG, no 140-kd band was detected but ~125- and ~110-kd bands as well as several smaller bands were visible (not shown).

From these results we conclude that when glomerular extracts are solubilized in neutral detergents (especially Triton X-100 and to a much less extent the SDS-containing RIPA buffer), (*a*) the 140-kd band is susceptible to degradation, and (*b*) the 125- and 110-kd bands are degradation products of the 140-kd band. Since the antibody recognizes primarily if

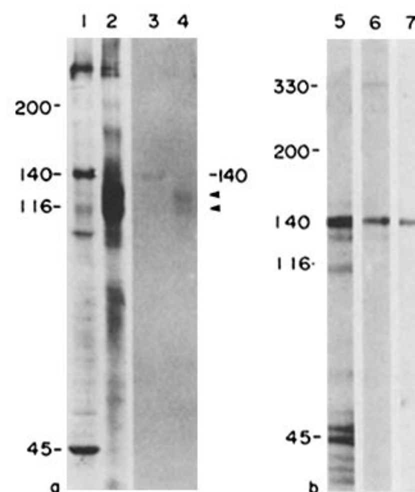

FIGURE 2 Protein patterns of glomerular extracts, showing the results obtained when glomeruli were directly extracted in SDS PAGE sample buffer (lanes *1* and *3*), or in 0.2% Triton–Tris-buffered saline followed by fractionation on WGA-Sepharose (lanes *2* and *4*). The gels were stained first in Stains All to detect sialoproteins (lanes *3* and *4*), the position of the blue band was marked by a punch (white dot) in the gel, and silver stain was subsequently applied (lanes *1* and *2*). In the Stains-All preparation of the SDS-extracted material (lane *3*), the 140-kd band is prominent, whereas in the Stains-All preparation of the WGA-purified Triton extract, two smaller bands, ~125 and 110 kd (arrows), are seen which are assumed to be degradation products of the 140-kd band. (*b*) Characterization of anti-sgp140 IgG by immunoprecipitation and immuneoverlay. For immunoprecipitation, isolated glomeruli were radiolabeled by the periodate-[^{3}H]borohydride procedure (to label sialic acid) and extracted in RIPA buffer. Lane *5*: Fluorogram of glomerular lysate showing the pattern of tritium labeling of glomerular sialoglycoproteins obtained. Lane *6*: Fluorogram of an immunoprecipitate prepared from the material in lane *5* with antiserum obtained from a rabbit immunized with the WGA-Sepharose eluate. The predominant band precipitated is the 140-kd band. In addition, faint bands with apparent M_r of ~330,000, ~125,000, and ~110,000 are also detected. The two latter are considered degradation products of the 140-kd band. Lane *7*: Immuneoverlay on a nitrocellulose transfer with the same IgG as above which had been affinity adsorbed against gp330. In this case isolated glomeruli were lysed immediately in SDS sample buffer before SDS PAGE, thereby avoiding proteolytic degradation, and only the 140-kd band is seen.

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not exclusively the 140-kd band in undegraded preparations, the antibody is referred to as anti-sgp140.

Immunocytochemical Results

LIGHT MICROSCOPY: In 0.5 μm frozen sections of fixed rat kidney, incubated with anti-sgp140 by indirect immunoperoxidase (Fig. 3), it was apparent that staining was concentrated at the surfaces of the visceral glomerular epithelial cells or podocytes. In addition, a faint staining of endothelial cells, both those of glomerular and peritubular capillaries, was seen. Pretreatment of the sections with neuraminidase did not alter the signal. Some staining was also seen in the proximal tubule brush border, but this was eliminated when IgG was adsorbed against gp330, a strong brush border antigen (31).

A preliminary survey of other organs indicates that among epithelia, sgp140 is restricted exclusively to the visceral glomerular epithelium, whereas endothelial staining is rather general since it was detected in capillaries of all organs surveyed (heart, exocrine pancreas, parotid, and small intestine). There was no staining of other cell types, erythrocytes included.

IMMUNOPEROXIDASE: By electron microscopy (Fig. 4), a thick layer of reaction product was seen on the entire exposed membrane surface of the glomerular epithelial cells, down to the slit diaphragms. A similar, but thinner layer of reaction product was also found along the soles of the foot processes which abut the basement membrane. In addition, there was a light staining of the endothelium of glomerular and peritubular capillaries. Occasionally some granular reaction product was also visible within the basement membrane, which is believed to result from the diffusion of diaminobenzidine reaction product and its readsorption onto the glomerular basement membrane (36). In heavily reacted specimens, reaction product was also detected in some Golgi cisternae and in some endoplasmic reticulum cisternae, including the perinuclear cisterna.

