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Isolation and some structural analyses of a proteodermatan sulphate from calf skin

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A proteodermatan sulphate was isolated from 0.15 M-NaCl and 0.45 M-NaCl extracts of newborn-calf skin. The proteoglycan was separated from collagen and hyaluronic acid by precipitation with cetylpyridinium chloride and CsCl-density-gradient centrifugation. Further purification was performed by ion-exchange, affinity and molecular-sieve chromatography. The proteoglycan bound to concanavalin A–Sepharose in 1 M-NaCl. It gave a positive reaction with periodic acid/Schiff reagent and contained 8.3% of uronic acid. The dermatan sulphate, the only glycosaminoglycan component, was composed of 74% iduronosylhexosamine units and 26% glucuronosylhexosamine units. The M_r was assessed to be 15000–20000 by gel chromatography. The core protein was found to be a sialoglycoprotein that had *O*-glycosidic oligosaccharides with *N*-acetylgalactosamine at the reducing termini. The molar ratio of oligosaccharide chains to dermatan sulphate was approx. 3:1. From these results the proposed structure of proteodermatan sulphate is: one dermatan sulphate chain (average M_r 17500), three *O*-glycosidic oligosaccharide chains and probably *N*-glycosidic oligosaccharide chain(s) bound to one core-protein molecule (M_r 55000).

Proteoglycan and collagen are essential structural components of connective tissues. Tissue analyses suggest a special relationship between dermatan sulphate and collagen accumulation (Kondo *et al.*, 1971; Nakamura & Nagai, 1980). A more recent ultrastructural study suggests that dermatan sulphate-rich proteoglycan plays an important role in collagen-fibre formation (Scott & Orford, 1981). Proteoglycans containing dermatan sulphate have been isolated from skin (Miyamoto & Nagase, 1980; Fujii & Nagai, 1981; Pearson & Gibson, 1982; Damle *et al.*, 1982), sclera (Cöster & Fransson, 1981) and aorta (Ehrlich *et al.*, 1975; Kapoor *et al.*, 1981). However, the structural components of proteodermatan sulphate in the native state were not fully characterized. The present paper describes the purification and some structural characterizations of proteodermatan sulphate from newborn-calf skin.

Experimental

Materials

A newborn calf was obtained from a local slaughter-house and the skin was frozen at −80°C before use. Sepharose, Sephadex, DEAE-Sephadex A-50 and concanavalin A–Sepharose 4B gels were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden; Ultrogel AcA-44 was from LKB Industrie Biologique Française, Paris, France; chondroitinase ABC (EC 4.2.2.4), chondroitinase AC-II (EC 4.2.2.5), *Streptomyces* hyaluronidase (EC 4.2.2.1), dermatan sulphate, chondroitin 4-sulphate and heparin were obtained from Seikagaku Kogyo Co., Tokyo, Japan; Pronase P was from Kaken Kagaku Co., Tokyo, Japan; cellulose acetate membrane (Separaphore III) was from Gelman Instrument Co., Ann Arbor, MI, U.S.A.; [^{3}H]acetic anhydride (500 mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K.; NaB^3H_4 (200 mCi/mmol) was from C.E.A. (Gif-sur-Yvette, France); En^{3}Hance was from New England Nuclear, Boston, MA, U.S.A.; Nonion HS-210 was from Nippon Yushi Co., Tokyo, Japan. Hyaluronic acid was generously given by Dr. R. Hata, Tokyo Medical and Dental University, Tokyo, Japan. Heparan sulphate was prepared from guinea-pig liver as described by Schiller *et al.* (1961). All other chemicals were analytical grade.

Analytical methods

Uronic acid was determined by the carbazole method (Bitter & Muir, 1962). Glycosaminoglycans

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were determined by the method of Hata & Nagai (1973). Radioactivity was measured with a liquid-scintillation spectrometer (Beckman LS-9000). Samples (0.5ml) were mixed with 5ml of scintillation 'cocktail' [toluene/Nonion HS-210 (2:1, v/v) containing 5mg of 2,5-diphenyloxazole and 0.1mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene per ml]. Hydroxyproline was determined as described by Kivirikko *et al.* (1967). Amino acid analysis was performed in an autoanalyser after hydrolysis of the samples in 6M-HCl at 110°C for 22h.

