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^{1}H-n.m.r. investigation of naturally occurring and chemically oversulphated dermatan sulphates

Identification of minor monosaccharide residues

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The ^{1}H-n.m.r. spectra of various dermatan sulphate preparations present, besides the major signals of the basic disaccharide unit, several other minor signals. We have assigned most of them by n.m.r., using two-dimensional proton–proton double-quantum-correlation and nuclear-Overhauser-effect spectroscopy experiments. This allowed us to identify 2-*O*-sulphated L-iduronic acid and D-glucuronic acid residues as well as 6-sulphated *N*-acetylgalactosamine (presumably 4-*O*-sulphated as well). 2-*O*-Sulphated iduronic acid was present to similar extents (6–10% of total uronic acids) in pig skin dermatan sulphate and pig intestine dermatan sulphate, whereas glucuronic acid represented 17% of the uronic acid of pig skin dermatan sulphate and was virtually absent (1%) from the other preparation. 6-*O*-Sulphated *N*-acetylgalactosamine was present in minor amounts in pig intestine dermatan sulphate only. The influence of sulphation of iduronic acid units on their conformation was assessed by using chemically oversulphated pig intestine dermatan sulphate. Introduction of sulphate groups in this unit in dermatan sulphate tends to shift the conformational equilibrium towards the $^1C_4$ conformer.

INTRODUCTION

Dermatan sulphate proteoglycans are components of the extracellular matrix of several tissues (skin, tendon, sclera, cornea, cartilage, bone, aortic wall), where they interact with collagen and fibronectin. Recent studies indicate that they are also present at the cell surface of several cell types, particularly endothelial cells, and that they might be involved in the regulation of cell growth and tissue repair after injury (Kinsella & Wight, 1988).

Dermatan sulphate glycosaminoglycan, the polysaccharide part of dermatan sulphate proteoglycans, has been shown to activate the plasma protein heparin cofactor II, a serine-proteinase inhibitor, thereby inducing a considerable increase in its thrombin-inhibitory potency (Tollefsen *et al.*, 1982). This is the rationale for developing dermatan sulphate as a new antithrombotic drug (Pangrazzi & Gianese, 1987).

The glycosaminoglycan polymer consists of the repetition of a basic disaccharide made up of *N*-acetyl-D-galactosamine and a uronic acid (Fig. 1). Most of the *N*-acetylgalactosamine units are ester-sulphated at position 4, but ester sulphates at position 6 have also been reported. The major uronic acid found in dermatan sulphate is iduronic acid, although glucuronic acid may be present as well. Some iduronic acid residues are sulphated at position 2 (for a review see Rodén, 1980).

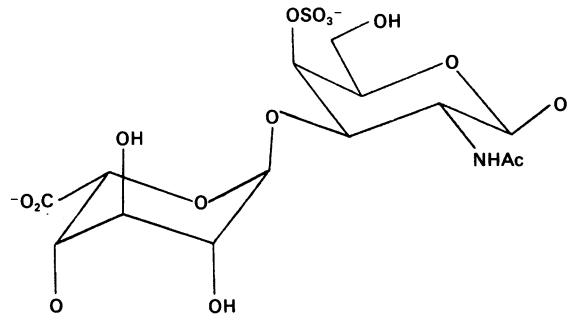

Fig. 1. Basic disaccharide repeating unit of dermatan sulphate
→4)-α-L-IdoA-(1→3)-β-D-GalNAc-4-OSO$_3$-(1→

It is highly probable that the biological function of dermatan sulphate molecules (and also other glycosaminoglycans) is connected with these structural heterogeneities, which affect local charge density and also local conformation, i.e. localization of these anionic charges in space (Casu *et al.*, 1988).

In this respect, specific sequences have been suggested to interact with heparin cofactor II (Tollefsen *et al.*, 1986; Munakata *et al.*, 1987), but no definitive evidence has been provided so far. Other studies demonstrate that a high proportion of iduronic acid in dermatan sulphate promotes binding with collagen (Lindahl & Höök, 1978; Gallagher *et al.*, 1983). Artificially oversulphated derivatives of dermatan sulphate have been shown to possess increased heparin-cofactor-II-mediated antithrombin activity (Sié *et al.*, 1986; Ofosu *et al.*, 1987; Scully *et al.*, 1988), but conflicting data have been reported concerning inhibition of Factor Xa (Dol *et al.*, 1988; Scully *et al.*, 1988) and the antithrombotic effect (Dol *et al.*, 1988; Van Ryn-McKenna *et al.*, 1989).

