Isolation and characterization of dermatan sulphate proteoglycans from bovine sclera

Lars CÖSTER and Lars-Åke FRANSSON
Department of Physiological Chemistry 2, University of Lund, S-220 07 Lund, Sweden

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1. Proteoglycans were extracted from sclera with 4M-guanidine hydrochloride in the presence of proteinase inhibitors and purified by ion-exchange chromatography and density-gradient centrifugation. 2. The entire proteoglycan pool was characterized by compositional analyses and by specific chemical (periodate oxidation) and enzymic (chondroitinases) degradations. The glycan moieties of the molecules were exclusively galactosaminoglycans (dermatan sulphate—chondroitin sulphate co-polymers). In addition, the preparations contained small amounts of oligosaccharides. 3. The scleral proteodermatan sulphates were fractionated into one larger (I) and one smaller (II) component by gel chromatography. Proteoglycan I was eluted in a more excluded position on gel chromatography in 0.5M-sodium acetate than in 4.0M-guanidine hydrochloride. Reduced and alkylated proteoglycan I was eluted in the same position (in 0.5M-sodium acetate) as was the starting material (in 4.0M-guanidine hydrochloride). The elution position of proteoglycan II was the same in both solvents. Proteoglycans I and II had $s_{20,w}^2$ values of $2.8 \times 10^{-13}$ and $2.2 \times 10^{-13}$'s respectively in 6.0M-guanidine hydrochloride. 4. The two proteoglycans differed with respect to the nature of the protein core and the co-polymeric structure of their side chains. Also proteoglycan I contained more side chains than did proteoglycan II. The dermatan sulphate side chains of proteoglycan I were D-glucuronic acid-rich (80%), whereas those of proteoglycan II contained equal amounts of D-glucuronic acid and L-iduronic acid. Furthermore, the co-polymeric features of the side chains of proteoglycans I and II were different. The protein core of proteoglycan I was of larger size than that of proteoglycan II. The latter had an apparent molecular weight of 46000 (estimated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis), whereas the former was $>100000$. In addition, the amino-acid composition of the two core preparations was different. 5. As proteoglycan I altered its elution position on gel chromatography in 4M-guanidine hydrochloride compared with 0.5M-sodium acetate it is proposed that a change in conformation or a disaggregation took place. If the latter hypothesis is favoured, aggregation may be due to self-association or mediated by an extrinsic molecule, e.g. hyaluronic acid.

Fibrous connective tissues contain an abundance of collagen fibres and, in the interfibrillar matrix, characteristic macromolecular glycoconjugates, which include dermatan sulphate proteoglycans. These molecules are difficult to extract quantitatively in an undegraded state. By extraction with urea at elevated temperatures Toole & Lowther (1965, 1968) succeeded in isolating proteodermatan sulphates from heart valves, skin and tendon. By a similar approach Öbrink (1972) prepared a proteoglycan from pig skin. Proteoglycans have also been extracted from tendon, arterial wall and sclera using 4M-guanidine (Anderson, 1975; Antonopoulos et al., 1974; Ehrlich et al., 1975; Eisenstein et al., 1975; Radhakrishnamurthy et al., 1977). More recently, Damle et al. (1979) separated pig skin proteoglycan into one proteochondroitin sulphate and one proteodermatan sulphate. These studies have demonstrated that dermatan sulphate is covalently linked to a non-collagenous protein.

By the introduction of efficient procedures for the extraction of fibrous connective tissues (Antonopoulos et al., 1974) and the utilization of proteinase inhibitors (Oegema et al., 1975) it became possible to isolate proteoglycans quantitatively from such tissues in a more native state. The present study is concerned with the extraction, purification and characterization of proteoglycans from bovine...
sclera. Preliminary reports have been published (Cöster & Fransson, 1975, 1976).

**Experimental**

**Materials**

Eyes from cows were obtained from the local slaughterhouse and the sclerae were excised at 4°C within 6 h and immediately frozen in liquid N₂.