Electrophoresis

Two-dimensional electrophoresis was performed on cellulose acetate membranes (Hata & Nagai, 1972).

After electrophoresis, the acetate membranes were stained with Alcian Blue, with Amido Black or with periodic acid/Schiff reagent.

Sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis was performed in 10% gels by the procedure of Laemmli (1970). The gels were stained with Coomassie Brilliant Blue. The radioactive glycoproteins were detected fluorographically by the method of Bonner & Laskey (1974), and the labelled glycosaminoglycans were located by autofluorography with En^{3}Hance (T. Nakamura & H. Shinkai, unpublished work).

High-voltage paper electrophoresis was performed in 0.06M-sodium tetraborate/HCl buffer, pH9.5 (Takasaki & Kobata, 1974), for determination of sugar components. Radioactive peaks were monitored with a radiochromatoscanner (Packard model 7220).

Gel chromatography

Gel chromatography of proteoglycans was performed on Sephadex G-200 in 1M-NaCl/0.05M-Tris/HCl buffer, pH7.4, and on Ultrogel AcA-44 and Sepharose CL-4B in 4M-guanidinium chloride/0.05M-Tris/HCl buffer, pH7.4. For glycosaminoglycans, Sepharose 6B in 1M-NaCl/0.05M-Tris/HCl buffer, pH7.4, or Sepharose CL-4B in 4M-guanidinium chloride/0.05M-Tris/HCl buffer, was used.

Isolation of proteodermatan sulphate

All procedures for isolation of proteodermatan sulphate except density-gradient centrifugation were performed at 4°C.

Newborn-calf skin (100g dry wt.) was powdered under liquid N_2. The powdered skin was extracted with 1 litre of 0.5M-NaCl/0.05M-Tris/HCl buffer, pH7.5, containing proteinase inhibitors (5mM-benzamidine hydrochloride, 25mM-EDTA, 0.1mM-phenylmethanesulphonyl fluoride, 2.5mM-*N*-ethylmaleimide, 2mM-*trans*-4-aminomethylcyclohexanecarboxylic acid and 10μM-L-*trans*-epoxysuccinic acid) at 4°C for 16h. The suspension was centrifuged at 10000*g* for 30min. This process was repeated three times. The pellet was further extracted with 1 litre of 0.45M-NaCl/0.05M-Tris/HCl buffer, pH7.5, containing the same proteinase inhibitors, and this process was also repeated three times. Each extract was mixed with cetylpyridinium chloride to final concentration of 0.1% and centrifuged at 10000*g* for 30min. The pellet was washed three times with 10% (w/v) potassium acetate in 95% (v/v) ethanol and then dissolved in 60ml of 4M-guanidinium chloride/0.05M-sodium acetate buffer, pH5.8, containing proteinase inhibitors (5mM-benzamidine hydrochloride, 10mM-EDTA, 0.1mM-phenylmethanesulphonyl fluoride, 2mM-*trans*-4-aminomethylcyclohexanecarboxylic acid, 10μM-L-*trans*-epoxysuccinic acid and 3.6μM-pepstatin). The extract was adjusted to a density of 1.49g/ml by addition of solid CsCl and centrifuged at 100000*g* for 48h at 15°C in a Sorvall OTD 65 ultracentrifuge (AH-627 rotor). Fractions of volume 3ml were collected from the bottom and were analysed for uronic acid and protein. The fractions with density 1.4–1.6g/ml were pooled and dialysed against 0.1M-NaCl/0.02M-sodium phosphate buffer, pH7.0, then ion-exchange chromatography was performed on DEAE-Sephadex A-50. Effluent fractions in peak II (Fig. 1) were pooled and adjusted to 1.0M-NaCl concentration.

The proteodermatan sulphate-containing fractions were applied to a column (1cm × 11cm) of concanavalin A–Sepharose 4B, which was washed with 1M-NaCl/0.02M-sodium phosphate buffer, pH7.0. When the absorption of effluent at 280nm returned to base-line, the columns were washed with the same buffer containing 0.1M-methyl α-mannoside. Bound and unbound fractions were dialysed against water and freeze-dried.

Proteodermatan sulphate was purified by molecular-sieve chromatography after affinity chromatography on concanavalin A–Sepharose.

