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Evidence for the presence and structure of asparagine-linked oligosaccharide units in the core protein of proteodermatan sulphate

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The *N*-glycosidically linked oligosaccharides were liberated by hydrazinolysis from purified proteodermatan sulphate from newborn-calf skin and reduced with NaB^3H_4 at the reducing terminal sugar. One asparagine-linked oligosaccharide chain was linked to one core-protein molecule in proteodermatan sulphate. Structural sequences were analysed by using exoglycosidase digestion. These oligosaccharides were composed of di- and tri-antennary oligosaccharide structures of complex type.

Dermatan sulphate is the major component of glycosaminoglycans in the dermis and is abundantly present in conjunction with collagen bundles (Breen *et al.*, 1972; Fleishmajer *et al.*, 1972; Scott & Orford, 1981).

We have suggested that proteodermatan sulphate from newborn-calf skin contains two distinct classes of oligosaccharides in addition to dermatan sulphate, because proteodermatan sulphate that binds to concanavalin A–Sepharose contains sialic acid residues as non-reducing terminals of oligosaccharides that are resistant to β-elimination with 0.15 M-NaOH, and two alditols derived from xylose and *N*-acetylgalactosamine were detected as reducing terminal sugar obtained by alkaline-borohydride digests of the proteodermatan sulphate (Nakamura *et al.*, 1983).

Structural analysis of *N*-linked oligosaccharides of proteoglycans from the Swarm rat chondrosarcoma (Nilsson *et al.*, 1982) has been performed, and evidence that the core protein from proteodermatan sulphate contains mannose residues has been reported (Cöster & Fransson, 1981; Fujii & Nagai, 1981; Kapoor *et al.*, 1981).

The present study was undertaken to analyse the structural sequence of asparagine-linked oligosaccharides from proteodermatan sulphate of newborn-calf skin by hydrazinolysis, reduction with NaB^3H_4 and sequential exoglycosidase digestion.

Materials and methods

Enzymes and chemicals

Sialidase from *Arthrobacter ureafaciens* was purchased from Nakarai Chemicals (Kyoto, Japan). α-Mannosidase and β-*N*-acetylhexosaminidase were purified from jack-bean meal (Li & Li, 1972). β-Galactosidase was purified from jack-bean meal (Arakawa *et al.*, 1974) and from the culture medium of *Diplococcus pneumoniae* (Hughes & Jeanloz, 1964). α-L-Fucosidase from *Charonia lampas* was purchased from Seikagaku Kogyo Co. (Tokyo, Japan). One unit of glycosidase activity was defined as the amount of activity that released 1 μmol of the monosaccharide from the substrate/min. NaB^3H_4 (200 mCi/mmol) was obtained from C.E.A. (Gif-sur-Yvette, France). Anhydrous hydrazine was purified from hydrazine hydrate by distillation in the presence of CaO and toluene at 95°C.

Oligosaccharides

Oligosaccharide a, Gal(β1–4)GlcNAc(β1–2)-Man(α1–6)[Gal(β1–4)GlcNAc(β1–2)Man(α1–3)]-Man(β1–4)GlcNac(β1–4)GlcNactol, was prepared from human complement subcomponent Clq (Mizuochi *et al.*, 1978). Oligosaccharide b, Gal-(β1–4)GlcNAc(β1–2)Man(α1–6)[Man(α1–3)]Man-(β1–4)GlcNAc(β1–4)GlcNActol, was prepared by incubating a mixture of β-galactosidase and β-*N*-acetylhexosaminidase with NeuAc(α2–6)Gal-(β1–4)GlcNAc(β1–2)Man(α1–6)[Gal(β1–4)Glc-NAc(β1–2)Man(α1–3)]Man(β1–4)GlcNAc(β1–4)-GlcNActol from human complement subcomponent Clq and then digestion of the product with sialidase. Oligosaccharide c, Man(α1–6)[Man-(α1–3)]Man(β1–4)GlcNAc(β1–4)GlcNActol, was obtained by β-galactosidase and β-*N*-acetylhexosaminidase digestion of oligosaccharide b. Oligosaccharide d, Man(β1–4)GlcNAc(β1–4)GlcNActol, was prepared by α-mannosidase digestion of oligosaccharide c. *N*-Acetylglucosaminitol, *N*-acetylgalactosaminitol, xylitol and galactitol were isolated by reduction of *N*-acetylglucosamine, *N*-acetyl-

Abbreviation used: GlcNActol (in sequences), *N*-acetylglucosaminitol.

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galactosamine, xylose and galactose respectively with NaB^3H_4 (Takasaki & Kobata, 1974). Glucose oligomers were prepared from dextran hydrolysate (Nishigai *et al.*, 1978).

