Biochem. J. (1991) 274, 415–420 (Printed in Great Britain)

Purification and *N*-terminal amino acid sequence of a chondroitin sulphate/dermatan sulphate proteoglycan isolated from intima/media preparations of human aorta

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A proteoglycan (PG) was purified to homogeneity from intima/media preparations of human aorta specimens by the following chromatographic steps: Sepharose Q anion exchange, Sepharose CL-4B size exclusion, hydroxyapatite, MonoQ anion exchange and TSK G 4000 SW size exclusion. The purity of the preparation was established by SDS/PAGE using direct staining by silver or Dimethylmethylene Blue, as well as by Western blots of biotin-labelled samples. The electrophoretic mobility of the native PG was less than that of a 200000-M_r standard protein. After treatment with chondroitin sulphate lyase ABC, a core protein of M_r 15000 was revealed. The M_r of the glycosaminoglycan (GAG) peptides was less than 24000, by comparison with a keratan sulphate peptide. The composition of the GAG chains was determined by differential digestion of the PG by chondroitin sulphate lyases AC/ABC or chondroitin sulphate lyase AC alone followed by anion-exchange chromatography of the resulting disaccharides. The GAG chains are composed of approximately one-third of dermatan sulphate and two-thirds chondroitin sulphate disaccharide units. The sequence of the 20 *N*-terminal amino acids is identical with the sequence previously reported for PG I isolated from human developing bone [Fisher, Termine & Young (1989) J. Biol. Chem. **264**, 4571–4576]. The assignment of glycosylation sites to the serine residues in positions 5 and 10 was confirmed. The findings indicate that the chondroitin sulphate/dermatan sulphate PG is a major PG in intima/media preparations of human aorta and represents a biglycan-type PG.

INTRODUCTION

Changes in the distribution of proteoglycans (PGs) seem to play an important role in the pathogenesis of atherosclerosis (Berenson *et al.*, 1986). These macromolecules are involved in some of the key events which lead to the formation of atherosclerotic lesions, e.g. binding of apolipoprotein B-containing plasma lipoproteins, platelet aggregation, binding of growth factors, growth inhibition of smooth muscle cells and precipitation of calcium (Wight, 1985; Ross, 1986; Berenson *et al.*, 1986).

It has been reported that the content of chondroitin sulphate (CS)/dermatan sulphate (DS) glycosaminoglycans (GAGs) is significantly increased in atherosclerotic lesions (Ylä-Herttuala *et al.*, 1986). Since DS chains show high-affinity binding to low-density lipoproteins *in vitro*, this change may be important in the development of atheromas. In order to understand the molecular basis of these findings, it is important to characterize the core protein(s) to which CS/DS GAGs are bound. Most of the structural studies on CS and/or DS PGs from aorta have been performed with non-human systems (Heinegård *et al.*, 1985). Less information is available on these PGs in humans (Lindblom *et al.*, 1989, and references therein on human PGs).

Here we report on the isolation, characterization and *N*-terminal amino acid sequence of a CS/DS PG isolated from intima/media preparations of human aorta. The *N*-terminal amino acid sequence is identical with the sequence deduced from a cDNA encoding PG I, a PG isolated from developing human bone (Fisher *et al.*, 1987, 1989).

EXPERIMENTAL

Materials

Human aortae were obtained at autopsy within 48 h. Specimens used for biosynthetic labelling were obtained within 12 h. Sulphate-depleted Dulbecco's modified Eagle's medium (DMEM) was from Gibco and carrier free $H_2{}^{35}SO_4$ was from New England Nuclear. Gels for column chromatography and columns were obtained from Pharmacia/LKB (Sepharose Q, MonoQ, Sepharose CL-4B; TSK G 4000 SW, TSK G SWP) and from Bio-Rad (hydroxyapatite, Bio-Gel HTP; Bio-Sil Amino 5 S). Preformed gels for PAGE (8–25% gradient) from Pharmacia/LKB were used. Zeta-Probe blotting membranes were obtained from Bio-Rad. High-M_r standards were from Sigma, and low-M_r standards were from Pharmacia/LKB. CS lyase AC (EC 4.2.2.5) and CS lyase ABC (EC 4.2.2.4) were purchased from Seikagaku, Tokyo, Japan. Sulphosuccinimidyl 6-(biotinamido)hexanoate [(NHS-LC)-biotin] was from Pierce. Sequencing reagents (Applied Biosystems) were of Sequenal grade, and the remaining reagents (Merck) were of analytical grade. Keratan sulphate peptide was kindly provided by Professor H. Stuhlsatz (University of Technology, Aachen, Germany).