Preparation and analysis of glycosaminoglycans

Glycosaminoglycans were prepared essentially as described by Hata & Nagai (1973).

Glycosaminoglycans were digested with chondroitinase ABC, chondroitinase AC-II or *Streptomyces* hyaluronidase, and were also treated with $NaNO_2$ as previously described (Saito *et al.*, 1968; Ohyama & Kaneko, 1970; Kraemer, 1971). The resultant reaction mixtures were evaluated by electrophoresis on a cellulose acetate membrane.

Determination of M_r of the core protein

Proteodermatan sulphate (60μg) was digested with chondroitinase ABC (0.1 unit) in the presence of proteinase inhibitors (Oike *et al.*, 1980). The

reaction mixtures were subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

Determination of reducing terminal sugar

Proteodermatan sulphate (1 mg) was dissolved in 100 μl of 0.1 M-NaOH, and liberated sugar chains were reduced with NaB^3H_4 (250 μCi) for 24 h at room temperature. The reaction was stopped by the addition of acetic acid, and borate was converted into volatile methyl borate by addition of methanol. The reaction mixtures were evaporated to dryness. The resultant products were digested with Pronase in 0.01 M-Tris/HCl buffer, pH 7.8, and applied to a Dowex 50 W (H^+ form) column (2 ml volume). The column was washed with 10 ml of water. The eluates and washings were combined and evaporated to dryness.

^{3}H-labelled sugar chains (3×10^6 d.p.m.) were subjected to electrophoresis on a cellulose acetate membrane. Two radioactive areas that corresponded to spots on the fluorogram were cut out from the cellulose acetate membrane, and radioactive sugars were eluted with water. The sugar chains obtained were hydrolysed with HCl as previously described (Porter, 1975). Radioactive alditols were determined as reducing terminal sugar by high-voltage paper electrophoresis (Takasaki & Kobata, 1974).

Labelling of sialic acid

Proteodermatan sulphate (200 μg) and the core protein prepared from 200 μg of proteodermatan sulphate were each oxidized with periodate and then reduced with NaB^3H_4 (100 μCi) for labelling of the terminal sialic acid (Van Lenten & Ashwell, 1971). The resultant products (8×10^4 d.p.m.) were subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography.

Determination of the disaccharide units of dermatan sulphate

Dermatan sulphate chains prepared from proteodermatan sulphate were de-acetylated with anhydrous hydrazine and re-acetylated with [^{3}H]acetic anhydride (2 mCi) (Höök *et al.*, 1982). Radioactive dermatan sulphate (3×10^4 d.p.m.) was digested with chondroitinase ABC and chondroitinase AC-II. The resultant products were subjected to paper chromatography to separate unsaturated disaccharides (Saito *et al.*, 1968). The fluorescent spots corresponding to authentic unsaturated disaccharides were eluted with 0.01 M-HCl and assayed for radioactivity.

Results

Isolation of proteodermatan sulphate

Determination of glycosaminoglycans in the skin extracts and residues indicated that 30% of the total dermatan sulphate and 80% of the total hyaluronic acid were recovered from 0.15 M-NaCl-soluble and 0.45 M-NaCl-soluble fractions. The extracts also contained heparan sulphate, chondroitin sulphate and collagen. Subsequent precipitation with cetylpyridinium chloride showed that almost all the extracted proteodermatan sulphate was co-precipitated with collagen. Most of the hyaluronic acid (90% of the total) remained in solution. No appreciable dermatan sulphate was detected in the supernatant. The remaining collagens were removed by CsCl-density-gradient centrifugation: most of collagens were found as aggregates at the top of the tubes. Determination of glycosaminoglycans in each fractions (density-gradient range 1.32–1.51 g/ml) indicated that dermatan sulphate was concentrated in the bottom fractions; therefore the fractions with densities greater than 1.34 g/ml were combined and subjected to ion-exchange chromatography on DEAE-Sephadex A-50. There were two uronic acid-containing peaks (Fig. 1). Determination of glycosaminoglycans showed that the fractions indicated by the horizontal bar in Fig. 1 contained dermatan sulphate. Affinity chromatography on concanavalin A–Sepharose 4B of the dermatan sulphate-containing fractions resolved the material into two fractions (Fig. 2). One ran through the column (unbound fraction), and the other was released with methyl α-mannoside (bound fraction). In the bound fraction dermatan sulphate only was detected as a glycosaminoglycan component, whereas in the unbound fraction dermatan sulphate, hyaluronic acid, heparan sulphate and chondroitin sulphate were detected (inserts in Fig. 2). On electrophoresis on a cellulose acetate membrane the dermatan sulphate of the unbound fraction migrated to the same position as authentic dermatan sulphate, without being subjected to β-elimination reaction. The ratio of dermatan sulphate in the bound fraction to that in the unbound fraction was 1.7:1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the bound fraction gave positive staining with Coomassie Brilliant Blue at a migration position similar to that of dimeric bovine serum albumin; however, the M_r of this bound fraction was decreased by chondroitinase ABC digestion (results not shown). Since bound fractions contained small amounts of impurities with lower M_r (less than that of ovalbumin), further purification was carried out by successive gel chromatography.