IMMUNOGOLD: After indirect immunogold labeling with anti-sgp140, a heavy concentration of gold particles was detected on the exposed surfaces of the glomerular epithelial cells where they face the urinary spaces (Fig. 5). The gold particles did not penetrate very deeply into the filtration slits, nor did they bind to the base of the foot processes, presumably owing to the limited diffusion of these large particles. Gold particles were also localized in clusters along the luminal membrane of both the glomerular endothelium and the endothelium of peritubular capillaries.

CHEMICAL COMPOSITION OF THE 140-KD SIALOPROTEIN: When the 140-kd band was electroeluted and checked for purity by rerunning it on SDS PAGE, a single band was seen at the 140-kd position. Analyses of the eluted 140-kd band indicated that it contains ~20% total hexose and 4.5% sialic acid/mg protein.

DISCUSSION

In this investigation we have identified a single sialoprotein with an apparent M_r of 140,000 (in 5–10% gradient SDS gels) as the predominant sialoprotein present in glomerular extracts. Moreover, we have obtained evidence that this protein is the biochemical equivalent of the histochemically defined "epithelial polyanion." Identification is based on the fact that this sialoprotein shares the following characteristics with the "epithelial polyanion" in situ: (*a*) it is intensively stained with

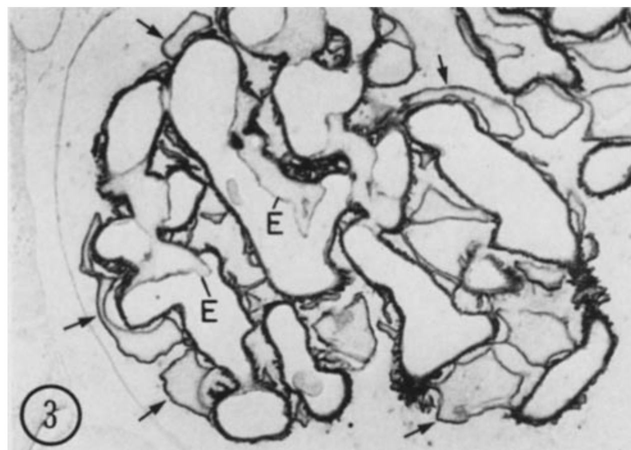

FIGURE 3 Light micrograph showing immunoperoxidase localization with anti-sgp140 IgG, on a 0.5-μm frozen section (34) of a fixed kidney. The surfaces of the visceral glomerular epithelial cells are heavily stained. Arrows indicate staining on the cell bodies of several epithelial cells where they face the urinary spaces. There is also a fainter staining of the endothelial cell surfaces (*E*), but the parietal epithelium is not stained. × 800.

cationic dyes; (*b*) it has a high affinity for WGA and fails to bind this lectin after neuraminidase treatment; and (*c*) it binds peanut lectin after neuraminidase treatment. Furthermore, using an antibody that specifically recognizes the 140-kd protein, it was found to be localized on the entire surface of the glomerular epithelium by immunocytochemistry. Of the large number of proteins resolved on SDS gels from glomerular lysates, only the140-kd band has the properties of the "epithelial polyanion." We have called this protein "podocalyxin" since it is a main constituent of the cell coat or glycocalyx of podocytes.

An unexpected finding is that podocalyxin contains most of the protein-bound sialic acid of the glomerulus. This conclusion relies on the facts that (*a*) it is the only band in SDS gels of glomerular extracts that is stained intensely blue by the cationic dyes, alcian blue, and Stains All (16, 17); (*b*) it is virtually the only band that binds [^{125}I]WGA in nitrocellulose transfers, and WGA binding is prevented by pretreatment with neuraminidase, indicating that sialic acid is responsible for the binding (28); and (*c*) it is the most prominent band in fluorograms of glomerular lysates after selective labeling of sialic acid residues by the periodate-[^{3}H]borohydride method (30). Moreover, it contains 4.5% sialic acid by the Warren assay, being comparable to fetuin in this respect (37). The high efficiency of extraction of sialic acid from glomeruli (~85–95%) obtained under the conditions used indicate that we are dealing with a major glomerular sialoprotein rather than a minor component selectively enriched by the extraction procedure.

