The Copolymeric Structure of Pig Skin Dermatan Sulphate

CHARACTERIZATION OF D-GLUCURONIC ACID-CONTAINING OLIGOSACCHARIDES ISOLATED AFTER CONTROLLED DEGRADATION OF OXYDERMATAN SULPHATE

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Selective periodate oxidation of unsubstituted L-iduronic acid residues in copolymeric dermatan sulphate chains was followed by reduction-hydrolysis or alkaline elimination. By this procedure the glucuronic acid-containing periods were isolated in oligosaccharide form; general formula:

Further degradation of these oligosaccharides with chondroitinase-AC yielded three types of products: (a) sulphated trisaccharide containing an unsaturated uronosyl moiety in the non-reducing terminal and a C₄ fragment in the reducing terminal, $\Delta\text{UA-GalNAc}(-\text{SO}_4)-\text{R}$; (b) monosulphated, unsaturated disaccharide, $\Delta\text{UA-GalNAc-SO}_4$ when n is greater than or equal to 2; and (c) *N*-acetylglactosamine with or without sulphate. Oligosaccharides containing a single glucuronic acid residue ($n = 1$) comprised more than half of the glucuronic acid-containing oligosaccharides. The terminal *N*-acetylglactosamine moiety of the shortest oligosaccharide was largely 4-sulphated, whereas higher oligosaccharides primarily contained 6-sulphated or unsulphated hexosamine moieties in the same position. Moreover, IdUA-SO₄-containing oligosaccharides were encountered. These oligosaccharides were resistant to the action of chondroitinase-ABC.

The principal repeating disaccharide unit of dermatan sulphate may be abbreviated as IdUA-GalNAc-4S* (Mathews, 1958; Hoffman *et al.*, 1960; Stoffyn & Jeanloz, 1960). Also various proportions of GlcUA-GalNAc-S are present in the molecule (Fransson & Rodén, 1967; Fransson & Havsmark, 1970). In pig skin dermatan sulphate the latter units form 1–2 block regions/chain. However, a considerable amount

* Abbreviations: GalNAc-S, sulphated *N*-acetylglactosamine; GalNAc-4S and GalNAc-6S, 4- and 6-sulphated *N*-acetylglactosamine, respectively; UA, uronic acid; ΔUA , 4,5-unsaturated uronic acid; IdUA-GalNAc-4S, a repeating disaccharide unit composed of L-iduronic acid and 4-sulphated *N*-acetylglactosamine; GlcUA-GalNAc-S, analogous repeating unit composed of D-glucuronic acid and a sulphated *N*-acetylglactosamine; Di-OS, unsulphated disaccharide (type UA-GalNAc); $\Delta\text{Di-OS}$, unsulphated disaccharide with a 4,5-unsaturated uronosyl moiety; Di-4S and Di-6S, 4-sulphated and 6-sulphated disaccharides, respectively; $\Delta\text{Di-4S}$ and $\Delta\text{Di-6S}$, 4-sulphated and 6-sulphated disaccharides with 4,5-unsaturated uronosyl moieties, respectively; Di-monoS and Di-diS, mono- and di-sulphated disaccharides, respectively; IdUA, L-iduronic acid; GlcUA, D-glucuronic acid.

of the GlcUA-containing periods are also present as single repeating units, particularly in the vicinity of the carbohydrate-protein link (Fransson & Malmström, 1971). These features were demonstrated after enzymic degradation of copolymeric molecules. The GlcUA-containing regions were cleaved selectively by the action of testicular hyaluronidase and chondroitinase-AC.