Analytical methods

H.p.l.c. and high pressure gel-permeation chromatography (h.p.g.p.c.) were performed on a LC 31 A chromatography system from Bruker (Bremen, Germany). The radioactivity was measured using a LKB-Wallach scintillation counter (Typ 1217

Abbreviations used: GAG, glycosaminoglycan; PG, proteoglycan; CS, chondroitin sulphate; DS, dermatan sulphate; DMEM, Dulbecco's modified Eagle's medium; DMB, Dimethylmethylene Blue; h.p.g.p.c., high performance gel-permeation chromatography.

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Rack Beta) with Lumagel (Lumac, Basel, Switzerland) as scintillator.

Biosynthetic labelling

Two aorta specimens were obtained at autopsy within 12 h. The intima/media was dissected from the adventitia. Biosynthetic labelling was performed in organ culture using sulphate-depleted DMEM containing 0.1 mCi of $H_2{}^{35}SO_4$/ml for 8 h at 37 °C. Subsequently the specimens (approx. 25 g of tissue) were washed twice with sulphate-depleted DMEM. The following processing was carried out as described for the non-labelled specimens.

Processing of tissue and extraction

The intima/media layer was dissected from the adventitia and kept frozen at −78 °C until further processing. Tissue from non-labelled aortae and the material from two biosynthetically labelled aortae were combined (180 g wet wt.) and thawed in extraction buffer. The extraction buffer was a 0.05 M-acetate buffer containing 4 M-guanidinium chloride/0.1 M-6-aminohexanoic acid/0.05 M-benzamidine hydrochloride/5 mM-phenylmethanesulphonyl fluoride/0.05 M-EDTA/0.01 M-*N*-ethylmaleimide/0.5% (w/v) CHAPS, pH 5.8. Extraction and purification were performed at 4 °C. The tissue was passed through a mincer and treated with extraction buffer (5 ml/g wet wt.). After stirring of the mixture for 24 h, the supernatant was removed. Fresh extraction buffer (400 ml) was added and the extraction procedure was repeated twice. After centrifugation of the combined extracts (1.7 litres; 19000 *g*, 2 h), the supernatant was removed and processed further. The volume of the solution was reduced to 700 ml over a YM 5 membrane (Amicon) and the extraction medium was exchanged against 7 M-urea in 0.05 M-acetate buffer, pH 5.8, containing 0.05 M-benzamidine hydrochloride/2 mM-phenylmethanesulphonyl fluoride/0.03 M-EDTA/5 mM-*N*-ethylmaleimide/0.5% (w/v) CHAPS.

Purification of CS/DS PG

Sepharose Q ion-exchange chromatography. The extract (700 ml) was applied to a Sepharose Q anion-exchange column (4.6 cm × 20 cm) equilibrated with 7 M-urea in 0.05 M-acetate buffer, pH 5.2, containing 0.05 M-NaCl/0.05 M-LiCl/2 mM-benzamidine hydrochloride/1 mM-phenylmethanesulphonyl fluoride/1 mM-*N*-ethylmaleimide/0.2% (w/v) CHAPS. The column was washed with equilibration buffer using 1.5 times the column volume. The bound material was eluted by a gradient to 4 M-NaCl added to the equilibration buffer. Gradient elution was performed at a flow rate of 4 ml/min with two linear NaCl gradients [0.05–1.1 M (450 ml) and 1.1–4.0 M (450 ml)]. The gradient was formed by an h.p.l.c. pump system (Bruker). The sugar components in the eluted fractions were monitored by orcinol and carbazole reactions (Bitter & Muir, 1962; Feather & Harris, 1973), and by the scintillation counting of ^{35}S. Fractions eluted between 1.5 and 2.5 M-NaCl were pooled (150 ml), concentrated approx. 6-fold over a YM 5 membrane and immediately submitted to size-exclusion chromatography.

Sepharose CL-4B size-exclusion chromatography. A Sepharose CL-4B column (3.5 cm × 125 cm) was equilibrated with 7 M-urea in 0.05 M-acetate buffer, pH 5.8, containing 0.3 M-NaCl/2 mM-benzamidine hydrochloride/1 mM-phenylmethanesulphonyl fluoride/0.01 M-EDTA/0.2% (w/v) CHAPS. The sample (25 ml) was subjected to chromatography at a flow rate of 0.9 ml/min. Fractions eluting with a $K_{av.} > 0.11$ were pooled and concentrated 2-fold over a YM 5 membrane.