Preparation of proteodermatan sulphate

Proteodermatan sulphate was extracted from newborn-calf skin with 0.15 M-NaCl/50 mM-Tris/HCl buffer, pH 7.0, containing proteinase inhibitors and purified as described by Nakamura *et al.* (1983). The purity of the preparations was monitored by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis by the procedure of Laemmli (1970) and two-dimensional electrophoresis on a cellulose acetate membrane (Hata & Nagai, 1972).

Liberation of asparagine-linked oligosaccharides by hydrazinolysis

Dried proteodermatan sulphate (8 mg) was suspended in 0.4 ml of anhydrous hydrazine and heated at 100°C for 12 h. The reaction mixture was evaporated to dryness under reduced pressure over conc. H_2SO_4. The residue was dissolved in saturated $NaHCO_3$ and was completely acetylated with acetic anhydride. The reaction mixture was passed through a Dowex 50W (H^+ form) column (5 ml volume), and then the column was washed with 30 ml of water. The eluates and washings were combined and evaporated to dryness. The residue was dissolved in small amount of water and spotted on Whatman 3MM paper, and the paper was developed with ethyl acetate/pyridine/acetic acid/water (5:5:1:3, by vol.) for 24 h at room temperature. Oligosaccharides were recovered from the region from the origin to 5 cm of paper by elution with water. The oligosaccharide was reduced with 100 μmol of NaB^3H_4 in 200 μl of 0.05 M-NaOH at 30°C for 4 h as reported by Mizuochi *et al.* (1978). The reaction was stopped by addition of acetic acid, and the reaction products were passed through the small column of Dowex 50W (H^+ form). The column was washed with 5 bed volumes of water. Eluate and washing were combined and then evaporated to dryness. The residue was dissolved into a small amount of water, spotted on Whatman 3MM paper and subjected to chromatography with butan-1-ol/ethanol/water (4:1:1, by vol.) for 2 days. The migration positions of radioactive material were monitored with a radiochromatoscanner (Packard model 7220). The major radioactive oligosaccharides, which remained at the origin, were eluted with water.

Fractionation of oligosaccharides by paper electrophoresis

The radioactive oligosaccharides were spotted on Whatman 3MM paper, and subjected to paper electrophoresis in pyridine/acetic acid/water (3:1:387, by vol.), pH 5.4, at 80 V/cm for 90 min. The radioactive oligosaccharides were eluted with water and evaporated to dryness.

Identification of reducing terminal sugar residue

The radioactive oligosaccharides were hydrolysed in 4 M-HCl at 100°C for 1 h. The hydrolysates were analysed by paper electrophoresis in 0.06 M-borate buffer, pH 9.5, for 2.5 h at 40 V/cm, with radioactive galactitol, xylitol and *N*-acetylglucosaminitol as authentic standards (Takasaki & Kobata, 1974).

Molecular-sieve chromatography

The radioactive oligosaccharides and dextran hydrolysates were mixed, and the mixture was subjected to chromatography on a Bio-Gel P-4 (200–400 mesh) column (2.5 cm × 90 cm) at 50°C equilibrated with water. Fractions of volume 3.5 ml were collected at a flow rate of 10 ml/h. A differential refractometer (Knauer type 98; Knauer, Berlin, West Germany) was used for the detection of internal standard sugars that were eluted from the column.

Results

Liberation of the asparagine-linked oligosaccharides from proteodermatan sulphate and fractionation of the oligosaccharides

Oligosaccharides were liberated by hydrazinolysis from proteodermatan sulphate, and reducing terminal sugars were labelled by reduction with NaB^3H_4. The yields of radioactive oligosaccharides from proteodermatan sulphate were calculated for the neutral component (N) and the acidic components (A-1, A-2 and A-3) after electrophoresis as shown in Fig. 1. The molar proportions of the N, A-1, A-2 and A-3 components, calculated on the basis of their radioactivities, were 17.6:7.7:1.0:2.3. The amounts of these components were calculated, from the specific radioactivity of NaB^3H_4, to be as shown in Table 1. These data suggested that one asparagine-linked oligosaccharide chain binds to one proteodermatan sulphate molecule, because the recovery rate of radioactivity was 85% of spotted samples by this method.