Sepharose and Sephadex gels, as well as a protein calibration kit, were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. ECTEOLA-cellulose was purchased from Serva, Heidelberg, Germany. Dialysis tubing (nominal molecular-weight cut-off 12000–14000) was a product of Spectrum Medical Inc., Los Angeles, CA, U.S.A. Chondroitinase-AC and chondroitinase-ABC (EC 4.2.2.5) were obtained from Miles Laboratories, Elkhart, IN, U.S.A. Papain (twice-crystallized, 16–40 BAEE units/mg) and guanidine hydrochloride (practical grade) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Stock solutions of guanidine hydrochloride (7 M) were treated with activated charcoal under stirring overnight. After filtration through filter paper the A₂₈₀ of the clarified solution was 0.05 (with water as blank). Stock solutions of urea (8 M) were passed through a mixed anion-cation exchange resin to remove traces of cyanate immediately before use. Dithiothreitol (‘for biochemistry’) was bought from Merck, Darmstadt, Germany. All other chemicals were analytical grade.

**Analytical methods**

Hexosamine was quantified by a modified Elson–Morgan procedure (Antonopoulos et al., 1964). Hexose was estimated by the method of Goa (1955). Hexuronic acid was determined by the carba-zole-borate method (Bitter & Muir, 1962) and protein by the method of Lowry et al. (1951). Automated versions of these methods were also employed (Heinegård, 1973).

Amino acids, glucosamine and galactosamine were estimated by using a Durrum automatic amino-acid analyser. Hydrolysis was performed (under argon) in 6 M-HCl at 110°C for 24 h (for amino acids) or in 8 M-HCl at 95°C for 3 h (for hexosamines). Norleucine was used as the internal standard. The amounts of cysteine and cystine were determined as cysteic acid (on the amino-acid analyser) after oxidation with performic acid (Moore, 1963). Hydroxyproline was quantified by the method of Stegemann & Stalder (1967).

Neutral sugars were determined by g.l.c. of their alditol acetates after hydrolysis in 2 M-trifluoro-acetic acid at 100°C for 4 h under N₂ (Axelsson & Heinegård, 1975).

**Ultracentrifugation**

Sedimentation velocity measurements were performed in an MSE Centriscan 75 ultracentrifuge by using the Schlieren detection system. Serial dilutions of stock solutions were each dialyzed with stirring against the buffer to achieve Donnan equilibrium. Sedimentation coefficients were determined as described by Schachman (1957). The S₂₀,w values were obtained by extrapolation to infinite dilution.

**Degradations and modifications**

The glycan side chains of proteoglycans were released by alkaline elimination of the xylose–serine linkage or by exhaustive proteolytic digestion of the protein core. Alkaline cleavage was performed in 0.1 M-LiOH (2 mg of proteoglycan/mg) at 4°C for 72 h. The reaction was terminated by neutralization with 0.5 M-acetic acid. Proteolysis was carried out with papain (0.1 mg/10 mg of substrate in 1 ml of 1.0 M-NaCl/0.05 M-EDTA (disodium salt)/0.01 M-cysteine hydrochloride/0.05 M-sodium phosphate buffer, pH 7.0] at 65°C for 24 h.

Periodate oxidation-alkaline elimination (selective cleavage of L-iduronic acid residues) at pH 3.0 and 4°C was used to degrade the liberated dermatan sulphate side chains as described previously (Fransson, 1974; Fransson & Carlstedt, 1974; Fransson & Cöster, 1979).

Cleavage of hexosaminidic bonds to D-glucuronic acid or to all hexuronic acids in free chains or proteoglycans was accomplished by chondroitinase-AC or chondroitinase-ABC digestions. Samples (2 mg/ml) were dissolved in 0.1 M-Tris acetate, pH 7.3, and treated with enzyme at 37°C for 4 h. The amounts of enzyme used were 0.1 unit/mg (chondroitinase-AC) and 0.01 unit/mg (chondroitinase-ABC) respectively.

