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Bovine aortic chondroitin sulphate- and dermatan sulphate-containing proteoglycans

Isolation, fractionation and chemical characterization

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1. Guanidinium chloride (4 M) in the presence of proteinase inhibitors extracted 90% of bovine aorta galactosaminoglycans as proteoglycans that were subsequently purified by ion-exchange and gel chromatography. 2. Fractionation of the calcium salts of the purified proteoglycans with increasing concentration of ethanol yielded fractions PG-25 (28%), PG-35 (45%) and PG-50 (37%). 3. Fraction PG-50 contained proteochondroitin 6-sulphate, whereas fractions PG-25 and PG-35 were proteodermatan sulphates of greatly different carbohydrate composition; the molar proportions of L-iduronate–*N*-acetylgalactosamine 4-sulphate, D-glucuronate–*N*-acetylgalactosamine 4-sulphate and D-glucuronate–*N*-acetylgalactosamine 6-sulphate were 75:18:7 in fraction PG-25 and 14:46:40 in fraction PG-35. 4. The presence of alternating or mixed sequences with L-iduronate- and D-glucuronate-containing repeating disaccharides was indicated by the formation of tetrasaccharides after chondroitinase AC digestion (single L-iduronate residues) and by the release of fragments containing four or five consecutive D-glucuronate–*N*-acetylgalactosamine repeats after periodate oxidation and alkaline elimination. 5. The amino acid compositions of fractions PG-25 and PG-35 were similar and markedly different from that of fraction PG-50, which also contained more side chains.

The arterial wall contains scattered fibroblasts and smooth-muscle cells in a matrix composed of elastic and collagenous fibres embedded in an amorphous ground substance, which contains glycosaminoglycans (proteoglycans). Most of the known glycosaminoglycans (hyaluronate, heparan sulphate, keratan sulphate, chondroitin sulphate, dermatan sulphate and heparin) seem to be represented in the arterial wall from a variety of species, including man (Kaplan & Meyer, 1960; Sirek *et al.*, 1964; Muir, 1965; Kumar *et al.*, 1967; Madhavan & Chandra, 1971; Eisenstein *et al.*, 1975; Picard *et al.*, 1975). The co-polymeric nature of the chondroitin sulphate/dermatan sulphate chains was first described for horse aorta by Fransson & Havsmark (1970).

Early attempts to isolate proteoglycans from aortic tissue were made after extractions with water (Buddecke *et al.*, 1963), with EDTA (Kresse *et al.*, 1971) or with 3 M-$MgCl_2$ (Ehrlich *et al.*, 1975) or after treatment with collagenase (Radhakrishnamurthy *et al.*, 1977). The molecular weights of these preparations ranged from 72000 to 1.2×10^6. By the introduction of efficient procedures for the extraction of fibrous connective tissues (Antonopoulos *et al.*, 1974) and the utilization of proteinase inhibitors, such as EDTA, benzamidine and 6-aminohexanoic acid (Oegema *et al.*, 1975), it became possible to isolate proteoglycans from aorta in a more native state. Accordingly, Oegema *et al.*, (1979) isolated, from a 4 M-guanidinium chloride extract of bovine aorta, a chondroitin sulphate- and dermatan sulphate-containing proteoglycan fraction containing 18% of protein. The material, which was

Abbreviations used: GalNAc, *N*-acetylgalactosamine; HexA, hexuronic acid; ΔHexA, 4,5-unsaturated hexuronic acid; IdoA, L-iduronic acid; GlcA, D-glucuronic acid; $-SO_4$, ester sulphate.

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shown to be heterogeneous by several techniques, had a weight-average molecular weight in the range 1.5×10^6–2.0×10^6. At least 10% of the proteoglycan interacted with hyaluronate in a manner similar to that shown by the aggregating proteoglycan found in hyaline cartilage. Furthermore, two proteoglycan–hyaluronate complexes have been isolated from bovine aorta (McMurtrey *et al.*, 1979). More recently (Gardell *et al.*, 1980) it has been demonstrated that proteoglycans from arterial wall cross-react with antisera raised against the hyaluronate-binding region, the chondroitin sulphate–peptides and the link proteins purified from cartilage.

The present study is mainly concerned with fractionation of galactosaminoglycan-carrying proteoglycans obtained from 4M-guanidinium chloride extracts of aorta in the presence of proteinase inhibitors. The proteoglycans were fractionated into one separate proteochondroitin sulphate population and two pools of proteodermatan sulphates. The amino acid composition and the co-polymeric structure of the side chains were determined.

Experimental

Materials

Fresh bovine aortae were obtained from the local abattoir and packed in ice for transport to the laboratory. The intimal and medial layers were collected by dissection, washed with cold 0.15M-NaCl, defatted with cold diethyl ether, air dried and stored at −20°C until further use.

Sepharose and Sephadex gels were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. DEAE-cellulose was from Whatman, Maidstone, Kent, U.K., and mixed-bed resin MB-3 was from BDH Chemicals, Poole, Dorset, U.K.