When components A-1 and A-2 were digested with sialidase (20 munits/20 μl of 0.1 M-sodium acetate buffer, pH 5.0, for 24 h at 37°C), these acidic components were completely converted into neutral fractions (Figs. 1*b* and 1*c*). By mild sialidase digestion of component A-3 (1 munit/20 μl of 0.1 M-acetate buffer, pH 5.0, for 30 min at 20°C), four bands were detected on paper electrophoresis. One remained at the origin, and other three corresponded to components A-1, A-2 and A-3 (Fig. 1*d*). These results indicated that component A-3 is

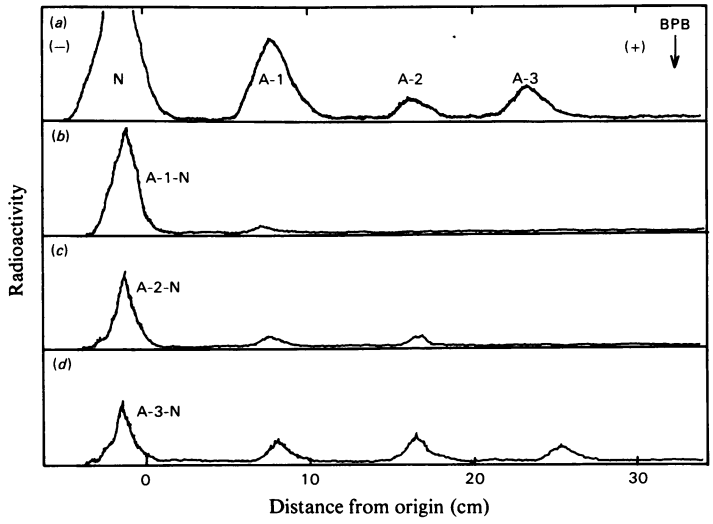


Fig. 1. *Radioelectrophoretogram of the oligosaccharides liberated from proteodermatan sulphate by hydrazinolysis*
Oligosaccharides ^{3}H-labelled at reducing terminals were subjected to electrophoresis on paper at pH 5.4 at 80 V/cm for 90 min. The arrow indicates the position of Bromophenol Blue (BPB). (*a*) Intact oligosaccharides; (*b*) sialidase digestion of A-1 component; (*c*) sialidase treatment of A-2 component; (*d*) mild sialidase digestion of A-3 component.

Table 1. *Contents of asparagine-linked oligosaccharide chains in proteodermatan sulphate*
The M_r of proteodermatan sulphate was calculated on the M_r values for the core protein (55000) and dermatan sulphate (15000) (Nakamura *et al.*, 1983).

M_r	70000
Amount of sample used	8 mg (114 nmol)
Radioactivity	
In N fraction	112.6×10^5 d.p.m. (51.2 nmol)
In A-1 fraction	49.1×10^5 d.p.m. (22.3 nmol)
In A-2 fraction	6.4×10^5 d.p.m. (2.9 nmol)
In A-3 fraction	14.7×10^5 d.p.m. (6.7 nmol)
Total	182.8×10^5 d.p.m. (83.1 nmol)
Oligosaccharide/proteodermatan sulphate	0.73

more acidic than are components A-2 and A-1. Desialylated oligosaccharides from the acidic components after digestion with sialidase were recovered from the electrophoresis paper by elution with water (named A-1-N, A-2-N and A-3-N).

A portion of each neutral component (3×10^4 d.p.m.) was hydrolysed with 4 M-HCl, and the hydrolysates were analysed for identification of the reducing terminal sugar of oligosaccharides by high-voltage paper electrophoresis with the borate buffer system. The position of each radioactive component accorded with that of authentic *N*-acetylglucosaminitol (results not shown). These results indicated that the reducing terminal end of these oligosaccharides is *N*-acetylglucosamine.

Analysis of the structural sequence of oligosaccharides

Since sialic acid-containing oligosaccharides were eluted in the void volume of the Bio-Gel P-4 column, desialylated oligosaccharides were analysed by sequential exoglycosidase digestion and subjected to molecular-sieve chromatography.

The oligosaccharide fraction A-3-N was directly subjected to Bio-Gel P-4 column chromatography, and two radioactive peaks were detected with mobilities corresponding to those of oligosaccharides containing 17.2 and 16.2 glucose units (Fig. 2*a*). These materials were converted by jack-bean β-galactosidase into materials that ran as peaks with mobilities corresponding to those of oligosaccharides containing 14.5 and 13.5 glucose units (Fig. 2*b*). Since one galactose residue behaves approximately as one glucose unit on this column, these results suggested that jack-bean β-galactosidase liberated three galactosyl residues that were linked to branching chains as non-reducing terminal sugar.