Ratios between L-iduronic acid and D-glucuronic acid were obtained by measuring the amount of unsaturated glycuronosyl residues formed after chondroitinase digestions by the periodate-thiobarbiturate assay of Hascall et al. (1972).

Reduction and alkylation of proteoglycans (10 mg/ml) was performed in 4 M-guanidine hydrochloride/0.005 M-dithiothreitol/0.05 M-Tris/HCl, pH 8.5. After incubation at 37°C for 5 h iodoacetamide was added to a final concentration of 0.015 M. The solution was kept in the dark at 4°C overnight. The sample was then dialysed against several changes of 0.5 M-sodium acetate, pH 7.0.

**Chromatography**

Gel chromatography of proteoglycans, polysaccharides and oligosaccharides was performed on Sepharose CL4B, Sephadex G-200 or G-50 in 0.5 M-sodium acetate, pH 7.0, or 4 M-guanidine
hydrochloride/0.05 M-Tris/HCl, pH 7.0. Samples (3–5 mg/0.5 ml) were dissolved in the elution buffer and centrifuged to remove, if present, small amounts of insoluble material before chromatography. For details see the legends to the appropriate Figures.

Separation of glycopeptides (oligosaccharide-peptides) and peptidoglycans (glycosaminoglycan-peptides) was accomplished by chromatography on ECTEOLA-cellulose of papain digests of the proteoglycans (Anseth, 1961).

Polyacrylamide-gel electrophoresis was conducted on 5 or 8% (w/v) gels in 0.1% sodium dodecyl sulphate by the procedure of Neville (1971). The gels were stained with 0.25% Kenacide R and scanned with a Zeineh soft-laser densitometer.

**Isolation and fractionation of proteoglycans**

Sclera was powdered under liquid N₂ and extracted with 15 vol. of 4 M-guanidine hydrochloride/0.05 M-sodium acetate, pH 5.8, containing proteinase inhibitors (0.10 M-6-aminocaproic acid/0.01 M-disodium EDTA/0.005 M-benzamidine hydrochloride) at 4°C under gentle shaking for 15 h. The suspension was centrifuged at 18,000 g for 45 min and the pellet was re-extracted once. The extract was concentrated by ultrafiltration in an Amicon cell with a PM 10 filter, dialysed against 7 M-urea/0.05 M-Tris/HCl, pH 6.5, with inhibitors, and passed through a DEAE-cellulose column (Antonopoulos et al., 1974) equilibrated with the same buffer. The column was eluted with (a) the starting buffer, (b) 0.15 M-NaCl in the same buffer and (c) 2 M-NaCl in the same buffer. The 2 M-NaCl fraction yielded a crude proteoglycan preparation free of collagen. Further purification was achieved by density-gradient centrifugation.

**Density-gradient centrifugation**

A 4 M-guanidine hydrochloride/0.05 M-sodium acetate, pH 5.8/CsCl solution containing proteinase inhibitors with a starting density of 1.30 g/ml was prepared and the crude proteoglycan preparation (5 mg/ml) was dissolved in this solution. Centrifugation was performed in an MSE Superspeed 65 centrifuge fitted with an 8 × 25 ml angle rotor at 10°C and 34,000 rev./min for 48 h. Tubes were emptied (2 ml fractions) with the aid of an MSE tube piercer. The densities of the fractions were determined with a 200 μl pipette as pycnometer. Uronic acid was estimated directly on the fractions and protein was estimated by measuring the A₂₈₀ after diluting 10-fold with water. Material was recovered by dialysis against several changes of water followed by freeze-drying. Final purification and fractionation of the proteoglycan was performed by gel chromatography on a column (16 mm × 1300 mm) of Sepharose CL 4B, which was eluted with 0.5 M-sodium acetate, pH 7.0, or 4 M-guanidine hydrochloride/0.05 M-Tris/HCl, pH 7.0, at a rate of 13 ml/h; fraction size, 3.5 ml.

