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Characterization of chondroitin sulfate and dermatan sulfate proteoglycans of extracellular matrices of human umbilical cord blood vessels and Wharton's jelly

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The chondroitin sulfate/dermatan sulfate proteoglycans (CS/DSPGs) of the human umbilical cord vein, arteries and Wharton's jelly matrices were characterized and localized by immunohistochemical analysis. The CS/DSPGs were found to be decorins and biglycans with 43–48 kDa core proteins and are distributed throughout the umbilical cord. A truncated form of decorin having only the ∼14 kDa NH_2-terminal portion of the core protein was found exclusively in the vein. The proteoglycans, regardless of their locations, have two types of CS/DS chains, one with ∼90% CS and ∼10% DS and the other with ∼65% CS and ∼35% DS. The glycosaminoglycan (GAG) chains of the truncated decorin consist of ∼53% CS and ∼47% DS. Both decorin and biglycan including the truncated form of decorin could efficiently bind collagen I and fibronectin. The decorin and biglycan with ∼10% DS and ∼90% CS were loosely bound in the extracellular matrices, whereas those with ∼35% DS bound strongly. Together, these data demonstrate that, the GAG chains with 35–47% DS but not those with 10% DS, interact strongly with the matrix. Our data also show that the GAG chain composition is a significant factor in binding of the decorin and biglycan to matrix proteins. The expression of decorin and biglycan with distinctively different CS/DS proportions implies specific biological functions for these PGs in the umbilical cord. The occurrence of the truncated form of decorin exclusively in the umbilical vein suggests a specific functional role.
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Keywords: **umbilical cord, vein and arteries, proteoglycans, decorin and biglycan, chondroitin/dermatan sulfate, tissue distribution**

Abbreviations: **GAG, glycosaminoglycan; CS, chondroitin sulfate; CSPG, chondroitin sulfate proteoglycan; C4S, chondroitin 4-sulfate; C6S, chondroitin 6-sulfate; DS, dermatan sulfate; DSPG, dermatan sulfate proteoglycan; HA, hyaluronic acid; HS, heparan sulfate; BSA, bovine serum albumin; BD, blue dextran; Glc, glucose; HexN, hexosamine; GalN, galactosamine; GlcN, glucosamine; CsBr, cesium bromide; HRP, horseradish peroxidase; DEAE, diethylaminoethyl; EDTA, ethylenediamine tetraacetic acid; GdnHCl, guanidine hydrochloride; NEM, *N*-ethylmaleimide; PBS, phosphate buffered saline; PMSF, phenylmethylsulfonyl fluoride; TLCK, *N*-∝-tosyl-L-lysine chloromethyl ketone; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; PAGE, polyacrylamide gel electrophoresis; CAPS, 3-(cyclohexylamino)propanesulfonic acid; PVDF, polyvinylidene difluoride.**

Introduction

Proteoglycans (PGs) are complex macromolecules consisting of glycosaminoglycan (GAG) chains—chondroitin sulfate (CS), dermatan sulfate (DS) or heparan sulfate (HS)—ranging in size from 10 to 100 kDa, covalently linked to proteins. PGs comprise a heterogeneous family found ubiquitously in animal tissues, predominantly as components of the extracellular matrix and as integral cell surface molecules [1–3]. The extracellular matrix of some tissues, notably the cartilage contains hyaluronic acid (HA) that binds to PGs to form large aggregates. PGs and HA have long been perceived as inert materials providing structural integrity and mechanical strength to tissues by binding collagens, fibronectin, and other matrix proteins [4–9]. However, in recent years, both cell surface and

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extracellular matrix PGs have also been found to serve as recognition molecules for diverse and important biological functions such as cell-cell and cell-matrix interactions, cell proliferation, differentiation and migration [10–13]. Further, the GAG chains of PGs have been shown to be involved in mobilization of growth factors, chemokines, cytokines, cell adhesion molecules, and minerals that are critical for the normal function of tissues [3,11].

The extracellular matrices of the connective tissues such as skin and tendon, and bone contain high levels of two structurally related small chondroitin sulfate/dermatan sulfate proteoglycans (CS/DSPGs) called decorin and biglycan. The core proteins of these PGs have extensive sequence similarities and they predicted to have been produced by gene duplication [14]. Decorins and biglycans apparently have important roles in the tissue organization and cellular function. The DS but not CS chains of decorin and biglycan have been proposed to undergo self-association, which may profoundly influence the matrix assembly [15,16]. In addition to their ability to interact with matrix proteins such as collagens, fibronectin, and thrombospondin, decorins and biglycans are also known to bind growth factors such as TGF-β and epidermal growth factor, and a variety of proteins including cytokines and chemokines [4,6–9,11]. These properties are likely to be responsible for the suggested involvement of decorin and biglycan in processes such as the regulation of cell migration, cell proliferation, and blocking of cell adhesion [3,17].

Umbilical cord is comprised of a vein and two arteries, which are surrounded and protected by tissue enriched in a gelatinous material referred as Wharton's jelly, rich in HA. Previous studies have reported the presence of chondroitin 4-sulfate/chondroitin 6-sulfate (C4S/C6S) copolymers in the umbilical cord arteries, and CS/DS copolymers in the Wharton's jelly [18–22]. Recently, it has been reported that Wharton's jelly contains decorin and biglycan but these have not been characterized in detail [19]. Moreover, practically nothing is known about the nature of PGs of umbilical vein and arteries.

To investigate the involvement of umbilical blood vessel chondroitin sulfate proteoglycans (CSPGs) in the binding of *Plasmodium falciparum*-infected red blood cells (IRBCs) entering the umbilical cord from the infected placentas, by transplacental passage, we studied the CSPGs of the vein, arteries and tissue of umbilical cord [23]. It was found that while a versican CSPG is present in the vein, arteries and the tissue around the blood vessels (Wharton's jelly), a serglycin CSPG occurs exclusively in the vein. The structural characterization, tissue distribution, and IRBC binding characteristics of these CSPGs have been reported [23]. During our studies on these CSPGs and *P. falciparum* IRBC binding to umbilical cord, we noted that the umbilical cord blood vessels and Wharton's jelly contain distinctive types of CS/DSPGs with GAG chains consisting of different proportions of CS and DS, and were differentially bound in the tissue matrix; these may have important biological relevance. Here, we report the characterization of these CS/DSPGs and their tissue distribution.

Materials and methods

Materials

Proteus vulgaris chondroitinase ABC, protease-free *P. vulgaris* chondroitinase ABC (120 units/mg), *Arthrobacter aurescens* chondroitinase AC-II (87 units/mg), *Flavobacterium heparinum* chondroitinase B (25 units/mg), *F. heparinum* heparitinase (113 units/mg), *Streptomyces hyalurolyticus* hyaluronidase (2000 turbidity reducing units/mg), monoclonal antibodies against Δdi-6S (IgM) and Δdi-4S (IgG), sturgeon notochord C4S, and shark cartilage C6S were purchased from Seikagaku America (Falmouth, MA). Monoclonal antibody (clone CS-56, IgM) that recognizes intact C4S and C6S but not DS, bovine pancreas RNase (74 units/mg), C4S (bovine trachea), alkaline phosphatase-conjugated goat anti-rabbit IgG, PMSF, NEM, and benzamidine were from Sigma. DNase I (grade II, 2000 units/mg), TLCK, and TPCK were from Roche Molecular Biochemicals. Sepharose CL-6B, Sepharose CL-4B, DEAE-Sephacel, and blue dextran were from Amersham Biosciences. Bio-Gel P-6, alkaline phosphatase substrate kit, and 4–15% gradient Tris-HCl polyacrylamide mini gels were from Bio-Rad. HPLC-grade 6 M HCl, and modified Lowry protein assay kit were from Pierce. Protein molecular weight standards for SDS-PAGE, and bovine plasma fibronectin were from Invitrogen. Bovine placenta type I collagen (collagen I) was from Southern Biotechnology Inc. The rabbit antisera LF-136 and LF-112, respectively, against the synthetic peptides GIGPEVPDDRDF and GVLDPDSVTPTYSA sequences at the NH_2-terminal ends of the human decorin and biglycan core proteins were generous gifts from Dr. Larry Fisher, Craniofacial and Skeletal Disease Branch, NIDR, NIH, Bethesda.