Low-M_r glycoproteins of the bound fraction from the concanavalin A–Sepharose 4B chromatography were separated from proteodermatan sulphate by chromatography on Sephadex G-200 in 1 M-NaCl (Fig. 3*a*), and a small amount of impurities ($K_{av.} = 1.0$) absorbing at 280 nm was further removed by gel chromatography on Ultrogel AcA-44 in 4 M-guanidinium chloride (Fig. 3*b*). The final

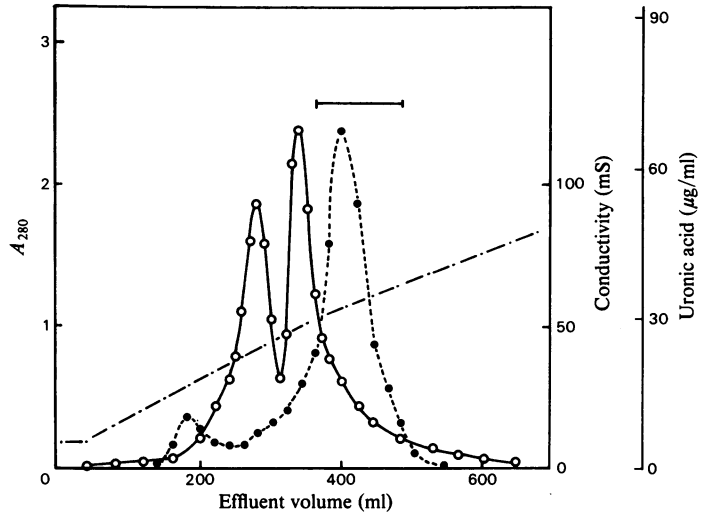

Fig. 1. *Ion-exchange chromatography of skin proteodermatan sulphate preparation*
After CsCl-density-gradient centrifugation (see the text), a sample (10.6 mg as uronic acid) was applied to a column (3 cm × 15 cm) of DEAE-Sephadex A-50 equilibrated with 0.1 M-NaCl/0.02 M-sodium phosphate buffer, pH 7.0. Elution was performed with a linear gradient of 0.1–2.1 M-NaCl; the total elution volume was 1200 ml, and the flow rate was 40 ml/h. O—O, Uronic acid; ●---●, protein (A_{280}); —·—, conductivity.