A procedure has been described which allows selective oxidation of unsubstituted IdUA residues in dermatan sulphate by periodate (Fransson, 1974). Subsequently, controlled degradation of oxydermatan sulphate is achieved either by reduction-hydrolysis or by alkaline elimination (Fransson & Carlstedt, 1974). By using these procedures, the GlcUA-containing as well as the IdUA-SO₄-containing regions of copolymeric chains were isolated in oligosaccharide form. The present paper is primarily concerned with a complete characterization of the former oligosaccharides. One conspicuous finding was that *N*-acetylglactosamine residues in the sequence -IdUA-GalNAc-GlcUA- were frequently 6-sulphated or unsulphated.

Experimental

Materials

Chondroitinase-AC-II from *Arthrobacter aurescens*, chondroitinase-ABC from *Proteus vulgaris* (chondroitin sulphate lyases, EC 4.2.99.6) and chondro-4-sulphatase from *Proteus vulgaris* were products of Seikagaku Fine Biochemicals, Tokyo, Japan.

N-Acetylgalactosamine was purchased from Calbiochem., Los Angeles, Calif., U.S.A. *N*-Acetylgalactosamine 4- or 6-sulphate (GalNAc-4S and GalNAc-6S) were prepared by sequential degradation of chondroitin 4- or 6-sulphate tetrasaccharide with β -glucuronidase and chondroitinase-AC as described elsewhere (Fransson & Havsmark, 1970). *N*-Acetylchondrosine (Di-OS), 4- or 6-sulphated disaccharide (Di-4S and Di-6S) and 4,5-unsaturated 4- or 6-sulphated disaccharide (Δ Di-4S and Δ Di-6S) were the same preparations as described previously (Fransson & Malmström, 1971). Unsulphated, 4,5-unsaturated disaccharide (Δ Di-OS) and disulphated, 4,5-unsaturated disaccharide (Δ Di-diS) were obtained after digestion with chondroitinase-AC of chemically desulphated chondroitin sulphate and oversulphated chondroitin sulphate, respectively (Malmström & Fransson, 1971). A disulphated trisaccharide, GalNAc(-SO₄)-GlcUA-GalNAc(-SO₄), was obtained by digestion with β -glucuronidase of a chondroitin sulphate tetrasaccharide.

Pig skin dermatan sulphate (preparation PS-25; Fransson, 1968a) was oxidized with periodate at pH 3 and 4°C as described previously (Fransson, 1974). The resulting oxydermatan sulphate was cleaved by reduction-hydrolysis or by alkaline elimination (Fransson & Carlstedt, 1974). Oligosaccharides with the general carbohydrate sequence GalNAc-(UA-GalNAc)_n-R were resolved by gel chromatography on Sephadex G-50 (Fransson & Carlstedt, 1974); in the formula UA connotes a periodate-insensitive hexuronic acid residue, either GlcUA or IdUA-SO₄, and R represents the residual, C₄ fragment of an oxidized-degraded IdUA residue.

Analytical methods

The procedures used for the determination of hexosamine and hexuronic acid have been listed elsewhere (Fransson & Carlstedt, 1974). For analyses of hexosamine, oligosaccharides were hydrolysed with 4M-HCl at 100°C for 6 h. U.v.-absorption spectra were recorded on a Beckman DK-2 spectrophotometer in aqueous solutions at pH 7.0.

Chromatographic methods

Analysis of the hexuronic acid composition was performed by hydrolysis and ion-exchange chromatography as described previously (Fransson *et al.*, 1968).

Descending paper chromatography was carried out on Whatman 3MM paper in isobutyric acid-0.5M-NH₃ (5:3, v/v) for 48 h. High-voltage paper electrophoresis was done on the same paper in 0.1M-pyridine acetate buffer, pH 5.0 (40V/cm; 1.5h). Papers were stained with aniline hydrogen phthalate (Partridge, 1949).