Isolation of PGs from the human umbilical cord vein, arteries and Wharton's jelly

All procedures were performed on ice or at 4°C. The tissue extracts were centrifuged at 12,000 g for 30 min in a Sorvall centrifuge using SS-34 rotor. Human normal umbilical cords collected from term placentas were dissected to separate vein, arteries and the tissue surrounding the blood vessels (Wharton's jelly). The tissue (40 g each) were cut into pieces and separately extracted with 250 ml of PBS, pH 7.2, containing 10 mM EDTA, 0.1 mM PMSF, 0.1 mM TLCK, 0.25 mM TPCK, 1 mM benzamidine, and 0.1 mM NEM (buffer A) as described previously [24]. The pellets were then extracted three times with 250 ml of buffer A containing 0.5% Triton X-100. The tissue pellets were finally suspended in 250 ml of buffer A containing 0.5% Triton X-100 and 6 M urea. The suspensions were homogenized for 30 min using Polytron, stirred at 4°C for 6 h, and centrifuged. The pellets were extracted two more times as above,

centrifuged, and the supernatants pooled. The different extracts from each tissue were separately chromatographed on DEAE-Sephacel columns (2.5 × 15 cm). The columns were washed first with 25 mM Tris-HCl, 10 mM EDTA, 0.15 M NaCl, pH 8.0, followed by 20 mM NaOAc, 0.25 M NaCl, pH 5.5. In each case, column was washed extensively with 0.25 M NaCl-containing buffer to remove most of the associated HA. The columns were then equilibrated with 50 mM NaOAc, 0.1 M NaCl, pH 5.5, containing 4 M urea, and the bound materials were eluted with a linear gradient of 0.1–1.0 M NaCl in 50 mM NaOAc, 4 M urea, pH 5.5. Ten-ml fractions were collected and the elution of the PGs monitored by absorption at 260 and 280 nm, and by analyzing uronic acid content [25]. The uronic acid-containing fractions were combined, dialyzed, and lyophilized.

Purification of PGs by gel filtration on Sepharose CL-4B

The PGs (20–25 mg) obtained from the DEAE-Sephacel columns contained significant level of nucleic acids and, therefore, treated with DNase (300 units/ml) and RNase (10 units/ml) as described [24]. The PGs containing significant levels of glucosamine were incubated with *S. hyalurolyticus* hyaluronidase (100 turbidity reducing units/ml) at 60°C for 2 h [26], dialyzed, lyophilized, and then treated with heparitinase (50 milliunits/ml) at 43°C for 18 h [27]. The reaction mixtures were then separated on Sepharose CL-4B (1.5 × 83 cm) in 20 mM Tris-HCl, 0.15 M NaCl, 4 M GdnHCl, pH 7.6. Fractions (2 ml) were monitored for proteins and uronic acid content [25]. The uronic acid-containing fractions were pooled, dialyzed, and lyophilized.

Purification of PG by cesium bromide density gradient centrifugation

The PGs obtained after Sepharose CL-4B chromatography were dissolved (2 mg/ml) in 25 mM sodium phosphate, pH 7.2, containing 50 mM NaCl, 4 M GdnHCl and 42% (w/w) CsBr. The solutions were centrifuged in a Beckman 50 TI rotor at 44,000 rpm for 65 h at 14°C [28]. Gradients were collected from the bottom of the tubes into 15 equal fractions, absorption at 280 nm measured, and the uronic acid-containing fractions were combined, dialyzed, and lyophilized.

Release of GAG chains from PGs

The purified PGs (200–400 μg) were treated with 0.5 ml of 0.1 M NaOH, 1 M $NaBH_4$ at 45°C for 18 h under nitrogen [29]. The solution was cooled in an ice bath, neutralized with 1 M acetic acid, and dried in a rotary evaporator. Boric acid was removed by repeated evaporation after adding 0.1% acetic acid in methanol. The residue, dissolved in 0.2 M NaCl, was chromatographed on a Sepharose CL-6B column (1 × 49 cm) in 0.2 M NaCl. Fractions (0.67 ml) were collected and aliquots assayed for uronic acid content. The uronic acid-containing fractions were combined, dialyzed (molecular weight cut-off 3,500), and lyophilized.

CS/DS compositional analysis

The PGs or their GAG chains (200–300 μg each) were treated with GAG degrading enzymes as follows: Chondroitinase ABC (20 milliunits) in 50 μl of 100 mM Tris-HCl, pH 8.0, containing 30 mM NaOAc at 37°C for 5 h [30]; chondroitinase AC-II (200 milliunits) in 50 μl of 100 mM NaOAc, pH 6.0, containing 0.01% BSA at 37°C for 30 min [31]; chondroitinase B (20 milliunits) in 50 μl of 50 mM Tris-HCl, pH 8.0, containing 0.05% BSA at 30°C for 2 h as described earlier [24]. The products were analyzed by gel filtration on columns of Bio-Gel P-6 (1 × 50 cm) and Sepharose CL-6B (1 × 49 cm) in 0.2 M NaCl. The fractions (0.67 ml) were collected, dried in a Speed-Vac, and analyzed for uronic acid [25].

SDS-PAGE

The core proteins of the PGs were released by the treatment with protease-free chondroitinase ABC as described above [30]. The untreated PGs and core proteins were electrophoresed on 4–15% gradient polyacrylamide gels under reducing conditions [32]. The gels were stained sequentially with Coomassie Blue, Alcian Blue, and then with ammoniacal silver [33].

Western blotting

The purified PGs (10–15 μg) were treated with protease-free chondroitinase ABC [30] and the released core proteins were electrophoresed on 4–15% gradient polyacrylamide gels under reducing conditions and electroblotted onto PVDF membranes. The membranes were blocked with 1% BSA in 50 mM Tris-HCl, 0.15 M NaCl, pH 8.0, containing 0.1% Tween 20 and incubated with 1:1000 diluted rabbit antisera against the synthetic peptides corresponding to specific peptide motifs of decorin and biglycan [34]. The immunoreactivity was visualized using 1:5000 diluted alkaline-phosphatase-conjugated goat anti-rabbit IgG as the secondary antibody and nitro blue tetrazolium/5-bromo-4-chloro-3-indoyl phosphate as colour developing reagent.

Hexosamine compositional analysis

The PGs or GAGs (10–20 μg) were hydrolyzed with 4 M HCl at 100°C for 6 h. The hydrolysates were dried in a Speed-Vac and analyzed on a CarboPac^{TM} PA10 high pH anion-exchange column (4 × 250 mm) using Dionex BioLC HPLC with pulsed amperometric detection [35]. The elution was performed with 20 mM sodium hydroxide. Hexosamine contents were determined by using standard sugar solutions.

GAG chain disaccharide compositional analysis

The PGs or GAGs (25–50 μg) were digested with chondroitinase ABC as described above [30], the proteins were precipitated with four volumes of cold methanol, and removed by centrifugation. The supernatant was dried in a Speed-Vac and the unsaturated disaccharides in the residues were analyzed on a 4.6 × 250 mm amine-bonded silica PA03 column as reported previously [24].

NH_2-terminal sequencing of the core proteins

The PGs (100 μg) were treated with protease-free chondroitinase ABC [30], and electrophoresed on 4–15% gradient SDS-polyacrylamide gels (25–30 μg/well) under reducing conditions [32]. The protein bands in the gel were electroblotted onto PVDF membranes using 10 mM CAPS, pH 11.0 containing 10% methanol. The membranes were briefly stained with Coomassie Blue, the protein bands were cut out and analyzed by NH_2-terminal sequencing at the Section of Research Resources, Pennsylvania State University College of Medicine, Hershey.

Identification of core proteins by mass spectrometry

The proteins of PGs released by chondroitinase ABC were electrophoresed as above. The gels were briefly stained with Coomassie Blue and the proteins bands in the gels were cut out were digested with 0.02 μg/μl sequencing grade trypsin in 50% acetonitrile, 40 mM ammonium carbonate, pH 8.0. The solution was desalted using ZipTip SCX tips and the peptides were analyzed using the Applied Biosystems 4700 MALDI-TOF mass spectrometer at the Section of Research Resources, Pennsylvania State University College of Medicine, Hershey.

Immunohistochemistry

The immunohistochemical staining was performed as reported recently [36]. Briefly, 1 × 1 cm pieces of umbilical cord were fixed with 2% formalin/0.5% glutaraldehyde followed by heating to 45°C in a microwave oven [37], transferred to ice-cold PBS, pH 7.2, and stored at 4°C until used. The tissues were sectioned (5-μm thickness) onto glass slides, processed and treated with protease-free chondroitinase ABC (for staining with mAbs) and/or with 0.3% H_2O_2 to inactivate endogenous peroxidase, and then blocked with 1:60 diluted normal goat or horse serum in PBS, pH 7.2, for 20 min. The sections were incubated with the purified 1:100 diluted monoclonal antibodies against GAGs or 1:200 rabbit antisera against proteoglycan peptide motifs, in PBS, pH 7.2. After 1 h, sections were washed, incubated with 1:200 diluted biotinylated goat anti-mouse IgM or horse anti-mouse IgG for tissue sections treated with mouse monoclonal antibodies. For staining with rabbit antisera, sections were incubated with 1:200 biotinylated goat anti-rabbit IgG. The bound secondary antibodies were detected with HRP-conjugated avidin and DAB (3,3′-diaminobenzidine tetrahydrochloride) color developing reagent. Sections not treated with chondroitinase ABC (for monoclonal antibodies) or those treated with pre-immune rabbit serum were used as controls [37]. The tissue sections were counterstained with methyl green, mounted under glass cover slips using permount, examined under microscope, and photographed.