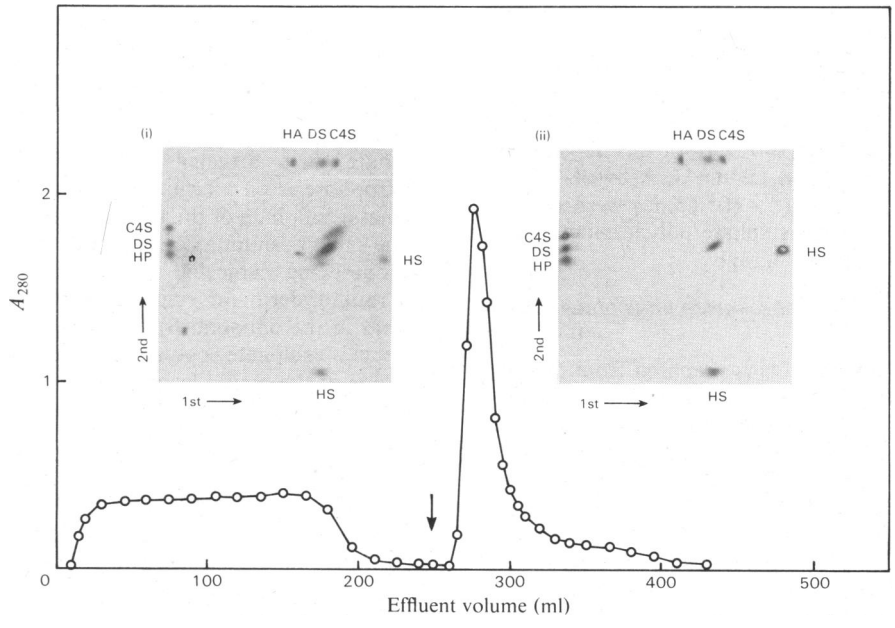

Fig. 2. *Affinity chromatography of proteodermatan sulphate on concanavalin A–Sepharose 4B*
Effluent fractions from the DEAE-Sephadex A-50 chromatography (indicated by the horizontal bar in Fig. 1) were applied to a column (1 cm × 11 cm) of concanavalin A–Sepharose 4B. The column was washed with 1 M-NaCl/0.02 M-sodium phosphate buffer, pH 7.0. When the absorbance at 280 nm returned to base-line, the elution was started (arrow) with the same buffer containing 0.1 M-methyl α-mannoside; the flow rate was 1 ml/h. O——O, Protein (A_{280}). The inserts show two-dimensional electrophoretic patterns of glycosaminoglycans in the unbound fraction (i) and the bound fraction (ii). Key: HA, hyaluronic acid; DS, dermatan sulphate; HS, heparan sulphate; HP, heparin; C4S, chondroitin 4-sulphate.

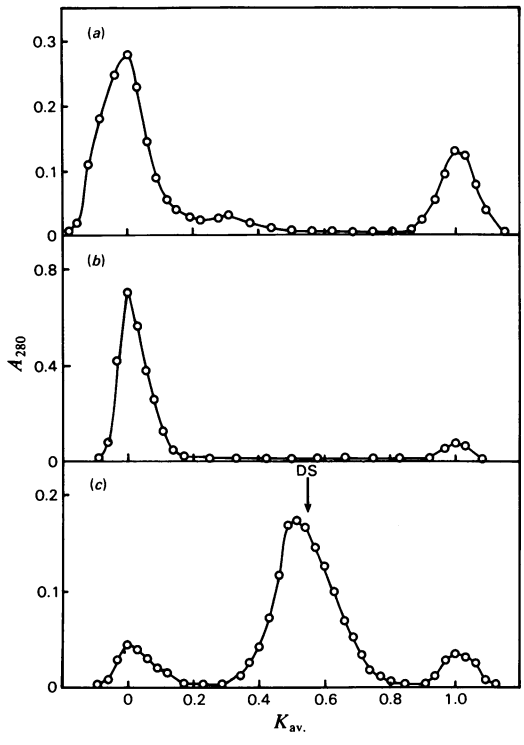

Fig. 3. *Elution profile of proteodermatan sulphate on molecular-sieve chromatography*

The bound fractions (20mg dry wt.) from the concanavalin A–Sepharose 4B chromatography (see Fig. 2) were applied on a column (1.5cm × 90cm) of Sephadex G-200 in 1M-NaCl/0.05M-Tris/HCl buffer, pH7.4, at a rate of 9ml/h (*a*). The void fractions were then applied on a column (1.5cm × 45cm) of Ultrogel AcA-44 in 4M-guanidinium chloride/0.05M-Tris/HCl buffer, pH7.4, at a rate of 5ml/h (*b*). The void fractions were then further chromatographed on a column (1.5cm × 50cm) of Sepharose CL-4B in 4M-guanidinium chloride/0.05M-Tris/HCl buffer, pH7.4, at a rate of 5.5ml/h (*c*). O—O, Protein (A_{280}); the arrow indicates the elution position of dermatan sulphate ($K_{av.} = 0.53$).

step for the purification of protodermatan sulphate was performed by gel chromatography on Sepharose CL-4B in 4M-guanidinium chloride. The major peak ($K_{av.} = 0.51$) contained dermatan sulphate.