Chondroitinase and chondrosulphatase digestion of oligosaccharides

Material was dissolved in 0.5M-Tris-HCl buffer, pH 8.0 (10mg/ml) and digested with chondroitinase-AC or chondroitinase-ABC (0.2 unit of enzyme/10mg of substrate) at 37°C for 3 h. To ensure that the digestion had been exhaustive another 0.1 unit of enzyme was added after the initial digestion. When no further increase of *E*₂₃₂ was observed (Yamagata *et al.*, 1968), it was concluded that the enzymic cleavage had proceeded to completion. Finally, the digests were chromatographed on columns (1.1cm×230cm) of Sephadex G-25 (superfine), which were eluted with 0.2M-pyridine acetate, pH 5.0, at a rate of 12ml/h. The effluents were analysed for uronic acid (Bitter & Muir, 1962; Heinegård, 1973) and for reducing power (Park & Johnson, 1949). Appropriate fractions were pooled, freeze-dried and dried over P₂O₅ under decreased pressure.

Incubations with chondro-4-sulphatase were performed as described by Yamagata *et al.* (1968).

Results

Pig skin dermatan sulphate contains three types of hexuronic acid residues, i.e. IdUA, IdUA-SO₄ and GlcUA (Malmström & Fransson, 1971). Treatment with periodate at low temperature and pH selectively oxidizes the unsubstituted IdUA residues (Fransson, 1974). Further degradation of oxydermatan sulphate by way of reduction-hydrolysis or alkaline elimination yields a number of oligosaccharide fragments with the carbohydrate sequence GalNAc-(UA-GalNAc)_n-R where *n* = 1–4 (Fransson & Carlstedt, 1974). These oligosaccharide fragments include (I) sequences containing IdUA-SO₄, (II) sequences containing GlcUA, and (III) linkage-region glycopeptides (Scheme 1). GlcUA-containing oligosaccharides (II in Scheme 1) may be identified by degradation with chondroitinase-AC. As shown in Scheme 2 these oligosaccharides should be cleaved into monosaccharide, a varying number of disaccharides and trisaccharide.

Gel-chromatography patterns of various periodate-resistant oligosaccharides digested with chondroitinase-AC are shown in Fig. 1. The various oligosaccharides produced by alkaline elimination (Figs. 1a–1d) and by Smith degradation (Fransson & Carlstedt, 1974) (Figs. 1e–1h) yielded similar patterns.

Scheme 1. Controlled degradation of dermatan sulphate

(a) Copolymeric structure for pig skin dermatan sulphate; (b) degradation products obtained after alkaline treatment or reduction-hydrolysis of oxydermatan sulphate. I, oligosaccharides containing IdUA-SO₄; II, oligosaccharides containing GlcUA; III, linkage-region glycopeptides [the xylose residue appears to resist periodate oxidation under the present conditions (L.-Å. Fransson, unpublished work)]; IV, *N*-acetylgalactosamine sulphate substituted with R = residual C₄ fragment of an oxidized L-iduronic acid residue.

For details see the text.

Chondroitinase-AC degradation of the tetrasaccharides gave three components corresponding to undegraded material (a), trisaccharide (b) and mono-

saccharide (d). No disaccharide peak was observed, which is to be expected from a tetrasaccharide with the carbohydrate sequence GalNAc-GlcUA-GalNAc-R. As well as tri- and mono-saccharides, the higher oligosaccharides (hexa, octa and deca) yielded disaccharide components (c) after digestion with chondroitinase-AC. The yield of disaccharide increased with increasing chain length of the degraded oligosaccharide. Moreover, the higher oligosaccharides contained considerable proportions of material which was resistant to chondroitinase-AC (peaks a in hexa and octa and peak v₀ in deca, Fig. 1). The yield of the various oligosaccharides obtained after Smith-degradation (Figs. 1e-1h) are shown in Table I. It is seen that almost one-third of the repeating units in dermatan sulphate were obtained as periodate-insensitive oligosaccharides (A in Table I). This is in

Fig. 1. Gel chromatography on Sephadex G-25 of chondroitinase-AC digests of oligosaccharides obtained after controlled degradation of dermatan sulphate