When the peaks with mobilities corresponding to those of oligosaccharides containing 14.5 and 13.5 glucose units were incubated with jack-bean β-*N*-acetylhexosaminidase, the enzyme liberated three *N*-acetylglucosamine residues from each peak (Fig. 2*c*), on the basis that one *N*-acetylglucosamine residue behaves approximately as two glucose units on chromatography on a Bio-Gel P-4 column equilibrated with water. These resulting radioactive materials with mobilities corresponding to those of

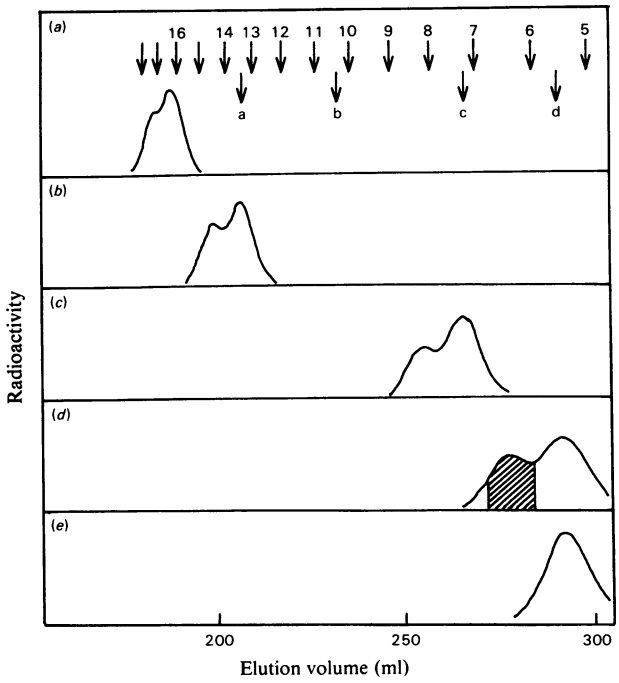


Fig. 2. *Chromatographic patterns of oligosaccharides liberated by sequential exoglycosidase digestion of A-3-N oligosaccharides*

The mixture of radioactive oligosaccharides and dextran hydrolysates as internal standard was subjected to Bio-Gel P-4 column chromatography. The arrows indicate the elution positions of oligosaccharides (numbers indicate the glucose unit equivalents, and a–d indicate the authentic oligosaccharides: oligosaccharide a, Gal(β1–4)GlcNAc(β1–2)Man(α1–6)[Gal(β–4)GlcNAc(β1–2)Man(α1–3)]Man(β1–4)GlcNAc(β1–4)GlcNactol; oligosaccharide b, Gal(β1–4)GlcNAc(β1–2)Man(α1–6)[Man(α1–3)]Man(β1–4)GlcNAc(β1–4)GlcNActol; oligosaccharide c, Man(α1–6)[Man(α1–3)]Man(β1–4)GlcNAc(β1–4)GlcNActol; oligosaccharide d, Man(β1–4)GlcNAc(β1–4)GlcNActol. (*a*) Intact A-3-N oligosaccharides; (*b*) A-3-N oligosaccharides treated with 2 units of jack-bean β-galactosidase in 50μl of 0.1 M-citrate/phosphate buffer, pH 3.5, for 16h at 37°C; (*c*) material in radioactive peaks shown in (*b*) incubated with 1 unit of jack-bean β-*N*-acetylhexosaminidase in 50μl of 0.1 M-citrate/phosphate buffer, pH 5.0, for 16h at 37°C; (*d*) material in radioactive peaks shown in (*c*) digested with 0.5 unit of jack-bean α-mannosidase in 50μl of 0.1 M-acetic acid/NaOH buffer, pH 5.0, containing 0.5mg of galactono-1,5-lactone at 37°C for 40h; (*e*) material in radioactive peak of hatched area shown in (*d*) treated with 10 munits of *Charonia lampas* α-L-fucosidase in 0.1 M-citrate/phosphate buffer, pH 5.0, at 55°C for 16h.

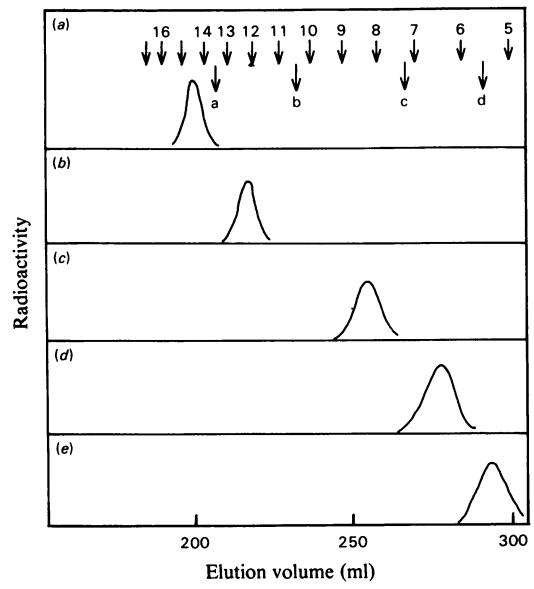


Fig. 3. *Chromatographic patterns of oligosaccharides liberated by sequential exoglycosidase digestion of A-2-N oligosaccharide*

