Glycoconjugate Journal (1991) **8**:108–115

Oligosaccharide mapping of proteoglycan-bound and xyloside-initiated dermatan sulfate from fibroblasts

LARS-ÅKE FRANSSON*, ARTUR SCHMIDTCHEN, LARS CÖSTER and ANDERS MALMSTRÖM
Department of Physiological Chemistry, University of Lund, P.O. Box 94, S-221 00 Lund, Sweden
Received 21 November 1990

The copolymeric structure of dermatan sulfate chains synthesized by skin fibroblasts has been examined. Chains initiated onto exogeneous *p*-nitrophenyl-β-D-xylopyranoside or attached to protein in a large proteoglycan, PG-L, and two small proteoglycans, PG-S1 and PG-S2, have been compared by using high resolution electrophoresis and gel chromatography of oligosaccharides generated by specific enzymatic or chemical degradations. The results confirm that chains attached to PG-L are glucuronate-rich, whereas novel findings indicate that chains attached to either of the two PG-S variants yield closely similar oligosaccharide maps, have approximately equal glucuronate and iduronate content and contain over 90% 4-sulfated disaccharide repeating units. Dermatan sulfate chains built onto xyloside at concentrations of 50 μM and below have a copolymeric structure similar to that of chains from the two PG-S variants. These findings indicate that the polymer-modifying machinery can generate chains with extended iduronate-containing repeats also when the xylose primer is not linked to core protein.

Keywords: Proteoglycans, dermatan sulfate, oligosaccharide mapping, xylosides

Proteoglycans are complex macromolecules which consist of a protein core that is substituted with sulphated glycosaminoglycans (GAG), such as keratan sulfate (KS), chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), or heparin. They have a wide tissue distribution and occur mainly in the interstitial extracellular matrix, but also in basement membranes, at cell surfaces and in intracellular storage granules (see reviews [1–6]). CS/DS-containing proteoglycans with a large core polypeptide (M_r greater than 200 kDa) are found in cartilage but also in other fibrous connective tissues such as sclera, aorta, tendon, and skin [3,7]. The aortic proteoglycan is probably related to a recently cloned large fibroblast proteoglycan (PG-L or versican) [8].

Many tissues, including cartilage and bone, also contain small CS/DS-proteoglycans (PG-S) with core proteins in the size-range 40–45 kDa [3,7]. Two homologous but different gene-products (PG-S1 or biglycan, and PG-S2 or decorin) have been identified; the former generally contains two CS/DS-chains, whereas the latter contains only one chain [3,7,9–11].

The large fibroblast-derived proteoglycan, PG-L (probably related to versican), is generally considered to be substituted with CS [a polymer composed of a glucuronate (GlcA)-*N*-acetylgalactosamine (GalNAc) backbone]. However, the human fibroblast-derived [13] and the bovine, scleral [14] PG-L carry side-chains which contain a small but significant number of iduronate(IdoA)-GalNAc disaccharides, and could therefore be classified as DS. The uronate composition of the DS-chains in the various forms of small proteoglycans (PG-S) varies considerably depending on the type of tissue and the stage of maturation. In bone and tracheal cartilage the chains are virtually IdoA-free, i.e., CS [3], in articular cartilage PG-S1 and PG-S2 chains contain 28 and 42% IdoA, respectively [9], whereas in skin, sclera and the uterine cervix 60–80% of the hexuronates in PG-S are IdoA [9, 14, 15]. The hexuronate composition of the DS chains affects the ability of the proteoglycan to self-associate [16] and to bind to proteins, such as anti-proteinases [17]. The basis for variations in the copolymeric structure of DS is not generally understood; there could be regulatory signals in the core protein or cell-specific arrangements of the GAG-synthesizing machinery.

The first step in the biosynthesis of the glycan portion of a proteoglycan is the xylosylation of specific serine residues (for reviews, see [1, 5, 6]. The xylose is then extended with the sequence GlcA-Gal-Gal- followed by polymerization of the main chain by repeated, alternating additions of GalNAc and GlcA residues (in the case of CS/DS). DS is formed when certain GlcA residues are converted to IdoA via C-5

* To whom correspondence should be addressed.

0282-0080/91 $03.00+.12

epimerization prior to sulfation [18]. β-D-Xylosides can compete with xylosylated core proteins and serve as alternative acceptors for the assembly of GAG chains (for reviews, see [5]). Uncoupling of protein synthesis from GAG assembly permits an assessment of the role of the core protein in controlling the copolymeric structure of the newly synthesized DS chains.

As shown in a preceding report [19] xylosides added to fibroblast cultures induce a progressive inhibition of proteoglycan synthesis and stimulate formation of free GAG-chains with full effect above 100 μM xyloside. Disaccharide analyses of PG-bound and xyloside-initiated chains indicated that the GlcA content of the xyloside-bound GAG-chains is similar to that of chains in the PG-S pool and remains fairly constant up to approx. 60 μM. Above that level, the GlcA-content in xyloside-GAGs increases [19]. In the present report we have extended these studies and examined the individual DS-chains bound to either PG-L, PG-S1 or PG-S2 or initiated onto xyloside at moderate concentrations of this compound. The copolymeric structure of these chains has been studied by oligosaccharide mapping by using degradation with chondroitin AC-I lyase followed by polyacrylamide gradient gel electrophoresis [20, 21].