With the view to better understanding of some of these properties we have undertaken structural studies on dermatan sulphate. As a first step we have used high-field n.m.r. spectroscopy to identify the different monosaccharide units present. We have investigated two natural dermatan sulphate preparations, one from pig skin and the other from pig intestinal mucosa. Studies were also performed on chemically partially oversulphated dermatan sulphate and extensively oversulphated

Abbreviation used: n.O.e., nuclear Overhauser effect.

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dermatan sulphate to determine the positions of newly introduced sulphate esters. In a second step we assessed the influence of these structural features on the conformation of the molecules.

Several publications devoted to n.m.r. characterization of the intact dermatan sulphate polymer have appeared (Perlin *et al.*, 1970; Gatti *et al.*, 1979; Sanderson *et al.*, 1989). In the present paper we report new experiments using two-dimensional n.m.r. techniques of proton–proton double-quantum-correlation and nuclear-Overhauser-effect (n.O.e.) spectroscopy, which were used in order to assign signals of some monosaccharide residues present in minute amounts only.

MATERIALS AND METHODS

Dermatan sulphate from pig intestinal mucosa was obtained as a by-product during heparin production and had a sulphate/carboxylate (OSO_3^-/CO_2^-) ratio of 1.06:1. It was purified according to classical methods (Rodén *et al.*, 1972). Pig skin dermatan sulphate was from Sigma Chemical Co. (batch 64F0403) and had an OSO_3^-/CO_2^- ratio of 1.02:1.

Partially oversulphated dermatan sulphate was prepared from intestinal dermatan sulphate as follows: dermatan sulphate sodium salt (2 g) was passed through a Dowex 50 (H^+ form) column (2 cm × 20 cm), and the solution was neutralized with tributylamine and freeze-dried, yielding 3 g (90%) of tributylammonium salt. This salt (0.76 g) was then dissolved in dimethylformamide (25 ml) and cooled to 0 °C, and SO_3–pyridine complex (1.9 g; corresponding to 4 equivalents per OH group) was added. After 15 min at 0 °C water (20 ml) was added, the pH was adjusted to 9 with 2 M-NaOH, and a saturated solution of sodium acetate in ethanol (150 ml) was added. The precipitate was washed with ethanol, dissolved in water (50 ml) and dialysed (M_r cut-off 3500) against water. The sodium salt was obtained by passage through a Dowex 50 (H^+ form) column followed by neutralization with NaOH. After freeze-drying a white powder was obtained (0.64 g). The OSO_3^-/CO_2^- ratio of the preparation was determined by conductimetry (Casu & Gennaro, 1975) to be 2.15:1. Extensively oversulphated dermatan sulphate was obtained in the same way (except that the sulphation reaction lasted for 24 h at room temperature) and had an OSO_3^-/CO_2^- ratio of 3.66:1. Gel-permeation chromatography–h.p.l.c. analysis of starting material and oversulphated compounds showed that no depolymerization occurred during sulphation.

For n.m.r. experiments, dermatan sulphate (20 mg) was dissolved in ^2H_2O (0.3 ml); 2 M-NaCl in ^2H_2O (30 μl) was added and the pH was adjusted to 7.0 (meter-reading). After two freeze-dryings from ^2H_2O (99.95% ^2H; C.E.A., Saclay, France) the residue was dissolved in 0.4 ml of ^2H_2O and transferred to the n.m.r. tube. All experiments were performed at 500 MHz, at 310 K, with a Bruker WM500 spectrometer upgraded with an Aspect 3000 computer, a process controller and an array processor. One-dimensional spectra were obtained by co-addition of 32 scans and 16k data points. Chemical shifts of the various dermatans are given relative to internal trimethylsilyl-[$^2H_4$]propionate. Proton–proton double-quantum-correlation spectroscopy experiments were performed with the use of a delay of 20 ms to create the double-quantum coherence; 256 t_1 incremented free induction decays were co-added. The phase-sensitive n.O.e. spectroscopy experiment (Bodenhausen *et al.*, 1984; Wagner & Wüthrich, 1979) was performed with the use of a mixing time of 50 ms to avoid spin diffusion. This mixing time was chosen after an n.O.e. build-up experiment that showed that up to 200 ms the n.O.e. remains linear (Bossennec *et al.*, 1988). Quantitative analysis of uronic acid residues was performed at 330 K with a delay of 5 times the longest t_1, estimated from an inversion-recovery experiment.