The proteoglycans obtained here were confirmed to be proteodermatan sulphate because they gave a single spot stained with Alcian Blue (Fig. 4*a*) and Amido Black (Fig. 4*b*) at the same migration position on electrophoresis on a cellulose acetate membrane. The glycosaminoglycan component of the purified proteoglycan had the same electrophoretic mobilities as authentic dermatan sulphate (Fig. 4*c*), and it was susceptible to chondroitinase ABC digestion but resistant to chondroitinase AC-II digestion, *Streptomyces* hyaluronidase digestion and nitrous acid deamination (results not shown). The proteodermatan sulphate also gave a positive staining with periodic/Schiff reagent (Fig. 4*d*).

Characterization of proteodermatan sulphate

As shown by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 5*a*), the proteodermatan sulphate migrated as a broad band but was not affected by reduction with dithiothreitol (results not shown). The core protein, obtained by chondroitinase ABC digestion, showed a single peak with M_r 55000. The amino acid composition of proteodermatan sulphate is given in Table 1. Aspartate, glutamate and leucine contents were high, in agreement with reports by other workers. When proteodermatan sulphate was oxidized with periodate and reduced with NaB^3H_4, radioactivity was detected at the migration positions of protodermatan sulphate and core protein (Fig. 5*b*). This suggests that the core protein of proteodermatan sulphate is a sialoglycoprotein. Therefore reducing terminal sugars were determined, after they had been liberated by alkaline NaB^3H_4 treatment. Two radioactive peaks were detected on electrophoresis on a cellulose acetate membrane (Fig. 6). One corresponded to the position of authentic dermatan sulphate (peak II), and the other migrated more slowly than authentic hyaluronic acid (peak I). The ratio of radioactivity (peak I/peak II) was approx. 3:1. Radioactive alditols derived from peak I and peak II corresponded to *N*-acetylgalactosaminitol and xylitol respectively.

The dermatan sulphate chains were homogeneous with average M_r 17500 on chromatography on Sepharose CL-6B in 4M-guanidinium chloride [column calibrated with hyaluronic acid (M_r 84000 and 50000) and dermatan sulphate (M_r 15000) (T. Nakamura & H. Shinkai, unpublished work) and were eluted at $K_{av.} = 0.53$ on Sepharose CL-4B in 4M-guanidinium chloride (Fig. 3).

The dermatan sulphate from purified proteodermatan sulphate contained 74% of iduronosylhexosamine units and 26% of glucuronosylhexosamine units, as determined by chondroitinase ABC and chondroitinase AC-II digestions.

Purified proteodermatan sulphate (1mg dry wt./ml) gave an absorption of 0.432 at 280nm and contained 8.3% of uronic acid.

Discussion

Proteodermatan sulphate was extracted from calf skin. Further purification, based on CsCl-density-gradient centrifugation, ion-exchange chromatography, affinity chromatography and molecular-

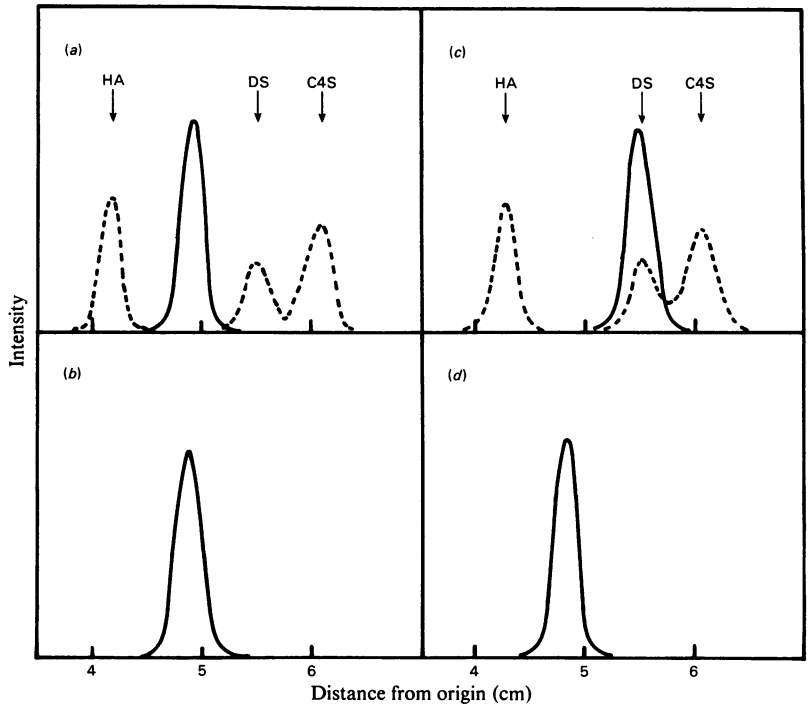

Fig. 4. *Electrophoresis of proteodermatan sulphate on a cellulose acetate membrane*