Hydroxyapatite chromatography. A hydroxyapatite column (4.6 cm × 12 cm) was equilibrated with 0.01 M-sodium phosphate buffer, pH 6.8. The fractions pooled after size-exclusion chromatography were applied to the column. The column was washed with equilibration buffer using 1.2 times the column volume. The bound material was eluted at a flow of 4 ml/min by increasing the phosphate concentration to 0.8 M (pH 7.2). Two linear gradients [0.01–0.48 M-phosphate (320 ml) and 0.48–0.8 M-phosphate (100 ml)] were formed by the h.p.l.c. system described above. Fractions that were eluted between 0.30 and 0.33 M-phosphate were pooled and immediately lyophilized.

MonoQ anion-exchange chromatography. A MonoQ anion-exchange column (Typ HR 10/10) was equilibrated with a 0.02 M-sodium phosphate buffer, pH 4.5, containing 0.1 M-NaCl/0.1 M-LiCl/0.05% (w/v) CHAPS. A 200 mg portion of the lyophilized sample was dissolved in 8 ml of water and applied to the column. After application, the column was run with 45 ml of equilibration buffer. The bound material was eluted at a flow rate of 3 ml/min using three linear NaCl gradients [0.1–1.2 M-NaCl (240 ml), 1.2–2.0 M-NaCl (80 ml) and 2.0–4.0 M-NaCl (40 ml)]. Fractions eluting between 0.9 and 1.3 M-NaCl were pooled, concentrated 4-fold over a YM 5 membrane and stored at −20 °C.

TSK G 4000 SW size-exclusion chromatography. A TSK G 4000 SW column (300 mm × 7.5 mm) with a TSK G SWP precolumn (75 mm × 7.5 mm) was equilibrated with a 0.01 M-sodium phosphate buffer containing 0.3 M-NaCl, pH 6.0. The sample (50 μl) was applied to the column and eluted at a flow rate of 0.6 ml/min. Absorbance was monitored at 210 and 280 nm. The fractions that were eluted between 11 and 14 min were used for further characterization of the purified PG. Fractions of several runs were combined and stored at −20 °C.

Characterization of purified CS/DS PG

PAGE and Western blots. For characterization and purity control of the preparation, two different electrophoretic techniques were applied. (i) The sample was submitted to SDS/PAGE under reducing conditions in minigels of an acrylamide gradient of 8–25% using the Phast System (Pharmacia). Buffers and electrophoretic conditions followed the instructions of the manufacturer. Silver staining of the gel used the protocol by Heukeshoven & Dernick (1988). Dimethylmethylene Blue (DMB) staining of the gel was performed after fixation of the gel with ethanol/acetic acid (9:1, v/v) for 15 min. After removal of the fixation solution staining was performed with DMB solution (Farndale *et al.*, 1986; DMB concentration was elevated to 0.5 mg/ml) for 15 min. Destaining of the gel was carried out with ethanol/acetic acid/water (9:1:10, by vol.) under visual control. (ii) Characterization of the biotin-labelled PG by electrophoretic separation on minigels and blotting on to a nylon membrane before and after enzymic digestion was performed as described (Stöcker *et al.*, 1989). Briefly, NHS-LC-biotin was used for labelling. For the specific degradation of the glycan chains, chondroitin sulphate lyases AC and ABC, heparinase/heparitinase and keratanase were used in the presence of proteinase inhibitors (Oike *et al.*, 1980; Kato *et al.*, 1985). Electrophoresis of enzyme-treated and non-treated samples was performed as described above. After transfer to nylon membranes, the biotin-labelled compounds were visualized by the use of avidin–peroxidase conjugate and H_2O_2/diaminobenzidine tetrahydrochloride as substrate.

Ion-exchange chromatography of disaccharides. A Bio-Sil Amino 5 S column (250 mm × 4.6 mm) was equilibrated with 0.1 M-sodium phosphate buffer, pH 3.9. For disaccharide analysis, digestion of the sample was performed with chondroitin sulphate lyases ABC and/or AC (0.1 mg of CS/DS PG/0.1 unit of enzyme) in 0.1 M-sodium phosphate buffer, pH 7.0, for 4 h at 37 °C. Equal amounts (about 0.04 mg) of the different digested samples were applied to the column and were eluted at a flow rate of 1 ml/min. The absorbance was monitored at 232 nm.