Materials and methods

Materials

Dermatan sulfate preparation DS-36 from pig skin was the same preparation as used elsewhere [22]. Cell culture medium was obtained from Nord Vacc AB, Sweden. D-[6-^{3}H]Glucosamine (20–40 Ci/mmol), Amplify™-spray and Hyperfilm™-MP were purchased from The Radiochemical Centre, Amersham, UK. Chondroitin AC-I lyase (EC 4.2.2.5) and chondroitin ABC lyase (EC 4.2.2.4) were from ICN Biomedicals, Inc., USA. DE-53 DEAE-cellulose was from Whatman, and Sephacryl S-500 HR, Superose 6 HR 10/30, Octyl Sepharose CL-4B, MonoQ HR 5/5, gel-casting stand, gradient mixer, and power supply (Macrodrive 1) were from LKB-Pharmacia, Sweden. The semi-dry electroblotter was manufactured by Ancos A/S, Denmark, and the electrophoresis unit in the workshop at Malmö General Hospital. Zetaprobe nylon was from Bio-Rad, USA, and acrylamide and *N,N'*-methylene bisacrylamide (2×) from Serva, Germany. 3-(3-Cholamidopropyl)dimethylammonio]propane sulphonate (CHAPS) was bought from Fluka AG, Switzerland and guanidinium chloride, urea (both technical grade) and *p*-nitrophenyl-β-D-xylopyranoside were obtained from Sigma, USA. Stock solutions of guanidinium chloride (6 M) and urea (8 M) were treated as described elsewhere [19]. Mulgophene [tridecyloxypoly(ethyleneoxy)ethanol] was purchased from GFA Corp., Johanneshov, Sweden. Films were developed with a Gevamatic 60 (Agfa-Gevaert) and scanning was performed with a videodensitometric system developed by Mr. Lars Kopp (Makab, Göteborg, Sweden).

Cell culture and metabolic labelling

Fibroblast cultures were established from human embryonic skin and grown as monolayers in Earle's minimal essential medium supplemented with 10% (v/v) donor calf serum, 2 mM L-glutamine, penicillin (100 units/ml) and streptomycin (100 μg/ml). Confluent cultures (75 cm^2 dishes) between passages 5 and 15 were used for the experiments and incubated with 25 μCi/ml of [^{3}H]glucosamine for 24 h in the absence or presence of *p*-nitrophenyl-β-D-xyloside (see the appropriate Figure). No pre-incubations with xyloside were carried out as this has been shown to be unnecessary [19].

Isolation of proteoglycans and free chains

After incubation with radioactive precursor the culture medium was decanted and the cell layer was washed gently with 3 × 5–10 ml of phosphate-buffered saline (0.13 M NaCl; 50 mM sodium phosphate buffer, pH 7.4). The medium and the washings were adjusted to pH 5.8 with HOAc and applied to columns (1 ml) of DE-53 DEAE-cellulose which were equilibrated with 6 M urea/10 mM *N*-ethylmaleimide/0.2 M NaOAc, pH 5.8. The columns were washed with 5 bed volumes of the equilibrating buffer, followed by the same buffer containing 0.5 M NaOAc (without *N*-ethylmaleimide). Polyanionic material was then displaced by elution with 5 × 1 bed volumes of 4 M guanidinium chloride/10 mM *N*-ethylmaleimide/50 mM NaOAc, pH 5.8.

Separation of PG-L, PG-S1, PG-S2, and xyloside-bound GAG by gel chromatography

The [^{3}H]glucosamine-labelled polyanionic material from the medium was subjected to ion-exchange FPLC on Mono Q HR 5/5 as described [23]. This procedure separated the material grossly into three fractions, i.e., hyaluronan, HS-containing, and CS/DS-containing material. The latter was diluted three-fold with water and recovered by chromatography on DEAE-cellulose (0.2 ml columns) as described above. The eluted polyanionic material was supplied with 50 μg of carrier dermatan sulfate, precipitated with 5 volumes of ethanol, and dissolved in 200 μl of 4 M guanidinium chloride/0.1% (v/v) mulgophene/50 mM NaOAc buffer, pH 5.8. It was then fractionated according to size into PG-L, PG-S and xyloside-bound CS/DS by chromatography on Superose 6 HR 10/30 eluted with the same solvent at a flow-rate of 0.4 ml/min using an LKB-Pharmacia FPLC system. One minute fractions were collected and analysed for radioactivity in a scintillation counter from LKB-Wallach using ReadySafe (Beckman) as a scintillator. Pooled material was diluted and recovered by chromatography on DEAE-cellulose (see above). The overall recovery was 60–70%.

Separation of PG-S1 and PG-S2 by hydrophobic interaction chromatography

The [^{3}H]glucosamine-labelled polyanionic material from the medium was first separated into large and small

proteoglycans by chromatography on Sephacryl S-500 HR [24]. The small proteoglycans were recovered by passage over DEAE-cellulose as described above and eluted with 4 M guanidinium chloride/50 mM NaOAc buffer, pH 5.8. After dialysis, the material was subjected to ion exchange FPLC on Mono Q [23,24]. The CS/DS-enriched fraction was pooled and again passed over DEAE-cellulose. After ethanol precipitation and solubilization in 4 M guanidinium chloride/50 mM NaOAc, pH 5.8, samples were subjected to hydrophobic interaction chromatography [9] on Octyl Sepharose CL-4B (0.5 × 10 cm) by using the LKB-Pharmacia FPLC system. The column was equilibrated with 4 M guanidinium chloride/50 mM NaOAc buffer, pH 5.8, and eluted with a linear gradient of CHAPS (0–1.5%) in the same solvent at a rate of 0.1 ml/min. Fractions (0.5 ml) were collected and analysed for radioactivity. Between runs the column was washed with n-butanol and ethanol according to the recommendations of the manufacturer.

Preparation of CS/DS chains

CS/DS chains bound to xyloside were recovered directly from the chromatography on Superose 6, whereas chains bound to a core protein were released by treatment of the proteoglycan with alkali (0.5 M NaOH/0.1 M $NaBH_4$ at 20 °C overnight), followed by neutralization (0.1 M HOAc) and recovered by chromatography on DEAE-cellulose (see above). Prior to analysis, all chains were converted to their sodium salts by precipitation with ethanol saturated with sodium acetate, washed with absolute ethanol, dried and dissolved in 10 µl of water.