Besides the presence of sialic acid groups, the fact that peanut lectin binds to podocalyxin after (but not before) neuraminidase treatment suggests the presence of penultimate Gal-GalNAc or Gal groups which are unmasked by removal of terminal sialic acid residues (38). These results could suggest that at least some of the sialic acid groups may be O-glycosidically linked because preterminal Gal-GAlNac groups frequently occur in O-glycosidically linked oligosaccharide chains of glycoproteins, but are rare or absent in N-linked oligosaccharides (39). In addition, amino acid analysis of the

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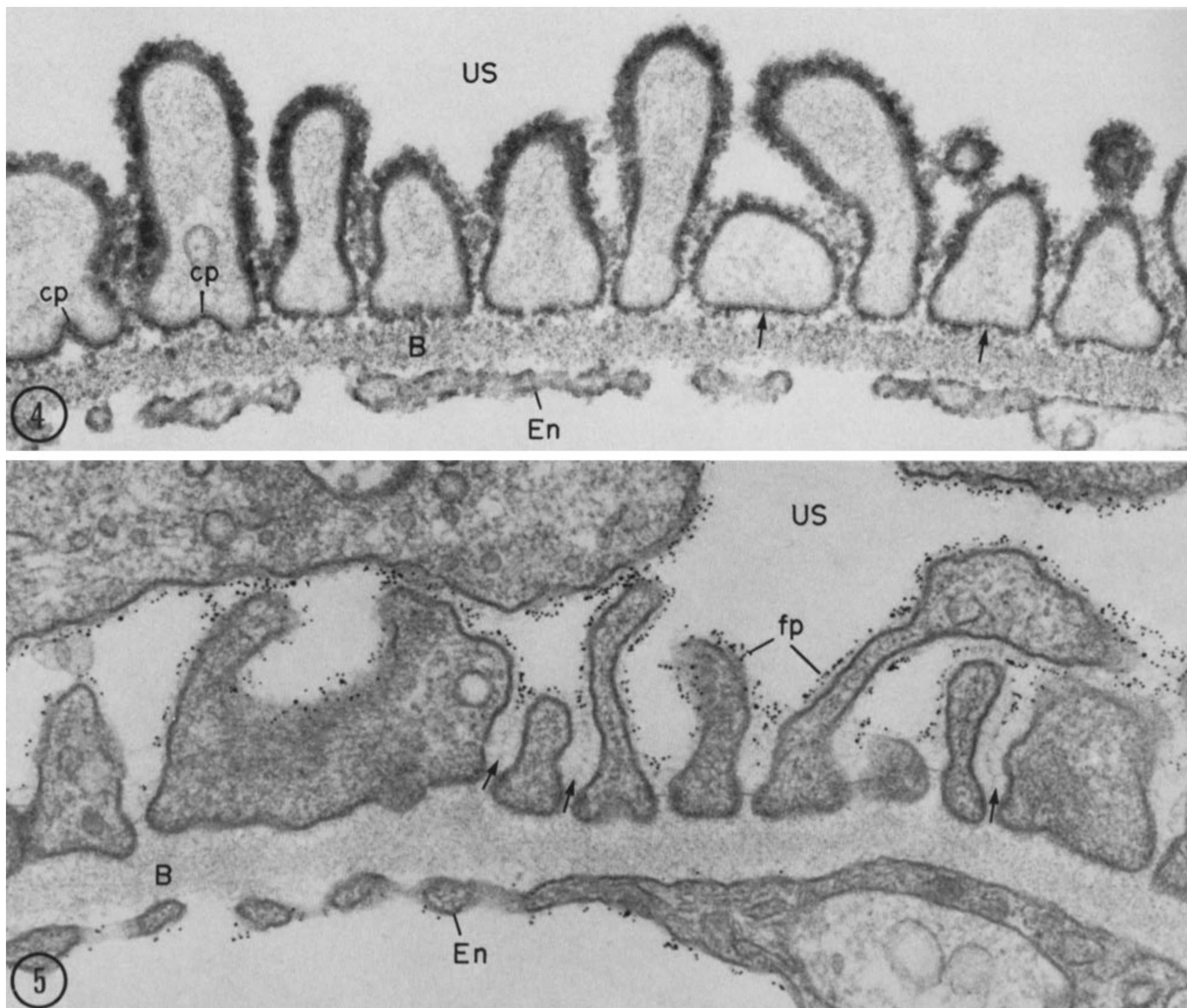

