Domain structure elucidation of human decorin glycosaminoglycans

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INTRODUCTION

PGs (proteoglycans) are composed of one or more GAG (glycosaminoglycan) chains with MMs (molecular masses) in the range 5–40 kDa, covalently attached to a protein core [1]. PGs make up a large proportion of the extracellular matrix, performing a variety of roles in the excretory system, respiratory system, circulatory system and skeletal system, and are involved in multisystem diseases of aging and cancer [2]. The GAG chains are responsible for many of the PG biological functions, and subtle variations in the GAG structure can have a pronounced effect on the organism physiology and pathophysiology [3].

Decorin is one of the simplest cellular or pericellular matrix PGs belonging to the SLRP (small leucine-rich proteoglycan) family. Decorin consists of a protein core containing multiple leucine repeats with flanking cysteine-rich disulfide domains and a single CS (chondroitin sulfate) or DS (dermatan sulfate) GAG chain [4]. Decorin maps to human chromosome 12 and, under the influence of a responsive promoter, is biosynthesized in the endoplasmic reticulum as a nascent protein containing a signal peptide and propeptide [5]. The single GAG attachment site in decorin is located near its N-terminus (Ser34; Swiss-Prot accession number P07585), and several N-linked oligosaccharides are present [6].

Key words: decorin, dermatan sulfate (DS), LC-MS, proteoglycan, structural domain, structural motif.
Following the biosynthesis of the core protein, the linkage region is biosynthesized and the nascent GAG is extended with a simple 4→β-D-GlcA (1→3)β-D-GalNAc(1→) repeating disaccharide unit (chondroitin). Subsequent chain modification can result in up to 16 different disaccharide units in chondroitin/DS. Symbols correspond to xylose (stars), galactose (grey circles), N-acetylgalactosamine (white squares), GlcA (black-and-white diamonds) and iduronic acid (white-and-grey diamonds).

Previously, we reported MM and composition analysis of intact bikunin GAG using a high-resolution MS technique, Fourier-transform ion cyclotron resonance MS [14]. However, this approach has limitations in handling the higher MM and greater structural complexity of the decorin GAG. Thus the MM analysis of the intact decorin GAG and high-MM products of its enzymatic degradation was performed using PAGE. A series of selective enzymatic degradation steps was used for the preparation and characterization of decorin GAG oligosaccharides in a bottom-up type sequencing approach. Information obtained in the process of oligosaccharide mapping was combined with the data obtained from NMR spectroscopy and LC-MS disaccharide compositional analyses and used for reconstructing the decorin GAG sequence motifs.

**MATERIALS AND METHODS**

**Materials**

Decorin was generously provided by Life Cell Corporation. Actinase E [EC (Enzyme Commission) 3.4.24.4] was from Kaken Biochemicals. CS-A was purchased from Celsus Laboratories. Endolytic chondroitinase ABC from *Proteus vulgaris* (EC 4.2.2.4), endolytic chondroitinase ACI from *Flavobacterium heparinum* (EC 4.2.2.5), exolytic chondroitinase ACII from *Arthrobacter aurescens* (EC 4.2.2.5), endolytic chondroitinase B from *F. heparinum* (EC 4.2.2.x), and 4,5-unsaturated CS/DS disaccharide standards [ΔUA-GalNAc (0S, where S is sulfo), ΔUA-GalNAc4S (4S), ΔUA-GalNAc6S (6S), ΔUA2S-GalNAc (2S), ΔUA2S-GalNAc4S (2S4S), ΔUA2S-GalNAc6S (2S6S), ΔUA-GalNAc4S6S (4S6S), ΔUA2S-GalNAc4S6S (2S4S6S)] were from Seikagaku (Associates of Cape Cod). CS-C, urea, CHAPS, Aiclan Blue and tributylamine were from Sigma. Electrophoresis-grade acrylamide, N,N-methylenebis(acrylalamide), glycine, TEMED (N,N,N,N-tetramethylethylenediamine), APS (ammonium persulfate), Tris and sodium EDTA were from Bio-Rad Laboratories. All solvents were HPLC grade.