Enzyme-linked immunosorbent assay

The 96-well microtiter plates (Pro-Bind from Becton Dickinson) were coated with 100 μl of bovine placenta collagen I or bovine plasma fibronectin (5 μg/ml in PBS) at 4°C overnight. The wells were blocked with 200 μl of 1% BSA in PBS for 2 h at room temperature, and incubated with 100 μl of the purified CS/DSPGs (10 μg/ml) for 2 h, washed with PBS, and incubated with 1:1000 diluted anti-decorin or anti-biglycan antibodies in PBS/0.05% Tween 20 (PBST) for 2 h. The plates were washed with PBST, and incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (1:5000 dilution in 1% BSA/PBST) for 1 h. After washing, the plates were incubated with *p*-nitrophenyl phosphate substrate solution and the developed color was measured at 405 nm. All data are presented as mean ± SD.

Other analytical procedures

The uronic acid content was determined by the Dische method [25]. Protein was estimated using Lowry protein assay kit according to the manufacturer's procedure. Statistical analysis was done by student 't' test [38]. The P values <0.05 were considered statistically significant.

Results

Isolation and purification of the human umbilical cord PGs

We have recently reported that the umbilical cord vein, arteries, and Wharton's jelly that surrounds the blood vessels contain versican, decorin and biglycan PGs [23]. In addition, a serglycin type PG was found to be present only in the vein but not in the arteries or Wharton's jelly [23]. DEAE-Sephacel chromatography of the extracts of the tissues with an isotonic buffer gave PG fractions, VPG-II, APG-II and WPG-II. These were fractionated by gel filtration on Sepharose CL-4B, after removing the co-isolated hyaluronic acid by enzymatic digestion. In each case, two distinct PG types designated VPG-IIa, VPG-IIb, APG-IIa, APG-IIb, WPG-IIa, and WPG-IIb were obtained (Figures 1 and 2A, and not shown). Of these PGs, VPG-IIa, APG-IIa, and WPG-IIa were found to be versican and their structural characterization has been reported [23]. In the case of umbilical vein, DEAE-Sephacel chromatography of the buffer extract gave an additional distinct PG fraction (VPG-I), which was identified as a serglycin type PG. Serglycin is present only in the vein but not in arteries and Wharton's jelly [23]. The

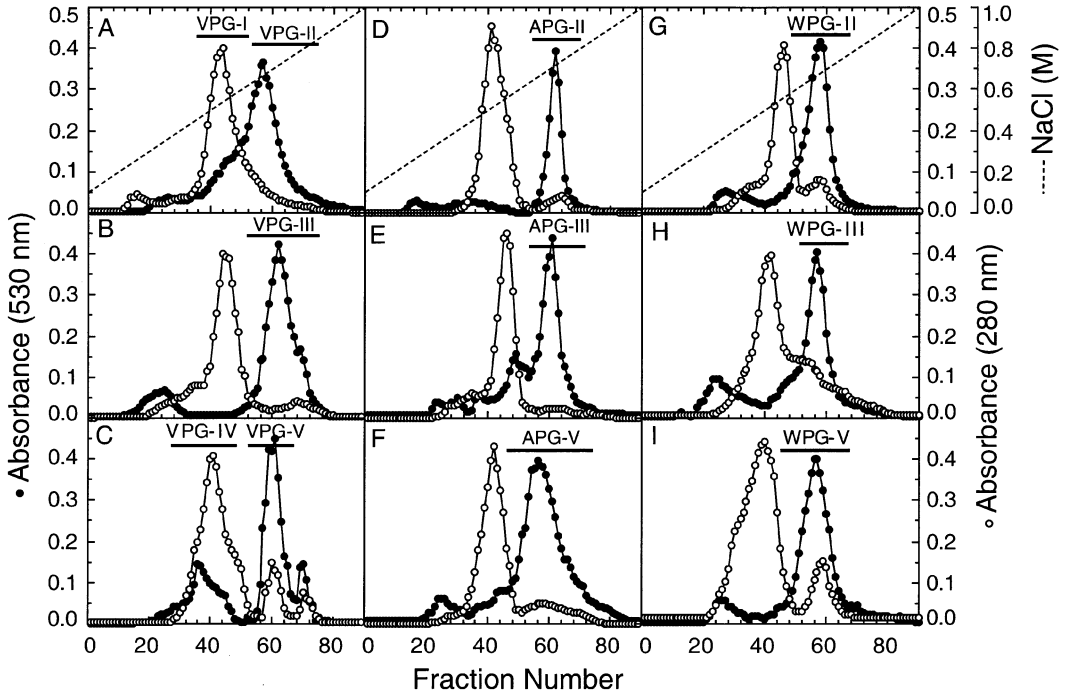


Figure 1. Isolation of the CS/DSPGs from the extracts of human umbilical cord by DEAE-Sephacel chromatography. The PGs extracted from the umbilical vein, arteries and Wharton's jelly were separately chromatographed on DEAE-Sephacel columns (2.5 × 15 cm) and eluted with a linear gradient of 0.1 to 1.0 M NaCl. Ten-ml fractions were collected, absorption at 280 nm was measured and aliquots (50–100 μl) were assayed for uronic acid (530 nm). Panel A, D, and G: Isotonic buffer extracts of the vein, arteries, and Wharton's jelly, respectively; panel B, E, and H: Buffer/detergent extracts of the vein, arteries, and Wharton's jelly, respectively; panel C, F, and I: Buffer/detergent/urea extracts of the vein, arteries, and Wharton's jelly, respectively. The uronic acid-containing fractions were pooled as indicated by the horizontal bars, dialyzed, and lyophilized to recover PGs. NaCl gradients are similar in all panels.

fractions, VPG-IIb, APG-IIb, and WPG-IIb comprised of decorin and biglycan. The characterization of these PGs and the PGs obtained by the extraction of tissues with buffer containing Triton X-100 followed by buffer containing Triton X-100 and urea is described in this paper. Since the respective PGs purified from the umbilical vein, arteries and Wharton's jelly are similar to one another, only the PGs from umbilical vein are described in detail. The yield, composition and structural characteristics of the PGs from the arteries and Wharton's jelly are summarized in Tables 1–2.

The procedures for the isolation and purification of the PGs are illustrated in Scheme 1. The extraction of the tissue pellets (see above) with PBS containing 0.5% Triton X-100 solubilized the PGs associated with cells and those weakly bound in the extracellular matrices. Final extraction of the tissue pellets with PBS containing 0.5% Triton X-100 and 6 M urea solubilized the

Table 1. Proteoglycan types extracted from the vein, arteries and Wharton's jelly of human umbilical cord

	Procedures used for the extraction of PGs and PG types extracted					
Umbilical Cord tissue Component	*PBS CSPG Serglycin*[a]	*PBS CSPG Versican*[b]	*PBS CS/DSPG Decorin/biglycan*	*Detergent/PBS CS/DSPG Biglycan/decorin*	*Urea/PBS CS/DSPG Decorin (truncated)*	*Urea/PBS CS/DSPG Decorin/biglycan*
Vein	VPG-I[c]	VPG-IIa	VPG-IIb	VPG-III	VPG-IV	VPG-V
Arteries	NP	APG-IIa	APG-IIb	APG-III	NP	APG-V
Wharton's jelly	NP	WPG-IIa	WPG-IIb	WPG-III	NP	WPG-V

NP, not present.
[a,b]The characterization of these PGs have been studied (Ref. 23).
[c]See text for designation of the PGs.

