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Sulfate contributes to the negative charge of podocalyxin, the major sialoglycoprotein of the glomerular filtration slits

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ABSTRACT **Podocalyxin is the major sialoprotein of the rat glomerulus. Its function is to maintain the filtration slits of the glomerular epithelium open by virtue of its high net negative charge. We have used biosynthetic labeling and oligosaccharide analysis to characterize the anionic-charge-carrying moieties on this protein. Kidney slices from 2-day-old rats were biosynthetically labeled with [^{35}S]Cys, [^{3}H]Man, [^{3}H]GlcN, and $^{35}SO_4$, after which podocalyxin was immunoprecipitated and purified by SDS/PAGE. All these labels were incorporated into podocalyxin. Immunoprecipitates were subjected to digestion with specific glycosidases or digested with Pronase followed by chromatographic analysis of the released glycopeptides. Podocalyxin was susceptible to digestion with N-Glycanase and O-Glycanase, indicating the presence of both N- and O-linked oligosaccharides. Approximately 30% of the [^{3}H]GlcN-labeled glycopeptides bound to Con A, confirming the presence of high mannose, hybrid, or biantennary N-linked structures; alkaline borohydride treatment confirmed the presence of O-linked oligosaccharides. Analysis of the $^{35}SO_4$-labeled glycopeptides indicated that both the N- and O-linked structures were sulfated. We conclude that in newborn rat kidney (*i*) podocalyxin contains both O- and N-linked oligosaccharides [high mannose or hybrid type, biantennary, and complex (sialylated) type], (*ii*) podocalyxin is sulfated, and (*iii*) sulfate is located on both O-linked oligosaccharides and on glycopeptides carrying tri- or tetrantennary N-linked structures. These results indicate that the net negative charge of podocalyxin is most likely derived from sulfate as well as from sialic acid residues.**

The epithelium of the renal glomerulus is unique in that the intercellular spaces between interdigitating cell processes are normally open to allow passage of the glomerular filtrate, rather than being sealed off by typical tight junctions as is usually the case with epithelia in other locations. Maintenance of the patency of the intercellular spaces or filtration slits is dependent on the presence of highly negatively charged groups on the epithelial cell surface because, when the cell surface charge is neutralized by infusion of polycations, the filtration slits collapse and the adjoining cell membranes become closely opposed (1). Considerable evidence suggests that podocalyxin—a 140-kDa membrane glycoprotein present on the surfaces of the glomerular epithelium facing the urinary spaces (see Fig. 1)—plays a key role in maintaining the epithelial slits open. During development, the appearance of podocalyxin on the glomerular epithelium coincides with the opening of the intercellular spaces (2) and the disappearance of the tight junctions (3), which mark the transformation of this epithelium from a typical polarized epithelial cell layer to its adult arrangement.

We have previously shown that podocalyxin is the major sialoprotein of the rat glomerulus and accounts for >50% of the total glomerular sialic acid content (4). Moreover, we found that the sialic acid content of podocalyxin is reduced to one-third of the normal value in puromycin aminonucleoside nephrosis (5), a rat experimental model of human minimal change nephrosis. The latter is associated with changes in the organization of the foot processes and closure of the filtration slits. The close connection between the presence of podocalyxin, normal glomerular epithelial cell morphology, and open filtration slits led us to examine further the nature and location of oligosaccharide and other anionic-charge-carrying moieties on podocalyxin. We here provide data that podocalyxin from newborn rat kidneys contains N- and O-linked oligosaccharides that are both sialylated and sulfated.

MATERIALS AND METHODS

Materials. [^{35}S]Cys (>900 Ci/mmol; 1 Ci = 37 GBq) and $Na_2{}^{35}SO_4$ (carrier free) were from ICN; D-[2,6-^{3}H]Man (30–60 Ci/mmol) and D-[6-^{3}H]GlcN (20–40 Ci/mmol) were from Amersham. Tissue culture supplies were from GIBCO. N-Glycanase (peptide:*N*-glycosidase F) and O-Glycanase (α-*N*-acetylgalactosaminidase from chicken liver, EC 3.2.1.49) were from Genzyme, and neuraminidase (type X) was from Sigma.