**Preparation of the protein core**

Isolation of the protein core of the proteoglycans was accomplished by digestion with chondroitinase-ABC followed by gel chromatography (see above). Keiser & Hatcher (1977) claim that commercial chondroitinase-ABC preparations contain small amounts of proteinases. Therefore, chondroitinase-ABC digests of proteoglycans were carried out on a time scale of 3, 6, 12, 24 and 48 h. In addition, digests were performed in the presence of 2 mM-di-isopropyl fluorophosphate.

**Results**

**Isolation and characterization of scleral proteoglycans**

Analyses of tissue extracts and residues by ECTEOLA-cellulose chromatography of papain digests (Anseth, 1961) indicated that at least 85% of the tissue glycosaminoglycans were extracted by 4 M-guanidine hydrochloride. The crude proteoglycan preparation (2 M-NaCl fraction from DEAE-cellulose chromatography) was obtained in a yield of approx. 80%. This fraction was further purified by density-gradient centrifugation in 4 M-guanidine hydrochloride/CsCl. Fractions 1−5 from the bottom of the tube (densities ≥1.30 g/ml) corresponded to 90% of the hexuronic acid content of the material subjected to centrifugation. The purified proteoglycan contained 16% hexuronic acid and 50% protein. No hydroxyproline was detected. The overall yield after these steps was approx. 60%.

The purified proteoglycan was characterized by gel chromatography on Sepharose 2B and 4B (Figs. 1a and 1b). Two proteoglycan components were observed, the larger of which was included on Sepharose 2B (Fig. 1a) but partially excluded from Sepharose 4B (Fig. 1b). To demonstrate the proteoglycan nature of these components, chromatography was performed after release of the glycan side chains by alkali (Fig. 1c) or by papain digestion (Fig. 1d). The polysaccharides were eluted in a more retarded position than the proteoglycans. The results suggest that the smallest proteoglycans (Vₑ = 40−55 ml in Fig. 1b) contained very few glycan side chains.

The carbohydrate portion of the proteoglycan consisted of 87% glycosaminoglycan and 13% small neutral oligosaccharides (ECTEOLA-cellulose chromatography of papain digests and expressed as hexosamine). In the glycosaminoglycan portion the hexosamines were exclusively galactosamine. Thus no heparan sulphate could be present. To ascertain the uronic acid composition the galactosamino-
The proteoglycan was purified by DEAE-cellulose chromatography and density-gradient centrifugation (bottom fractions 1–5). Alkaline elimination and papain digestion were performed as described in the Experimental section. The column (9 mm × 1300 mm) contained Sepharose 2B (a) or 4B (b–d), with 0.5 M-sodium acetate, pH 7.0, as eluent at a rate of 4 ml/h. Solid lines, Uronic acid (carbazole); ———, protein (Folin). A minor portion of the alkali-treated material (c) had the elution volume of proteoglycans, although the protein content of this material was very low. We have no adequate explanation for this result.

Fig. 1. Gel chromatography of purified proteoglycan (a and b), alkali-treated proteoglycan (c) and papain-treated proteoglycan (d)

The proteoglycan side chains were treated with periodate at pH 3.0 and 4°C (selective oxidation of L-iduronic acid) followed by scission in alkali (Fig. 2). As most of the material was eluted in a more retarded position compared with the untreated chains it may be concluded that all side chains contained L-iduronic acid and the original proteoglycan may be classified as a dermatan sulphate proteoglycan. This was confirmed by chondroitinase-ABC digestion of the side chains. They were completely degraded to disaccharides (see below).