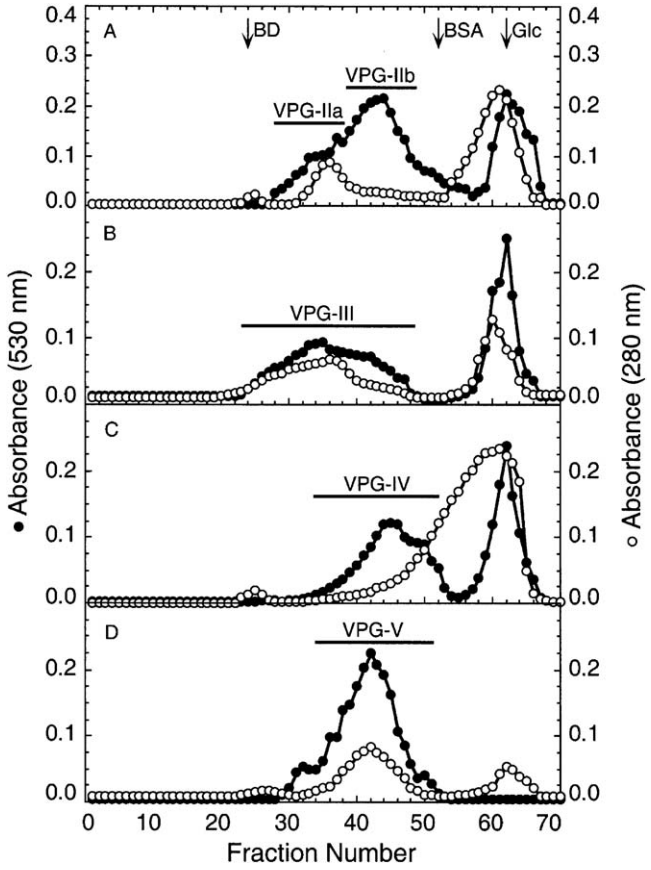


Figure 2. Purification of the CS/DSPGs of human umbilical cord by gel filtration on Sepharose CL-4B columns. The umbilical cord PG fractions obtained by DEAE-Sephacel chromatography (see Figure 1) were digested with *S. hyalurolyticus* hyaluronidase and heparitinase, and then chromatographed on Sepharose CL-4B columns (1.5 × 83 cm). Fractions of 2 ml were collected, screened for protein (280 nm) and aliquots assayed for uronic acid content (530 nm). Fractions were pooled as indicated by the horizontal bars. Panels A–D: The elution patterns of VPG-II, VPG-III, VPG-IV, and VPG-V, respectively. The chromatographic patterns of the PGs isolated from the arteries and the Wharton's jelly were similar to those from the vein (not shown). The elution positions of blue dextran (BD), BSA, and Glc, are indicated.

PGs that are tightly bound in the extracellular matrices. The PGs in these extracts were separately isolated by chromatography on DEAE-Sephacel columns (Figure 1).

DEAE-Sephacel chromatography of the PBS/detergent extract gave, in each case, a single uronic acid-containing peak, VPG-III, APG-III, and WPG-III (Figure 1B, E and H). These were recovered and treated with *S. hyalurolyticus* hyaluronidase and heparitinase. About 20–30% of the GAG chains were digested into oligosaccharides, which were removed by Sepharose CL-4B chromatography (Figure 2B, and data not shown), and the enzyme-resistant fractions, VPG-III, APG-III and WPG-III contained GalN as the predominant hexosamine.

DEAE-Sephacel chromatography of the PBS/detergent/urea-extracts of the vein separated the PGs into two major, well-resolved peaks, VPG-IV and VPG-V (Figure 1C). The minor uronic acid-containing PG fraction in Figure 1C contained predominantly GlcN. The GAG chains of this minor PG were completely susceptible to heparitinase, indicating that it is heparan sulfate proteoglycan, and it was not studied further. VPG-IV contained high levels of GalN and GlcN, whereas VPG-V had predominantly GalN. On treatment with *S. hyalurolyticus* hyaluronidase and heparitinase, ∼44% of the GAG chains in VPG-IV were degraded into oligosaccharides. The enzyme-resistant VPG-IV, when chromatographed on Sepharose CL-4B, eluted as a broad non-symmetrical peak (Figure 2C), and this fraction had predominantly GalN. VPG-V was resistant to both *S. hyalurolyticus* hyaluronidase and heparitinase confirming the absence of hyaluronic acid and heparan sulfate chains, and eluted on Sepharose CL-4B column mainly as a single peak with minor amounts of high molecular weight species (Figure 2D). In contrast to the vein, the DEAE-Sephacel chromatography of buffer/detergent/urea extract of the arteries and Wharton's jelly, each gave one major PG fraction predominantly containing GalN (APG-V and WPG-V); PGs similar to VPG-IV was absent in the arteries and the Wharton's jelly (Figure 1F and I).

The various PGs isolated from the human umbilical cord vein, arteries and Wharton's jelly, and their characteristics are summarized in Table 1. These PGs were further purified by CsBr density gradient centrifugation. In all the cases, the PGs sedimented to the high-density regions ($\rho = 1.43$–1.55 g/ml) separated from the associated protein contaminants, which remained in the low-density fractions ($\rho = 1.30$–1.35 g/ml) at the top of the CsBr gradients (data not shown). The yield and compositions of the purified PGs are summarized in Table 2. Consistent with their PG nature, all fractions contained high levels of proteins, hexosamine, and uronic acid. The presence of 92–96% GalN and 4–8% GlcN indicated that the PGs have CS, DS, or CS/DS chains.

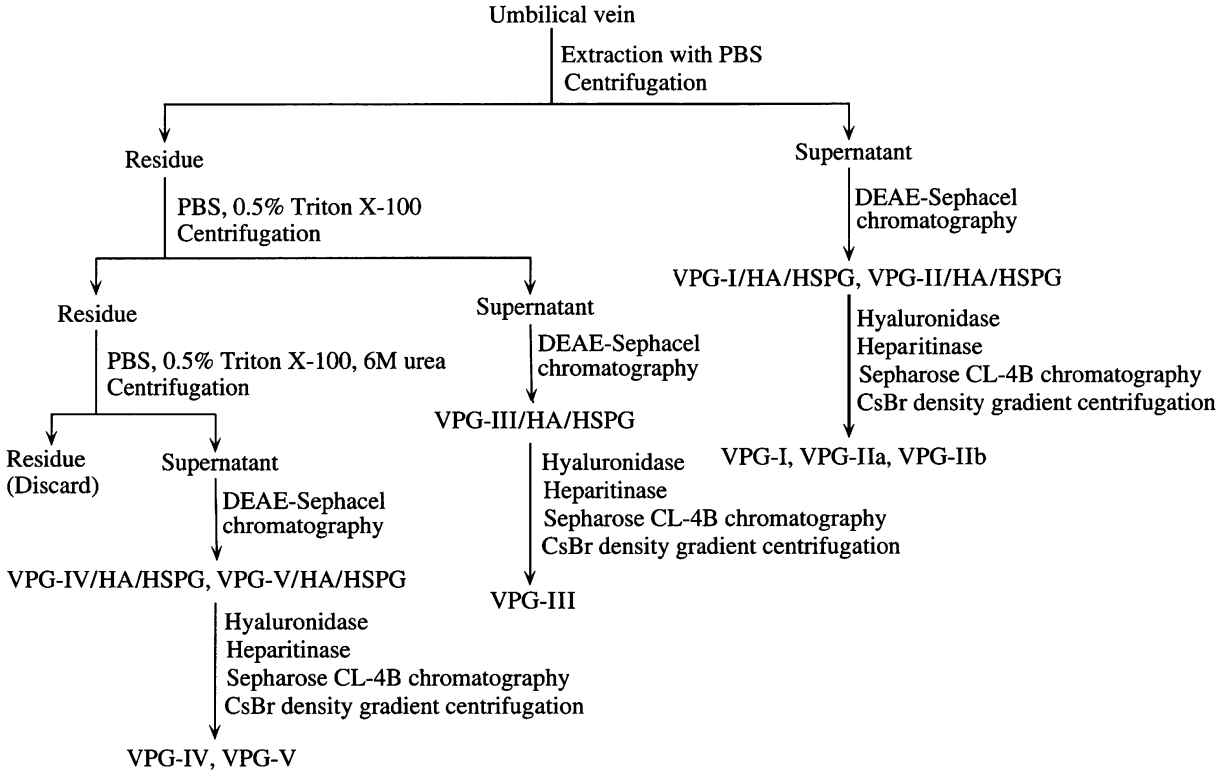
Characterization of the GAG chains of human umbilical cord PGs

The GAG chains of the various PGs from umbilical cord vein, arteries and Wharton's jelly, obtained by the β-elimination using NaOH/$NaBH_4$, were chromatographed on Sepharose CL-6B columns calibrated with GAG standards. In all cases, the GAG chains eluted on the Sepharose CL-6B column at similar elution volumes, corresponding to an estimated molecular weight of ∼60,000 (Figure 3A–C, and data not shown). In agreement with hexosamine compositions (Table 2), chondroitinase ABC completely digested the GAG chains of all these PGs into unsaturated disaccharides. HPLC analysis of the disaccharides formed revealed that the GAG chains of all these PGs consisted of mainly 4-sulfated and 6-sulfated disaccharide repeats with minor amounts of non-sulfated disaccharide residues (Table 2). On treatment with chondroitinase AC-II,

Table 2. Yield and composition of the proteoglycans purified from the human umbilical cord vein, arteries, and Wharton's jelly.