Immunoelectron Microscopy. Immunogold labeling of ultrathin cryosections was carried out as described (2).

Metabolic Labeling of Rat Kidney Slices. Kidney slices were prepared from newborn (2-day-old) Sprague–Dawley rats as described (6, 7). For [^{35}S]Cys labeling, the slices were rinsed in cysteine-free RPMI 1640 (wash medium) and incubated in a shaking waterbath at 37°C under continuous gasing with 95% O_2/5% CO_2. Two and one-half milliliters of medium was used for the slices obtained from four kidneys. After an equilibration period of 15 min, [^{35}S]Cys (200–400 μCi/ml) was added for 15 min after which 0.3% bovine serum albumin and cysteine (5 mg/liter) were added and the incubation was continued for 3 hr. For continuous labeling with $^{35}SO_4$, [^{3}H]Man, and [^{3}H]GlcN, the same conditions were used, except that the RPMI wash media were sulfate- or glucose-free and the labeling media contained reduced $MgSO_4$ (1 mg/liter) or glucose (100 μM), respectively; 100 μM sodium pyruvate was also added to the low glucose medium. In some cases, slices were pulse-labeled for 15 min with [^{35}S]Cys followed by chase incubation (15 min–6 hr) in the same medium containing an excess of unlabeled cysteine.

Extraction of Kidney Slices. At the end of the labeling period, slices were washed with ice-cold 20 mM Tris-buffered saline (pH 7.3) containing protease inhibitors (antipain, pepstatin A, leupeptin, diisopropyl fluorophosphate, and benzamidine each at 1 mM), and the slices were either stored at −70°C or homogenized three times at high speed at 4°C for 20 sec with a Tissuemizer or sonicated for 5 min and centrifuged (500 × *g* for 10 min). The resulting postnuclear supernatant was centrifuged (100,000 × *g* for 1 hr), and the pellet was extracted in 0.2% Triton X-100 in Tris-buffered saline (pH 7.3) with protease inhibitors (3 hr). The cortical extract was cleared by centrifugation (25,000 × *g* for 15 min), assayed for protein (Pierce bicinchoninic acid assay), and used for immunoprecipitation.

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Abbreviation: RCA, *Ricinus communis* agglutinin.

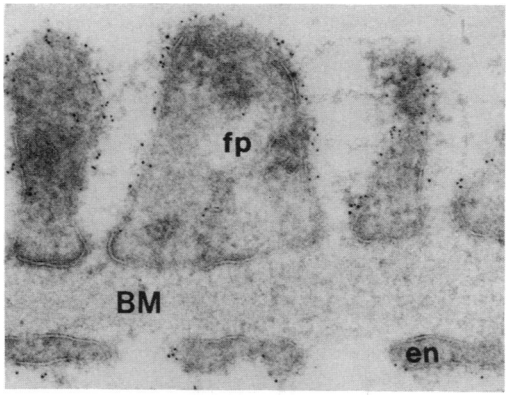

FIG. 1. Podocalyxin resides in glomerular filtration slits. Immunogold labeling of a mature rat glomerulus (ultrathin frozen section) with monoclonal anti-podocalyxin IgG demonstrating the localization of podocalyxin. Gold particles are present on those surfaces of the epithelial foot processes (fp) of the glomerular epithelium (podocytes) facing the urinary spaces, but they are not seen along their basal surface facing the basement membrane (BM). Podocalyxin is also detected on the luminal surface of the glomerular endothelium (en). (×46,800.)

Immunoprecipitation. Extracts were precleared overnight with protein A-Sepharose (5 mg/mg of extracted protein) after which mouse monoclonal C287.1A anti-podocalyxin IgG (25 μg/mg of protein) (7) or rabbit anti-podocalyxin serum (50 μg/mg of protein) (4) was added for 4 or 6 hr followed (in the case of monoclonal IgG) by rabbit anti-mouse IgG (2 hr) and protein A-Sepharose (2 hr). Rabbit anti-mouse IgG, nonimmune rabbit IgG, and monoclonal and polyclonal anti-gp330 IgGs served as controls (7).