The radioactivity in each tube was determined with a liquid-scintillation spectrometer (Beckman LS-900). Analytical conditions and the arrows are the same as indicated in Fig. 2. (*a*) Intact A-2-N oligosaccharide; (*b*) A-2-N oligosaccharide digested with jack-bean β-galactosidase; (*c*) material in radioactive peak shown in (*b*) digested with jack-bean β-*N*-acetylhexosaminidase; (*d*) material in radioactive peak shown in (*c*) incubated with α-mannosidase; (*e*) materials in radioactive peak shown in (*d*) treated with *Charonia lampas* α-L-fucosidase.

oligosaccharides containing 8.3 and 7.3 glucose units were sensitive to jack-bean α-mannosidase, and the resulting radioactive peaks ran with mobilities corresponding to those of oligosaccharides containing 6.5 and 5.5 glucose units (Fig. 2*d*). When the hatched peak indicated in Fig. 2(*d*) was digested with *Charonia lampas* α-L-fucosidase, the radioactivity was recovered in a peak with mobility corresponding to that of an oligosaccharide containing 5.5 glucose units, which accorded with authentic Man(β1–4)GlcNAc(β1–4)GlcNActol (Fig. 2*e*). From the above data it is clear that a fucose residue may bind to the trisaccharide that is the core oligosaccharide, that two mannose residues bind via α-linkages to the non-reducing terminal mannose residue of the core oligosaccharide and that three Galβ–GlcNAc chains bind via β-linkages to the $Man\alpha_2$–Manβ–GlcNAcβ–(±Fucα)–GlcNActol. Therefore A-3-N oligosaccharides have a tri-antennary structure of complex type.

When intact A-2-N oligosaccharide fraction was subjected to Bio-Gel P-4 column chromatography, the radioactivity was recovered in a peak with mobility corresponding to that of an oligosac-

charide containing 14.5 glucose units (Fig. 3*a*). Treatment of this material with jack-bean β-galactosidase led to the appearance of a peak with mobility corresponding to that of an oligosaccharide containing 12.2 glucose units (Fig. 3*b*). These results indicated that the non-reducing terminal sugar is galactose and that two galactose residues were released by β-galactosidase from the A-2-N oligosaccharide. When the resulting radioactive oligosaccharides were incubated with jack-bean β-*N*-acetylhexosaminidase, the radioactivity was eluted in a position corresponding to that of an oligosaccharide containing 8.3 glucose units (Fig. 3*c*). This result indicated that two *N*-acetylglucosamine residues were liberated from the degalactosylated oligosaccharides shown in Fig. 3(*b*). Therefore each galactose residue links to *N*-acetylglucosamine via a β-linkage.

When the material in the radioactive peak in Fig. 3(*c*) was treated with α-mannosidase, the radioactivity was recovered in a position corresponding to that of an oligosaccharide containing 6.5 glucose units (Fig. 3*d*). This oligosaccharide was sensitive to α-L-fucosidase, and the resulting radioactive peak ran with a mobility corresponding to that of an oligosaccharide containing 5.5 glucose units, which accorded with that of the authentic trisaccharide (Fig. 3*e*). These data suggested that the A-2-N oligosaccharide must possess a di-antennary structure and that a fucose residue links to the core trisaccharide chain.