**Preparation and purification of decorin PG**

Recombinant human decorin PG was expressed as a polyhistidine fusion protein in a stably transfected HEK (human embryonic kidney)-293-EBNA cell line using a Celligen Plus bioreactor (New Brunswick Scientific) as previously described [15]. Initial
purification of recombinant decorin was performed by passing HEK-293-cell conditioned medium over a nickel-charged HiTrap chelating column (GE Healthcare) followed by elution with a gradient of 0–250 mM imidazole in 20 mM Tris/HCl, pH 8.0, 500 mM NaCl and 0.2 % CHAPS. Decorin PG was further purified by applying to a HiTrap Q anion-exchange column (GE Healthcare) and eluting with a gradient of 0–2 M NaCl in 20 mM Tris/HCl, pH 8.0, and 0.2 % CHAPS.

**Preparation of decorin pG (peptidoglycan)**

A 25 % (w/v) aqueous solution of actinase E (100 μl) was added to 1 ml of 5 mg/ml decorin PG aqueous solution. The proteolysis reaction was allowed to proceed at 50 °C overnight, after which 800 mg of urea and 36 mg of CHAPS were added to the reaction mixture to give a 1.7 ml solution [2 % (w/v) CHAPS and 8 M urea]. The mixture was loaded on to a SAX (strong-anion exchange) spin column (Vivapure Maxi Q; Sartorius Stedim) pre-equilibrated with 3 ml of 8 M urea containing 2 % (w/v) CHAPS, and centrifuged at 500 g for 5 min. The bound pG was washed once with 3 ml of 8 M urea containing 2 % (w/v) CHAPS and three times with 3 ml of 100 mM NaCl. Decorin pG was released with three 1 ml volumes of 2 M NaCl, desalted using a 10 kDa MWCO (molecular-mass cut-off) centrifugal filter (YM-10; Millipore), lyophilized and stored at −20 °C.

**Preparation of GAG**

The GAG component of decorin pG was released by base-catalysed β-elimination under reducing conditions. Decorin pG was dissolved in a 0.5 M NaOH solution containing 0.5 M NaBH₄. The reaction was allowed to proceed overnight at 4 °C and neutralized with 1 M HCl. The resulting GAG mixture was purified using a 10 kDa MWCO centrifugal filter (YM-10; Millipore).

**PAGE analysis**

Products of the decorin GAG enzymatic depolymerization were analysed by native PAGE using 0.75 mm × 6.8 cm × 8.6 cm minigels cast from 15 % T resolving gel monomer solution and 5 % T stacking gel monomer solution [16]. Heparin oligosaccharide ladder and bikunin GAG CS-A-type chains of known MMs were used as molecular markers [17]. The mini-gels were subjected to electrophoresis at a constant 200 V for 30 min and visualized with 0.5 % (w/v) Alcian Blue in 2 % (v/v) aqueous acetic acid. Intact decorin GAG was analysed using precast 4–15 % gradient Tris/HCl mini-gels (Ready-gels; Bio-Rad Labaratories). The gradient gels were subjected to electrophoresis at 200 V for 20 min. A 25 % (v/v) ethanol and 10 % (v/v) acetic acid solution was used for washing the gels prior to the Alcian Blue staining for 1 h at room temperature (23 °C) and for destaining. Silver-ammine staining of the Alcian Blue-stained gels was used to improve visualization [18]. MM analysis was performed with the aid of UNSCANIT software (Silk Scientific) using the logarithmic relationship between the GAG MM and its migration distance.