O-Glycanase and N-Glycanase Digestions of [^{35}S]Cys and $^{35}SO_4$-Labeled Podocalyxin. Washed immunoprecipitation beads were aliquoted and resuspended in the appropriate digestion buffers (8). Aliquots were resuspended in 50 mM sodium acetate buffer (pH 5.5), 10 mM $CaCl_2$, and protease inhibitors (50 μl) and digested (18 hr at 37°C) with neuraminidase (2 units/ml). O-Glycanase was added to a final concentration of 80 milliunits/ml, and the incubations were continued for 24 hr. For N-Glycanase digestions, the beads were boiled for 3 min in 0.5% SDS and 0.1 M 2-mercaptoethanol; cooled to room temperature; adjusted to 0.17% SDS, 0.2 M sodium phosphate, 10 mM phenanthroline hydrate, 1.25% Nonidet P-40, and protease inhibitors (30 μl); and digested with N-Glycanase (50-20 units/ml; 18 hr at 37°C). For sequential neuraminidase/N-Glycanase digestions, the beads were resuspended in 50 mM phosphate buffer (pH 5.5), 10 mM $CaCl_2$, and protease inhibitors (30 μl) and digested (18 hr at 37°C) with neuraminidase (2 units/ml). The samples were adjusted to N-Glycanase conditions as above (100 μl) and digested (24 hr at 37°C). Controls consisted of beads boiled in SDS sample buffer directly after immunoprecipitation and those incubated in buffer without enzymes. Samples were analyzed by SDS/PAGE to detect changes in the electrophoretic mobility of podocalyxin.

SDS/PAGE. Freshly isolated glomeruli (4), cortical extracts, and labeled immunoprecipitates were heated in SDS sample buffer, reduced and alkylated with 25 mM iodoacetic acid (15 min in the dark), electrophoresed on 5–10% gradient gels, and analyzed by fluorography (7).

Acid Treatment of $^{35}SO_4$-Labeled Podocalyxin. Aliquots of $^{35}SO_4$-labeled immunoprecipitates and cortical extracts were separated by SDS/PAGE and treated with hot (95°C) 1 M HCl both in the gel and after transfer to an Immobilon membrane (Millipore), followed by fluorography with 2,5-diphenyloxazole/dimethyl sulfoxide and 2,5-diphenyloxazole/toluene, respectively (9).

Isolation and Structural Analysis of [^{3}H]GlcN and $^{35}SO_4$-Labeled Glycopeptides. The radiolabeled podocalyxin band was located on the gel from the fluorograph, excised, cut into smaller sections, and digested with Pronase (10). The resulting glycopeptides were desalted (Sephadex G-25), and the excluded glycopeptides were dried by rotary evaporation. They were then fractionated by Con A and *Ricinus communis* agglutinin (RCA) I affinity chromatography, gel-filtration chromatography (Sephadex G-25 and Bio-Gel P-6), and ion-exchange chromatography (Amberlite MB 3 and Dowex 50-H^+) as described (10, 11). Neuraminidase and endo-β-*N*-acetylglucosaminidase H digestions were performed as described (11). Neutral high-mannose-type oligosaccharides were size fractionated by HPLC using an AX-5 micropak column (12). Glycopeptides to be treated by mild base hydrolysis were dried and resuspended in 1 M $NaBH_4$ in 50 mM NaOH (16 hr at 45°C) (10); the deionized samples were spotted on Whatman no. 1 paper and developed by descending chromatography in ethyl acetate/pyridine/acetic acid/water (5:5:1:3, vol/vol).