Purified proteodermatan sulphate (10μg dry wt.) (see Fig. 3) was applied on a cellulose acetate strip. Electrophoresis was in 0.1M-pyridine/0.47M-formic acid buffer, pH 3. Densitometric profiles of proteodermatan sulphate stained with Alcian Blue (*a*), Amido Black (*b*) or periodic acid/Schiff reagent (*d*) are shown, as is also the Alcian Blue staining of the glycosaminoglycan component prepared from proteodermatan sulphate (*c*). ------, Alcian Blue staining of authentic glycosaminoglycan mixtures: HA, hyaluronic acid; DS, dermatan sulphate; C4S, chondroitin 4-sulphate.

sieve chromatography, yielded effective separation of concomitant proteins and free and/or peptide-bound dermatan sulphate from proteodermatan sulphate. Dermatan sulphate could not be separated from proteodermatan sulphate by CsCl-density-gradient centrifugation in dissociative conditions and DEAE-Sephadex A-50 ion-exchange chromatography. Molecular-sieve chromatography on Sepharose CL-4B after Sephadex G-200 gel filtration did not achieve effective separation of dermatan sulphate ($K_{av.}$ 0.53) and proteodermatan sulphate ($K_{av.}$ 0.51) in the presence of 4M-guanidinium chloride, as shown in Fig. 3.

Concanavalin A–Sepharose 4B was used for the isolation of proteodermatan sulphate from bovine achilles tendon (Anderson, 1975). The advantage of the procedure of affinity chromatography on concanavalin A–Sepharose 4B is the separation of proteodermatan sulphate and peptide-bound and/or free dermatan sulphate. As shown Fig. 2(*b*), dermatan sulphate was detected as the components of glycosaminoglycans on electrophoresis on a cellulose acetate membrane after digestion by Pronase of the concanavalin A-bound fraction. Alcian Blue-positive material from the bound fraction was retained at origin on the cellulose acetate membrane if Pronase digestion or β-elimination with NaOH was omitted (results not shown); on the other hand, Alcian Blue-positive material in the unbound fraction migrated at the positions of hyaluronic acid, dermatan sulphate, chondroitin sulphate and heparan sulphate without Pronase digestion and β-elimination (Fig. 2*a*). The purified proteodermatan sulphate migrated between authentic hyaluronic acid and dermatan sulphate, as an Alcian Blue-positive, periodic acid/Schiff-reagent positive and Amido Black-positive spot (Fig. 4). Low-M_r proteins (M_r 30000 and 15000 determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis), which co-migrated with proteodermatan sulphate in the bound fraction obtained by concanavalin A–Sepharose chromatography, were separated from macromolecules by molecular-sieve chromatography at high salt concentration. These

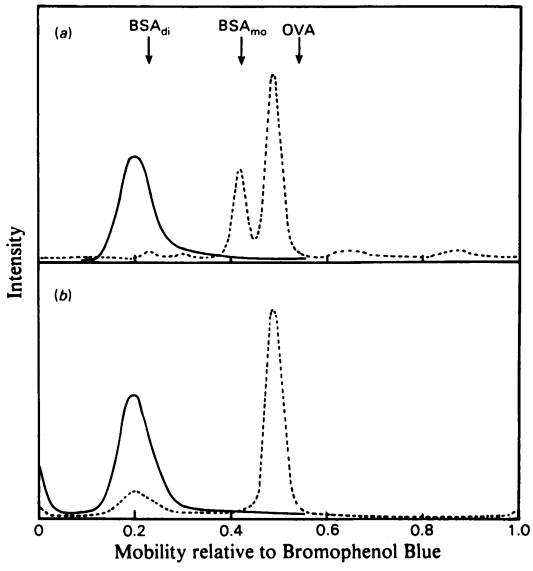

Fig. 5. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of proteodermatan sulphate*
Densitometric profiles are shown of proteodermatan sulphate (60μg dry wt.) stained with Coomassie Brilliant Blue (*a*) and fluorograms of radioactive proteodermatan sulphate (*b*), labelled with NaB^3H_4 as described in the Experimental section. ——, Before digestion with chondroitinase ABC; ------, after digestion with chondroitinase ABC. Markers: BSA_{di} and BSA_{mo}, dimeric and monomeric bovine serum albumin; OVA, ovalbumin.