Amino acid and amino sugar analysis. For amino acid and hexosamine analysis, the sample was treated with 3 M-HCl at 105 °C for 15 h. Analysis was performed on a LKB-Alphaplus amino acid analyser.

Amino acid sequence analysis. Amino acid sequence analysis was performed by automated Edman degradation in a 470A protein sequencer (program: 03RPTH) connected on-line to a 120A phenylhydantoin analyser (sequencer and analyser from Applied Biosystems). The yield of amino acids in each cycle was calculated by adding the overlap of the following cycle and subtracting the background of the previous cycle.

Before sequencing, the dried protein sample was incubated with 50 μl of performic acid (freshly prepared using 95 parts of formic acid to 5 parts of H_2O_2) for 1 h at 0 °C. Subsequently the peroxidized protein was dried in a SpeedVac concentrator, dissolved in 50% (v/v) formic acid and applied to a polybrene treated glass-fibre disc for sequence analysis (Henschen, 1986).

RESULTS AND DISCUSSION

Purification of CS/DS PG

Intima/media preparations were prepared from human aorta specimens. Preparations from two specimens were labelled biosynthetically with ^{35}S and combined with the non-labelled preparations. Isolation and purification of PGs was performed as shown in Fig. 1. After extraction and centrifugation, the volume of the supernatant was reduced and the sample was applied to a Sepharose Q anion-exchange column. By the addition of LiCl to the elution buffer the resolution of the ion-exchange chromatography was improved (results not shown). The bound material was eluted by using two linear NaCl gradients. As shown in Fig. 2(*a*), three distinct ^{35}S peaks were obtained. Peaks detected by the orcinol reaction (Fig. 2*c*) were superimposable with the ^{35}S peaks. In comparison with the orcinol elution pattern, the carbazole reaction (Fig. 2*b*) showed a lower staining for the eluted compounds in peak 2. These findings are probably due to non-uronic-acid-containing sugar components (i.e. keratan sulphate or glycoproteins) in peak 2. The fractions eluted between 1.5 and 2.5 M-NaCl (peak 3) were pooled and processed further. As shown by the carbazole reaction, these fractions were rich in uronic acid. The pooled fractions were passed over a Sepharose CL-4B column and the included ^{35}S-positive material was eluted as a homogeneous peak (results not shown). The fractions corresponding to this peak ($K_{av.} > 0.11$) were pooled and submitted to hydroxyapatite chromatography. This purification step was chosen on the basis of previous findings which indicate that different types of glycosaminoglycans show different affinities for Ca^{2+}(Buddecke & Drzeniek, 1962). The elution profile of 0.01–0.8 M-phosphate gradient chromatography is demonstrated in Fig. 3. A peak which was eluted at maximum phosphate concentration was separated from two partially resolved peaks. The fractions indicated by the bar in Fig. 3 were pooled. As the next purification step anion-exchange chromatography (MonoQ) was used. Fractions corresponding to a baseline-separated peak which was eluted between 0.9 and 1.3 M-NaCl (Fig. 4, bar) were pooled. H.p.g.p.c. over TSK G 4000 SW was the final purification step (Fig. 5). Characterization and purity control were performed