RESULTS

[^{35}S]Cys Labeling of Podocalyxin in Newborn Rat Kidney Slices. We have used kidney slices from the newborn rat to study the biosynthesis of podocalyxin, taking advantage of the fact that in the rat glomerular development continues after birth (13) and that synthesis of glomerular proteins occurs at a high rate (Fig. 2). It was necessary to use the kidney slice system, because we have not detected podocalyxin in cultured glomerular epithelial cells or in a variety of cultured endothelial cell lines. When immunoprecipitations were carried out after labeling with [^{35}S]Cys for 30 min–6 hr, a major band at 125 kDa and a minor band at 215 kDa were seen (Fig. 2). Sometimes another (115-kDa) band was also seen. Podocalyxin is susceptible to degradation under nondenaturing extraction and immunoprecipitation conditions, yielding bands of 125 kDa and sometimes 115 kDa, rather than the 140-kDa band seen by immunoblotting (4). The band at 215 kDa most likely represents a dimer of podocalyxin, as it is recognized by monoclonal and polyclonal anti-podocalyxin after immunoblotting (data not shown). To detect radiolabeled podocalyxin, a minimal incubation period of 30 min was necessary. Earlier precursor forms were not recognized by any of the antibodies. No changes in the migration of the podocalyxin bands were seen during chase periods up to 6 hr.

^{3}H-Labeled Monosaccharides and [^{35}S]Sulfate Are Incorporated into Podocalyxin. When immunoprecipitations were

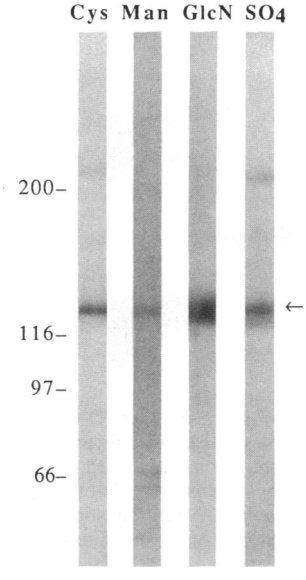

FIG. 2. Incorporation of ^{3}H-labeled monosaccharides and $^{35}SO_4$ into podocalyxin. Newborn rat kidney slices were metabolically radiolabeled with [^{35}S]Cys, [^{3}H]Man, [^{3}H]GlcN, and $^{35}SO_4$. Immunoprecipitations were carried out with monoclonal (Cys and Man) or polyclonal (GlcN and SO_4) anti-podocalyxin, and the immunoprecipitates were subjected to SDS/PAGE and fluorography. The major podocalyxin band is marked with an arrow. Molecular mass standards (in kDa) are indicated.

carried out after continuous labeling (3–4 hr) of kidney slices with [^{3}H]Man, [^{3}H]GlcN, or $^{35}SO_4$, the major 125-kDa podocalyxin band and minor bands at ≈115 and 215 kDa (Fig. 2) were precipitated. These data suggest (*i*) that podocalyxin contains N-linked oligosaccharides (based on incorporation of [^{3}H]Man), which is compatible with earlier results (5), and (*ii*) that it is sulfated (based on incorporation of $^{35}SO_4$).

Podocalyxin Is Susceptible to N- and O-Glycanase. To obtain further information on the nature of the glycans associated with podocalyxin, we analyzed the susceptibility of $^{35}SO_4$- or [^{35}S]Cys-labeled podocalyxin immunoprecipitates to glycosidase digestion. After desialylation by neuraminidase (Fig. 3, lane 3), podocalyxin migrated more slowly (≈165 kDa) than the mock-digested sample (125 kDa) (Fig. 3, lane 4). This anomalous migration behavior due to loss of negative charge also applies to podocalyxin from the adult kidney and is not unusual for heavily sialylated glycoproteins (5). After digestion with N-Glycanase, podocalyxin showed an increase in mobility from 125 to 115 kDa (data not shown). This indicates the presence of N-linked oligosaccharides, in keeping with earlier results (5). After sequential digestion with neuraminidase and N-Glycanase (Fig. 3, lane 1), podocalyxin shifted to ≈120 kDa. After digestion with O-Glycanase without prior desialylation, podocalyxin migrated slightly faster than the undigested control, resulting in broader bands (Fig. 3, lane 5). No further change in mobility could be detected when podocalyxin was digested with neuraminidase before O-Glycanase treatment (Fig. 3, lane 2). Since O-Glycanase from chicken liver hydrolyzes O-glycosidic linkages between the nonsialylated GalNAc or Gal(β1-3)GalNAc and serine or threonine residues (14), the susceptibility of newborn rat podocalyxin to direct O-Glycanase treatment indicates the presence of nonsialylated O-linked GalNAc or the disaccharide Gal(β1-3)GalNAc.