Papain was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and chondroitinases AC (EC 4.2.2.5) and ABC (EC 4.2.2.4) were from Seikagaku Kogyo, Tokyo, Japan. Glycosaminoglycans used as reference standards were from the Chicago collection.

All reagents were of analytical grade, except for guanidinium carbonate, urea and acetone, which were reagent grade. Guanidinium chloride was prepared by the method of Nozaki (1972). Stock solutions of urea (8M) were passed through MB-3 resin to remove traces of cyanate.

Analytical methods

Total hexosamines were determined by a modification of the Elson–Morgan reaction (Gardell, 1958), *N*-acetylhexosamine by the Morgan–Elson reaction (Reissig *et al.*, 1955), hexuronic acid by an automated procedure (Heinegård, 1973) of the carbazole/borate method (Bitter & Muir, 1962), protein by the method of Lowry *et al.* (1951), with bovine serum albumin as standard, and total sulphate by the method of Terho & Hartiala (1971). Amino acids, glucosamine and galactosamine were determined by using a Locarte amino acid analyser consisting of a single-column elution system. Hydrolysis was performed (under N_2 in sealed ampoules) in 6M-HCl at 100°C for 16h (for amino acids) or in 4M-HCl at 100°C for 8h (for hexosamines). No corrections were made for the destruction of hexosamines during hydrolysis. Neutral sugars were determined by g.l.c. of their alditol acetates after hydrolysis in 2M-trifluoroacetic acid at 100°C for 4h under N_2 (Axelsson & Heinegård, 1975) or by methanolysis followed by formation of trimethylsilyl derivatives (Bhatti *et al.*, 1970). The instrument used was a Pye Unicam 104.

Degradation methods

Nitrous acid deamination of glycosaminoglycans (specific for heparin and heparan sulphate) was performed as described by Lindahl *et al.* (1973). Periodate oxidation of iduronate residues in galactosaminoglycans was performed at pH3.0 (50mM-formate) and 4°C for 24h. After dialysis, the oxidized product was cleaved with alkali (pH12; 20°C; 30min).

Cleavage of bonds between *N*-acetylgalactosamine and glucuronate or between *N*-acetylgalactosamine and either hexuronate in galactosaminoglycans was accomplished by chondroitinase AC and chondroitinase ABC digestions respectively (Yamagata *et al.*, 1968). Approx. 0.01 unit of enzyme was used per mg of substrate. The amounts of disaccharides (ΔHexA–GalNAc-SO_4) obtained in the two cases were quantified by the periodate/thiobarbiturate method of Hascall *et al.* (1972).

Separation methods

Degradation products of glycosaminoglycans were separated on Sephadex G-50 (superfine grade) in 0.2M-pyridine/acetate buffer, pH5.0. For details see the legend to Fig. 2. Glycosaminoglycan chains were chromatographed on a column (7mm × 140mm) of DE-52 DEAE-cellulose, which was eluted with a linear gradient from 0.10M- to 2.5M-sodium acetate buffer, pH5.0, at a flow rate of 4ml/h. Fractions (1ml) were analysed for hexuronate. Polyacrylamide-gel electrophoresis was conducted on 7% (w/v) gels containing 0.1% sodium dodecyl sulphate by the procedure of Neville (1971). Gels were stained with 0.25% Kenacide R and scanned at 605nm.

Isolation of proteoglycans

Aortic tissue was minced and extracted with 10vol. of various solvents (1–5M-guanidinium chloride, -urea, -$CaCl_2$ or -$MgCl_2$) for 5 days at

4°C or with 4M-guanidinium chloride for different lengths of time. Extracts were centrifuged in an MSE High Speed 18 centrifuge at 20000*g* for 30min at 4°C. Samples of the supernatant were analysed for uronic acid directly and for glycosaminoglycan composition after papain digestion and ion-exchange chromatography. The effluents were analysed for uronic acid by carbazole measurements (see above).

Preparative extraction and isolation of proteoglycans were performed in accordance with the principles described by Antonopoulos *et al.* (1974). A 1kg batch of minced aorta was extracted with 10 litres of 4M-guanidinium chloride/50mM-sodium acetate buffer (pH5.8)/10mM-Na_2EDTA/10mM-benzamidinium chloride/10mM-6-aminohexanoic acid for 5 days at 4°C. The suspension was filtered through cheese-cloth, and the residue was washed with 5 litres of 0.3M-NaCl. The washings and the filtrate were combined and centrifuged in an MSE High Speed 18 centrifuge at 20000*g* for 1h at 4°C. The supernatant was then concentrated 5–7-fold by ultrafiltration in an Amicon cell with a PM 10 filter, dialysed against 6M-urea/50mM-Tris/HCl buffer, pH6.5, with inhibitors, and passed (in two batches) through a column (50mm × 400mm) of DE-23 DEAE-cellulose equilibrated with the same buffer. The columns were then eluted with 2–4 bed volumes of (1) 6M-urea, (2) 0.15M-NaCl/6M-urea and (3) 2M-NaCl/6M-urea, all in 50mM-Tris/HCl buffer, pH6.5. The last fraction (3) yielded a crude proteoglycan preparation free of collagen.