A, Oligosaccharides obtained after alkaline elimination of oxydermatan sulphate are: (a) tetra; (b) hexa; (c) octa and (d) deca. Oligosaccharides obtained after reduction and hydrolysis of oxydermatan sulphate are: (e) tetra; (f) hexa; (g) octa and (h) deca. —, Carbazole; ----, reducing power. The various oligosaccharides have the general carbohydrate sequence GalNAc-(UA-GalNAc)_n-R and may vary from tetrasaccharide ($n = 1$, tetra) to decasaccharide ($n = 4$, deca). Since the residual C₄ fragment (R) obtained after Smith-degradation (e-h) contains no aldehyde group, assay for reducing power was only performed on the monosaccharide peaks (d). Before analysis for reducing power, the buffer salts were removed by evaporation; $v_0 = 82$ ml; $v_t = 210$ ml.

Table 1. Yield of oligosaccharides obtained after Smith degradation (oxidation-reduction-hydrolysis) of dermatan sulphate

The various oligosaccharides were obtained by Smith degradation of dermatan sulphate followed by preparative gel chromatography on Sephadex G-50. The general formula is:

When $n = 1$ the saccharide is referred to as tetra, $n = 2$ is a hexa, $n = 3$ is an octa and, finally $n = 4$ represents a deca.

Oligosaccharide	After Smith degradation* (A)	Material resistant to chondroitinase-AC† (B)	Material digestible with chondroitinase-AC (A-B)
Tetra	9.9	4.9	5.0
Hexa	5.9	3.9	2.0
Octa	6.4	5.4	1.0
Deca	6.9	5.3	1.6
Sum ...	29.1	19.5	9.6

* Expressed as recovered hexosamine in per cent of starting material (dermatan sulphate submitted to oxidation). Total recovery [including fragment $\text{GalNAc}(\text{SO}_4)\text{-R}$] was 97%.

† Material recovered as tetrasaccharides or larger oligosaccharides after digestion with chondroitinase-AC (see Figs. 1e-h).

Fig. 2. U.v.-absorption spectra

(a) $\text{GalNAc}(\text{SO}_4)\text{-R}$ (where R is a C_4 , acidic, α, β -unsaturated aldehyde); (b) an unsaturated disaccharide ($\Delta\text{UA-GalNAc-SO}_4$, fraction hexa-c in Fig. 1b), and (c) an unsaturated trisaccharide [$\Delta\text{UA-GalNAc}(\text{SO}_4)\text{-R}$, fraction hexa-b in Fig. 1b)]. For details of preparation see the text.

agreement with previous estimations, which indicated that approx. 30% of the hexuronic acid residues were periodate-resistant (Fransson, 1974). A large proportion of the periodate-insensitive oligosaccharides were also resistant to digestion with chondroitinase-AC. It was found that approx. 20% of the hexuronic acid residues in dermatan sulphate were resistant both to periodate and to chondroitinase-AC (B in Table 1). Consequently, it may be calculated that the GlcUA-containing oligosaccharides comprised almost 10% of the starting material (A - B in Table 1). This value is somewhat higher than estimates based on carbazole-orninol ratios (0.36; corresponding to approx. 6-7% GlcUA of total hexuronic acid). The reason for this discrepancy will be discussed below. Although the exact quantity is somewhat uncertain at the present stage it may be concluded that a large

part of the GlcUA-GalNAc-S repeating units of pig skin dermatan sulphate occur as single units. The yield of larger oligosaccharides (clusters of up to 4 units were observed) decreased with increasing chain length. When more GlcUA-rich dermatan sulphate preparations were degraded by the present procedure the GlcUA-containing oligosaccharides were considerably larger; oligosaccharides with as much as 7 repeating units have been isolated (L.-Å. Fransson, unpublished work).