Newborn Rat Podocalyxin Contains N-Linked Biantennary and High Mannose Oligosaccharides. When ^{3}H-labeled glycopeptides released from [^{3}H]GlcN-labeled podocalyxin by Pronase digestion were chromatographed on Con A-Sepharose, 28% of the radioactivity bound to the lectin affinity column; of the bound radiolabeled glycopeptides, 68% and 32% were eluted sequentially with 10 mM α-methyl-D-glucoside and 100 mM α-methyl-D-mannoside, respectively (Fig. 4*A*). Thus, podocalyxin contains N-linked biantennary-type glycopeptides (eluted with α-methylglucoside) as well as

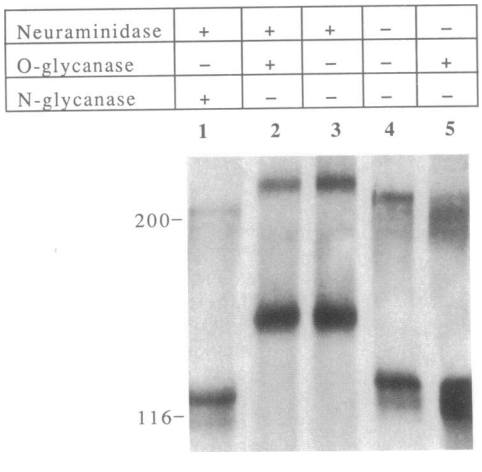

FIG. 3. Podocalyxin is susceptable to neuraminidase, O-Glycanase, and N-Glycanase digestion. Newborn rat kidney slices were labeled with $^{35}SO_4$, extracted, and immunoprecipitated with monoclonal anti-podocalyxin IgG. Immunoprecipitates were digested or received no enzyme treatment as indicated. After digestion the immunoprecipitates were subjected to SDS/PAGE and fluorography. Molecular mass standards (in kDa) are indicated.

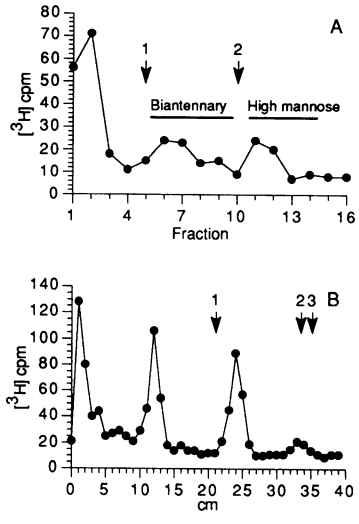

FIG. 4. (*A*) Podocalyxin contains high mannose and biantennary oligosaccharides. [^{3}H]GlcN-labeled podocalyxin glycopeptides prepared from newborn rat kidney slices were fractionated on a Con A-Sepharose column. Bound glycopeptides were eluted sequentially as indicated with 10 mM α-methylglucoside (arrow 1) and 100 mM α-methylmannoside (arrow 2). Aliquots of each fraction were assayed for radioactivity, and fractions were pooled as indicated by the horizontal bars. (*B*) Podocalyxin contains O-linked monosaccharides, disaccharides, and tri- or tetrasaccharides. The unbound [^{3}H]GlcN-labeled glycopeptides in the Con A-Sepharose flow-through (from *A*) were treated with neuraminidase and NaOH/$NaBH_4$ and analyzed by descending paper chromatography. One-centimeter sections of the chromatogram were assayed for radioactivity. Standards: 1, lactose; 2, *N*-acetylgalactosaminitol (GalNAcitol); 3, *N*-acetylglucosaminitol (GlcNAcitol).

high-mannose-type oligosaccharides (eluted with α-methylmannoside) (10). To investigate further the nature of the high mannose-type glycopeptides, they were treated with endo-β-*N*-acetylglucosaminidase H, and the released neutral oligosaccharides were separated from charged species by Amberlite MB 3 chromatography. The neutral high mannose oligosaccharides were separated by HPLC and found to consist of a mixture of species containing five to nine mannose residues (data not shown).