The crude proteoglycan was then subjected to ion-exchange chromatography on a column (15mm × 400mm) of DE-52 DEAE-cellulose that was eluted with a linear gradient of 0.1–2.0M-sodium acetate buffer, pH5.8, containing 6M-urea, at a rate of 16ml/h. Fractions (1.5ml) were analysed for uronic acid and A_{280}. The main uronic acid peak was pooled and dialysed, and, finally, a u.v.-absorbing impurity was removed by gel chromatography on a column (40mm × 1500mm) of Sephadex G-75 that was eluted with 0.5M-sodium acetate buffer, pH7.0, at a rate of 50ml/h. Fractions (25ml) were analysed for uronic acid, and the main peak, which occurred in the void volume, was pooled and dialysed and material was recovered by freeze-drying.

Subfractionation of proteoglycans

Proteoglycans (1–5mg/ml) were dissolved in 5% (w/v) calcium acetate/0.5M-acetic acid, pH5.0, and the solution was kept overnight at 4°C. After removal of a small amount of undissolved material by centrifugation at 20000*g* for 30min at 4°C, the supernatant was treated with ethanol. Cold ethanol was added dropwise under stirring to give a final concentration of 25% (v/v). Precipitate formed after 12h at 4°C was collected by centrifugation, and the ethanol concentration of the supernatant was adjusted in stages to 35%, 50% and 70% (v/v). Each precipitate was collected by centrifugation, washed with the corresponding ethanol/water mixture, dissolved in water, dialysed and freeze-dried.

Proteolysis

Samples of fresh tissue, extracted residues or purified proteoglycans were digested with papain essentially as described by Scott (1960). Papain was purified by the method of Kimmel & Smith (1954), and additions of 0.5–1mg were made every 6h to the samples, which were incubated for 12–16h at 65°C in 5mM-Na_2EDTA/5mM-cysteine hydrochloride/100mM-sodium phosphate buffer, pH6.5. Oligosaccharide–peptides and glycosaminoglycan–peptides were isolated by ion-exchange chromatography on a column (7mm × 140mm) of DE-23 DEAE-cellulose that was eluted stepwise with 20mM-HCl, 0.3M-NaCl and 2M-NaCl. The oligosaccharides were obtained from the first fraction and the glycan chains from the last fraction after dialysis against water and freeze-drying of the dialysis residue. The quantification of oligo- and poly-saccharides was made by hexosamine analyses of the eluates.

Results

Extraction and isolation of proteoglycans

To assess the extractability of aortic proteoglycans intima and media were treated with different solvents. It was observed that 4M-guanidinium chloride was a better extractant than urea, $CaCl_2$ or $MgCl_2$ at any concentration in the range 1–5M. After 5 days of extraction with 4M-guanidinium chloride in the presence of proteinase inhibitors, only 60% of the total hexuronate content of the tissue was solubilized. Prolonged extraction did not increase the yield. The whole tissue, the extract and the residue were individually digested with papain, and the glycosaminoglycan content was assessed by ion-exchange chromatography on DEAE-cellulose (gradient elution; carbazole analyses; results not shown). The results indicated that most (approx. 90%) of the chondroitin sulphate/dermatan sulphate pool was solubilized by 4M-guanidinium chloride, whereas a considerable proportion of the hyaluronate and most of the heparan sulphate remained in the tissue after extraction.

The 4M-guanidinium chloride extract was centrifuged, concentrated, dialysed against 6M-urea and chromatographed on DEAE-cellulose (Antonopoulos *et al.*, 1974). The hexuronate-containing material was distributed as follows: 6M-urea, 15%; 0.15M-NaCl, 10%; 2M-NaCl, 70%. Thus 42% of the

total tissue hexuronate was recovered in the crude proteoglycan fraction. The latter material was then rechromatographed on DEAE-cellulose with an NaCl concentration gradient (Fig. 1). Minor impurities such as hyaluronate and nucleic acids were removed. This proteoglycan fraction contained material absorbing at 260nm, which could be removed by chromatography on Sephadex G-75