**1H-NMR spectroscopy**

Decorin pG was dissolved in 0.4 ml of [2H]H₂O (99.96 %; Sigma), freeze-dried three times from [2H]H₂O, and re-dissolved in 0.4 ml of [2H]H₂O for one-dimensional and two-dimensional NMR experiments. One-dimensional 1H-NMR and two-dimensional H-H COSY spectra were recorded on a Bruker Avance II 600 MHz spectrometer equipped with a cryogenically cooled HCN probe with a z-axis gradient. All NMR experiments were recorded at 40 °C (313 K), because at 25 °C the chemical shift of the Idoo H1 overlaps HO²H signal. One-dimensional 1H spectra were recorded with 128 scans with a spectral width of 12 kHz and an acquisition time set to 2.7 s. Two-dimensional H-H COSY spectra were recorded with 16 scans with a spectral width of 7.4 kHz and an acquisition time set to 1.1 s.

**Enzymatic depolymerization of decorin GAG for PAGE analysis**

Two 10 μg portions of decorin GAG were exhaustively treated with 5 m-units of chondroitin ACII endolyase in 50 mM ammonium bicarbonate buffer, pH 8, at 37 °C overnight to obtain DS-type domains, or 5 m-units of chondroitin B endolyase in 50 mM ammonium bicarbonate buffer, pH 8, at 37 °C overnight to obtain CS-type domains. Products of the depolymerization reactions were analysed by isocratic 15 % T PAGE using approx. 5 μg/lane.

**Enzymatic depolymerization and analysis of the non-reducing end**

A 200 μg portion of decorin GAG was exhaustively digested with 50 milli-units of chondroitin ACII exolyse in 50 mM Tris/HCl, pH 8, and 60 mM sodium acetate buffer. After an overnight incubation, the enzyme was denatured by heating in a boiling water bath for 5 min and precipitated by centrifugation at 12 000 g. The digestion mixture was passed through a 30 kDa MWCO centrifugal filter (YM-30; Millipore) to separate the disaccharides (flow-through) from the high-MM components (retentate) of the digestion mixture. The disaccharide composition of the NRE (non-reducing end) was determined by LC-MS. The retentate containing ACII exolyse-resistant reducing ends of decorin GAG was analysed by isocratic (15 % T) and gradient (4–15 % T) PAGE.

**Decorin GAG domain mapping**

The overall workflow of the mapping experiment consisted of: (i) exhaustive degradation of the decorin DS-type domains with chondroitin B endolyase and the LC-MS analysis of digestion products; (ii) treatment of the resulting continuous CS-type domains with chondroitin ACII exolyse and the LC-MS analysis of digestion products removed at timed intervals; and (iii) exhaustive depolymerization of the remaining mixture with chondroitin ABC lyase and the LC-MS analysis of digestion products (Figure 2). The decorin GAG (1 ml of 0.4 mg/ml solution in deionized water) was treated with 0.1 unit of chondroitin B endolyase at 37 °C overnight. The enzyme was denatured by heating at 100 °C and removed by centrifugation at 12 000 g. The supernatant containing CS-type domains was treated with 5 milli-units of chondroitin ACII lyase at 37 °C. During the digestion, 100 μl aliquots were removed at 0, 10, 30, 90 and 120 min, corresponding to 0 %, 20 %, 40 %, 60 % and 100 % digestion completion; at each time-point, the enzyme was thermally denatured and removed by centrifugation. The aliquot removed at zero time contained disaccharides obtained by the exhaustive chondroitinase B treatment. The supernatants were lyophilized, reconstituted in water and analysed by LC-MS. After 2 h of chondroitinase ACII digestion, 50 milli-units of chondroitin ABC lyase was added to the remaining 500 μl of the digestion mixture, and the mixture was incubated at 37 °C overnight. The enzyme was thermally inactivated and removed by centrifugation, and the supernatant was lyophilized.
Action pattern and activity of the chondroitin ACII exolyase were verified using CS-A and CS-C standard polysaccharides. A 1 ml aliquot of a 10 mg/ml solution of CS-A or CS-C was treated with 0.4 unit of chondroitin ACII lyase at 37°C. The enzyme was thermally inactivated and removed by centrifugation. Each time, the enzyme was thermally inactivated and removed by centrifugation. The A235 of each supernatant was measured after 100-fold dilution.