Table 1. *Amino acid composition of proteodermatan sulphate*

	Content (residues/1000 residues)			
	Calf skin	Bovine skin*	Bovine sclera proteoglycan II†	Pig skin‡
Asx	130	126	123	128
Thr	42	39	49	42
Ser	67	68	68	65
Glx	100	108	122	104
Pro	77	69	74	72
Gly	72	81	84	76
Ala	53	49	54	49
CyS	9	13	9	15
Val	58	59	59	66
Met	12	9	7	6
Ile	63	60	55	56
Leu	121	122	115	127
Tyr	28	29	15	23
Phe	33	33	34	29
Lys	77	80	76	87
His	28	27	25	23
Arg	30	28	32	32

* Results from Pearson & Gibson (1982).
† Results from Cöster & Fransson (1981).
‡ Results from Damle *et al.* (1982).

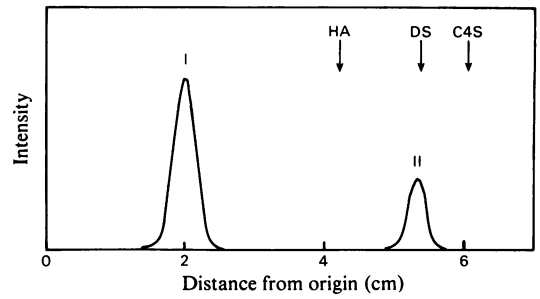

Fig. 6. *Fluorographic detection of ^3H-labelled oligosaccharide chains from proteodermatan sulphate*
Oligosaccharide chains were liberated from proteodermatan sulphate by alkaline-NaB^3H_4 treatment and electrophoresed on a cellulose acetate membrane. Electrophoresis was in 0.1 M-pyridine/0.47 M-formic acid buffer, pH 3. The arrows indicate the migration positions of authentic glucosaminoglycans: HA, hyaluronic acid; DS, dermatan sulphate; C4S, chondroitin 4-sulphate.

findings suggested that low-M_r proteins bound to proteodermatan sulphate and prevented the migration of proteodermatan sulphate on electrophoresis on a cellulose acetate membrane.

Proteodermatan sulphate has two types of *O*-glycosidic oligosaccharide chains, which were liberated by β-elimination with NaOH and have xylose and *N*-acetylgalactosamine as reducing terminal sugar respectively. For the detection of presence of *N*-glycosidic oligosaccharide chains, proteodermatan sulphate was oxidized with periodate and reduced with NaB^3H_4 for the labelling of terminal sialic acid residues. Radioactivity was found to be incorporated into the protein core that was obtained after treatment with chondroitinase ABC (Fig. 5) or β-elimination (results not shown) of tritiated proteodermatan sulphate. When the radioactive oligosaccharide chains from peak I in Fig. 6 were digested with sialidase, the major desialylated radioactive sugars migrated at the position of a trisaccharide on Bio-Gel P-4 column chromatography; this material was converted into a radioactive disaccharide by jack-bean β-galactosidase (H. Shinkai, T. Nakamura & E. Matsunage, unpublished work). These findings suggest that proteodermatan sulphate has sialic acid–galactose–*N*-acetylgalactosamine chains and *N*-glycosidic oligosaccharide chain(s) in addition to the dermatan sulphate side chain.

From the above results the proposed structure of proteodermatan sulphate, obtained here, is one dermatan sulphate chain, three *O*-glycosidic oligosaccharide chains and probably *N*-glycosidic oligosaccharidic chains(s) bound to one core protein (M_r 55000), because the proteodermatan sulphate con-

tains 8.3% iduronic acid and the size of the average dermatan sulphate chain is M_r 17500.

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