Subfractionation of the proteoglycan

As indicated above (Fig. 1) the purified scleral proteoglycan was heterogeneous in size and two subpopulations could be discerned. On chromatography on Sepharose CL4B in 0.5 M-sodium acetate (Fig. 3a) the larger proteoglycan subpopulation was almost entirely excluded (proteoglycan I). However, on gel chromatography in 4 M-guanidine hydrochloride (Fig. 3b) proteoglycan I was partially included in the gel, whereas the position of proteoglycan II was unchanged. The two proteoglycan subfractions were isolated by preparative gel chromatography (see the Experimental section) either in 0.5 M-sodium acetate (Fig. 3a) or in 4 M-guanidine hydrochloride (Fig. 3b). In both cases the yields were 40 and 60% respectively, on a w/w basis.

Fig. 2. Gel chromatography on Sephadex G-200 of papain-digested proteoglycans before (a) and after (b) periodate oxidation-alkaline elimination

The purified proteoglycan was digested with papain (see the Experimental section) and chromatographed (a). The glycosaminoglycan-peptides were subsequently oxidized with periodate (L-iduronic acid residues), cleaved in alkali and chromatographed (b). Column size, 9 mm × 1400 mm; eluent, 0.5 M-sodium acetate, pH 5.0; rate, 5 ml/h; analysis, uronic acid.
Scleral proteodermatan sulphates

chemical composition was also the same whether the separation was carried out in acetate or guanidine (see below). Rechromatography of proteoglycan I isolated as in Fig. 3(a) on the same column eluted with 4 M-guanidine hydrochloride produced the profile of a partially included proteoglycan (results not shown). Conversely, rechromatography of proteoglycan I isolated as shown in Fig. 3(b) on the same column but in acetate resulted in exclusion of the material (as shown in Fig. 3a). Proteoglycan subfraction II was eluted in the same position in both solvents. After reduction and alkylation of the proteoglycans, fraction I was partially included in the gel (Fig. 3c) when chromatographed in 0.5 M-sodium acetate. The profile was not altered by chromatography in 4 M-guanidine hydrochloride (results not shown). The elution position of proteoglycan II was unaffected by reduction and alkylation.

Ultracentrifugation of the proteoglycans

Solutions of the two proteoglycan subfractions in 6 M-guanidine hydrochloride were studied by ultracentrifugation in the sedimentation-velocity mode. Both proteoglycans I and II showed the pattern of a polydisperse material, but neither were resolved into more components. The sedimentation coefficients obtained (2.8 x 10^{-13} and 2.2 x 10^{-13} s) suggest that both proteoglycans are small.

Chemical characterization of the proteoglycan species

Both the glycan side chains and the protein core of the two proteoglycan species were subjected to compositional analyses. As shown in Table 1 proteoglycan I had a higher glycan/protein ratio than did proteoglycan II. There were no significant differences in chemical composition between the preparations obtained under associative or dissociative conditions (Figs. 3a and 3b). Proteoglycan I contained a slightly smaller proportion of neutral carbazole reactivity of fraction II was higher in the manual procedure (b) than in the automated procedure (a). Although chondroitin sulphate gives the same colour yield in both procedures (Heinegård, 1973), dermatan sulphates with L-iduronic acid content should give lower colour yield in the automated procedure as the boiling time is shorter (7-8 min versus 20 min). As shown in Table 1 proteoglycan II contains two to three times more L-iduronic acid than proteoglycan I. Fractions (I and II in a and b) were pooled as indicated by the vertical lines and material was recovered by freeze-drying and weighed. The weight ratio of the proteoglycan subfractions was approx. 2:3, irrespective of whether the separation was performed in acetate (a) or guanidine (b).