LC-MS analysis

The LC-MS disaccharide analysis was performed as described previously [19] on an Agilent 1100 LC-MSD mass spectrometer equipped with an ion-trap mass analyser. An Agilent 1100 LC system was used for delivering a two-step mobile-phase gradient consisting of an isocratic 10 min segment of solution A followed by a linear gradient from 0 to 40 min of 0–50% solution B. Solutions A and B were 0% and 75% acetonitrile respectively, containing 15 mM n-hexylamine as an ion-pairing reagent and 100 mM 1,1,1,3,3,3-hexafluoro-2-propanol as an organic modifier. Disaccharides were separated on an Acquity UPLC BEH C18 column (2.1 mm × 150 mm, 1.7 μm; Waters). The column temperature was maintained at 45°C and the mobile-phase flow rate was 100 μl/min. The disaccharide elution was monitored by the A235 (4,5-unsaturated uronic acid) and by MS. Mass spectra were acquired in the positive ionization mode over a 350–2000 m/z range at a rate of 10 scans/s. Nitrogen was used as the drying and nebulizing gas.

RESULTS AND DISCUSSION

The recombinant decorin used in the present study was produced by culturing stably transfected, transformed, HEK cells (HEK-293-EBNA) and represents a single lot of PG sample. The N-terminal sequence of the recombinant decorin core protein contained six histidine residues (His6-tag), facilitating its purification. The decorin pG, a decorin core protein peptide with a GAG chain attached to Ser21, was prepared by treating the PG with a non-specific protease, actinase E. The GAG chain was then released from the purified pG by reductive β-elimination. The MM and dispersivity of the pG and GAG, examined using PAGE, were very similar, indicating that peptide represents a small percentage of the pG MM, which is consistent with the actinase E action pattern [20–22].

Intact decorin GAG

The MM of decorin GAG was analysed by PAGE (Figure 3) using a ladder of heparin oligosaccharides and bikunin GAG chains with known MMs as standards [16,17,23]. It should be noted that neither the heparin oligosaccharide ladder nor bikunin GAG polysaccharides used in the present study as the standards cover the entire gel range. MM values of decorin GAG and its degradation products with MM > 9 kDa were derived by extrapolating a linear function of log(MM) against migration distance plotted using available standards, which entails a fairly wide degree of uncertainty in the extrapolated MM distributions. The Mn (weight-average MM) of the decorin GAG chain was determined to be 30 kDa and the Mn (number-average MM) was 22 kDa, corresponding to the chain length of ~50 disaccharides based on the monosulfated disaccharide residue mass (459 Da).

The uronic acid epimers D-GlcA and L-IdoA are distinguishable by 1H-NMR. One-dimensional 1H-NMR and two-dimensional COSY spectra of the decorin pG were used to determine the ratio of GlcA to IdoA [24,25,26]. Based on the integration of the GlcA and IdoA H1 signals, the two-dimensional COSY was used to estimate their intensity [24,25,26]. Based on the integration of the GlcA and IdoA H1 signals, the two-dimensional COSY NMR spectra, the ratio of GlcA to IdoA...
Decorin structural motifs

**Figure 4** Disaccharide composition of the decorin GAG and GAG domains determined by LC-MS

(A) Disaccharide composition of continuous DS-type domains (black), CS-type domains resistant to chondroitinase B (dark grey) and overall disaccharide composition (light grey).

(B) Digestion of the chondroitinase B-resistant domains with exolytic chondroitinase ACII; the disaccharide compositions at 20%, 40%, 60% and 100% reaction completion are shown.

was determined to be 5:1. Because this ratio was obtained for a collection of GAG chains as opposed to a single GAG chain, it represents a statistical approximation rather than an exact number. Additional uncertainties in the NMR-based relative quantification experiments inevitably arise from varying the instrumental conditions and integration parameters. These deviations fall within ±7% of the mean value (results not shown).