Degradative methods

Cleavage of all GalNAc-GlcA/IdoA bonds in the CS/DS-chains was performed with chondroitin ABC lyase in 0.1 M Tris-Ac/10 mM EDTA, pH 7.3 or with 10 mM Tris-Ac/10 mM NaF, pH 7.3 at 37 °C (5–10 µg of substrate in 50 µl of buffer with 10 mIU of enzyme for 6 h). The same conditions were used for the cleavage of GalNAc-GlcA bonds by chondroitin AC-I lyase, except that NaF was omitted. Nonsulfated IdoA residues were selectively periodate-oxidized and alkali-cleaved as described [25]. HS-containing material was degraded with nitrous acid at pH 1.5 [26].

SDS-polyacrylamide gel electrophoresis

Electrophoresis was performed on 3–12% gels (T/C = 30/0.8), with a 3% stacking gel, in the buffer system of Laemmli [27] as described [23]. Samples were dissolved in 60 µl of 5% (w/v) sodium dodecylsulphate (SDS):20% (v/v) glycerol: 4 mM EDTA:0.04% (w/v) Bromophenol Blue/125 mM Tris-HCl, pH 6.8, boiled for 3 min and electrophoresed for approx. 30 h at a constant current of 10 mA. Afterwards the gels were stained with Coomassie, destained, soaked in Amplify NAMP-100 (Amersham), dried and exposed to Kodak XAR-5 film at −60 °C for 1 week.

Chromatography of CS/DS-oligo- and disaccharides

Oligosaccharides were separated by gel chromatography on a column (10 mm × 1000 mm) of Bio-Gel P-6 which was eluted with 0.5 M NH_4HCO_3 at a flow rate of approx. 5 ml/h. Fractions (0.8 ml) were assayed for radioactivity as described above. The GlcA content was estimated by the formula described previously [28]. Disaccharides were separated into 4- and 6-sulfated species by chromatography on a Lichrosorb-NH_2 column (4 mm × 250 mm, Merck) as described [19,29]. Non-, mono- and disulfated disaccharides were separated on a PartiSil 10 SAX column (6.35 mm × 250 mm, Whatman) by gradient elution with 0–0.6 M KH_2PO_4, pH 4.2, for 70 min at a rate of 1 ml/min. Both columns were connected to a Varian Vista 5500 HPLC system.

Electrophoresis of CS/DS-oligosaccharides

Separation was achieved by vertical polyacrylamide gradient gel electrophoresis which was carried out by a modification [22] of the methods of Rice *et al.* [20] and Turnbull and Gallagher [21]. The gels were 18 cm wide, 40 cm high and 0.75 mm thick, and consisted of a 20–30% gradient resolving gel and a 5% stacking gel with application wells. The upper reservoir and the wells contained a high ionic-strength pH 8.3 buffer, the stacking gel a lower ionic-strength pH 6.3 buffer, and the resolving gel and lower reservoir a pH 8.3 buffer of the same low ionic strength. Samples were freeze-dried, dissolved in 10 µl of 25% (w/v) sucrose in stacking gel buffer and layered onto the bottom of the wells. Electrophoresis was carried out for approx. 20 h with 600 V (constant voltage) and 8–10 mA. Standards were stained with Azure A. Radioactive samples were transferred from the gels to Zetaprobe nylon by semi-dry electroblotting [30]. The trans-unit consisted of (a) six layers of filter paper soaked in 0.3 M Tris/NaOH, pH 10.3 (first anode buffer), (b) three layers of filter paper soaked in 25 mM Tris-NaOH, pH 10.4 (second anode buffer), (c) the Zeta-probe membrane wetted in distilled water, (d) the gel which had been equilibrated for 15 min in the second anode buffer, and (e) three filter papers soaked in 26 mM Tris/40 mM 6-amino-n-hexanoic acid, pH 9.4 (cathode buffer). Transfer was achieved at 0.8 mA/cm^2 for 40 min. The membranes were dried, sprayed with Amplify™, dried again and exposed to x-ray film for 1–2 weeks at −60 °C. After development of the film, tracks were scanned by videodensitometry in the transmittance mode.

Results and discussion

Isolation of proteoglycans and free chains

Confluent cell cultures were incubated with [^{3}H]glucosamine in the absence or presence of *p*-nitrophenyl-β-D-xylopyranoside (5 µM, 50 µM or 1 mM) for 24 h. The media were collected and hyaluronan and the bulk of the HS-material

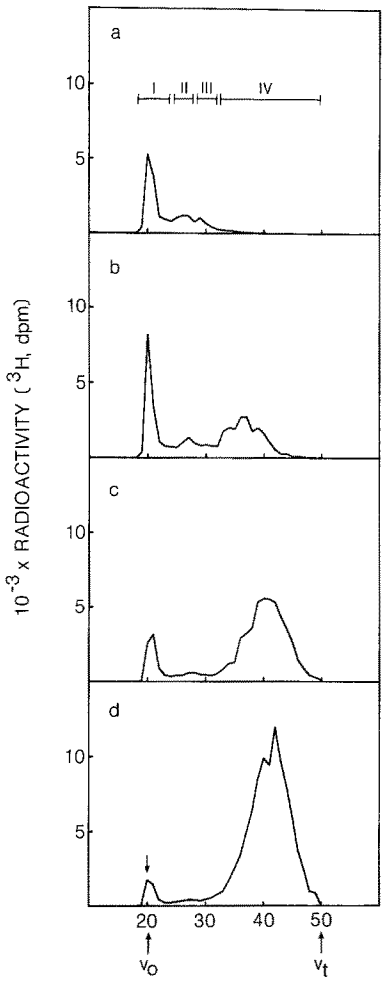

Figure 1. Gel chromatography on Superose 6 of [^{3}H]glucosamine-labelled CS/DS-proteoglycans and xyloside-bound CS/DS from the media of fibroblast cultures exposed to (a) 0, (b) 5 μM, (c) 50 μM, and (d) 1 mM *p*-nitrophenyl-β-D-xyloside. Confluent cultures were incubated with [^{3}H]glucosamine and various concentrations of xyloside as described in Materials and methods. The media were passed over columns of DEAE-cellulose to isolate polyanionic material. CS/DS-containing material was then separated from hyaluronan and HS-containing proteoglycans by ion exchange FPLC on MonoQ. The DS-containing material was recovered and chromatographed on Superose 6 as described in Materials and methods. Fractions I–IV were pooled as indicated by the bars; v_0, void volume; v_t, total volume.