Some of the fragments produced by digestion with chondroitinase-AC of periodate-insensitive oligosaccharides were characterized by measurements of their u.v.-absorption spectra. Chondroitinase-AC digestion of a hexasaccharide fraction (Fig. 1b) produced trisaccharide (b), disaccharide (c) and a monosaccharide (d). The u.v. spectra of the tri- and the di-saccharide together with that of $\text{GalNAc}(\text{SO}_4)\text{-R}$ are shown in Fig. 2. The compound $\text{GalNAc}(\text{SO}_4)\text{-R}$ displayed considerable absorbance in the 220-230 nm range (Fig. 2a) and the disaccharide around 232 nm (Fig. 2b), whereas the trisaccharide showed considerable absorbance in both regions (Fig. 2c). These findings indicate that the trisaccharide (hexa-b) contained both a 4,5-unsaturated uronic acid moiety and an α, β -unsaturated aldehydic residue. It is concluded that the structure is $\Delta\text{UA-GalNAc}(\text{SO}_4)\text{-R}$.

Tri-, di- and mono-saccharide fragments obtained after chondroitinase-AC digestion of various periodate-insensitive oligosaccharides (Fig. 1) were also characterized by paper electrophoresis and paper chromatography. As shown in Fig. 3(a) the trisaccharide fractions (-b) contained, with one exception, two components, one with a mobility slightly higher than that of Di-monoS and the other with the mobility of a disulphated trisaccharide. When trisaccharide fraction octa-b was hydrolysed with chondro-4-sulpha-

Fig. 3. Paper electrophoresis of various oligosaccharides

(a) Fragments produced by chondroitinase-AC digestion of oligosaccharides obtained after Smith-degradation (reduction-hydrolysis) of oxydermatan sulphate. (b) Paper electrophoresis of components octa-b (a trisaccharide) and octa-c (a disaccharide) after hydrolysis with chondro-4-sulphatase. CR, spots which appear cherry-red after staining with aniline hydrogen phthalate. The two components which were obtained from deca-a are considered to represent glycopeptides derived from the carbohydrate-protein link. Approx. 20–50 μ g of each sample was applied to the paper. After incubation with chondro-4-sulphatase in a small volume (20 μ l) samples were directly spotted on the paper.

tase (Fig. 3b) both trisaccharide components were desulphated by this enzyme, indicating the presence of a 4-sulphated GalNAc in each compound. The slow-moving trisaccharide component, which was present in all cases, should correspond to the trisaccharide Δ UA-GalNAc(4-SO₄)-R. The nature of the fast-moving component is uncertain, but its staining properties suggest that one of the sulphate groups is located to an IdUA residue (Yamagata *et al.*, 1968). It is tentatively proposed that this component has the carbohydrate sequence GalNAc-IdUA-GalNAc,

with sulphate substituents on the IdUA residue and on one of the GalNAc residues. Since the latter trisaccharide was included in the 'chondroitinase-AC-digestible material' (Table 1 hexa, octa and deca; column A – B) this resulted in a slight overestimation of GlcUA-containing periods in dermatan sulphate (see above).

The disaccharide components (-c) obtained after digestion with chondroitinase-AC contained only monosulphated species (Fig. 3a). One of the disaccharides (octa-c) was quantitatively converted

Fig. 4. Paper chromatography of various mono- and disaccharides

Standards are: I, Di-6S; II, Δ Di-6S; III, Δ Di-4S; IV, Di-0S; V, Δ Di-0S; VI, GalNAc-4S; VII, GalNAc-6S; VIII, GalNAc. These samples were also subjected to paper electrophoresis (see Fig. 3).

into unsulphated disaccharide by chondro-4-sulphatase (Fig. 3b). The monosaccharides released by chondroitinase-AC from the periodate-insensitive oligosaccharides comprised both GalNAc and GalNAc-S (Fig. 3a).