LC-MS analysis of products of the exhaustive depolymerization with chondroitinase ABC afforded overall disaccharide composition of decorin GAG: 12% 0S, 23% 6S, 63% 4S, 1% 2S6S, 1% 2S4S and 0.4% 4S6S (Figure 4A). Based on the results of PAGE, NMR and LC-MS disaccharide-composition analyses, an average hypothetical decorin GAG chain consists of 50 disaccharides, 42 of which contain GlcA and eight contain IdoA, six disaccharides are unsulfated, 43 disaccharides are monosulfated and one disaccharide is disulfated (Figure 5).

**DS-type domains**

Exhaustive treatment of the decorin GAG mixture with endolytic chondroitinase ACI afforded IdoA-rich DS-type domains, resistant to depolymerization by this enzyme. The products of the depolymerization reaction were analysed by PAGE (Figure 3B, lane 5) using heparin oligosaccharides of known MM as standards. The results of PAGE analysis indicate that continuous DS-type domains in the decorin GAG chain contain 3−15 disaccharides. Based on the proportion of DS-type disaccharides determined by NMR, the majority of IdoA-containing disaccharides appear to be present in the continuous DS-type domains.

Exhaustive treatment of decorin GAG with endolytic chondroitinase B affords disaccharides originating from continuous DS-type domains. The disaccharide composition of these DS-type domains was determined using LC-MS (Figure 4A). Continuous DS-type domains of decorin GAG contained 28% 0S, 28% 6S, 36% 4S and 8% 2S4S disaccharides. Neither 2S6S nor 4S6S disaccharides were detected in DS-type domains. Since exhaustive treatment of decorin GAG with chondroitinase ABC shows that 2S4S disaccharide constitutes 1% of the overall decorin GAG disaccharide composition, its presence in the continuous DS-type domains at a relatively high proportion of 8% suggests that essentially all 2S4S disaccharides originate from continuous DS-type domains.

**CS-type domains**

GlcA-containing disaccharides constitute 83% of decorin GAG, according to the NMR analysis, suggesting that there is a high probability of finding continuous CS-type domains in a decorin GAG chain. Exolytic chondroitinase ACII acts on CS by removing one GlcA-containing disaccharide at a time from the NRE of GAG, and does not act on IdoA-containing disaccharides, i.e. DS-type domains [27,28]. Exhaustive treatment of decorin GAG with exolytic chondroitinase ACII followed by the analysis of products demonstrated that most decorin GAG chains are terminated with a CS-type NRE and allowed us to estimate the average length of the CS-type domain at the NRE and to determine its disaccharide composition.

The low-MM products of chondroitinase ACII digestion were resolved using isocratic 15% PAGE, whereas 4−15% gradient PAGE was used for determining the size of the high-MM products (Figures 3C, lane 2, and 3A, lane 6). The downward shift in chain size from Mn 22 kDa to Mn 12 kDa (Mw 28 kDa) after the exolyase treatment suggests that the NRE consists of up to 20 CS-type disaccharides. The low-MM products of the ACII exolyase treatment contain oligosaccharides with dp6 (degree of polymerization 6, i.e. hexasaccharide) and higher, as evident from the isocratic PAGE analysis (Figure 3C, lane 2). These oligosaccharides could originate from the reducing end and either contain a penultimate DS-type disaccharide or, in case of dp6, represent the LR (linkage-region) hexasaccharide which is usually obtained by exhaustive enzymatic depolymerization of a GAG [11]. This suggests that some decorin GAG chains may be entirely composed of CS-type building blocks.