As shown in Fig. 4(*a*), the labelled oligosaccharides of intact A-1-N fraction ran with mobilities corresponding to those of oligosaccharides containing 15.5 and 14.5 glucose units. When a portion of these oligosaccharides was digested with jack-bean β-galactosidase, the radioactivities appeared at positions corresponding to those of oligosaccharides containing 12.2 and 11.2 glucose units (Fig. 4*b*). These degalactosylated oligosaccharides were sensitive to jack-bean β-*N*-acetylhexosaminidase digestion, and the resulting two radioactive peaks had mobilities corresponding to those of oligosaccharides containing 8.3 and 7.3 glucose units (Fig. 4*c*). The material in the former radioactive peak was converted by incubation with *Charonia lampas* α-L-fucosidase into the material with mobility corresponding to that of an oligosaccharide containing 7.3 glucose units, which accorded with authentic Man_3–GlcNAc–GlcNActol (Fig. 4*c*). When the remaining radioactive oligosaccharides shown in Fig. 4(*a*) were incubated with a mixture of *Diplococcus* β-galactosidase and β-*N*-acetylhexosaminidase, the radioactive peaks obtained had mobilities corresponding to those of oligosaccharides containing 12.2, 11.2, 8.3 and 7.3 glucose units (Fig. 4*d*). The first two of these radioactive oligosaccharides were sensitive to jack-bean β-galactosidase, and the resulting radioactive peaks ran with mobilities corresponding to those of oligosaccharides containing 10.2 and 9.2 glucose units (Fig. 4*e*). These data indicated that the equivalent of three glucose units were liberated from A-1-N oligosaccharides by digestion with a mixture of *Diplococcus* β-galactosidase and β-*N*-acetylhexosaminidase, that two galactose residues were further liberated from the degalactosyl and de-*N*-acetylglucosaminyl oligosaccharides by jack-bean β-galactosidase, and also that jack-bean β-galactosidase liberated three galactose residues from intact A-1-N oligosaccharides. Therefore A-1-N oligosaccharides have a Galβ–Galβ–GlcNAcβ chain that is resistant to *Diplococcus* β-galactosidase. The Galβ–Galβ–GlcNAcβ chain links to one non-reducing terminal mannose residue of the pentasaccharide$(Man\alpha)_2$–Manβ–GlcNAcβ–GlcNActol, and a Galβ–GlcNAcβ chain links to another non-reducing terminal mannose residue.

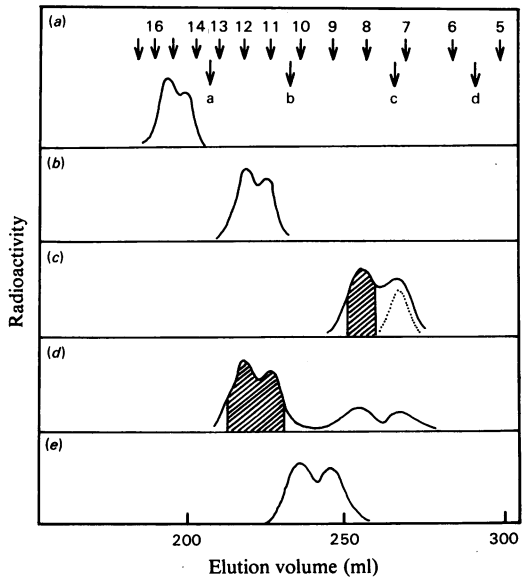


Fig. 4. *Chromatographic patterns of oligosaccharides liberated by sequential exoglycosidase digestion of A-1-N oligosaccharides*

Analytical conditions and the arrows are the same as indicated in Fig. 2. (*a*) Intact A-1-N oligosaccharides; (*b*) A-1-N oligosaccharides digested with jack-bean β-galactosidase; (*c*) material in radioactive peaks shown in (*b*) treated with jack-bean β-*N*-acetylhexosaminidase, and (·····) material in the hatched area after digestion with *Charonia lampas* α-L-fucosidase; (*d*) material in radioactive peaks shown in (*a*) incubated with a mixture of Diplococcus β-galactosidase and β-*N*-acetylhexosaminidase; (*e*) material in the hatched area shown in (*d*) digested with jack-bean β-galactosidase.