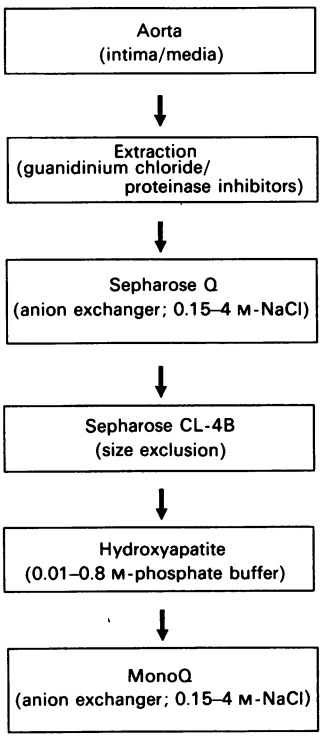

Fig. 1. Purification of PGs from human aorta

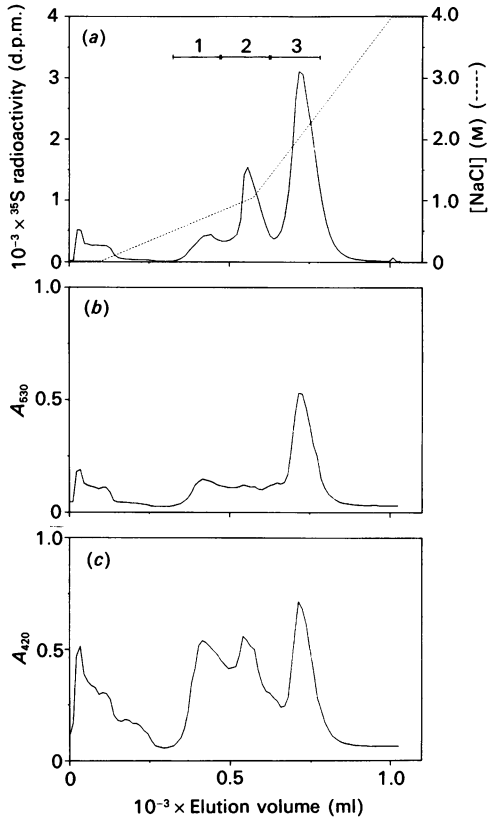

Fig. 2. Ion-exchange chromatography on Sepharose Q of intima/media extract from human aorta

Intima/media extract was exchanged in acetate buffer containing 7 M-urea/0.05 M-NaCl/0.05 M-LiCl/0.2% (w/v) CHAPS and proteinase inhibitors as described in the Experimental section. After application of the sample, the column was washed with 1.5 column vol. of equilibration buffer. Elution of the bound material was performed by using two linear NaCl gradients (0.05–1.1 M- and 1.1–4 M-NaCl). Fractions were analysed for sugar components by ^{35}S radioactivity (*a*), the carbazole reaction (*b*) and the orcinol reaction (*c*). Fractions that were eluted between 1.5 and 2.5 M-NaCl (see bar 3) were pooled, concentrated and submitted to Sepharose CL-4B size-exclusion chromatography.

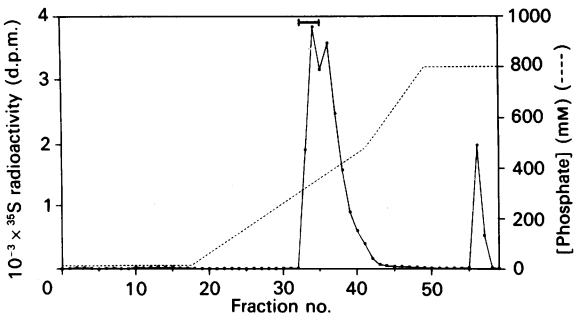

Fig. 3. Hydroxyapatite chromatography of fractions obtained after ion-exchange and gel-permeation chromatography

The sample obtained after gel-permeation chromatography was applied to a hydroxyapatite column (4.6 cm × 12 cm) and chromatographed as described in the Experimental section. Elution of the bound material was performed using two linear phosphate gradients [0.01–0.48 M-phosphate (320 ml) and 0.48–0.8 M-phosphate (100 ml)]. Fractions were analysed for ^{35}S radioactivity and those indicated by the bar were pooled.

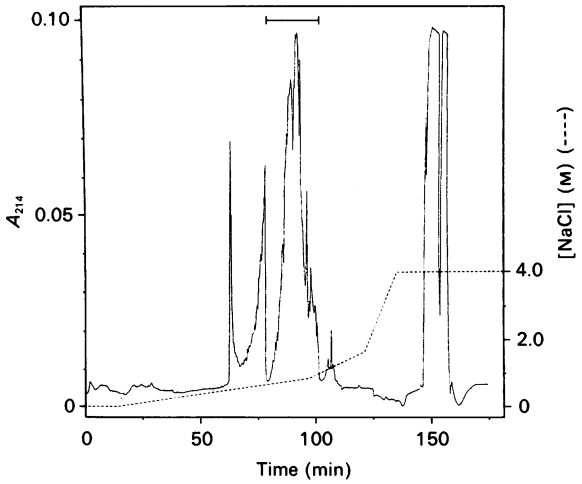

Fig. 4. Ion-exchange h.p.l.c. on MonoQ of PGs isolated by ion-exchange, gel-permeation and hydroxyapatite chromatography

A sample obtained after hydroxyapatite chromatography was applied to the column as described in the Experimental section. After eluting the column isocratically with 45 ml of equilibration buffer, three linear NaCl gradients [0.1–1.2 M-NaCl (240 ml), 1.2–2.0 M-NaCl (80 ml) and 2.0–4.0 M-NaCl (40 ml)] were applied. Absorbance was monitored at 214 nm. Fractions that were eluted between 0.9 and 1.3 M-NaCl (see bar) were pooled.

with the purified fractions that were eluted between 11 and 14 min. This material was also submitted to amino acid sequence analysis. Calculations on the basis of GalNAc content relative to total GalNAc-containing PGs isolated from the intima/media preparations indicate that the CS/DS PG described here constitutes at least 15% of the total amount of GalNAc-containing PGs.