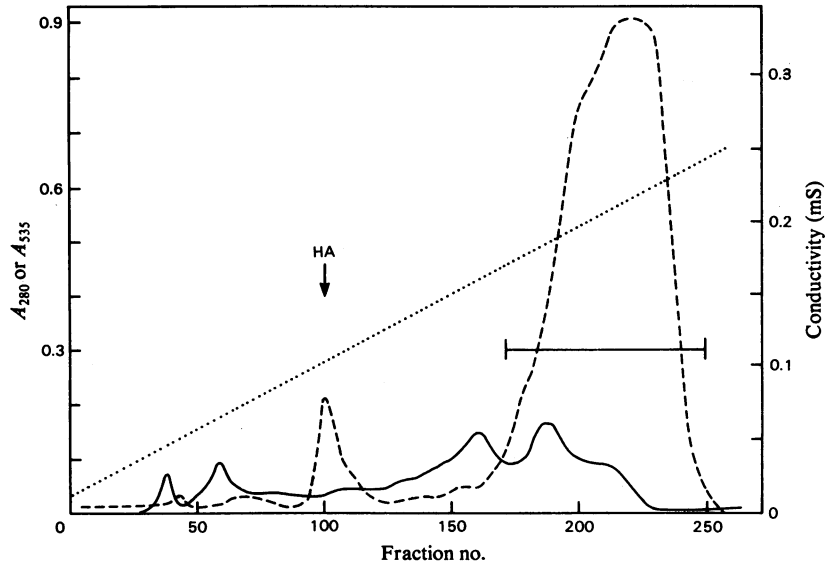

Fig. 1. *Ion-exchange chromatography of aortic proteoglycan preparation (2 M-NaCl fraction)*
Approx. 100mg of proteoglycan was dissolved in 0.10 M-sodium acetate buffer, pH 5.8, containing 6 M-urea and chromatographed on a column (15mm × 400mm) of DE-52 DEAE-cellulose that was precycled as recommended by the manufacturer and finally equilibrated with the starting buffer. Elution was performed with a linear gradient (0.10–2.00 M-sodium acetate buffer, pH 5.8, containing 6 M-urea; total elution volume, 1000ml), 2ml fractions being collected. The effluent was analysed for protein (——, A_{280}), uronic acid (– – – –, A_{535}; carbazole reaction) and conductivity (·····). Fractions were pooled as indicated by the horizontal bar, dialysed against water and freeze-dried. HA, Elution position of standard hyaluronate.

Table 1. *Analyses of aortic proteoglycan and its subfractions (PG-25, PG-35 and PG-50)*
The various proteoglycan preparations were obtained as described in the Experimental section. Oligosaccharides and glycosaminoglycans were released by papain treatment, separated by ion-exchange chromatography and quantified by hexosamine analyses. The uronic acid composition was determined after digestions with chondroitinase AC (glucuronate) and chondroitinase ABC (iduronate + glucuronate).

	Composition (%)			
Analyses	Proteoglycan	Fraction PG-25	Fraction PG-35	Fraction PG-50
Protein (Folin reaction)	23	29	21	12
Carbohydrate side group				
Oligosaccharide	15			
Glycosaminoglycan	85			
Hexosamine	28.5	27	31	27
Galactosamine	87			
Glucosamine	13			
Uronic acid (carbazole reaction)	36.5	25	34	38
L-Iduronate	20	75	13	0
D-Glucuronate	80	25	87	100
Sulphate	10.6	12	11	11
Xylose	0.4	0.3	0.4	0.4
Galactose	3.8	2.3	4.7	5.8
Mannose	0.9	0.6	0.5	1.0

(see the Experimental section). The purified proteoglycan accounted for approx. 40% of the total tissue hexuronate.

Characterization of aortic proteoglycan

The glycosaminoglycan and oligosaccharide prosthetic groups of the purified proteoglycan were released by papain treatment and isolated by ion-exchange chromatography (yield: 75% of the starting material). The ratio of the two forms was 3:17 (Table 1). The glycosaminoglycan chains had the same elution position on ion-exchange chromatography (results not shown) as did chondroitin sulphate/dermatan sulphate. As shown in Fig. 2(*a*), the side chains of the proteoglycan were resistant to deaminative cleavage, indicating the absence of heparan sulphate. As shown in Table 1, the glycan chains contained both iduronate and glucuronate (ratio 1:4), and, accordingly, they were extensively degraded by chondroitinase AC (Fig. 2*b*) and completely degraded to disaccharides by chondroitinase ABC (Fig. 2*c*). Thus it may be concluded that the side chains were galactosaminoglycans. The co-polymeric nature of these side chains is illustrated by the profile shown in Fig. 2(*b*). Besides the major disaccharide peak (ΔHexA–GalNAc-SO_4), there was also a tetrasaccharide, ΔHexA–GalNAc(-SO_4)–IdoA–GalNAc-SO_4, as well as longer oligosaccharides, ΔHexA–GalNAc(-SO_4)–[IdoA–GalNAc(-SO_4)]$_n$; the unsaturated hexuronate residue was originally a glucuronate residue.

The proteoglycan preparation had a galactosamine/xylose ratio of 60:1 (Table 1), giving a number-average molecular weight for the side chains of approx. 30000. The sulphate/galactosamine ratio of the preparation was close to 1:1. An oligosaccharide fraction isolated from the proteoglycan after proteolysis had the following molar proportions: GlcN, 1.00; GalN, 0.05; Gal, 1.10; Man, 0.41; Xyl, 0.01. All of the glucosamine and the mannose of the proteoglycan was in the oligosaccharide. In the galactosaminoglycan chain preparations the galactose/xylose ratio was 1.8:1.