Location in the cord/ proteoglycan	*Yield (mg/100 g tissue)*[a]	*Composition (wt.%)*		*HexN ratio (mol %)*			*Disaccharides (mol%)*[e]			*GAG type(%)*[f]	
		Protein[b]	*Uronic acid*[c]	*HexN*[d]	*GalN*[d]	*GlcN*[d]	*Δdi-0S*	*Δdi-4S*	*Δdi-6S*	*CS*	*DS*
Vein											
VPG-IIb	4.3	25	31	34	93	7	13	31	56	90	10
VPG-III	1.4	24	32	34	93	7	10	42	48	89	11
VPG-IV	2.5	20	33	36	94	6	7	62	31	53	47
VPG-V	5.3	24	30	35	95	5	8	55	37	65	35
Arteries											
APG-IIb	4.9	26	31	33	93	7	11	27	62	90	10
APG-III	2.1	27	30	35	94	6	28	21	51	91	9
APG-V	9.2	25	31	34	95	5	14	45	41	65	35
Wharton's jelly											
WPG-IIb	5.9	28	29	33	94	6	17	28	55	90	10
WPG-III	2.5	26	31	35	92	8	32	17	51	95	5
WPG-V	11.2	24	31	35	96	4	15	49	36	65	35

[a]Yield of PGs after final purification.
[b]By the modified Lowry protein assay.
[c]By the carbazole method.
[d]By the high pH anion-exchange HPLC.
[e]Determined by HPLC analysis of unsaturated disaccharides released by the digestion of the GAG chains with chondroitinase ABC. The molar response factors for sulfated and non-sulfated unsaturated disaccharides were considered equal and the peak areas of the HPLC detector response were used for calculating their percent mol proportions.
[f]Based on the analysis of the chondroitinase B and chondroitinase AC-II digestion products on Bio-Gel P-6 and Sepharose CL-6B columns.



Scheme 1. Flow chart illustrating the isolation and purification of CS/DSPGs from human umbilical cord. The umbilical cord vein, arteries and Wharton's jelly were extracted sequentially with isotonic buffer, buffer/detergent, and buffer/detergent/urea. The PGs in the extracts were isolated by DEAE-Sephacel chromatography (see Figure 1), and purified by gel filtration on Sepharose CL-4B (see Figure 2) and CsBr density gradient centrifugation (not shown). Shown is the chart for the isolation of PGs from the umbilical vein. Similar procedures were used for the isolation and purification of PGs from the umbilical cord arteries and Wharton's jelly.

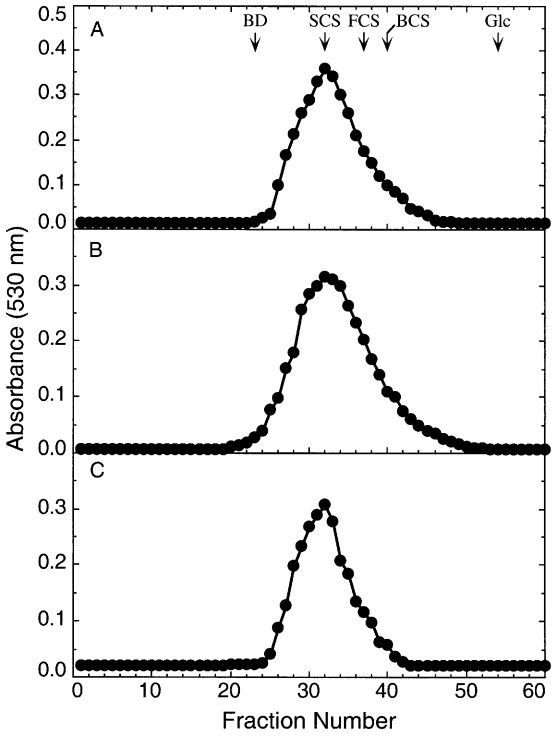


Figure 3. Determination of the GAG chain size of the human umbilical cord CS/DSPGs. The GAGs (80–120 μg), released by NaOH/$NaBH_4$ β-elimination of the umbilical cord CS/DSPGs, were chromatographed on Sepharose CL-6B columns (1 × 49 cm). Fractions of 0.67 ml were collected, and aliquots assayed for uronic acid (530 nm). Panels A–C: The elution pattern of GAG chains of VPG-IIb, VPG-IV, and VPG-V, respectively. The GAGs from VPG-III and those from the PGs of the umbilical arteries and Wharton's jelly exhibited similar elution patterns (not shown). The elution positions of BD, Glc, shark cartilage C6S with M_r 60,000 (SCS), sturgeon notochord C4S with M_r 37,000 (FCS), and bovine trachea C4S with M_r 25,000 (BCS) are indicated.

89–95% of the GAG chains of VPG-IIb, VPG-III, APG-IIb, APG-III, WPG-IIb, and WPG-III were converted into unsaturated disaccharides. Consistent with these results, chondroitinase B converted 5–11% of the GAG chains of these PGs into unsaturated di- and tetrasaccharides, and ~90% of GAGs into larger fragments. From these results, it was estimated that the GAG chains of these PGs have 89–95% CS and 5–11% DS structures (Table 2). In contrast, treatment with chondroitinase AC-II converted ~65% of the GAGs of VPG-V, APG-V and WPG-V into unsaturated disaccharides, whereas treatment with chondroitinase B converted ~35% of the GAG chains into unsaturated di- and tetrasaccharides. These data demonstrated that the GAGs of VPG-V, APG-V and WPG-V have ~65% CS and ~35% DS (Table 2). In the case of VPG-IV, chondroitinase AC-II converted ~53% of the GAGs into unsaturated disaccharides, while chondroitinase B converted ~47% of the GAG chains into unsaturated di- and tetrasaccharides. These results indicated that the GAGs of VPG-IV have ~53% CS and ~47% DS (Table 2). Analysis of the chondroitinase B-digestion products of the GAG chains on Sepharose CL-6B indicated that, in all proteoglycans, >90–95% of the enzyme resistant portions of the GAG chains were eluted at lower molecular weight regions compared with that of the untreated GAG chains. These data indicate that the GAG chains of the proteoglycans are present as CS/DS copolymers.

Characterization of the core proteins of human umbilical cord PGs

The core proteins obtained by treatment of the purified PGs with chondroitinase ABC were analyzed by SDS-PAGE, Western blotting, NH_2-terminal sequencing, and mass spectrometry.

On SDS-PAGE, the PG fractions VPG-IIb, APG-IIb, WPG-IIb, VPG-III, APG-III, WPG-III, VPG-V, APG-V, and WPG-V, each electrophoresed as a polydispersed band with mobility corresponding to an average M_r of ~150-kDa and a high molecular weight (>200 kDa) species (Figure 4, and data not shown). In each case, a significant amount of the material was eluted as a ~35–40-kDa species, which appears to represent the cell-associated GAGs co-purified with the PGs. Treatment with chondroitinase ABC released, in each case, 43–48-kDa multiple core proteins (Figure 4, and data not shown). Based on the

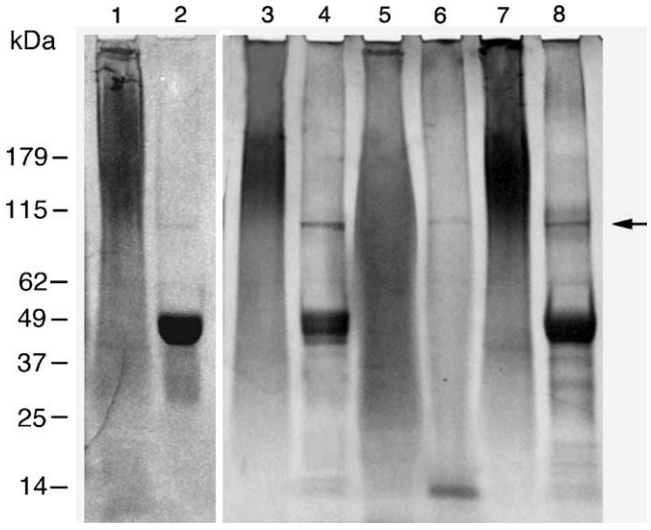


Figure 4. SDS-PAGE of the human umbilical cord CS/DSPGs and their core proteins. The purified PGs (30–40 μg) of the umbilical cord and the core proteins (15–20 μg) released by the treatment of the PGs with chondroitinase ABC were electrophoresed on 4–15% gradient SDS-polyacrylamide gels under reducing conditions. The gels were stained successively with Coomassie Blue, Alcian Blue, and ammoniacal silver. Lanes 1, 3, 5, and 7, untreated VPG-III, VPG-IIb, VPG-IV, and VPG-V, respectively; lanes 2, 4, 6, and 8, chondroitinase ABC-treated VPG-III, VPG-IIb, VPG-IV, and VPG-V, respectively. The positions of the protein molecular weight markers are indicated to the left. The arrow on the right indicates the position of chondroitinase ABC.