The position of the sulphate groups in the various di- and mono-saccharides was corroborated by paper chromatography (Fig. 4). Only the tetrasaccharide gave a 4-sulphated GalNAc (tetra-d), whereas the longer fragments afforded 6-sulphated GalNAc as well as unsulphated GalNAc. The yield of GalNAc (unsulphated) increased with increasing chain length of the original fragment. In contrast, most of the disaccharide components were 4-sulphated; only the decasaccharide produced significant quantities of 6-sulphated Di-monoS (deca-c).

Chondroitinase-AC-resistant oligosaccharides

Fig. 5. Gel chromatography on Sephadex G-25 of chondroitinase-ABC digests of oligosaccharides resistant to chondroitinase-AC

—, Carbazole; ----, reducing power; -.-.-, deca- v_0 after reoxidation with periodate; $v_0 = 82$ ml. The various chondroitinase-AC-resistant oligosaccharides were treated with chondroitinase-ABC as described in the text. Reoxidation of deca- v_0 with periodate was performed under the same conditions as the previous oxidation. (a) Tetra-a; (b) hexa-a; (c) octa-a; (d) deca- v_0 .

(tetra-a, hexa-a, octa-a and deca- v_0 in Figs. 1a-1d) were subjected to further degradation by chondroitinase-ABC as shown in Fig. 5. Surprisingly, most of the oligosaccharides were poorly degraded by this enzyme. Only the tetrasaccharide was efficiently degraded to trisaccharide (I) and monosaccharide (III) (Fig. 5a). The larger oligosaccharides (hexa-a, octa-a, and deca- v_0) were only partially degraded by chondroitinase-ABC to produce disaccharides (II) and monosaccharides (III) (Figs. 5b-5d); in these cases no trisaccharide was formed. The chondroitinase-ABC-resistant decasaccharide (deca- v_0 -I in Fig. 5d) yielded exclusively L-iduronic acid on hydrolysis and ion-exchange chromatography. Moreover, repeated periodate oxidation-alkaline treatment had little or no effect on the molecular size (Fig. 5d).

Fig. 6. Paper electrophoresis

(a) Paper electrophoresis of fragments obtained after chondroitinase-ABC digestion of various oligosaccharides. (b) Paper electrophoresis of a tri- and a di-saccharide after hydrolysis with chondro-4-sulphatase.

The various saccharides released by chondroitinase-ABC were subjected to paper electrophoresis (Fig. 6). The trisaccharide obtained after digestion with chondroitinase-ABC of a tetrasaccharide (tetra-a-I) had a mobility similar to that of trisaccharide $\Delta\text{UA-GalNAc}(-\text{SO}_4)\text{-R}$ (cf. Figs. 6a and 3a). However, the chondroitinase-ABC-released trisaccharide was unaffected by chondro-4-sulphatase (Fig. 6b) suggesting that it had the structure $\Delta\text{UA}(-\text{SO}_4)\text{-GalNAc-R}$. The monosaccharide obtained in the same experiment (tetra-a-III in Fig. 6a) had the mobility of a sulphated GalNAc. The higher oligosaccharides (hexa-a, octa-a and deca-v0) gave in addition to GalNAc-S (hexa-a-III in Fig. 6a), disulphated as well as monosulphated disaccharides (hexa-a-II, octa-a-II and deca-v0-II). Octa-a-II yielded only Di-monoS on

digestion with chondro 4-sulphatase (Fig. 6b). Presumably, the original Di-monoS component of octa-a-II had the structure $\Delta\text{UA}(-\text{SO}_4)\text{-GalNAc}$. The structure of the disulphated component should be $\Delta\text{UA}(-\text{SO}_4)\text{-GalNAc-4-SO}_4$. Finally, the chondroitinase-ABC-resistant oligosaccharides (hexa-a-I, octa-a-I and deca-v0-I) appeared heterogeneous on electrophoresis (Fig. 6a).