Fig. 3. Gel chromatography on Sepharose CL 4B of purified proteoglycans in (a) 0.5 M-sodium acetate, pH 7.0, (b) 4 M-guanidine hydrochloride/0.05 M-Tris/HCl, pH 7.0 and (c) reduced and alkylated proteoglycan in 0.5 M-sodium acetate, pH 7.0

Reduction and alkylation was performed as described in the Experimental section. Samples were dissolved in 4 M-guanidine hydrochloride and dialysed against the elution buffer before chromatography. For column size and technical details, see the legend to Fig. 1. ——, Uronic acid (automated carbazole in a and c, manual carbazole in b); ———, protein (Folin in a and c, A_{440} in b). The
oligosaccharide side chains than did proteoglycan II (Tables 1 and 2). Both oligosaccharide-peptides contained large amounts of D-mannose and D-glucosamine. The composition of neutral sugars was quite similar. However, oligosaccharide I had a significantly higher D-galactosamine content than did oligosaccharide II.

The glycan side chains of the individual proteoglycans were chromatographed on Sephadex G-200 as in Fig. 2a (results not shown). Both preparations showed a similar degree of polydispersity. The dermatan sulphate chains of proteoglycan I were D-glucuronic acid-rich, whereas in proteoglycan II the side chains contained almost equal proportions of the two uronic acids (Table 1).

Selective periodate oxidation of L-iduronic acid residues in the glycan side chains followed by scission in alkali and gel chromatography afforded the elution profile shown in Fig. 4. In accordance with the high content of D-glucuronic acid, chains from proteoglycan I were moderately degraded (Fig. 4a). The degradation products that were excluded from the gel (fraction 1) were further degraded with chondroitinase AC (Fig. 4b). The vast majority of the material was degraded to disaccharides. The periodate-degradation products that were partially included in the gel (fraction 2 in Fig. 4a) yielded both disaccharides and oligosaccharides on chondroitinase-AC digestion (Fig. 4c). These oligosaccharides represent sequences where the L-iduronic
Scleral proteodermatan sulphates

Fig. 4. Gel chromatography on Sephadex G-50 of degradation products derived from the dermatan sulphate side chains of (a–c) proteoglycan I and (d–f) proteoglycan II

Proteoglycan subfractions I and II (Fig. 3a) were digested with papain (see the Experimental section) and the liberated side chains (isolated by ECTEOLA-cellulose chromatography) were subsequently degraded by periodate oxidation-alkaline elimination (a and d). The excluded and the partially included degradation products (fractions 1 and 2 respectively) were separately digested with chondroitinase-AC and re-chromatographed on the same column (the results for fraction 1 are shown in b and e and those for fraction 2 in c and f). Column size 8 mm × 1500 mm; eluent, 0.2 M-pyridine/acetate, pH 5.0; rate, 6 ml/h; analysis, carbazole.

Acid residues are O-sulphated (Cöster et al., 1975). Thus the side chains of proteoglycan I are characterized by the presence of large block regions composed of D-glucuronic acid-containing repeats. Periodate oxidation-alkaline degradation of the side chains of proteoglycan II produced largely oligosaccharide fragments (Fig. 4d). The fragments eluted with the void volume (fraction 1) contained an appreciable quantity of chondroitinase-AC-resistant material (Fig. 4e). The main oligosaccharide fraction obtained after periodate oxidation (fraction 2 in Fig. 4d) was further degraded by chondroitinase-AC (Fig. 4f) into disaccharides and higher oligosaccharides. The latter saccharides contain O-sulphated L-iduronic acid residues. A comparison between the two sets of results indicates that segments containing L-iduronic acid O-sulphate were more frequent in the dermatan sulphate side chains of proteoglycan II than in those of proteoglycan I. In the present scheme of degradation regions composed of alternating short segments of L-iduronic acid- and D-glucuronic acid-containing repeats
would yield short oligosaccharides of the general carbohydrate structure \(N\)-acetylgalactosamine-(D-glucuronic acid-N-acetylgalactosamine)\(_R\), where \(R\) is the remnant of an oxidized and degraded l-iduronic acid residue. Such oligosaccharides should be present in fraction 2 in Figs. 4(a) and 4(d). Although the side chains of proteoglycan II afforded large amounts of saccharides containing periodate-resistant uronic acids (Fig. 4d) a considerable proportion of these saccharides contained both l-iduronic acid \(O\)-sulphate and \(\alpha\)-glucuronic acid (Fig. 4f). The corresponding saccharide fraction from proteoglycan I (fraction 2 in Fig. 4a) contained largely \(\alpha\)-glucuronic acid residues (Fig. 4c).