The protein content of the aortic proteoglycan preparation was 23% (Table 1). A protein core preparation was obtained by depolymerizing the galactosaminoglycan side chains with chondroitinase ABC. Chromatography of the digest on Sepharose CL-2B gave the result shown in Fig. 3. The position of the main protein peak was close to the total volume, which is the same position as that seen for a core preparation of cartilage proteoglycans (Heinegård & Axelsson, 1977). The core preparation of aortic proteoglycan did not appreciably penetrate a 7% polyacrylamide gel in electrophoresis. This is also seen with the corresponding material from cartilage (Axelsson & Heinegård, 1975; Heinegård, & Axelsson, 1977; Hascall & Heinegård, 1979).

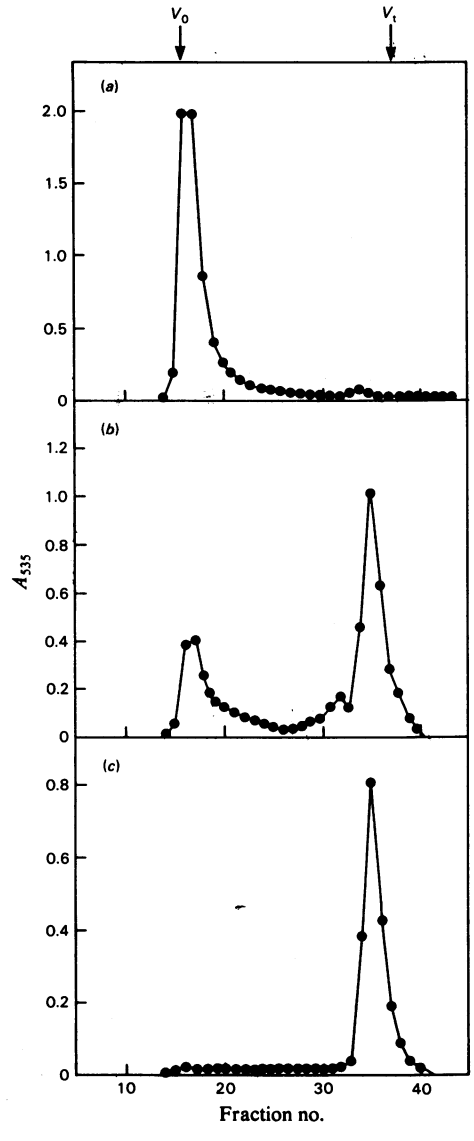

Fig. 2. *Gel chromatography on Sephadex G-50 of glycosaminoglycan side chains derived from aortic proteoglycan after (a) nitrous acid degradation, (b) chondroitinase AC digestion and (c) chondroitinase ABC digestion*

Glycosaminoglycan side chains were released from the proteoglycan by proteolysis and subjected to degradation. For details see the Experimental section. The column size was 5mm × 1500mm, the eluent was 0.2M-pyridine/acetate buffer, pH 5.0, the elution rate was 5.8ml/h and the fraction size was 2ml. Analysis was by using the carbazole reaction. V_0, Elution volume of Blue Dextran; V_t, elution volume of $Na_2{}^{35}SO_4$.

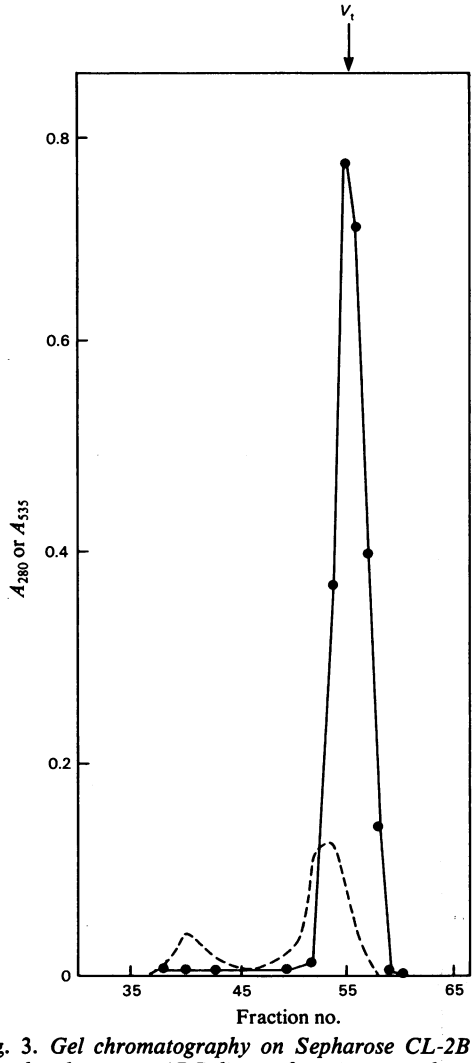

Fig. 3. *Gel chromatography on Sepharose CL-2B of a chondroitinase ABC digest of aortic proteoglycan*
Aortic proteoglycan was treated with chondroitinase ABC as described in the Experimental section and chromatographed on a column (5mm × 1500mm) of Sepharose CL-2B. The eluent was 0.15M-NaCl/5mM-sodium phosphate buffer, pH 7.4, the elution rate was 3ml/h and the fraction size was 1.2ml. Analysis was by using the carbazole reaction (——, A_{535}) and by measuring the protein content (– – –, A_{280}).