Discussion

Structure of D-glucuronic acid-containing regions of pig skin dermatan sulphate

The present paper describes the isolation of GlcUA-containing regions of pig skin dermatan sulphate in oligosaccharide form. These oligosaccharides were

Fig. 7. Formulae of some GlcUA-containing oligosaccharide fragments released from pig skin dermatan sulphate after oxidation and degradation

found to have the structures shown in Fig. 7. It was shown that more than half of the GlcUA-containing units occurred as single units (corresponding to oligosaccharide $n = 1$ in Fig. 7). Since the starting material had an average GlcUA-content of 6–7% and a molecular weight (M_N) of approx. 20000 (Fransson & Carlstedt, 1974), the average number of GlcUA-containing periods should be 2–3. The starting material [a 0–25% (w/v) ethanol precipitate] was undoubtedly heterogeneous and the GlcUA-content could theoretically vary from 0 to 14%. Consequently, the dermatan sulphate chains might contain as much as six GlcUA residues. However, dermatan sulphate fractions recovered between 25 and 40% ethanol usually have a GlcUA-content between 15 and 20% (L.-Å. Fransson, unpublished work). Therefore, the GlcUA-content of the present preparation should vary between 2 and 12%, corresponding to 1–5 GlcUA residues/chain. These calculations illustrate that copolymeric dermatan sulphate molecules with a considerable variation in hexuronic acid composition exist within one tissue. Some copolymeric chains contain only a few, single GlcUA-GalNAc-SO₄ periods, whereas other chains contain two or more cluster-like arrangements of the GlcUA-containing periods. This is in agreement with earlier findings (Fransson & Rodén, 1967), which also indicated that in almost half of the chains one of these clusters is located in the vicinity of the carbohydrate-protein link.

Previous studies have indicated that single GlcUA-GalNAc(SO₄) units of pig skin dermatan sulphate are preferentially located in sequences like:

(Fransson & Malmström, 1971). The present paper indicated that single units appear to be largely 4-sulphated, whereas long segments also contain unsulphated or 6-sulphated GalNAc residues (see also Fig. 7). It should be noted that internal periods of long GlcUA-containing segments were largely 4-sulphated. It is possible that the GalNAc residue in the sequence -IdUA-GalNAc-GlcUA- became sulphated at a later stage of the biosynthetic process. Studies on the biosynthesis of chondroitin sulphate *in vitro* have shown that the extent of 4-sulphation falls as the reaction proceeds (Robinson, 1969). At later stages of the reaction most of the sulphation occurs on the 6-position.

L-Iduronic acid-containing, periodate-insensitive oligosaccharides

Digestion with chondroitinase-AC of periodate-insensitive oligosaccharides produced tri-, di- and mono-saccharide as well as undegraded material. The yields of the former components were not entirely equimolar. It was noted that the yield of mono-saccharide (GalNAc and GalNAc-SO₄) was lower, and that the yield of trisaccharide was higher than expected. Moreover, the trisaccharide fraction usually contained two components, ΔUA-GalNAc(SO₄)-R and a disulphated trisaccharide. The latter component, which was found in oligosaccharides with 2 or more repeating units, is thought to contain a sulphated IdUA residue. Although the present results suggest that units containing sulphated IdUA are often located adjacent to GlcUA-containing segments, further studies are required to clarify this point.

Periodate- and chondroitinase-AC-resistant oligosaccharides were extremely poor substrates for chondroitinase-ABC. Only the smallest oligosaccharide was cleaved into GalNAc-SO₄ and ΔUA(SO₄)-GalNAc-R to any major extent. The larger oligosaccharides, which comprised approx. 15% of the repeating units of the molecule, were only partially degraded by this enzyme despite the presence of IdUA in this material. The split products comprised considerable proportions of Di-diS and ΔUA(SO₄)-GalNAc, suggesting that the sulphated IdUA residues of dermatan sulphate were recovered in these oligosaccharides. The absence of trisaccharide among the split products is again consistent with the presence of segments that contain IdUA(SO₄)-GalNAc or IdUA(SO₄)-GalNAc(SO₄) units adjacent to GlcUA-GalNAc(SO₄) units.