FIGURES 4 and 5 Fig. 4 shows the localization of anti-sgp140 IgG by immunoperoxidase. The foot processes of the glomerular epithelial cells show a thick layer of diaminobenzidine-reaction product on their surface coats where they face the urinary spaces (*US*). A similar but thinner layer is present on the membrane at the base of the foot processes (arrows) where they face the basement membrane (*B*) including along some coated pits (*cp*). A small amount of granular reaction product is seen in the baement membrane (*B*) and on the endothelium (*En*). Fig. 5 shows the localization of anti-sgp140 IgG on the surfaces of glomerular epithelial cells and foot processes (*fp*) by an immunogold technique. The protein A-gold particles are restricted to those portions of the epithelial cell body and foot processes that face the urinary spaces (*US*). A filamentous meshwork (arrows) presumably consisting of the surface coats cross-linked by the antibodies is visible in the filtration slits above the slit diaphragms. A patchy (clustered) distribution of gold particles is also observed on the luminal surface of the endothelium (*En*). × 55,000.

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140-kd band (unpublished data) shows a relatively high content of serine and threonine residues which could serve as sites of O-glycosylation. The binding of Con A to the 140-kd band may indicate the simultaneous presence of mannose-containing N-linked oligosaccharide chains, which would make podocalyxin similar to glycophorin (40) in regard to its pattern of glycosylation.

The determination of the accurate molecular weights of glycoproteins by SDS PAGE is complicated by their well-known anomalous migratory behavior caused by inefficient SDS binding (41). A more reliable molecular weight determination can be achieved on gradient SDS gels (42), as used in this study. Clearly, the precise molecular weight determination of podocalyxin awaits additional analysis by alternative methods, e.g., as done for human glycophorin (43). Another problem encountered with sialoglycoproteins from murine erythrocytes is their tendency to form aggregates (35). To avoid this problem, we solubilized glomerular samples and separated them under conditions previously found to counteract aggregation (35). When the 140-kd sialoprotein was electroeluted and rerun, it banded again in the 140-kd position. From these data we conclude that 140,000 may be close to the actual M_r of podocalyxin, and that it consists of a single molecule.

Recently, Nevins and Michael (13) have raised antibodies to two of the sialopeptides (~100 kd and a band of undetermined kd) released by trypsin from isolated rat glomeruli. By immunocytochemistry, both the resultant antibodies produced intense staining of the visceral glomerular epithelium and less intense focal staining of the glomerular endothelium.

We found only a single 140-kd sialoglycoprotein in extracts of glomeruli rapidly solubilized in SDS, but after extraction in neutral detergents, no 140-kd band was detectable and it was replaced by two bands of higher electrophoretic mobility (125 and 110 kd) on SDS gels that react by immuneoverlay with the specific antibodies we raised. We have concluded that these lower molecular weight bands are generated as a result of proteolytic degradation of the 140-kd protein by proteases in the glomerular lysate that are still active in the presence of the inhibitors used. As was the case with the trypic sialopeptides isolated by Nevins and Michael (13), the fragments produced are rather large which is in keeping with the fact that heavily glycosylated glycoproteins are known to be protease resistant.

Besides staining the visceral glomerular epithelium, our antibody also produced wide staining of endothelial cells, not only of glomerular and peritubular capillaries, but also endothelia in several other rat organs—heart, pancreas, parotid, and small intestine. At present it is not known whether the same or cross-reactive molecules are responsible for the endothelial staining.

Podocalyxin can be added to the growing inventory of podocyte membrane constituents which includes C3 complement receptors (man and primates) (44); a leukemia lymphocyte antigen (man) (45, 46); a heparan-sulfate proteoglycan (rat) which cross-reacts with that isolated from liver cell membranes (47); gp330, the pathogenic antigen of Heymann nephritis (rats) (31) which is concentrated in coated pits (14), and several as yet uncharacterized antigens (rat) against which monoclonal antibodies have been generated (48).

With the identification of podocalyxin and the availability of specific antipodocalyxin antibodies, it will be possible to further characterize this sialoprotein and to gain insights into the role it plays in physiological and pathological processes.

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