Characterization of the PG

The fractions used for characterization corresponded to an included peak of $K_{av.}$ 0.12–0.31 obtained by size-exclusion chromatography on a TSK G 4000 SW column (Fig. 5). To check purity, approx. 1 μg of the sample was submitted to SDS/PAGE and was stained by a sensitive silver stain which

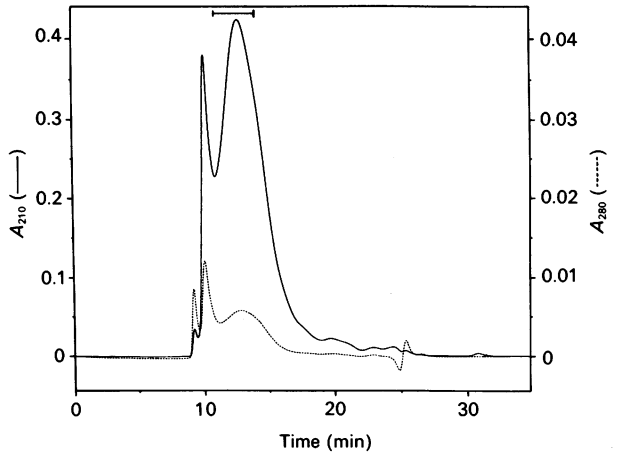

Fig. 5. Gel-permeation chromatography of the CS/DS PG on TSK G 4000 SW

The sample of purified CS/DS PG was submitted to size-exclusion chromatography as described in the Experimental section. Elution was monitored at 210 and 280 nm. Fractions that were eluted between 11 and 14 min were pooled and used for further characterization.

Table 1. Amino acid and hexosamine composition of a CS/DS PG isolated from human aorta

Protein content is calculated as the sum of amino acids.

Amino acid or amino sugar	Content (residues/1000 residues) or ratio
Asp	126
Thr	78
Ser	104
Glu	133
Pro	99
Gly	78
Ala	57
Val	51
Met	10
Ile	33
Leu	77
Tyr	22
Phe	37
His	25
Lys	50
Arg	20
HexN/protein	1.39
GlcNAc/protein	0.03
GalNAc/protein	1.36
GlcNAc/GalNAc	0.03

allowed the detection of down to 5 ng of standard proteins. In the PG preparation, a weak broad band in the high-M_r range was seen. No additional bands were present, indicating the absence of contaminating proteins (results not shown). The absence of contamination was further confirmed by electrophoretic separation and blotting of the biotin-labelled sample as described recently (sample 3a in Stöcker *et al.*, 1989).

The amino acid and amino sugar composition of the PG is shown in Table 1. The high relative content of GalNAc and the presence of GlcNAc in only trace amounts indicate that the GAG chains of the PG represent CS and/or DS.

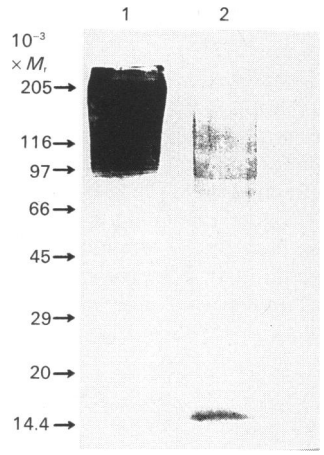

Fig. 6. Diffusion blot of native and enzymically digested biotinylated CS/DS PG from human aorta after SDS/PAGE under reducing conditions on 8–25% gradient gels

Lane 1, untreated CS/DS PG; lane 2, CS/DS PG after CS lyase ABC treatment. Detection of the compounds was performed by the use of an avidin–peroxidase conjugate and H_2O_2/diaminobenzidine tetrahydrochloride as substrate.