Podocalyxin Contains O-Linked Oligosaccharides. When the [^{3}H]GlcN-labeled podocalyxin glycopeptides present in the Con A flow-through were treated with neuraminidase and chromatographed on Sephadex G-25, 24% of the applied radioactivity was released and eluted in the included volume. This suggests that a fraction of the [^{3}H]GlcN had been metabolized into sialic acid residues. When the desialylated glycopeptides in the flow-through were subjected to mild base hydrolysis (to release O-linked oligosaccharides) and followed by descending paper chromatography, 63% of the applied radioactivity migrated away from the origin, indicating the presence of O-linked glycoconjugates. The base-released units migrated as three distinct species corresponding to a monosaccharide, a disaccharide, and a tri- and/or tetrasaccharide (Fig. 4*B*). The radiolabeled glycopeptides (37%) that remained near the origin most likely consist of branched, tri-, or tetrantennary N-linked oligosaccharides. These results demonstrate that newborn rat podocalyxin contains sialylated glycopeptides that carry a variety of O-linked oligosaccharides.

O-Linked Podocalyxin Oligosaccharides Are Sulfated. When $^{35}SO_4$-labeled glycopeptides (released by Pronase digestion) were applied to a Bio-Gel P-6 column, a single peak of radioactivity was eluted in the void volume (Fig. 5*A*). Prior work suggests that the high apparent molecular mass of the Pronase-digested glycopeptides may be due to the presence

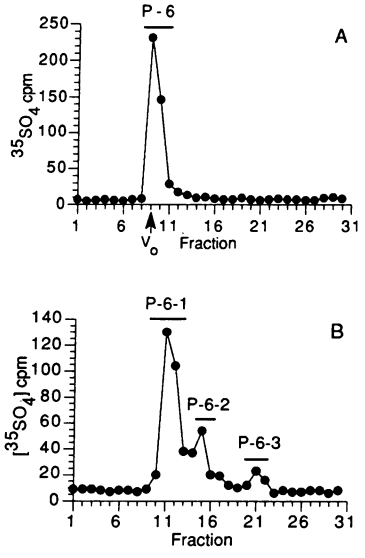

FIG. 5. O-Linked structures released by base hydrolysis are sulfated. (*A*) $^{35}SO_4$-labeled glycopeptides from newborn rat podocalyxin were applied to a Bio-Gel P-6 column. Fractions were pooled as indicated by the horizontal bar. (*B*) The pooled fractions were treated with NaOH/$NaBH_4$ and rechromatographed. Aliquots of the fractions were assayed for radioactivity.

of closely spaced O-linked oligosaccharides, which inhibit proteolytic cleavage (10).

When the $^{35}SO_4$-labeled glycopeptides recovered from the P-6 column were subjected to mild alkaline hydrolysis and rechromatographed (99% recovery), 69% of the radioactivity still eluted in the excluded volume (P-6-1), but two smaller, partially included peaks, P-6-2 (25%) and P-6-3 (≈6%), were also detected (Fig. 5*B*), indicating the presence of base-labile, sulfated O-linked structures.

To corroborate the existence of sulfated O-linked oligosaccharides on podocalyxin, the pooled peaks from the P-6 column were analyzed by descending paper chromatography. Prior to base hydrolysis, the $^{35}SO_4$-labeled glycopeptides remained near the origin of the chromatogram (Fig. 6*A*). After base hydrolysis, 45% of the applied radioactivity associated with P-6-1 (Fig. 6*B*), and all of that associated with P-6-2 (Fig. 6*C*) migrated away from the origin as expected for base-released O-linked oligosaccharides. When the P-6-1 glycopeptides were pretreated with neuraminidase and then chromatographed after base hydrolysis, the faster migrating species were shifted compared to an undigested control, indicating that many (45%) of the $^{35}SO_4$-labeled O-linked units are sialylated (data not shown).

In summary, at least 58% of the sulfate on newborn rat podocalyxin recovered after Bio-Gel P-6 chromatography (33% derived from pool P-6-1 and 25% from pool P-6-2) is associated with O-linked units.