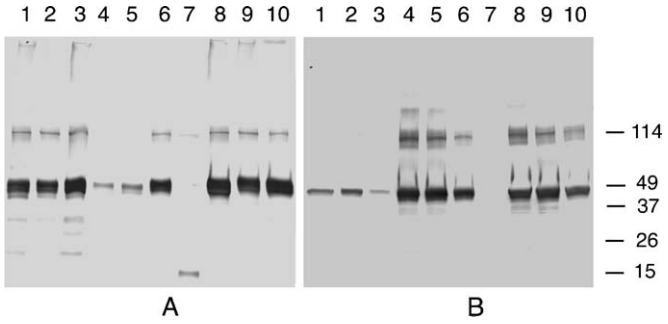


Figure 5. Western blot analysis of the core proteins of the purified umbilical cord CS/DSPGs. The core proteins (5–10 μg) released by chondroitinase ABC-treatment of PGs purified from the human umbilical vein, arteries, and Wharton's jelly were electrophoresed on 4–15% gradient SDS-polyacrylamide gels under reducing conditions. The protein bands in the gels were electroblotted onto PVDF membranes and probed with 1:1000 diluted anti-decorin or anti-biglycan antibodies. The bound antibodies were detected with alkaline phosphatase-conjugated goat anti-rabbit IgG using NBT and BCIP reagent. Panel A: Lanes 1–10, the core proteins of VPG-IIb, APG-IIb, WPG-IIb, VPG-III, APG-III, WPG-III, VPG-IV, VPG-V, APG-V, and WPG-V, respectively, probed with anti-decorin peptide antibodies. Panel B: Lanes 1–10, the core proteins as in (panel A), but probed with anti-biglycan peptide antibodies. The numbers on the right indicate the molecular mass (kDa). The 114-kDa bands in the immunoblot correspond to chondroitinase ABC. The cross-reactivity of the chondroitinase ABC band on the membranes with the antisera is likely due to the exposure of animals to bacteria that produces the enzyme.

size of the core proteins (43–48 kDa), and the estimated molecular weight of the GAG chains (~60,000), these PG species must be substituted with one or two GAG chains.

In the case of VPG-IV, SDS-PAGE showed two distinct polydispersed bands, a major ~100-kDa and a minor ~30-kDa species (Figure 4, lane 5); the ~30-kDa PG species likely represents the intracellular CS/DS chains lacking core proteins. Upon chondroitinase ABC treatment, VPG-IV, released a ~14 kDa-core protein (Figure 4, lane 6). Based on the estimated size of the major GAG chains (~60 kDa), the core protein of VPG-IV is likely to be substituted with one GAG chain. These results are consistent with VPG-IV being a truncated form of decorin (see below).

The core proteins of the PGs of the umbilical cord were also analyzed by Western blotting using antibodies against specific peptide motifs of decorin and biglycan core proteins [34]. The 43–48-kDa core proteins of VPG-IIb, APG-IIb, WPG-IIb, VPG-III, APG-III, WPG-III, VPG-V, APG-V, and WPG-V were immunoreactive to decorin and biglycan anti-peptide antibodies (Figure 5A and B). While 45 and 48 kDa bands were reactive to decorin anti-peptide antibodies, the 43- (minor) and 46-kDa (major) core proteins were immunoreactive to biglycan anti-peptide antibodies (Figure 5A and B). Interestingly, the CS/DSPGs that extracted with the isotonic buffer, VPG-IIb, APG-IIb, and WPG-IIb, contained mainly decorin and low proportions of biglycan (compare lanes 1–3 in Figure 5A and B). The CS/DSPGs obtained with detergent/urea-containing buffers, VPG-V, APG-V and WPG-V, have higher proportions of decorin than biglycan (compare lanes 8–10 in Figure 5A and B). In contrast, CS/DSPGs that extracted with detergent-containing buffer (VPG-III and APG-III) had higher proportions of biglycan than decorin (compare lanes 4 and 5 in Figure 5A and B); in the case WPG-III, however, decorin and biglycan are in nearly equal proportion (see lane 6 in Figure 5A and B). The 14-kDa core protein of VPG-IV was reactive to anti-decorin antibody but not to the anti-biglycan antibody (lane 7 in Figure 5A and B).

The NH_2-terminal amino acid sequences of the anti-decorin antibody-reactive 45- and 48-kDa core proteins of both VPG-IIb, and VPG-V were determined and the sequences of the 16 amino acids were identical (DEASGIGPEVPDDRDF). These NH_2-terminal sequences were identical to the NH_2-terminal sequences of the previously reported core proteins of the fetal membrane and fetal fibroblast cell decorin DSPGs [39,40] and partial homology with calf cartilage DSPGs [41]. The NH_2-terminus sequences of 20 amino acids of the 46-kDa core protein of VPG-III was found to be DEEASGADTSGVLD-PDSVTP, which is identical to the NH_2-terminal amino acid sequence deduced from the cloned human biglycan gene [14,42]. The NH_2-terminal sequences of the core proteins from the remainder of the PGs of umbilical vein, arteries and Wharton's jelly were not determined. However, based on similar electrophoretic mobility and immunoreactivity of the respective core proteins with anti-decorin and anti-biglycan antibodies (see above), the core proteins of the PGs, APG-IIb, WPG-IIb APG-III, WPG-III, APG-V, and WPG-V, were presumed to be very similar or identical to the core proteins of the corresponding PGs from umbilical vein.

Interestingly, the NH_2-terminal sequences of 14-kDa core protein of VPG-IV is similar to the 45- and 48-kDa core proteins of VPG-IIb and VPG-V decorins. The amino acid composition of core protein of VPG-IV (not shown) is similar to that of the NH_2-terminal 110 amino acid residues of the core protein of the fetal fibroblast cell DSPG [39]. These results indicate that the 14-kDa core protein of VPG-IV is a truncated form of the 45- and 48-kDa core proteins of decorin, likely formed by a specific proteolytic cleavage of the C-terminal portion.

The anti-decorin antibody reactive 48-kDa core protein of VPG-IIb, and the 46-kDa anti-biglycan antibody reactive core protein of VPG-III were further studied by mass spectrometry of the peptides obtained by the digestion with trypsin. As shown in Table 3, various peptides obtained from the 48-kDa core protein of VPG-IIb exactly matched the amino acid sequences in the corresponding regions of the decorin core protein [40]. Similarly, the sequences of the peptides derived from the 46-kDa core protein of VPG-III matched the amino acid sequence in the corresponding regions of the biglycan core protein (Table 4).

Table 3. Identification of the peptides obtained by the trypsin digestion of the human umbilical cord decorin core protein by mass spectrometry

Peptides[a]	*Calculated mass*	*Observed mass*	*Determined peptide sequence*	*Positions of amino acid in decorin core protein sequence*[b]
1	873.3818	873.4426	CQCHLR	58–63
2	1513.8737	1513.9871	VSPGAFTPLVKLER	120–133
3	971.5230	971.5959	ELPEKMPK	143–150
4	941.4686	941.5352	AHENEITK	157–164
5	1059.6156	1059.6718	ISRVDAASLK	233–242
6	1277.7324	1277.7871	LTRVPGGLAEHK	281–292
7	2763.3413	2763.5383	ASYSGVSLFSNPVQYWEIQPSTFR	322–345

[a]The 48 kDa core proteins of VPG-IIb were digested with trypsin and the peptides were analyzed by MALDI-TOF.
[b]The amino acid residues corresponding to the human decorin amino acid sequence (Ref. 40).

Table 4. Identification of the peptides obtained by the trypsin digestion of the human umbilical cord biglycan core protein by mass spectrometry

Peptides[a]	*Calculated mass*	*Observed mass*	*Determined peptide sequence*	*Position of amino acids in biglycan core protein sequence*[b]
1	1118.5874	1118.5361	VVQCSDLGLK	73–82
2	2286.1460	2286.0562	EISPDTTLLDLQNNDISELR	87–106
3	818.4882	818.4727	AFSPLRK	133–139
4	2027.1283	2027.0487	NHLVEIPPNLPSSLVELR	148–165
5	1787.8922	1787.8291	DLPETLNELHLDHNK	226–240
6	1322.7174	1322.6709	ELHLDNNKLAR	281–291
7	1526.7129	1526.6599	VGVNDFCPMGFGVK	315–328

[a]The 46 kDa core proteins of VPG-III were digested with trypsin and the peptides were analyzed by MALDI-TOF.
[b]The amino acid residues corresponding to the human biglycan amino acid sequence (Ref. 14).