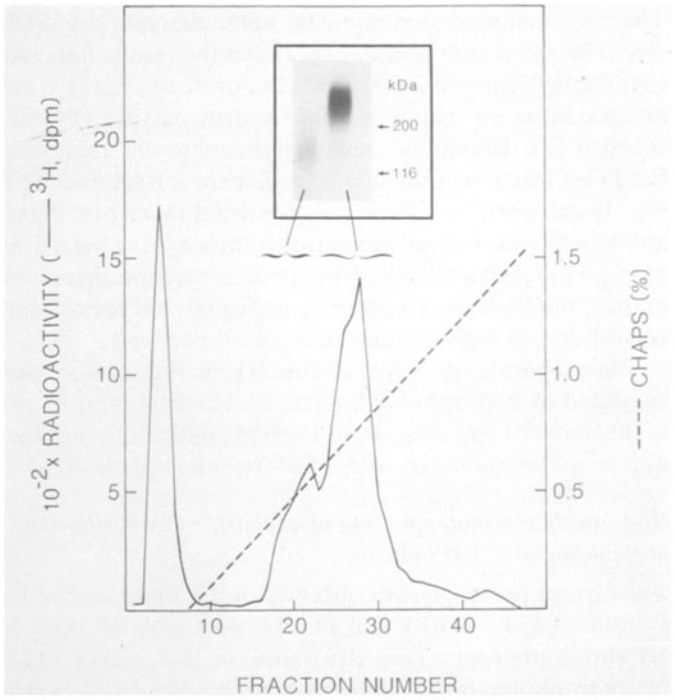

Figure 2. Hydrophobic interaction chromatography of small proteoglycans from the medium of non-treated cells. Confluent cultures were incubated with [^{3}H]glucosamine in the absence of xyloside. Polyanionic material was recovered from the medium by ion exchange chromatography on DEAE-cellulose and small and large proteoglycans were separated by gel chromatography on Sephacryl S-500 HR. The pool of small proteoglycans was finally subjected to chromatography on Octyl-Sepharose. The detergent gradient is indicated by a dashed line. Two retarded fractions were pooled (see brackets) and analysed by SDS-polyacrylamide gel electrophoresis (see inset).

were separated from CS/DS-material by ion exchange FPLC on Mono Q. The CS/DS-pool was resolved into proteoglycans and free chains by gel FPLC on Superose 6 (Fig. 1). The excluded material was pooled as fraction I and contained the large proteoglycan PG-L (analogous to versican) [19]. The more retarded components corresponding to small proteoglycans (PG-S) were pooled as two fractions (II and III). In the presence of xyloside the cell media contained free CS/DS-chains which were pooled as fraction IV (see Fig. 1(b–d)). As seen in Fig. 1(b), proteoglycan production was not markedly suppressed in the presence of 5 μM xyloside in accordance with previous findings [19]. As the xyloside concentration was further increased, first 10-fold (Fig. 1(c)) and then 200-fold (Fig. 1(d)), proteoglycan production decreased while formation of free chains was stimulated approx. three- and five-fold, respectively. It is also seen that the chain-length of free GAG-chains decreases with increasing xyloside concentration.

The small proteoglycans were also separated into PG-S1 and PG-S2 by hydrophobic interaction chromatography. In this case the entire pool of small proteoglycans (including HS-proteoglycan) was first isolated by gel filtration on Sephacryl S-500 HR and then subjected to ion-exchange FPLC on Mono Q (enrichment of CS/DS-species) followed by hydrophobic interaction chromatography on Octyl Sepharose (Fig. 2). One portion of the material passed straight through whereas the other portion consisted of two retarded components. The former material was resistant to digestion with chondroitin AC-I and chondroitin ABC lyase but sensitive to nitrous acid and heparan sulphate lyase (results not shown) indicating that it consisted of HS-proteoglycans.

The two retarded components were analysed by SDS-electrophoresis (see inset in Fig. 2) and the results indicated that the first one was a 100–200 kDa-proteoglycan (PG-S2) and the latter one was a >200 kDa-proteoglycan (PG-S1); see also [9]. Results of similar electrophoretic analysis of the PG-S fractions obtained from Superose 6 (II and III in Fig. 1) indicated that they, too, consisted mainly of PG-S1 and PG-S2, respectively (results not shown). The behaviour of PG-S1 and PG-S2 in hydrophobic interaction chromatography and SDS-electrophoresis indicated that they should correspond to biglycan and decorin, respectively.

The preparations of free chains (IV in Fig. 1) were also subjected to hydrophobic interaction chromatography. All of the material passed straight through, indicating that they were not contaminated with PG-S (results not shown).

Oligosaccharide mapping and disaccharide composition of protein-bound CS/DS-chains

The various proteoglycans obtained in the absence of added xyloside, PG-L, PG-S1 and PG-S2, were isolated both by gel chromatography (Fig. 1(a)) and, in the case of PG-S, by hydrophobic interaction chromatography (Fig. 2). The CS/DS-chains were released by base-catalysed elimination of the xylose–serine bonds and reisolated. The size of the PG-bound chains was assessed by gel FPLC on Superose 6. The chains from PG-S1 and PG-S2 both had a K_{av} of 0.5 (results not shown), i.e., similar to free chains formed in the presence of 5 μM xyloside (Fig. 1(b), IV).