Subfractionation of aortic proteoglycan

We attempted to fractionate the proteoglycan by ethanol precipitation in the presence of Ca^{2+} ions, a fractionation technique that is sensitive to the iduronate/glucuronate ratio of galactosaminoglycans (Fransson *et al.*, 1970). The aortic proteoglycans were subdivided into three fractions (25%, 35% and 50% ethanol), comprising 28%, 45% and 27% of the material respectively. Analyses of the individual fractions (Table 1) revealed a decreasing protein content with increasing ethanol solubility, and, simultaneously, marked changes in the uronic acid composition were noted. Whereas fraction PG-50 contained exclusively glucuronate and could be classified as a proteochondroitin sulphate, fractions PG-25 and PG-35 both contained iduronate and glucuronate, the former in the ratio 75:25 and the latter 13:87. The sulphate/galactosamine ratios were rather similar, and the galactosamine/xylose ratios were 62:1 for fractions PG-25 and PG-35 and 52:1 for fraction PG-50, suggesting that the chondroitin sulphate chains were somewhat shorter (mol.wt. 26000) than were the co-polymeric dermatan sulphate/chondroitin sulphate chains (mol.wt. 31000). The galactose and mannose content was higher in the proteochondroitin sulphate preparation than in the two proteodermatan sulphate fractions.

Further analyses of the galactosaminoglycan side chains of the three proteoglycan fractions were obtained after sequential chondroitinase AC and chondroitinase ABC digestion followed by determinations of 4-sulphate and 6-sulphate contents (Table 2). In fraction PG-25 93% of the repeating units were 4-sulphated, with a ratio between IdoA–GalNAc-4-SO_4 and GlcA–GalNAc-4-SO_4 of approx. 4:1. In contrast, fraction PG-35 contained 86% glucuronate-containing repeating units with almost equal proportions of 4-sulphate and 6-sulphate. Fraction PG-50 may be classified as proteochondroitin 6-sulphate (95%).

The co-polymeric features of the dermatan sulphate side chains of fractions PG-25 and PG-35 were also examined by periodate oxidation–alkaline elimination, which cleaves the chains at the iduronate residues. As shown in Fig. 4(*a*), the chains of fraction PG-25 were extensively degraded and the bulk of the products should be represented by oligosaccharides of the general structure GalNAc(-SO_4)–[GlcA/IdoA(-SO_4)–GalNAc-(-SO_4)]$_n$–R, where R is the remnant of an oxidized and degraded iduronate residue (Fransson *et al.*, 1974*a*,*b*). It was estimated that the weight distribution of *n* ranged between 1 and 8, with a maximum at 4–5. Although a considerable proportion of the degradation products of the fraction PG-35 chains was excluded from Sephadex G-50 and hence possibly represents proper chondroitin sulphate chains, at least half of the material corresponded to oligosaccharides with the features shown above. The chains of fraction PG-50 were almost completely excluded from Sephadex G-50 after periodate oxidation, in agreement with the fact that they are regular chondroitin sulphate chains.

The three proteoglycan fractions were also

Table 2. *Disaccharide composition of galactosaminoglycan chains derived from aortic proteoglycan subfractions (PG-25, PG-35 and PG-50)*

The galactosaminoglycan side chain that were released from the various proteoglycans by proteolysis were fragmented by chondroitinase AC digestion. The products were resolved by gel chromatography on Sephadex G-50 (Fig. 2*b*) into a void-volume fraction comprising the [IdoA–GalNAc-SO_4]$_n$ block regions and an included fraction containing largely the disaccharides ΔHexA–GalNAc-SO_4 derived from the GlcA–GalNAc-SO_4 repeating units. The yields of the two fractions were assessed from hexosamine analysis. The proportions of 4-sulphated and 6-sulphated *N*-acetylgalactosamine in the iduronate- and the glucuronate-containing repeating disaccharides were assessed by determination of 6-sulphated reducing terminal *N*-acetylgalactosamine (Morgan–Elson reaction; 4-sulphated *N*-acetylgalactosamine does not react in this assay). With the [IdoA–GalNAc-SO_4]$_n$ fragments the Morgan–Elson assays were conducted after complete degradation to disaccharides (ΔHexA–GalNAc-SO_4) by chondroitinase ABC. In the galactosaminoglycan chain preparations *N*-acetylgalactosamine accounted for 96% of all hexosamine; 93% of the xylose, 13% of the galactose and 0% of the mannose content of the original proteoglycan were associated with the chain preparations. The galactose/xylose molar ratio was 1.8:1. The proportions of non-sulphated GlcA–GalNAc repeats were not specifically determined.