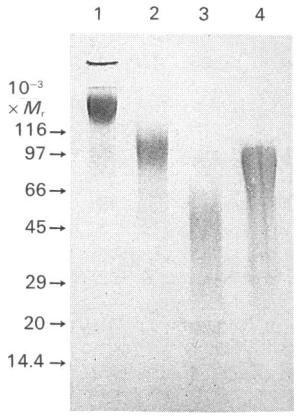

Fig. 7. SDS/PAGE on a 8–25% gradient gel of native and enzymically digested CS/DS PG

About 2 μg of native or digested PG per lane was applied to the gel. Electrophoresis was performed under reducing conditions. Staining of the gel with DMB was done as described in the Experimental section. Lane 1, native CS/DS PG; lane 2, CS/DS PG digested with trypsin; lane 3, CS/DS PG digested with papain; lane 4, keratan sulphate peptide (M_r 24700 according to compositional analysis; Stuhlsatz *et al.*, 1981).

The native biotin-labelled PG was treated by chondroitin sulphate lyase ABC, submitted to SDS/PAGE and blotted on to a nylon membrane (Stöcker *et al.*, 1989). As shown in Fig. 6, the band corresponding to the native sample (lane 1) disappeared after digestion. In the digested sample a sharp band of M_r 15000 corresponding to the core protein can be distinguished (lane 2). The residual staining in the higher-M_r range is probably a result of incomplete digestion.

The size of the glycan chains was estimated by proteolytic digestion of the native PG, SDS/PAGE and staining for GAGs by application of the DMB staining method (Farndale *et al.*, 1986) to the polyacrylamide gel (Fig. 7). The electrophoretic mobility of the samples was compared with that of a GAG peptide preparation which was characterized by compositional analysis (Stuhlsatz *et al.*, 1981). After trypsin treatment (lane 2),

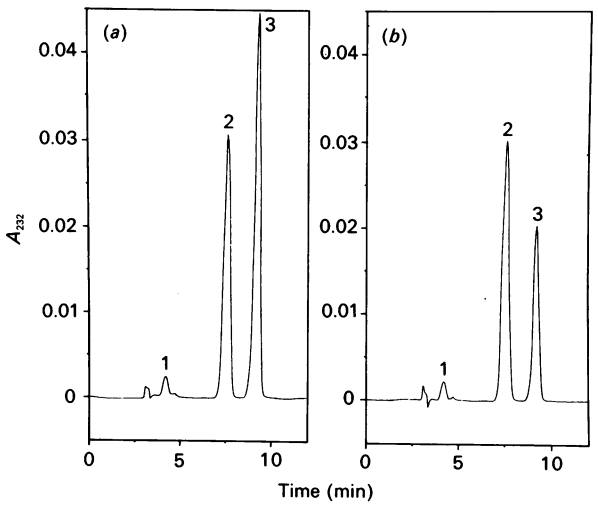

Fig. 8. Ion-exchange chromatography of the disaccharides of the purified CS/DS PG obtained after CS lyases ABC and/or AC treatment

Chromatography of the disaccharides was performed on a Bio-Sil Amino 5 S column (4.6 cm × 25 cm) as described in the Experimental section. The elution of the disaccharides was monitored at 232 nm; 1, Δ-di-0-sulphate; 2, Δ-di-6-sulphate; 3, Δ-di-4-sulphate. (*a*) CS/DS PG digested with CS lyases AC and ABC, (*b*) CS/DS PG digested with CS lyase AC only.

1 5 * 10 * 15 20
Asp-Glu-Glu-Ala-Ser-Gly-Ala-Asp-Thr-Ser-Gly-Val-Leu-Asp-Pro-Asp-Ser-Val-Thr-Pro

Fig. 9. *N*-Terminal amino acid sequence of a CS/DS PG isolated from human aorta

* The serine residues at positions 5 and 10 are glycosylated; at positions 5 and 10 this is probably a GAG chain.

an increased electrophoretic mobility was observed relative to the native PG (lane 1). Since the trypsin digest appears as a defined band, trypsin digestion seems not to be suited to release single GAG chains. The staining of the GAGs after papain digestion of the native sample (lane 3) indicates an electrophoretic mobility higher than that of the reference GAG preparation (lane 4). It is concluded that the M_r of the GAG chains is less than the value of 24000 determined for the reference preparation by compositional analysis (Stuhlsatz *et al.*, 1981).