The various CS/DS-chains were further analysed by polyacrylamide gradient gel electrophoresis (Fig. 3) before and after exhaustive degradation by chondroitin AC-I lyase, which cleaves the GalNAc-GlcA bonds. Upon electrophoresis of intact chains those from PG-S1 were somewhat more retarded than those from PG-S2 suggesting a large molecular size. (However, on Superose 6 they had the same K_{av}, see above). Chains from PG-L gave a very broad distribution. After digestion the PG-L chains yielded mainly di-, tetra-and hexasaccharide, whereas the PG-S chains afforded a series of fragments ranging in size from disaccharide to almost 100 unit polymers (corresponding to an M_r of 25,000). This indicated that PG-L chains contain very few IdoA-GalNAc repeats and that the number of consecutive such repeats seldom exceeds 2–3. In contrast, PG-S chains carry large amounts of IdoA-GalNAc repeats. There are probably also chains that consist entirely of such repeats. There were no significant differences between the chains derived from PG-S1 and PG-S2. We also degraded PG-S chains by selective oxidation-scission of non-sulfated IdoA followed by electrophoresis. There was extensive degradation (results not shown) indicating that these chains do not include variants with a low IdoA content like those in PG-L. It should be added that a linear relationship between the amount of a particular fragment and the autoradiographic response is difficult to obtain with this procedure. Blotting as well as formation of silver grains can be both nonuniform and nonlinear.

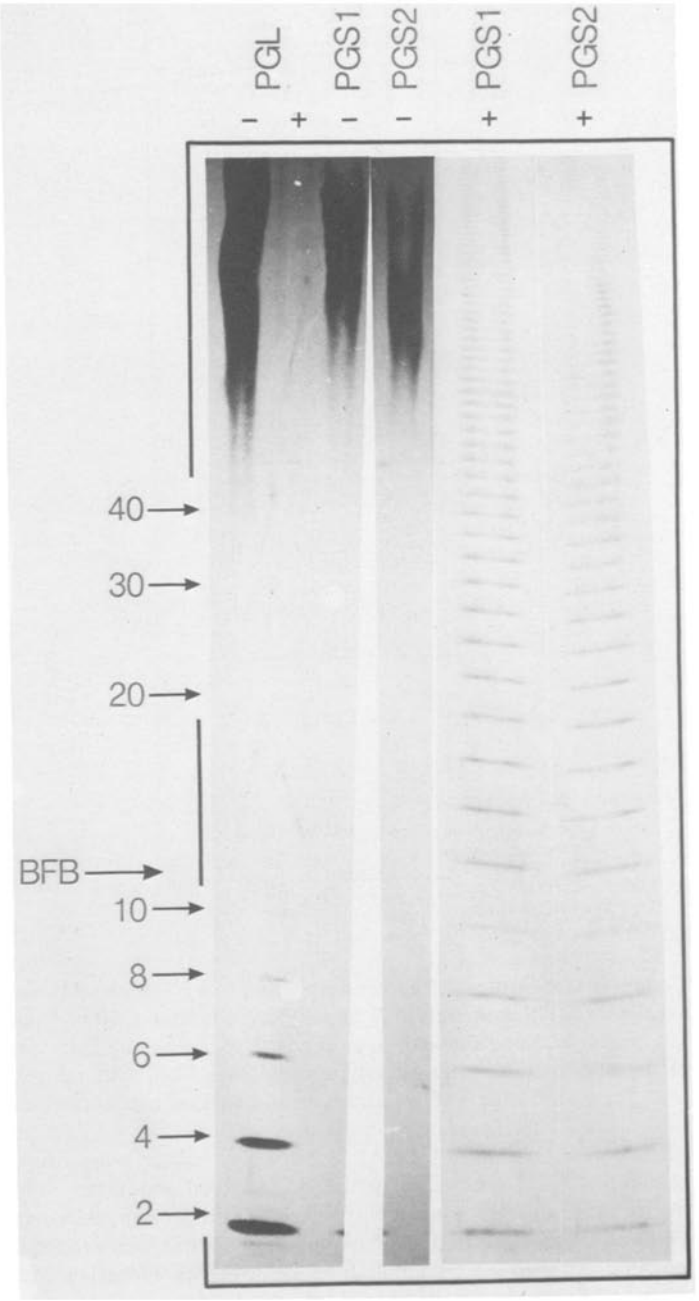

Figure 3. Electrophoresis of chondroitin AC lyase digests of CS/DS-chains derived from PG-L, PG-S1 and PG-S2. Confluent cultures were incubated with [^{3}H]glucosamine in the absence of xyloside. The various proteoglycans were isolated via Superose 6 (PG-L, see Fig. 1(a)) or via Sephacryl S-500 and Octyl-Sepharose (PG-S1 and PG-S2, see Fig. 2). The GAG-chains were released by alkali-borohydride treatment, purified by ion exchange chromatography, and subjected to electrophoresis before (−) or after (+) exhaustive digestion with chondroitin AC-I lyase. The positions of standard oligosaccharides (Di = 2, Tetra = 4, Hexa = 6, and so on) obtained by digestion of dermatan sulfate DS-36 with the same enzyme are indicated on the left. BFB, Bromophenol Blue marker.

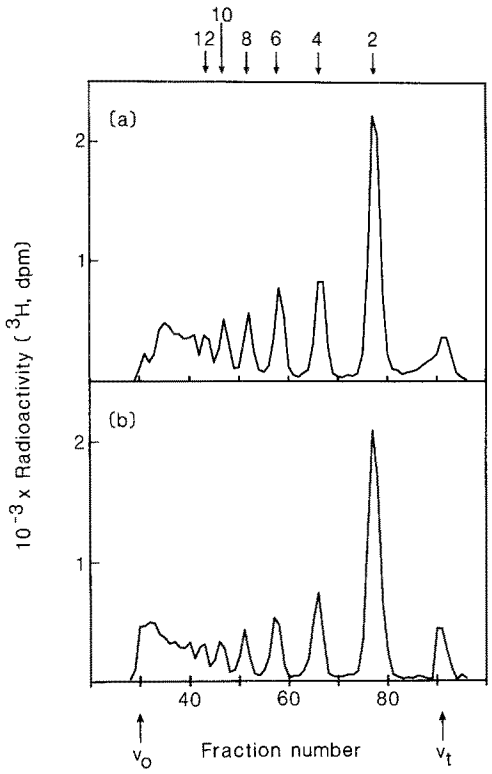

Figure 4. Gel chromatography on Bio-Gel P-6 of chondroitin AC lyase digests of CS/DS-chains derived from (a) PG-S1 and (b) PG-S2. The chains were obtained as described in the legend to Fig. 3 and digested exhaustively with the enzyme. The positions of standard oligosaccharides (Di = 2, Tetra = 4, Hexa = 6, and so on) are indicated above the top panel.