Lack of Evidence for Tyrosine Sulfation. The $^{35}SO_4$-labeled components not bound by Con A were desalted by Sephadex G-25 chromatography. The majority of the radioactivity eluted in the void volume as expected for glycopeptides; no radioactivity eluted in the included volume as would be expected for $^{35}SO_4$-labeled tyrosine residues. Furthermore, treatment of $^{35}SO_4$-labeled podocalyxin immunoprecipitates and cortical extracts with hot 1 M HCl after gel electrophoresis did not result in a major loss of bound $^{35}SO_4$ when compared to the untreated control. These data indicate that tyrosine sulfate is not a major contributor to the sulfate found on newborn rat podocalyxin (data not shown).

Podocalyxin Glycopeptides Carrying N-Linked Structures Are Sulfated. The desalted sulfate-labeled units not bound by Con A were treated with neuraminidase and subjected to

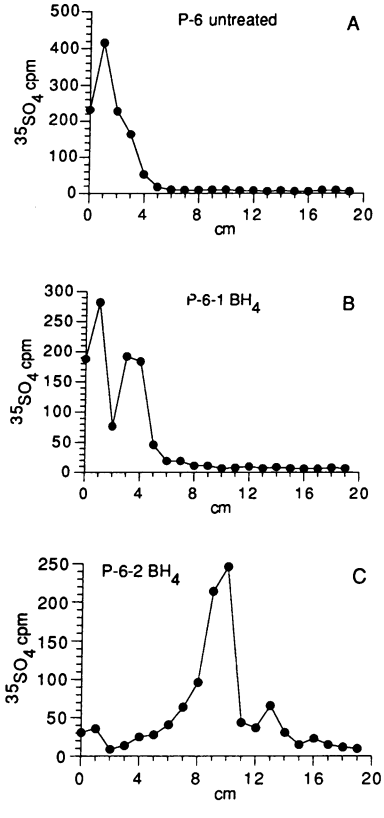

FIG. 6. Sulfated O-linked structures are detected by descending paper chromatography. (*A*) Glycopeptides in the pooled excluded fractions from Fig. 4*A* were analyzed by descending paper chromatography. (*B* and *C*) P-6-1 and P-6-2 oligosaccharides released by NaOH/$NaBH_4$ treatment were analyzed as in *A*. Standards were mannose (43 cm), mannose 6-phosphate (32 cm), and fucose (45 cm).

RCA I-agarose chromatography. Virtually all of the radioactivity bound to RCA I and was eluted as a single peak with 0.2 M galactose. Thus, all of the $^{35}SO_4$ appears to be associated with glycopeptides. RCA I binds branched N-linked oligosaccharides bearing terminal galactose residues with high affinity while it only weakly interacts with desialylated O-linked oligosaccharides (15). The simplest explanation for the binding of virtually all podocalyxin glycopeptides to RCA I-agarose is that they carry both N- and O-linked structures.

In summary, $^{35}SO_4$-labeled glycopeptides do not bind to Con A, bind to RCA I after desialylation, and are unaffected by alkaline borohydride treatment. The most likely candidates to fit this description are sulfated tri- and/or tetrantennary N-linked oligosaccharides.

DISCUSSION

We have investigated the nature of the oligosaccharide and negative-charge-carrying moieties of podocalyxin, which is the most abundant membrane sialoglycoprotein of the rat glomerulus and the biochemical equivalent of the histochemically defined "epithelial polyanion" (4). Via its negative charge, it is known to play a role in maintaining the intercellular spaces or filtration slits of the visceral glomerular epithelium open.

Previously we demonstrated that podocalyxin is sialylated. The most important additional result we obtained in this study is the finding that podocalyxin is also sulfated. This indicates that the negative charge of this glycoprotein is derived not only from sialic acid but also from sulfate residues. The data indicate that the majority of the sulfate is located on O-linked oligosaccharides and on glycopeptides carrying tri- or tetrantennary N-linked structures. No sulfate

was detected in association with high mannose, hybrid, or biantennary structures or with tyrosine sulfate. However, the presence of very small amounts of glycosaminoglycans or tyrosine-*O*-sulfate cannot be ruled out.