The disaccharide compositional profile of the NRE, as determined by LC-MS analysis of products obtained on chondroitinase ACII treatment, differs from the inner-chain CS-type disaccharide compositional profile in that the GlcA2S-containing...
disaccharides were not detected in the decorin GAG NRE domain, whereas 4S6S disaccharides were present in the highest proportion, 2% (Figure 4A) compared with 0.2% in the overall disaccharide profile (Figure 4B). These results suggest that virtually all 4S6S disaccharides originate from the NRE CS-type domain of decorin GAG. Other disaccharides present in the NRE domain included 42% 4S, 27% 6S and 29% 0S (Figure 4A).

Exhaustive depolymerization of the DS-type domains of decorin GAG with endolytic chondroitinase B generated DS-type disaccharides as described above, as well as CS-type domains, resistant to the degradation by chondroitinase B. These CS-type domains were then treated with exolytic chondroitinase ACII, and during the first 2 h of the digestion, aliquots were removed at timed intervals for LC-MS disaccharide composition analysis. LC-MS disaccharide profiles corresponding to various time-points throughout the digestion provided information about changes in disaccharide composition in going from the NRE towards the RE (reducing end) in these CS-type domains. The results obtained during this experiment are summarized in Figure 4(B), which shows, for example, that whereas the proportion of 6S disaccharide remain essentially unchanged, the proportion of 4S disaccharide doubles. Thus a more highly sulfated portion of the CS-type domain lies toward its RE.

**Domain map of a decorin GAG chain**

Based on the information gathered through sequential enzymatic treatment of decorin GAG with specific CS lyases and the analysis of the digestion products, a feature map of an ‘average’ decorin GAG chain was constructed (Figure 5). This chain is dp104 with the first four saccharide residues at the RE corresponding to the linkage region that attaches to Ser34 (Ser21 in the recombinant decorin) of the PG core protein. The dp100 chain that follows (residues 5–104) consists of three types of domain: isolated DS-type domains (residues 13–14), clustered DS-type domains (residues 21–26 and 53–60), and extended CS-type domains (residues 29–48 and 63–104). The clustered DS-type domains contain essentially all of the 2S4S disaccharides. The extended internal CS-type domain (residues 29–48) is enriched in 6S disaccharides, whereas the extended CS-type domain at the NRE (residues 63–104) is enriched in 4S and contains most of the 4S6S disaccharides. It should be noted that the chain drawn in Figure 5 is a composite average and may not correspond to any chain found in the decorin PG. Moreover, we see evidence of chains that are entirely CS in composition, containing no IdoA residues and devoid of a DS domain.

In conclusion, the present study provides an initial rough map of the GAG chain from decorin PG isolated from a single lot of cultured HEK cells. Biosynthesis of GAGs is a non-template process, and the structural features of CS/DS GAGs vary between different species; across a population of the same species, depending on age, environmental stimuli, physiological or pathophysiological state; within an organism, depending on tissue type; and, at a molecular level, depending on the type of PG. Thus the composite average chain of decorin GAG will vary depending on the source. The strategy described in the present work can be applied to constructing and comparing the domain maps of CS/DS chains isolated from different sources as well as to examining CS/DS components of other PGs. Future studies are underway to isolate individual GAG chains from decorin PG for structure and sequence determination.

**AUTHOR CONTRIBUTION**

Tatiana Laremore performed isocratic PAGE analysis of the chondroitinase ACI and chondroitinase B digestions of decorin GAG and interpreted and organized LC-MS and PAGE data. Mellisa Ly determined the decorin MM and polydispersity by PAGE, analysed products of decorin GAG enzymatic digestions by isocratic and gradient PAGE, and interpreted and organized PAGE results. Zhenqin Zhang performed enzymology studies and disaccharide analysis of the decorin GAG by LC-MS and analysed decorin GAG by one-dimensional and two-dimensional NMR to determine GlcA/IdoA ratios. Kemal Solakyildirim performed one-dimensional and two-dimensional NMR experiments.

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REFERENCES


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SUPPLEMENTARY ONLINE DATA
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Figure S1 NMR analysis of decorin peptidoglycan (one-dimensional 1H-NMR and two-dimensional H-H COSY)

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