The disaccharide composition of chains from PG-S1 and PG-S2 was quantified as follows. Chains were digested with chondroitin AC-I lyase followed by gel chromatography on Bio-Gel P-6 (Fig. 4). As this enzyme cleaves linkages to GlcA forming a 4,5-unsaturated glycuronic acid (ΔGlyA) the saccharides obtained have the general core structure ΔGlyA-GalNAc-(IdoA-GalNAc)$_n$ (sulfate positions not indicated). The proportion of GlcA in the original material was calculated from the yields of the various saccharides (disaccharide corresponds to $n = 0$; tetrasaccharide is $n = 1$ and so on). The chains from PG-S1 and PG-S2 had very similar GlcA content, 55% and 59%, respectively. After exhaustive digestion with chondroitin ABC lyase (in NaF-containing buffer), disaccharides were resolved into non-, mono-, and disulfated species by chromatography on PartiSil 10 SAX and into 4-*O*- and 6-*O*-monosulfated species by chromatography on Lichrosorb. Both chains yielded 97% monosulfated disaccharide. The majority of the monosulfated disaccharides were 4-sulfated: 94% and 97% in PG-S1 and -S2, respectively.

Comparative oligosaccharide mapping of protein-bound and xyloside-initiated CS/DS-chains

The various proteoglycans and free chains produced in the presence of xyloside were isolated by gel chromatography

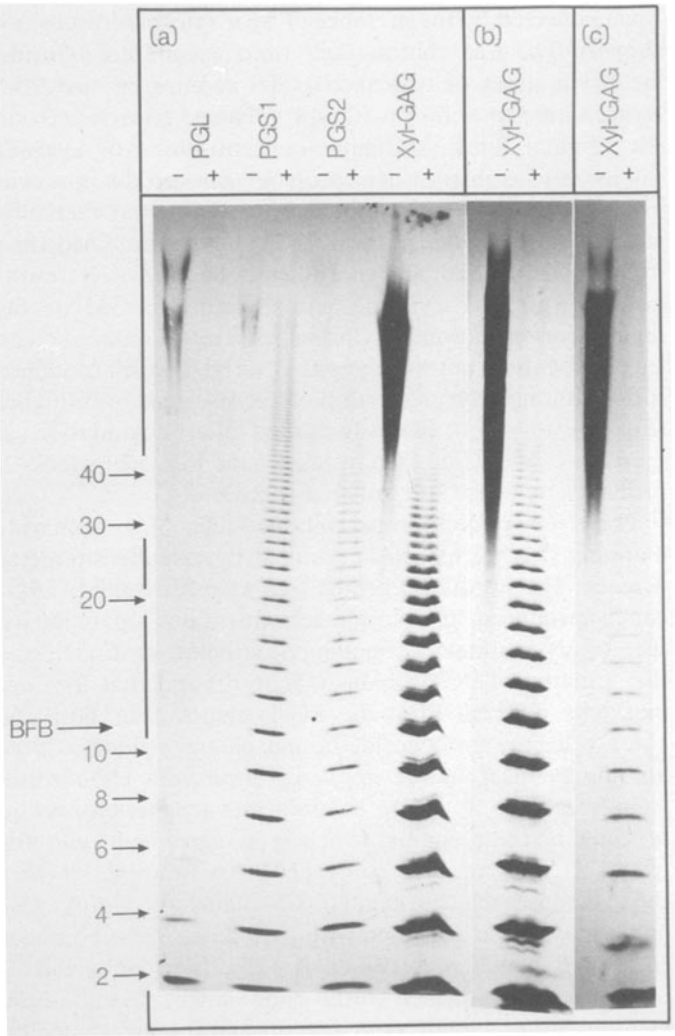

Figure 5. Electrophoresis of chondroitin AC lyase digests of CS/DS-chains derived from PG-L, PG-S1, or PG-S2, and xyloside bound CS/DS-chains formed in the presence of (a) 5 μM, (b) 50 μM and (c) 1 mM xyloside. Confluent cultures were incubated with [^{3}H]glucosamine in the presence of the indicated concentrations of xyloside. Proteoglycans (PG-L, PG-S1 and PG-S2) and free chains (Xyl-GAG) were isolated after chromatography on Superose 6 (Fig. 1). Proteoglycan-bound chains were released by alkali-borohydride treatment and purified by ion exchange chromatography. Proteoglycan-derived and xyloside-bound CS/DS-chains were electrophoresed before (−) and after (+) exhaustive digestion with chondroitin AC-I lyase (see Fig. 3).