The periodate- and chondroitinase-resistant oligosaccharides were rather heterogeneous in size and charge distribution. Therefore it cannot be excluded that unsulphated IdUA-GalNAc repeating units occur in this material. Consequently the possible existence of IdUA residues in the 1C chair conformation should be considered. The resistance to chondroitinase-ABC displayed by these oligosaccharides was unexpected. It is possible that this enzyme requires a substrate with fully sulphated GalNAc residues.

General conclusions

Previous studies have indicated that dermatan sulphate fractions obtained from various tissues are very heterogeneous in terms of the proportion and distribution of IdUA-GalNAc(4-SO₄) and GlcUA-GalNAc(4/6-SO₄) units (Fransson & Rodén, 1967; Fransson, 1968b; Fransson & Havsmark, 1970). Dermatan sulphate-chondroitin sulphate copolymers from pig skin contain a rather limited number of GlcUA-GalNAc(SO₄) units, whereas material obtained from horse aorta is extremely disperse. A similar series of variants have been isolated from the meniscus of human knee joint (Habuchi *et al.*, 1973). From these results it seems clear that complete sequence analyses can never be performed, since each population of copolymers must contain a very large number of different molecules. However, it seems worthwhile to search for possible general features among these molecules.

The present paper also suggests that the IdUA-GalNAc units surrounding GlcUA-containing sections are irregular. Disulphated disaccharide periods were found in close proximity to the GlcUA-containing regions. It appears that GlcUA- and IdUA-SO₄-containing periods are often located adjacent to each other. However, more definitive statements concerning the structural relation between these disaccharide periods will have to await further studies.

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References

- Bitter, T. & Muir, H. (1962) *Anal. Biochem.* **4**, 330-336
- Fransson, L.-Å. (1968a) *Biochim. Biophys. Acta* **156**, 311-316
- Fransson, L.-Å. (1968b) *J. Biol. Chem.* **243**, 1504-1510
- Fransson, L.-Å. (1974) *Carbohydr. Res.* **36**, in the press
- Fransson, L.-Å. & Carlstedt, I. (1974) *Carbohydr. Res.* **36**, in the press
- Fransson, L.-Å. & Havsmark, B. (1970) *J. Biol. Chem.* **245**, 4770-4783
- Fransson, L.-Å. & Malmström, A. (1971) *Eur. J. Biochem.* **18**, 422-430
- Fransson, L.-Å. & Rodén, L. (1967) *J. Biol. Chem.* **242**, 4170-4175
- Fransson, L.-Å., Rodén, L. & Spach, M. L. (1968) *Anal. Biochem.* **23**, 317-330
- Habuchi, H., Yamagata, T., Iwata, H. & Suzuki, S. (1973) *J. Biol. Chem.* **248**, 6019-6028
- Heinegård, D. (1973) *Chem. Scrip.* **4**, 199-201
- Hoffman, P., Linker, A., Lippman, V. & Meyer, K. (1960) *J. Biol. Chem.* **235**, 3066-3072
- Malmström, A. & Fransson, L.-Å. (1971) *Eur. J. Biochem.* **18**, 431-435
- Mathews, M. B. (1958) *Nature (London)* **181**, 421-423
- Park, J. T. & Johnson, M. J. (1949) *J. Biol. Chem.* **181**, 149-156
- Partridge, S. M. (1949) *Nature (London)* **164**, 443-445
- Robinson, H. C. (1969) *Biochem. J.* **113**, 543-549
- Stoffyn, P. & Jeanloz, R. W. (1960) *J. Biol. Chem.* **235**, 2507-2513
- Yamagata, T., Saito, H., Habuchi, O. & Suzuki, S. (1968) *J. Biol. Chem.* **243**, 1523-1535