	Composition (%)		
Disaccharide	Fraction PG-25	Fraction PG-35	Fraction PG-50
IdoA–GalNAc-4-SO_4	75	14	0
GlcA–GalNAc-4-SO_4	18	46	5
GlcA–GalNAc-6-SO_4	7	40	95

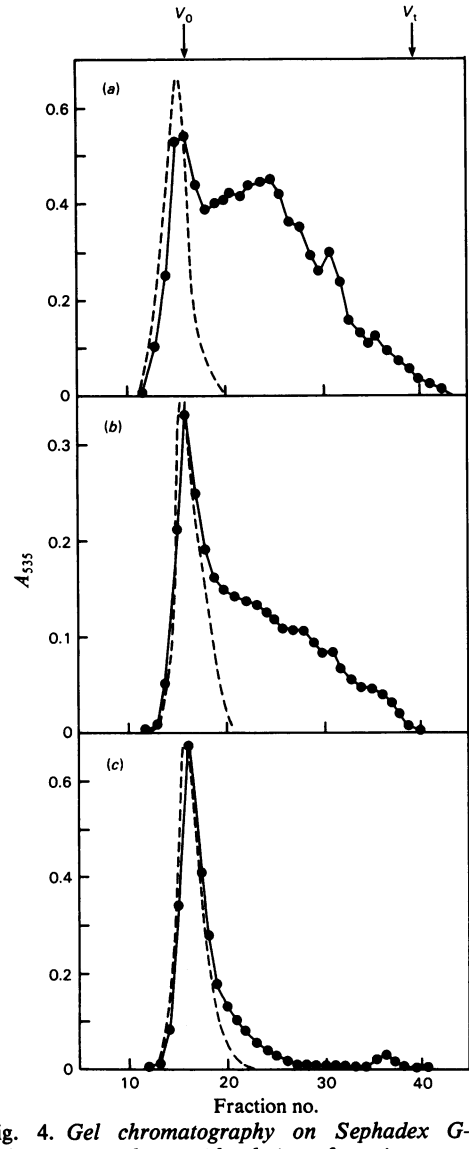

Fig. 4. *Gel chromatography on Sephadex G-50 of galactosaminoglycan side chains of aortic proteoglycan subfractions (a) PG-25, (b) PG-35 and (c) PG-50 after periodate oxidation and alkaline elimination*

The side chains were released from the proteoglycans by proteolysis as described in the Experimental section. After selective periodate oxidation of iduronate residues, dialysis and alkaline scission the fragments were chromatographed under the same conditions as in Fig. 2. The broken line indicates the elution profiles of the respective galactosaminoglycans before degradation.

analysed for their amino acid composition (Table 3). The two proteodermatan sulphates had quite similar amino acid compositions except for slightly higher proportions of threonine, glutamate/glutamine and proline and lower proportions of leucine in fraction PG-35. The proteochondroitin 6-sulphate had a markedly different amino acid composition and was higher in threonine, serine, glutamate/glutamine and proline contents and lower in glycine, leucine, histidine, lysine and arginine contents than the proteodermatan sulphate was.

Discussion

In the present study aortic intima and media were subjected to dissociative non-shear-dependent extraction in the presence of proteinase inhibitors. In this process most of the chondroitin sulphate- and dermatan sulphate-containing proteoglycans were extracted. The proteoglycans were purified by ion-exchange and gel chromatography. In this process, impurities such as hyaluronate and nucleic

Table 3. *Amino acid compositions of aortic proteoglycan and its subfractions (PG-25, PG-35 and PG-50)*
For details see the Experimental section.

	Composition (residues/1000 residues)			
	Proteoglycan	Fraction PG-25	Fraction PG-35	Faction PG-50
Asx	122	116	124	97
Thr	54	46	60	103
Ser	94	64	67	116
Glx	165	138	168	197
Pro	75	79	88	104
Gly	102	102	96	86
Ala	66	70	72	58
Cys	Trace	Trace	Trace	Trace
Val	50	48	47	58
Ile	24	36	30	23
Leu	98	131	105	41
Tyr	9	12	15	5
Phe	36	38	37	43
His	38	36	24	20
Lys	28	42	32	20
Arg	38	44	37	28

acids were removed. Although the final preparation contained 13% glucosamine, this could be entirely ascribed to the presence of oligosaccharides rich in glucosamine, mannose and galactose. The glycosaminoglycan side chains of the proteoglycan preparation were exclusively of the chondroitin sulphate and dermatan sulphate type. The presence of galactose and xylose in a ratio close to 2:1 suggests that the side chains are linked to the protein core with the common sequence –GlcA–Gal–Gal–Xyl–Ser<.