(Fig. 1(b–d)). The protein-bound chains were released by alkali treatment, whereas the xyloside-bound chains were analysed directly. After exhaustive digestion with chondroitin AC-I lyase, the oligosaccharide products were resolved by electrophoresis (Fig. 5). At low xyloside concentrations (5 μM), when proteoglycan synthesis was only marginally suppressed, a complete comparison could be made (Fig. 5(a)). The PG-L chains were still IdoA-poor, whereas the PG-S chains were IdoA-rich and afforded the full range of oligosaccharide sizes. Also, the size of PG-S-derived chains

was unaffected by the presence of 5 μM xyloside (results not shown). The free chains, built onto exogenous xyloside, yielded a series of oligosaccharides ranging in size from disaccharide to well into 80 unit polymers (clearly seen on the original film). At higher concentrations of xyloside (50 μM and 1 mM), when proteoglycan production was greatly inhibited and synthesis of free chains was markedly stimulated (Fig. 1(c,d)), the PG-S chains maintained their original chain length and continued to be IdoA-rich (results not shown). The xyloside-bound chains formed at the highest concentration of xyloside had a much reduced chain length (Fig. 1(d)) but still comprised variants which contained IdoA, although the proportion of GlcA seemed to be higher (Fig. 5(c)). Minor bands obtained after degradation of xyloside-GAGs (Fig. 5(a), track 8 and Fig. 5(b), track 2) probably represent low-sulfated saccharides.

The electrophoretograms obtained after oligosaccharide mapping (Fig. 5) were also scanned by videodensitometry (Fig. 6). The results confirmed that xyloside-bound GAG-chains produced at xyloside-concentrations up to 50 μM (Fig. 6(c,d)) maintain a copolymeric structure that resembles the structure of PG-S chains (Fig. 6(b)) and that they are markedly different from the PG-L chains (Fig. 6(a)). At 1 mM xyloside, the xyloside-bound chains yielded a large amount of disaccharide upon digestion with chondroitin AC-I lyase (Figs 5(c), 6(e)). This indicates a higher proportion of GlcA in these chains, which is in agreement with the result of the preceding study [19]. To examine whether these chains included regular CS-chains (no IdoA) this material was also degraded with periodate-oxidation under conditions which preserve GlcA [25]. Electrophoresis of these products indicated (results not shown) that all of the xyloside-bound chains were degraded to fragments smaller in size than the intact ones. Hence, even at 1 mM xyloside, the cells do not seem to synthesize regular CS-chains.

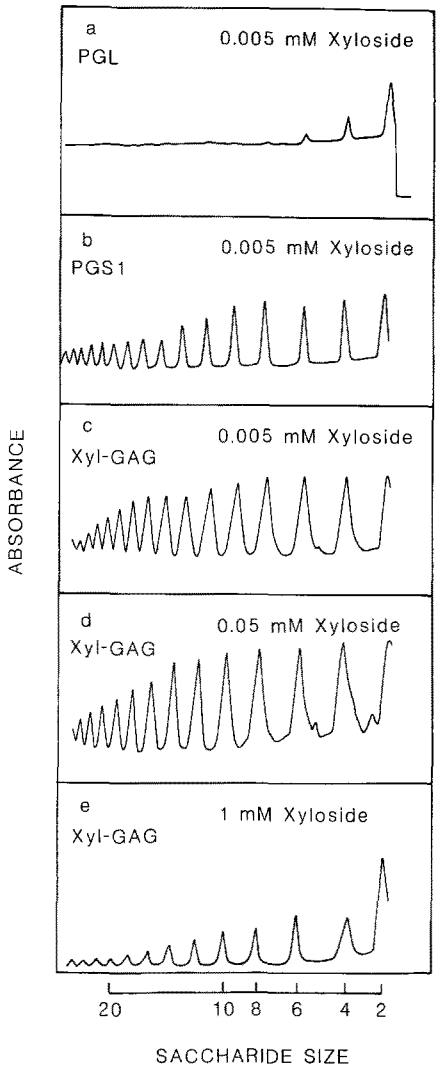

Figure 6. Densitometric scans of electrophoretograms obtained from chondroitin AC lyase-digests of proteoglycan-derived and xyloside-bound CS/DS-chains formed in the presence of various concentrations of xyloside. Tracks shown in Fig. 5 were scanned by videodensitometry. The origin of the CS/DS-chains and the concentration of xyloside used are indicated in the panels.

Conclusion

Proteoglycan synthesis occurs in two separate cellular compartments: the rough endoplasmic reticulum where the polypeptide is formed and the Golgi where GAG synthesis takes place. A particular protein can, in principle, be substituted with any one of a variety of GAGs including KS, CS, DS, HS or heparin. Although many cells can synthesize both HS- and CS/DS-chains, certain proteins become HS-proteoglycans, whereas others become CS/DS ones. Apparently, there is a selection mechanism which is believed to operate by recognizing sequences in the core protein [2]. However, there could conceivably also be other cell-specific control mechanisms which operate independently of core protein synthesis.

GAG chains, such as CS, DS, HS and heparin, are usually attached to serine residues which have adjacent glycines and, further away, acidic amino acids [2]. The single CS/DS-chain of PG-S2 (decorin) is attached to a serine in the sequence SGXG, which is also found in PG-L (versican) together with another type of sequence, (E/D)GSG(E/D) [8]. In PG-S1 (biglycan) the two potential CS/DS-attachment sites reside in the sequence EDDASGADTSGVL [12]. There is no obvious correlation between type of sequence and the nature of the attached DS-chain. Although the attachment sites in PG-S1 resemble the major type of sequence in PG-L, the two proteoglycans carry DS-chains with very different hexuronate composition. Conversely, the two PG-S forms which have different sequences in the GAG-attachment regions appear to be substituted with DS-chains that have very similar copolymeric structures, as indicated by the oligosaccharide mapping and disaccharide analysis performed in this study.

As shown elsewhere [19], proteoglycan as well as overall

protein syntheses in skin fibroblasts are not markedly suppressed at low concentrations of xyloside (5–50 μM). Under these conditions, the GAG-chains formed on the xyloside are similar to those in PG-S, both with respect to HexA composition and 4/6-*O*-sulfate ratios. At xyloside concentrations above 50 μM, proteoglycan synthesis is inhibited and the polymer-modifying enzyme activities (C-5′-epimerase and, in particular, 4-*O*-sulfotransferase) decline [19], which results in the formation of xyloside-initiated chains with higher proportions of GlcA-GalNAc repeats and 6-*O*-sulfate groups, whereas the chains on PG-S retain their copolymeric features. In the present study it is shown that the individual CS/DS-chains present in either the PG-S1 or the PG-S2 proteoglycans have similar copolymeric structure and that they maintain these features even at 1 mM xyloside. As discussed previously [19], the core protein may contain information that directs the precursor to a particular GAG-synthesizing multi-enzyme complex in the Golgi. Furthermore, the core protein may be required to fully activate the polymer-modifying enzymes.