Our data obtained by glycosidase digestion and chromatographic analysis of [^{3}H]Man- or [^{3}H]GlcN-labeled glycopeptides indicate that podocalyxin contains a complex mixture of both N- and O-linked oligosaccharides. The O-linked structures are in part sialylated or sulfated and, by descending paper chromatography after desialylation, were shown to consist of monosaccharides, disaccharides, and tri- or tetrasaccharides. The N-linked oligosaccharides consist of high mannose- or hybrid-type structures, as well as biantennary and highly branched complex-type units. The O-linked units may be very closely spaced on the peptide as in the case of the low density lipoprotein receptor (10, 14).

Previously it was demonstrated that podocalyxin from the adult rat kidney is highly anionic (binds cationic dyes), contains >50% of the total sialic content of the glomerulus, binds Con A and wheat germ lectins, and binds peanut lectin after neuraminidase treatment (4). The presence of N-linked oligosaccharides on podocalyxin was initially suspected based on Con A binding and was later deduced from its susceptibility to endo-β-*N*-acetylglucosaminidase F treatment and from the gas chromatographic analysis indicating the presence of Man, $GlcNH_2$, Gal, and sialic acid (5). The presence of O-linked oligosaccharides on podocalyxin was suggested by the earlier finding that podocalyxin binds peanut lectin [which binds Galβ(1-3)GalNAc in O-linked oligosaccharides after desialylation], but these results remained ambiguous until now because, for unknown reasons, GalNAc (the initiating sugar of O-linked oligosaccharides) was not detected by gas–liquid chromatographic analysis of sugars released from podocalyxin (5).

Recent work has indicated that sulfated N-linked oligosaccharides are present on the surfaces of a number of cultured cell lines (16, 17), including endothelial cells (18). They are also present in several membrane proteins [e.g., the low density lipoprotein receptor (10) and viral glycoproteins (19, 20)] and secretory proteins [e.g., pituitary hormones (21)]. Sulfated O-linked oligosaccharides have also been described in tracheobronchial mucus (22) and in allantoic fluid (23).

Podocalyxin is an unusual protein in that among epithelia it is expressed only on the visceral glomerular epithelium of the kidney and is not expressed by any other epithelium in the kidney or in other organs. In the developing kidney, this protein represents a differentiation marker for the glomerular epithelium (2). However, podocalyxin is also present in lesser amounts on the luminal surfaces of endothelial cells—i.e., on the glomerular endothelium, the peritubular capillary endothelium (4), and all other endothelia examined (24). In this study we have taken advantage of the fact that podocalyxin is synthesized at a high rate in the developing glomerulus. It can be metabolically radiolabeled in slices from the newborn rat kidney (7) and can be readily detected by immunoelectron microscopy in biosynthetic compartments of differentiating podocytes but not endothelial cells (2). The latter suggests that differentiating podocytes constitute the main source of radiolabeled podocalyxin in our slice system.

Anionic modification of glycans of glycoproteins are of interest because of the possibility that they confer biological specificity or function. Examples are the mannose 6-phosphate residues of lysosomal enzymes, which are the sorting signals responsible for targeting to lysosomes (25); leucosialin, which plays a key role in determining the cell surface charge of T cells (26, 27); the sialylated O-linked oligosaccharides of glycophorins, which are an integral part of blood groups antigens (28); the sulfated N-linked oligosaccharides of the pituitary glycoprotein hormone lutropin (21); and heparan sulfate proteoglycans, which play an important role in maintaining the restrictive permeability properties of the glomerular basement membrane (29). The presence of sulfate representing yet another anionic moiety on podocalyxin, which is already known to be heavily sialylated, would be expected to enhance the high net negative charge on the surface of podocytes. It also provides additional circumstantial evidence for the presumptive biological function of podocalyxin in the maintenance of an open filtration pathway between neighboring podocytes by electrostatic repulsion of adjacent cell membranes in the filtration slits. Because of the importance of charge-carrying moieties in glomerular function, in the future it will be important to determine the detailed structure of the sulfated oligosaccharides on podocalyxin.

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