The amino-acid compositions of the parent scleral proteoglycan, the subfractions I and II and proteoglycans prepared from skin, cornea and cartilage by other workers are given in Table 3. In proteoglycan I the serine, threonine, glutamate, proline, alanine and glycine contents were higher, whereas the aspartate, isoleucine, leucine and lysine contents were lower than in proteoglycan II; methionine was only found in the latter species, whereas cysteine was present in both.

The protein cores of the two subpopulations were prepared by chondroitinase-ABC digestion followed by preparative gel chromatography. As shown in Fig. 5 the protein core of proteoglycan I (Fig. 5a) was of a larger size than that of proteoglycan II (Fig. 5b). On polyacrylamide-gel electrophoresis intact proteoglycan II had a polydisperse appearance with an apparent average molecular weight of 85000 (results not shown). After chondroitinase-ABC digestion one distinct band was observed with an apparent molecular weight of 46000. The results were the same whether the chondroitinase-ABC digestion was carried out for 3h or 48h or if di-isopropyl fluorophosphate was present in the digestion mixture. Thus our preparation of chondroitinase-ABC did not seem to contain any proteinases active on the core protein. Identification of the core protein of proteoglycan I after gel electrophoresis was difficult owing to low affinity for the staining reagent. When large amounts of material were applied, core preparation I did not seem to penetrate into a 5% gel, suggesting a molecular weight \(\geq 100000\).

**Discussion**

Dermatan sulphate-containing proteoglycans have been isolated from heart valves, skin, tendon, sclera and arterial wall. The proteoglycans of the arterial wall are generally of higher buoyant density and higher carbohydrate content than those of the other tissues. Furthermore, the arterial wall (R. Kapoor, C. F. Phelps, L. Cöster & L.-Á. Fransson, unpublished work) and skin (Damle et al., 1979)

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**Table 3. Amino-acid composition of scleral proteoglycans**

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* Results from Öbrink (1972).
† Results from Damle et al. (1979).
‡ Results from Axelsson & Heinegård (1975).
§ Results from Heinegård (1972).
contain separate proteochondroitin sulphate and proteodermatan sulphate species. The proteoglycans of sclera, heart valves, tendon and skin resemble each other with respect to protein content and amino-acid composition. Attempts to determine the molecular weight of proteodermatan sulphates have yielded values ranging from approx. 10^6 (Toole & Lowther, 1968; Anderson, 1975; Damle et al., 1979) to 3 x 10^6 (Öbrink, 1972).

In the present investigation scleral proteoglycans (all of which are proteodermatan sulphates) were separated into two populations, one larger in size (I; \( s_{20, w}^2 = 2.8 \times 10^{-13} \)) and one smaller (II; \( s_{20, w}^2 = 2.2 \times 10^{-13} \)). The yield of the two components may vary if proteinase inhibitors are omitted from the tissue extractant and in some cases even free glycan chains may be obtained. However, in the presence of proteinase inhibitors the results were highly reproducible. Scleral proteoglycans are comparable with corneal proteoglycans in size (1.7S; \( M_w = 72000 \); Axelsson & Heinegård, 1978) whereas cartilage proteoglycans are considerably larger (25S; \( M_w = 2.5 \times 10^6 \); Hascall & Sajdera, 1970). The 2.1S scleral proteoglycan (II) contained 60% protein and the apparent molecular weight of the core protein was 46000. Thus a molecular weight of 80000 may be calculated for the intact proteoglycan II. This is to be compared with a value of 85000 obtained for the intact material by gel electrophoresis. It was not possible to obtain a molecular-weight estimate for the 2.8S proteoglycan (I) in gel electrophoresis. Results of gel chromatography and ultracentrifugation of proteoglycan I show that it is larger than proteoglycan II, but still considerably smaller than cartilage proteoglycan (cf. S values above; see also Figs. 1 and 5).