A comparison between xyloside-initiated chains produced in the presence of 0.05 and 1 mM xyloside indicated that the latter ones have a lower IdoA content which largely parallels the decline in C-5′-epimerase activity [19]. However, as the total amount of CS/DS-chains increases five-fold in going from 0.05 to 1 mM xyloside, rough calculations indicate that the total number of IdoA residues formed increases by 50%. It should be kept in mind that, when a large number of GAG chains are initiated on the exogenous primer, maximal modification and processing of each individual chain may not be possible.

The results obtained in this study suggest that the CS/DS-synthesizing machinery in fibroblasts can process chondroitin to DS containing extended IdoA-containing segments even when the xylose primer is not linked to core protein. Similar conclusions have been reached in other studies. Monocytes that differentiate into macrophage-like cells alter their production of CS-chains from 4-*O*-sulfated to 4,6-di-*O*-sulfated ones [31]. This transition also takes place on CS-chains that have been initiated on xylosides, suggesting that this differentiation process operates independently of any associations between polysaccharide and core protein syntheses. Other studies [32] have shown that a single gene is used to encode the core protein of an extracellular proteoglycan in rat L2 cells and an intragranular proteoglycan in rat RBL-1 cells. The former proteoglycan carries regular CS-chains, whereas the latter carries oversulfated CS or heparin. It may thus be concluded that the selection of the type of glycan chain that will be synthesized onto a particular protein core is a cell-specific event which is not exclusively dependent on the nature of the translated polypeptide.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council, National Board for Technical Development, and Cancer Trust, the Gustaf V:s 80-års fond, The Österlund, JA Persson, Bergvall, and Kock Foundations, and the Medical Faculty, University of Lund. We are grateful to Lena Åberg and Birgitta Havsmark for technical assistance, and to Birgitta Jönsson for art-work.

References

1. Fransson LÅ (1987) *Trends Biochem Sci* **12**:406–11.
2. Ruoslahti E (1988) *Annu Rev Cell Biol* **4**:229–55.
3. Heinegård D, Oldberg Å (1989) *FASEB J* **3**:2042–51.
4. Fransson LÅ (1989) In *Heparin* (Lane DA, Lindahl U, eds), pp 115–33. London: Edward Arnold.
5. Gallagher JT, Lyon M (1989) In *Heparin* (Lane DA, Lindahl U, eds), pp 135–58. London: Edward Arnold.
6. Lindahl U (1989) In *Heparin* (Lane DA, Lindahl U, eds), pp 159–89. London: Edward Arnold.
7. Mörgelin M, Paulsson M, Malström A, Heinegård D (1989) *J Biol Chem* **264**:12080–90.
8. Zimmermann DR, Ruoslahti E (1989) *EMBO J* **8**:2975–81.
9. Choi HU, Johnson TL, Pal S, Tang LH, Rosenberg L, Neame P (1989) *J. Biol Chem* **264**:2876–84.
10. Scott JE, Orford CR (1981) *Biochem J* **197**:213–16.
11. Krusius T, Ruoslahti E (1986) *Proc. Natl Acad Sci USA* **83**:7683–87.
12. Fisher L, Termine JD, Young MF (1989) *J Biol Chem* **264**:4571–76.
13. Cöster L. Carlstedt I, Malmström A (1979) *Biochem J* **183**: 669–81.
14. Cöster L. Fransson LÅ (1981) *Biochem J* **193**:143–53.
15. Uldbjerg N, Malmström A, Ekman G, Sheehan J, Ulmsten U, Wingerup L (1983) *Biochem J* **209**:497–503.
16. Cöster L, Fransson LÅ, Sheehan J, Nieduszynski IA, Phelps CF (1981) *Biochem J* **197**:483–90.
17. Tollefsen DM, Peacock ME, Monafo WJ (1986) *J Biol Chem* **261**:8854–58.
18. Malmström A (1984) *J Biol Chem* **259**:161–65.
19. Cöster L, Nernnäs J, Malmström A (1990) *Biochem J*, in press.
20. Rice KG, Rottink MK, Linhardt RJ (1987) *Biochem J* **244**: 515–22.
21. Turnbull JE, Gallagher JT (1988) *Biochem J* **251**:597–608.
22. Fransson LÅ, Havsmark B, Silverberg I (1990) *Biochem J* **269**:381–88.
23. Lindblom A, Carlstedt I, Fransson LÅ (1989) *Biochem J* **261**:145–53.
24. Schmidtchen A, Carlstedt I, Malmström A, Fransson, LÅ (1990) *Biochem J* **265**:289–300.
25. Fransson LÅ, Carlstedt I (1974) *Carbohydr Res* **36**:349–58.
26. Shively JE, Conrad HE (1976) *Biochemistry* **15**:3932–42.
27. Laemmli UK (1970) *Nature* **227**:680–85.
28. Malmström A, Carlstedt I, Åberg L, Fransson LÅ (1975) *Biochem J* **151**:477–89.
29. Hjerpe A, Antonopoulos CA, Engfeldt B, Nurminen M (1982) *J Chromatogr* **242**:193–95.
30. Kyhse-Andersen J (1984) *J Biochem Biophys Meth* **10**:203.
31. Kolset SO, Ehlorsson J, Kjellén L, Lindahl U (1986) *Biochem J* **238**:209–16.
32. Avraham S, Stevens RL, Gartner MC, Austen KF, Lalley PA, Weis JH (1988) *J Biol Chem* **263**:7292–96.