Binding of umbilical cord decorins and biglycans to collagen I and fibronectin

The differential extraction procedure used in this study revealed that the decorin and biglycans with ∼10% DS contents (VPG-IIb, APG-IIb, WPG-IIb, VPG-III, APG-III, and WPG-III) are bound loosely in the matrix (extracted with buffer alone or buffer with a non-ionic detergent), whereas those that contain 35–47% DS (VPG-IV, VPG-V, APG-V, and WPG-V) are bound tightly (need urea for extraction). To determine whether this is due to differential interactions of the PGs with matrix proteins, we measured the ability of decorins and biglycans including the truncated form of decorin to bind collagen I and fibronectin (Figure 6). The CS/DSPGs from vein, arteries, and Wharton's jelly exhibited similar binding to collagen I and fibronectin and therefore, only the results of umbilical cord vein CS/DSPGs are presented. The decorins with 10% as well as those with 35% DS could bind efficiently to both collagen I and fibronectin (Figure 6A). When probed with anti-biglycan antibody, the biglycan-rich PG fraction (VPG-III) exhibited high level of binding to collagen I as well as to fibronectin (Figure 6B). The fraction containing substantial amount of biglycan (VPG-V) also showed significant binding to fibroncetin and marginal binding to collagen I (Figure 6B). This is in agreement with the previous reports that biglycan also can bind collagen I and fibronectin [6–8,43]. These data are consistent with the results of previous studies that the leucine-rich repeats 4–5 of decorin core protein is mainly responsible for binding of decorin to collagen I [44]. However, the observed binding strength was significantly high ($p < 0.001$) for decorins and biglycans containing 35% DS compared to those with only 10% DS. Further, the truncated form of CS/DSPG (VPG IV) that lacks ∼two-third of the C-terminal portion of the core protein but contain 47% DS also bound collagen I and fibronectin (Figure 6A). Thus, these data demonstrate that, in addition to the C-terminal region of the core proteins of decorin and biglycan, the GAGs with high level of DS also interact with matrix proteins.

Tissue distribution of umbilical cord PGs

The distribution of CS/DSPGs in the umbilical cord was studied by immunohistochemical analysis of the tissue sections using a panel of antisera specific to PG core proteins and monoclonal antibodies specific to GAG chains. For analysis based on PG core proteins, rabbit antisera against the specific peptide motifs of decorin, and biglycan core proteins, and a rabbit antiserum

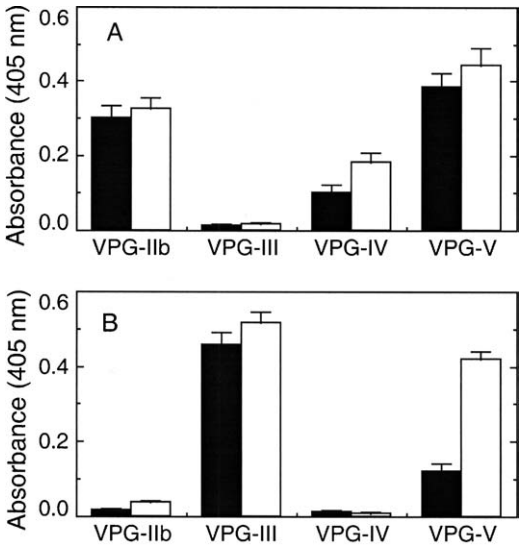


Figure 6. The binding of CS/DSPGs from human umbilical cord to fibronectin and type I collagen. The microtiter plates coated with 5 μg/ml of fibronectin or type I collagen were blocked and incubated with 10 μg/ml of CS/DSPGs for 2 h. The wells were washed with PBS, incubated with anti-decorin (panel A) or anti-biglycan (panel B) antibodies for 2 h, followed by incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG. The color was developed with *p*-nitrophenyl phosphate as substrate and the absorbance was measured at 405 nm. The dark bar indicates type I collagen and white bar indicates fibronectin in both the panels.

against the purified placental DS/CSPG-2 were used [34,36]. For the GAG chain based immunohistochemistry, a monoclonal antibody (CS-56) that is specific to the 4- or 6-sulfated chondroitin chains (C4S or C6S) but not DS, and two monoclonal antibodies that are specific to Δdi-4S and Δdi-6S stubs formed in PGs treated with chondroitinase ABC were used.

In the chondroitinase ABC-treated umbilical cord sections, the decorin and biglycan antisera strongly stained the extracellular matrices of the blood vessel walls, Wharton's jelly, and smooth muscle tissue of the arteries (Figure 7A-1–A-5 and Figure 7B-1–B-5). The intensity of staining of the smooth muscles of the umbilical vein and arteries was significantly higher with anti-biglycan antisera than anti-decorin antisera (compare Figure 7A-2 and A-4 with Figure 7B-2 and B-4). However, in the case tissue, the proportion of decorin is higher than biglycan. The antisera also stained the endothelial surface and matrices of the endothelial bed of the vein but not that of arteries. In the tissue sections not treated with chondroitinase ABC, however, the matrices of Wharton's jelly surrounding the blood vessels and blood vessel walls were lightly stained by both antisera, even though decorin and biglycan are present at high levels (not shown). The increase in immunostaining after treatment with chondroitinase ABC is because the peptide motifs of decorin and biglycan, used for antisera production, are in close proximity to the GAG attachment sites [42,45,46]. However, the staining in the matrices of smooth muscle tissue of the umbilical vein and arteries by decorin and biglycan anti-peptide antibodies was nearly comparable in intensity in untreated and chondroitinase ABC-treated tissue sections. Therefore, it appears that the matrices of the endothelial cell and smooth muscle cell layers contain significant levels of decorin and biglycan core proteins lacking the GAG chains and the decorin and biglycan substituted with GAG chains are either absent or present at low levels on the endothelial surface of the vein and arteries. The staining pattern of the umbilical cord sections (not shown) with rabbit antiserum raised against the previously characterized placental DS/CSPG-2 [24] was generally similar to that of the anti-decorin antisera (see above).

The umbilical cord sections treated with CS-56 mAb intensely stained the matrices of the blood vessel walls (Figure 7C-1 and C-3). The staining patterns in the walls of the umbilical vein and arteries resembled the extracellular space between the characteristic inner longitudinally and outer circularly layered distribution of smooth muscle cells in the endothelial beds of vein and arteries (Figure 7C-1 and C-3). The antibody also stained the extracellular matrix of the connective tissue of the Wharton's jelly (Figure 7C-5). Staining with anti-C4S/C6S monoclonal antibody was abolished upon preincubation of tissue sections with chondroitinase ABC (compare Figure 7D-1–D-5 with Figure 7C-1–C-5), suggesting that the staining was specific to the GAG chains of the umbilical cord PGs.

The monoclonal antibody specific to the Δdi-4S stubs, formed when the PGs were treated with chondroitinase ABC, intensely stained the matrices of the blood vessel walls, the smooth muscle tissues of the umbilical vein and arteries, and Wharton's jelly (Figure 7E-1–E-5). Similar staining pattern was observed with the monoclonal antibody that is specific to Δdi-6S (Figure 7F-1–F-5).

Discussion

The results described in this paper show that the human umbilical cord extracellular matrix contains high levels of decorin and biglycan. Both PG types exist in two distinct forms with different GAG chain composition, one with very low and the other with moderate levels of DS. Several lines of evidence identify these PGs as decorin and biglycan CS/DSPGs. These include: (1) The 45- and 48-kDa core proteins of the CS/DSPGs are immunoreactive to anti-decorin peptide antibodies, and the 43- and 46-kDa core proteins are cross-reactive to anti-biglycan peptide antibodies (Figure 5). (2) The 43- and 46-kDa as well as the 45-kDa and 48-kDa core proteins of the umbilical cord CS/DSPGs have N-linked complex type oligosaccharides (Valiyaveettil *et al*., unpublished data), which is a feature of biglycans and decorins [47]. The 46- and 48-kDa core proteins contain higher proportions of carbohydrate than those in the 43- and 45-kDa core proteins (Valiyaveettil *et al*., unpublished data). (3) The peptides formed by the treatment of core proteins matched