When intact N-component oligosaccharides were subjected to Bio-Gel P-4 column chromatography, the radioactive oligosaccharides were eluted at positions corresponding to those of oligosaccharides containing 15.5 and 14.5 glucose units (Fig. 5*a*). A portion of these radioactive oligosaccharides was incubated with jack-bean β-galactosidase, and the resulting radioactive peaks were recovered at positions corresponding to those of oligosaccharides containing 12.2 and 11.2 glucose units (Fig. 5*b*). This result indicated that three galactose residues were liberated from each N-component oligosaccharide. When the remaining radioactive oligosaccharides shown in Fig. 5(*a*) were incubated with a mixture of *Diplococcus* β-galactosidase and β-*N*-acetylhexosaminidase, the resulting radioactive peaks had mobilities corresponding to those of oligosaccharides containing 12.2, 11.2, 8.3 and 7.3 glucose units, although the last-mentioned two peaks were minor components (Fig. 5*c*). The size difference between the materials in the peaks shown in Fig. 5(*a*) and the major peaks in Fig. 5(*c*) was equivalent to three glucose units. Since one *N*-acetylglucosamine residue and one galactose residue behave as two glucose units and one glucose unit respectively, it is inferred that one *N*-acetylglucosamine and one galactose residue were liberated from each of the N-component oligosaccharides by a mixture of *Diplococcus* β-galactosidase and β-*N*-acetylhexosaminidase. When the material of the major peaks shown in Fig. 5(*c*) was incubated with jack-bean β-galactosidase, a further two galactose residues were liberated from each oligosaccharide (Fig. 5*d*). This result indicated that a galactose residue is a non-reducing terminal sugar and that it is resistant towards *Diplococcus* β-galactosidase. Therefore the presence of a Galβ–Galβ structure is also suggested in the N-component oligosaccharides. When the material of the radioactive peaks shown in Fig. 5(*d*) was treated with jack-bean β-*N*-acetylhexosaminidase, two glucose-unit equivalents (one *N*-acetylglucosamine residue) were liberated from each oligosaccharide (Fig. 5*e*). This result indicated that the Galβ–Gal chain links to *N*-acetylglucosamine via a β-linkage. When the radioactive oligosaccharides shown in Fig. 5(*e*) were incubated with *Charonia lampas* α-L-fucosidase, the resulting radioactive peak was at the position corresponding to that of an oligosaccharide containing 7.3 glucose units, which accorded with authentic Man(α1–6)[Man(α1–3)]Man(β1–4)GlcNAc(β1–4)-GlcNActol (Fig. 5*f*). From these data it is indicated that the major oligosaccharides in the N-component fraction have the same structural sequences as those of the A-1-N oligosaccharides.

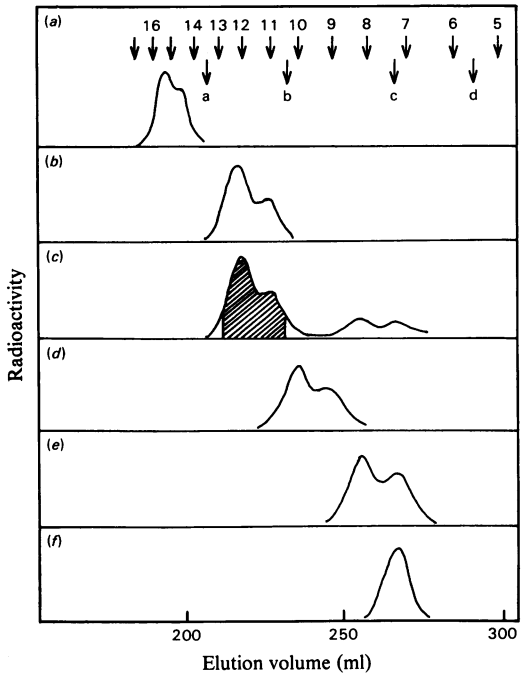


Fig. 5. *Chromatographic patterns of oligosaccharides liberated by sequential exoglycosidase digestion of N-component oligosaccharides*

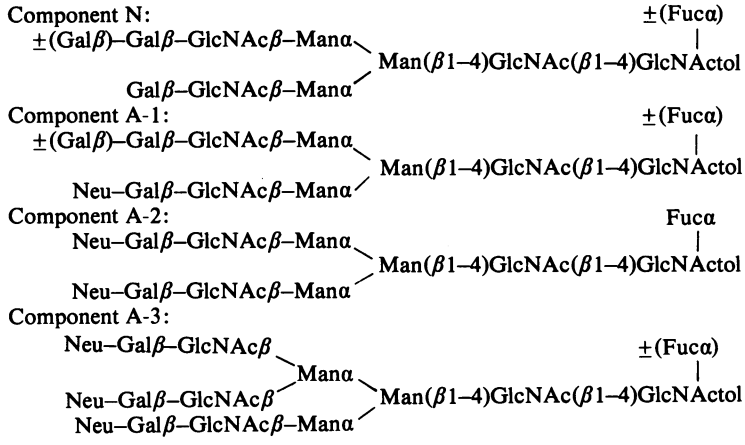
Analytical conditions and the arrows are the same as indicated in Fig. 2. (*a*) Intact N-component oligosaccharides; (*b*) N-component oligosaccharides digested with jack-bean β-galactosidase; (*c*) N-component oligosaccharides treated with a mixture of *Diplococcus* β-galactosidase and β-*N*-acetylhexosaminidase; (*d*) material in hatched area shown in (*c*) digested with jack-bean β-galactosidase; (*e*) material in radioactive peaks shown in (*d*) incubated with jack-bean β-*N*-acetylhexosaminidase; (*f*) material in radioactive peaks shown in (*e*) digested with *Charonia lampas* α-L-fucosidase.