The composition of the GAG chains was determined by CS lyase digestion and subsequent anion-exchange chromatography of the disaccharides. The relative amount of DS was calculated from the difference between the total digest obtained by CS lyases AC/ABC treatment and the digest obtained by CS lyase AC treatment alone. According to the results presented in Fig. 8, the GAG chains are composed of 32% iduronic acid (DS)-containing and 68% glucuronic acid (CS)-containing disaccharide units.

The purified CS/DS PG was submitted to *N*-terminal amino acid sequence analysis. During automated Edman degradation only a slight increase in the amounts of dithiothreitol-serine (15–20% of expected values) and no increase of phenylhydantoin-serine were observed in positions 5 and 10. Since cysteine may produce some dithiothreitol-serine during Edman degradation, the presence of cysteine in positions 5 and 10 was excluded by peroxidation of the sample before sequence analysis. Two additional runs using a peroxidized sample gave results which were identical with those obtained with the non-treated sample. If cysteine was present in positions 5 and 10, a blank

should have been obtained in these positions. Thus the presence of cysteine can be excluded.

Since carbohydrate residues bound to serine only partially undergo β-elimination during Edman degradation, the relatively low yield of serine in positions 5 and 10 is most probably due to glycosylation. If phosphoserine residues were present in these positions, a quantitative yield of dithiothreitol-serine would have been observed (Meyer *et al.*, 1986). In both potential glycosylation sites, glycine is present next to serine. Thus the sequence requirements for glycosylation sites of small CS/DS PGs (Asp/Glu-Xaa-Ser-Gly) (Ruoslahti, 1988) are met at these positions. In contrast, valine is next to a serine residue at position 17. No indications for modifications at that position were observed during Edman degradation.

The *N*-terminal amino acid sequence (Fig. 9) is identical with the sequence recently deduced for the small bone PG I designated 'biglycan' (Fisher *et al.*, 1989). In accordance with our results, the glycosylation sites were assigned to the serine residues in positions 5 and 10. A partial *N*-terminal amino acid sequence of PG I was obtained from a PG isolated from developing human bone (Fisher *et al.*, 1987). After CS lyase ABC treatment of PG I, a core of M_r 46000 was obtained (Fisher *et al.*, 1987). The size of the core is significantly higher than the 15000-M_r core determined in the present study, in which no additional bands could be observed in spite of the application of sensitive detection methods (Stöcker *et al.*, 1989). This difference may be due to alternative splicing of the mRNA. However, although each of the purification steps was performed in the presence of proteinase inhibitors, it cannot be formally excluded that the low M_r of the core is a result of proteolytic cleavage, since the CS/DS PG was isolated from autopsy specimens.

Our results indicate that the PG isolated from human aorta contains GAG chains which are composed of approx. one-third DS and two-thirds CS.

This is the first report that a biglycan-type PG with an *N*-terminal amino acid sequence and glycosylation positions identical to those described recently for human bone (Fisher *et al.*, 1989) is present in intima/media preparations of human aorta. The purification procedure included several chromatographic steps including hydroxyapatite chromatography for selective binding of PGs. The DS/CS PG described here is a major GalNAc-containing PG in intima/media preparations of human aorta.

With respect to the function of CS/DS PGs in tissues, strong interactions of CS and DS PGs with fibrillar collagen could have been demonstrated (Scott & Orford, 1981; Scott, 1988). Since PG-S2 (decorin) is bound to collagen fibrils at defined periodicity, it has been suggested that decorin may regulate the thickness of collagen fibrils (Ruoslahti, 1988). In contrast, no *in vitro* formation of collagen type I and II fibrils could be observed for PG-S1 from bovine aorta/intima (Hedbom & Heinegård, 1989). However, the amount of CS/DS GAGs has been shown to increase significantly in atherosclerotic lesions as well as in aging (Ylä-Herttula *et al.*, 1986). Furthermore, an increase in type V collagen has been demonstrated in proliferative fibrotic processes in human aorta intima and media (Murata *et al.*, 1987; Murata & Motoyama, 1990). Although the function of the aorta CS/DS PG is not yet known, one should consider possible interactions of CS/DS PG with minor collagen types, especially those which may be correlated with changes in the tissue architecture. If this assumption is correct, age-related changes in the architecture of aortic tissue, e.g. loss of elasticity, should be accompanied by qualitative and/or quantitative differences in the distribution of CS/DS PG. Future studies will show if this assumption is correct.

This study was supported by a grant from the Fritz Thyssen Stiftung and by grant Wa 473/3-2 from the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

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Received 16 March 1990/12 October 1990; accepted 25 October 1990