Both proteoglycans from sclera seemed to carry two types of carbohydrate side groups, i.e. mannosamine-rich and dermatan sulphate rich. Results of the study are indicated in the respective panels. The anthrone-positive components of proteoglycans I and II were quantitatively recovered in the respective core fractions.
The two proteodermatan sulphates described in
the present report contained markedly different
protein cores, as indicated by distinctly different
amino-acid composition and molecular size (Fig. 5).
Proteoglycan I, which carried more side chains than
did proteoglycan II (60 and 45% carbohydrate
respectively) had a higher serine content. As shown
previously dermalan sulphate chains are linked to
protein via the sequence D-glucuronic acid-D-galac-
tose-D-galactose-D-xylose-serine (Fransson, 1968;
Stern et al., 1971). In general, proteoglycan I, which
had more chondroitin sulphate-like side chains, had
an amino-acid composition similar to the chon-
droitin sulphate-rich proteoglycans from skin
(Damle et al., 1979) and cartilage (Heinegård,
1972). In contrast, proteoglycan II, which had a
higher protein content than did proteoglycan I, was
more akin to the protein-rich corneal proteokeratan
sulphates described by Axelsson & Heinegård
(1975).

As proteoglycan I altered its elution position on
gel chromatography in 4 M-guanidine hydrochloride
compared with 0.5 M-sodium acetate it is proposed
that a change in conformation or a disappearance
took place. We favour the latter hypothesis, as
proteoglycan I appears smaller in 4.0 M-guanidine
and after reduction and alkylation. A conforma-
tional change induced by guanidine or by the
cleavage of disulphide bonds might rather expand
the molecule. Proteoglycan II showed no aggrega-
tion phenomenon under the present conditions. The
aggregation seen with proteoglycan I must be due to
self-association or mediated by an extrinsic mole-
cule. Self association may be mediated via the side
chains and/or protein core in three different ways,
I.e. carbohydrate-carbohydrate, carbohydrate-pro-
tein and protein-protein. One extrinsic molecule
known to multimerize proteoglycans is hyaluronate
(see Heinegård & Hascall, 1974). The finding that
reduction and alkylation abolished aggregation
might suggest that the protein core was involved.
The same treatment of cartilage proteoglycan
abolishes the interaction with hyaluronate (Harding-
ham & Muir, 1974; Heinegård & Hascall, 1974).
The scleral proteoglycan was of rather low buoyant
density. Therefore it cannot be excluded that
endogenous hyaluronic acid was present in the
purified material. As the proteoglycans were puri-
field by ion-exchange chromatography and den-
sity-gradient centrifugation in a dissociative medium
the presence of an aggregating protein seems rather
unlikely.

Self association of proteoglycan I could be medi-
ated by the dermalan sulphate side chains. Free
dermatan sulphate chains from various sources
self-associate (Fransson, 1976). These species of
dermatan sulphate possess alternating or mixed
sequences of L-iduronic acid- and D-glucuronic
acid-containing repeats. Non-aggregating chains
lack this feature and contain considerable amounts
of O-sulphated L-iduronic acid residues (Fransson &
Cöster, 1979). At present one cannot definitely
distinguish between the various possibilities for
multimerization of proteodermatan sulphate. Any
explanation, however, must take into account the
distinct chemical differences between scleral proteo-
dermatan sulphates I and II, both with regard to the
nature of the protein core and the co-polymeryl
features of their side chains.

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