Location of sialic acid residue(s) in acidic oligosaccharides

When the A-1, A-2 and A-3 components were digested with sialidase, the resulting oligosaccharides remained at the origin on paper electrophoresis. Therefore the acidic nature of the A-1, A-2 and A-3 components is due to the presence of sialic acid residue(s). When the A-3 and A-2 components were first incubated with a mixture of *Diplococcus* β-galactosidase and β-*N*-acetylhexosaminidase and then the enzymes were inactivated by heating at 100°C for 10 min, the radioactive peaks were detected at the same mobilities as those shown by intact A-3-N and A-2-N oligosaccharides respectively (results not shown). These results indicated that the non-reducing terminal residues are sialic acid residues.

When A-1-component oligosaccharides were

Fig. 6. *Proposed structural sequences of oligosaccharides from proteodermatan sulphate*



digested with a mixture of jack-bean β-galactosidase and β-*N*-acetylhexosaminidase and then secondarily digested with sialidase, the radioactive peaks were eluted at the positions corresponding to those of oligosaccharides containing 11.3 and 10.3 glucose units, which accorded with Gal(β1–4)GlcNAc(β1–2)Man(α1–6[Man(α1–3)]Man(β1–4)GlcNAc(β1–4)(±Fucα)GlcNActol (results not shown). This result indicated that sialic acid residue is bound to the non-reducing terminal galactose residue and not to the Galβ–Galβ–GlcNAc chain of the A-1-N oligosaccharides.

Proposed structures of oligosaccharides from proteodermatan sulphate

From above results obtained by sequential digestion with exoglycosidases, the structures of N, A-1, A-2 and A-3 components are proposed to be as shown in Fig. 6.

Discussion

In the preceding paper we suggested that *N*-glycosidic oligosaccharides are linked to the core protein of proteodermatan sulphate (Nakamura *et al.*, 1983), as also described for proteoglycans from the Swarm rat chondrosarcoma (De Luca *et al.*, 1980; Lohmander *et al.*, 1980).

In the present work fragmentation by hydrazinolysis was successfully used for the quantitative liberation of asparagine-linked oligosaccharides, as previously described for glycoproteins (Kobata, 1979).

The oligosaccharide chains of proteodermatan sulphate as presented in Fig. 6 are of the complex type found in bovine thymocyte plasma membrane (Yoshima *et al.*, 1980), bovine complement subcomponent Clq (Mizuochi *et al.*, 1982) and pN-collagen type III (H. Shinkai & Ch. M. Lapiere, unpublished work).

We have confirmed in the present work that one proteodermatan sulphate molecule contains one asparagine-linked oligosaccharide chain, as calculated from the results of experiments with NaB^3H_4 labelling (Table 1). The asparagine-linked oligosaccharides from proteodermatan sulphate were shown to be composed of four components, one neutral and three acidic. The neutral-component oligosaccharides were the major oligosaccharides (62% of the total oligosaccharides were *N*-glycosidically linked) and have a di-antennary structure. The structures of the N-component oligosaccharides in the present paper indicated that Galβ–Galβ is exclusively located on the side chain linked at the branching β-mannose residue.

A-1-component oligosaccharides with one sialic acid residue located on the outer chain formed 27% of the asparagine-linked oligosaccharides.

A-3-component oligosaccharides (8% of the total) possessed a tri-antennary structure with a sialic acid residue located on each outer chain. A-2-component oligosaccharides (3.5% of the total) possessed a di-antennary structure.

The tri-antennary structure has also been found in proteoglycan from the Swarm rat chondrosarcoma, which contained more than 70% of total *N*-glycosidically linked oligosaccharides (Nilsson *et al.*, 1982); however, in proteodermatan sulphate the structure was found in only 8% of the total asparagine-linked oligosaccharides.

Carbohydrate contents were determined by Cöster & Fransson (1981), Fujii & Nagai (1981) and Kapoor *et al.* (1981) from proteodermatan sulphate. If one molecule of proteodermatan sulphate contains one asparagine-linked oligosaccharide chain, as indicated in the present paper, three

mannose residues, four *N*-acetylglucosamine residues, at least two galactose residues and less than one sialic acid residue should be contained in the core protein of proteodermatan sulphate. These numbers are very close to the values for neutral sugar components determined by Cöster & Fransson (1981, calculated from their Table 2).

The linkage region of the asparagine-linked oligosaccharide on the core protein could not be analysed, although these oligosaccharides could not be detected in fractions not bound to concanavalin A–Sepharose that contained free or peptide-bound dermatan sulphate. The oligosaccharide chains described in the present paper may play a role in recognizing other connective-tissue molecules. This hypothesis is consistent with the view that proteodermatan sulphate associates with collagen fibrils at the d-band (Scott & Orford, 1981).

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