Oegema *et al.* (1979) purified and fractionated aortic proteoglycans by density-gradient centrifugation. This method separates proteoglycans mainly according to the number of side chains on the protein core. The various fractions obtained showed little difference in amino acid composition. One fraction that was characterized in more detail contained a proteoglycan with 18% protein and co-polymeric galactosaminoglycan side chains (7% IdoA–GalNAc-4-SO_4; 46% GlcA–GalNAc-4-SO_4; 47% GlcA–GalNAc-6-SO_4). In the present study the aortic proteoglycans were separated according to the iduronate and glucuronate contents of the cognate glycan chains. We obtain a separate proteochondroitin 6-sulphate pool (PG-50) and two proteodermatan sulphate fractions (PG-25 and PG-35). The fraction characterized by Oegema *et al.* (1979) had a chemical composition intermediate between those of our fractions PG-35 and PG-50 with regard to protein content, amino acid and uronate composition. The proteochondroitin 6-sulphate isolated in the present study was particularly enriched with keratan sulphate-like glycopeptides, as previously noted by Oegema *et al.* (1979). Apparently there is scope for at least three different kinds of glucosamine-containing substituents, keratan sulphate, mannose-rich *N*-glycosidically linked oligosaccharides and *O*-glycosidically linked mucin-type saccharides (Lohmander *et al.*, 1980).

The aortic proteochondroitin 6-sulphate was similar in amino acid composition to the cartilage-type proteoglycans (Heinegård, 1972), with some notable exceptions (lower in threonine and leucine contents and higher in glutamate/glutamine, proline and glycine contents than the cartilage material). Our material was also higher in glutamate/glutamine, proline and glycine contents than was the aortic proteoglycan isolated by Oegema *et al.* (1979). This observation, in conjunction with the presence of only traces of cysteine in our material, raises the possibility that the putative hyaluronate-binding region of the proteochondroitin 6-sulphate has been partially degraded. It was estimated by Oegema *et al.* (1979) that 10–25% of the aortic proteoglycans were able to interact with hyaluronate. It should be of interest to examine whether the proteochondroitin 6-sulphate is the only molecular species with affinity for hyaluronate or whether proteodermatan sulphates may also aggregate with hyaluronate.

In the present study the proteodermatan sulphate molecules were separated into two fractions, comprising 28% and 45% of the extracted material respectively. These materials had a higher protein content and somewhat longer side chains than did the proteochondroitin 6-sulphate. This indicates

that the protein core of the proteodermatan sulphates contains fewer side chains than the chondroitin sulphate-bearing counterpart. The amino acid composition of the latter core was significantly different from that of the two proteodermatan sulphates (PG-25 and PG-35). Their amino acid composition were akin to those of two scleral proteodermatan sulphates isolated in this laboratory (Cöster & Fransson, 1981), i.e. a PG-I fraction was comparable with fraction PG-35, and a PG-II fraction was comparable with fraction PG-25. Differences between the amino acid compositions of the two sets of proteodermatan sulphates were also noted, fractions PG-35 and PG-I being higher in threonine, glutamate/glutamine and proline contents and lower in leucine content than fractions PG-25 and PG-II respectively. Interestingly, the PG-35 and PG-I preparations both contain side chains that are glucuronate-rich (glucuronate/iduronate ratios 86:14 80:20 respectively), whereas the iduronate content is considerably higher in fractions PG-25 and PG-II (75% and 52% respectively of total hexuronate). Further studies on the amino acid sequences of different proteodermatan sulphates and proteochondroitin sulphates are of great importance, as they should form a basis for investigations into the possible role of the protein core in regulating the biosynthesis of proteoglycans, particularly the polymerization and modification processes during elongation of the side chains.

The dermatan sulphate side chains of both fraction PG-25 and fraction PG-35 contain alternating or mixed IdoA–GalNAc-4-SO_4 and GlcA–GalNAc-4/6-SO_4 repeats. It has been shown previously (Cöster & Fransson, 1979) that these features are correlated with self-association that enables proteoglycans carrying such chains to form large aggregates (Cöster *et al.*, 1981). It is clear that the proteodermatan sulphates of aorta are potentially capable of such interactions.

The aortic wall is under considerable and variable hydrostatic pressure, which leads to complicated stress–strain characteristics (Sumner *et al.*, 1969; Dobrin, 1978). The main elastic and tensile components are elastin and collagen, which are embedded in a matrix that contains proteoglycans. The cartilage-type proteoglycan aggregates could endow the matrix with a marked resistance towards compression and deformation. This might be essential in providing a mechanism by which the arterial wall can be extended without diminishing the thickness of the wall. To achieve this the components of the matrix would have to be dispersed, and some proteoglycan aggregates would have to yield to pressure. It is noteworthy in this context that the self-association displayed by dermatan sulphate side chains is particularly sensitive to shear forces (Fransson *et al.*, 1979). Therefore the proteodermatan sulphates of the aortic wall might physically separate as the pressure increases. In general, different biomechanical requirements of a particular connective tissue are reflected in a particular blend of components, i.e. collagen, elastin and proteoglycans (Phelps, 1975). In addition, the particular mixture of proteoglycans should be capable of providing further, finer, modulation by way of the properties of the co-polymeric dermatan sulphate side chains, as well as by hyaluronate-dependent aggregate-formation.

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