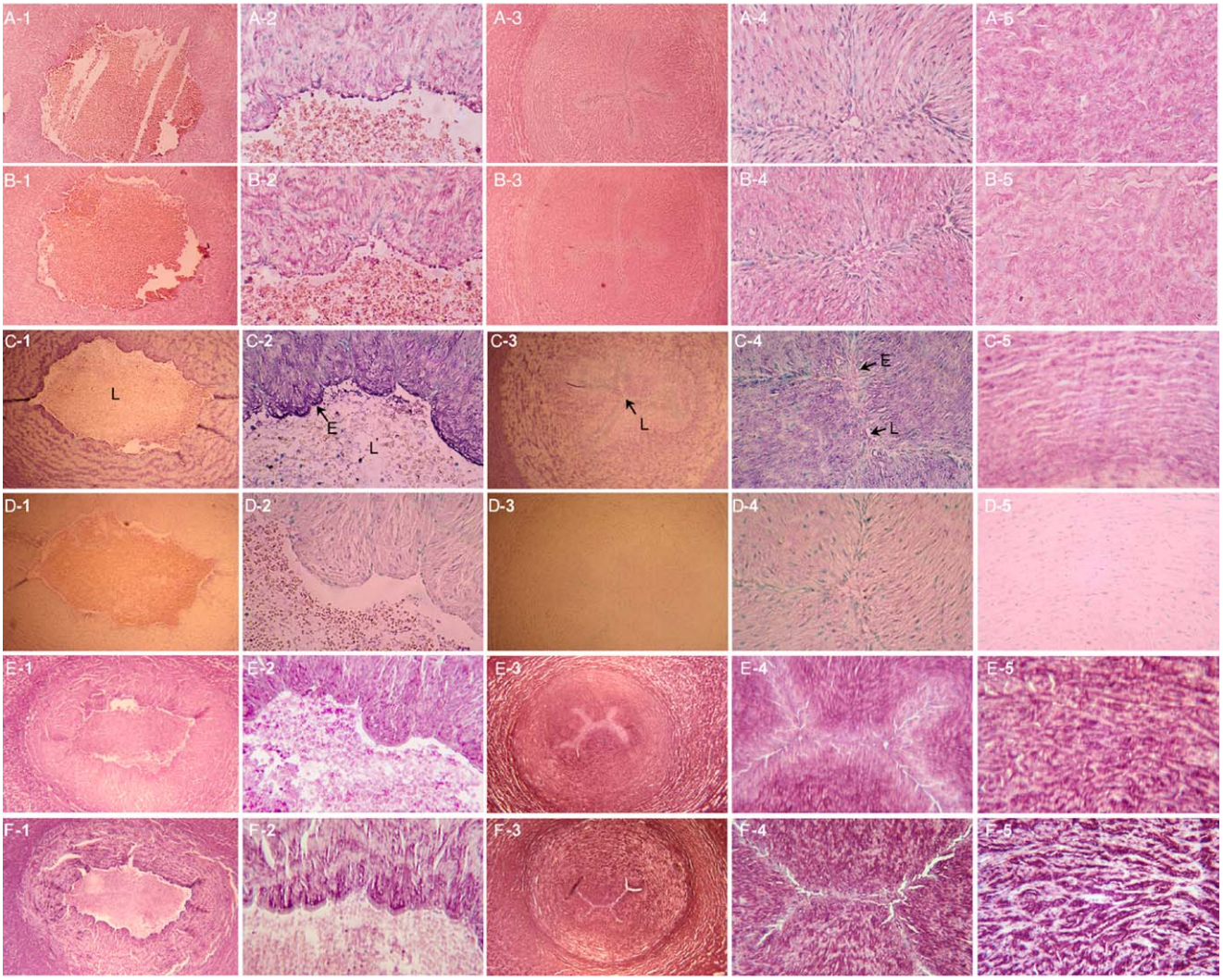


Figure 7. Immunohistochemical localization of the CS/DSPGs of human umbilical cord. The umbilical cord was fixed with formalin/glutaraldehyde followed by heat treatment with microwaves, sectioned, and stained with antisera against decorin and biglycan peptides, and with monoclonal antibodies against C4S and C6S. Panel A: Chondroitinase ABC-treated tissue sections stained with antisera against decorin. Panel B: Chondroitinase ABC-treated tissue sections stained with antisera against biglycan. Panel C and D: Tissue sections stained with monoclonal antibodies against C4S and C6S before (C) and after (D) treatment of the tissue sections with chondroitinase ABC. Panel E: Chondroitinase ABC-treated tissue section stained with the monoclonal antibody specific to Δdi-4S. Panel F: Chondroitinase ABC-treated tissue section stained with monoclonal antibody specific to Δdi-6S. Shown are the portions of the tissue sections. Umbilical vein (A-1, A-2, B-1, B-2, C-1, C-2, D-1, D-2, E-1, E-2, F-1, and F-2); umbilical arteries (A-3, A-4, B-3, B-4, C-3, C-4, D-3, D-4, E-3, E-4, F-3 and F-4); Wharton's jelly (A-5, B-5, C-5, D-5, E-5, and F-5). Panels A-1, B-1, C-1, D-1, E-1, F-1, A-3, B-3, C-3, D-3, E-3, and F-3 are at 4 $\times$ magnification; panels A-2, B-2, C-2, D-2, E-2, F-2, A-4, B-4, C-4, D-4, E-4, F-4, A-5, B-5, C-5, D-5, E-5, and F-5 are at 20X magnification. E, endothelial cell layers; L, lumen of the vein or the arteries.

exactly the peptide motifs of human decorin and biglycan core proteins [14,40]. (4) The NH_2-terminal sequence of the 46-kDa core proteins of the umbilical cord CS/DSPGs are identical to that of human biglycan, whereas NH_2-terminal sequences of 45- and 48-kDa core proteins are identical to human decorin previously characterised from a variety of sources, including, human bone cells, fetal fibroblast cell line IMR-90, human placenta, fetal membrane DSPG [24,39,40]. Collectively, these data conclusively establish that the PGs are decorin and biglycan.

Our data indicate that the decorins and biglycans with different DS and CS contents differentially interact with the matrix molecules. Based on the differential extraction of decorin and biglycans with different CS and DS contents, it is likely that the differences in GAG chain composition impart the PGs different abilities to interact with matrix proteins. Thus, the decorins and biglycans (with $\sim$10% DS and $\sim$90% CS) that were readily extracted by the isotonic buffer and the buffer containing mild detergent, respectively, are loosely bound to the matrix proteins.

In contrast, the CS/DSPGs with higher levels of DS required urea for their extraction, indicating that they are strongly interacting with the matrix components, and thus they must be tightly bound in the matrix.

Previous studies have shown that decorin can bind collagen I with high affinity through the interaction of leucine-rich repeat regions 4 and 5 of the core proteins [44]. Consistent with these results, the decorin isolated from various locations of the umbilical cord, irrespective of their CS and DS contents efficiently bound to collagen I as well as to fibronectin. However, the binding strength of the umbilical cord decorins and biglycans with higher levels of DS was significantly higher compared to those with very low levels of DS. This is despite the presence of identical core proteins. Therefore, components other than the core proteins of umbilical cord decorins and biglycans also must be interacting with the matrix proteins. It has been previously proposed that decorin GAG chains undergo self-association, which occurs optimally if the GAG chains contain alternative glucuronic acid- and iduronic acid-containing disaccharide units [48]. Therefore, it is possible that the GAG chains of the umbilical cord decorin and biglycan that contain 35–47% DS are suited for strong self-association, contributing to the tight binding of these PGs. Our observation that the truncated form of decorin, which lacks the ∼30-kDa C-terminal portion of the core proteins, also bound tightly in the matrix of the umbilical vein supports this conclusion.

The results of this study also show that a truncated form of decorin (VPG-IV) with 14-kDa core protein is present only in umbilical vein but not in the arteries and Wharton's jelly. The core protein of this truncated decorin corresponds to approximately one-third of the NH_2-terminal portion of the decorin core protein based on the NH_2-terminal amino acid sequence. The NH_2-terminal sequence of the truncated decorin core protein is identical to those of the 45- and 48-kDa decorin core proteins of VPG-IIb and VPG-V as well those of the previously studied fetal membrane DSPG, and human fibroblast cell DSPG [14,39,40]. The core protein of VPG-IV is immunoreactive to the anti-decorin antibodies that are directed against the peptide motif, GIGPEVPDDRDF, in the NH_2-terminal region of the decorin core protein [34]. Further, the amino acid composition of the 14-kDa core protein of VPG-IV closely resemble the amino acid composition calculated for the 110 amino acids from the NH_2-terminal end of the deduced amino acid sequence of the fetal fibroblast cell DSPG and human decorin. A previous study has reported the occurrence of a truncated form of decorin with 14.5-kDa in human post burn keloid scar [49]. Truncated form of decorin with ∼20-kDa core proteins have also been reported in newborn calf skin [50], rat submandibular gland [51], and bovine cornea [52]. Although the nature of these truncated forms of decorin was not determined in those studies, it appears likely that they all contain NH_2-terminal portion of the core protein because the GAG chain in decorin maps to its extreme NH_2-terminal end.

A recent study has shown that a truncated decorin PG (termed decorunt) with ∼17-kDa core protein is present in adult human skin but not in fetal skin [53]. The decorunt has been demonstrated to be the product of a specific proteolytic cleavage of C-terminal end of decorin [53]. However, based on the data presented here, it is not possible to conclude whether the core protein of VPG-IV is formed by a proteolytic removal of the C-terminal ∼30-kDa portion of the core protein of VPG-V or is a product of alternative splicing. Regardless, our data strongly suggest that VPG-IV also interact with matrix proteins, presumably through its CS/DS chains. The expression of the truncated form of decorin specifically in the umbilical vein suggests location-specific functional role for this PG.

Acknowledgments

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