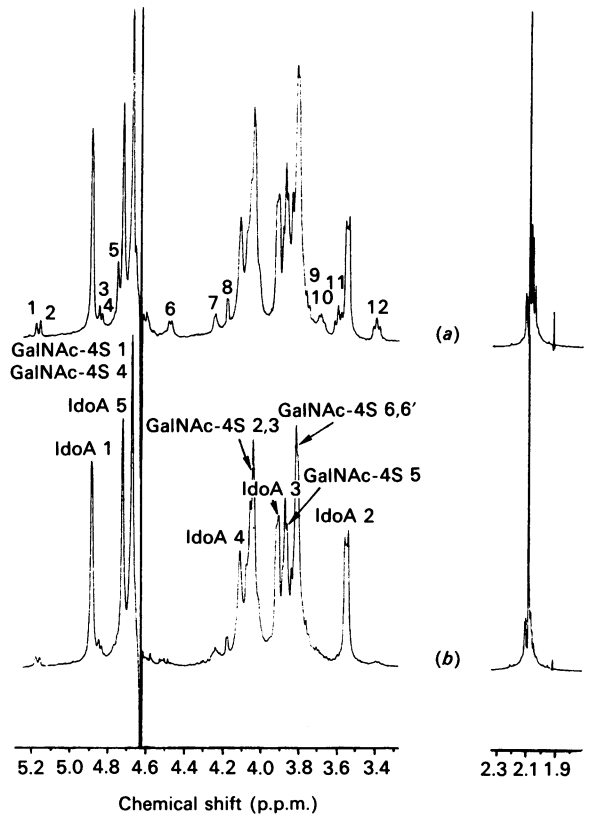

Fig. 2. One-dimensional ^1H-n.m.r. spectra of pig skin (*a*) and pig intestine (*b*) dermatan sulphate preparations

GalNAc-4S 2,3 and GalNAc-4S 6,6′ denote the H-2, H-3 and H-6,H-6′ protons of *N*-acetylgalactosamine 4-sulphate respectively. Strong coupling between H-2 and H-3 and between H-6 and H-6′ precludes a precise assignment.

RESULTS AND DISCUSSION

The ^1H-n.m.r. spectra of pig skin dermatan sulphate and pig intestine dermatan sulphate are shown in Fig. 2. Although major signals clearly indicate the presence in both preparations of the basic disaccharide unit IdoA1→3GalNAc-(4-OSO_3) shown in Fig. 1, it appears that minor signals (1 to 12 in Fig. 2) occur to different extents, thus indicating structural variations between pig skin dermatan sulphate and pig intestine dermatan sulphate. Since the signals corresponding to the basic disaccharide unit of dermatan sulphate were assigned previously (Perlin *et al.*, 1970; Gatti *et al.*, 1979; Bossennec *et al.*, 1988; Sanderson *et al.*, 1989) we paid particular attention to minor signals in order to elucidate the nature and substitution pattern of the corresponding monosaccharides. We thus had to detect correlations between these minor signals, and therefore we chose a two-dimensional approach based on the proton–proton double-quantum-correlation technique (Mareci & Freeman, 1983), which is very efficient in disclosing features of overcrowded spectra. This technique is applied here for the first time to a glycosaminoglycan. We also used two-dimensional n.O.e. spectroscopy to confirm some of the assignments, and to look at the influence of the nature of the substituents on the conformation of iduronic acid residues.

As shown on Fig. 4 for pig intestine dermatan sulphate, all the signals of iduronic acid and *N*-acetylgalactosamine can easily be localized in the two-dimensional spectrum. Direct connectivity peaks appear in pairs symmetrical with respect to the diagonal; the slope of the double-quantum diagonal is 2. The projection of the ω_2 axis is the proton spectrum. Since minor residues are

Table 1. Chemical shifts of uronic acid signals in pig skin dermatan sulphate and pig intestine dermatan sulphate and related structures

Measurements were made at pH 7 in 0.15 M-NaCl at 310 K.

	Chemical shift (p.p.m.)				
	H-1	H-2	H-3	H-4	H-5
IdoA in IdoA1→3GalNAc-(4-OSO_3)	4.85	3.51	3.62	3.95	4.64
IdoA in pig intestine dermatan sulphate (values from Bossennec *et al.*, 1988)	4.88	3.53	3.90	4.10	4.72
IdoA-(2-OSO_3) in pig intestine dermatan sulphate	5.16 (+0.28*)	4.17 (+0.64*)	4.23 (+0.33*)	4.06 (−0.04*)	4.85 (+0.13*)
IdoA-(2-OSO_3) in pig skin dermatan sulphate	5.16	4.17	4.23	4.06	4.85
GlcA in pig skin dermatan sulphate	4.47	3.38	3.58	3.78	3.66
GlcA in GlcA1→3GalNAc-(4-OSO_3)	4.49	3.38	3.48	3.55	3.67

* Difference from chemical shift with IdoA in pig intestine dermatan sulphate.

present in larger amounts in pig skin dermatan sulphate, we used this product to assign their signals.

Iduronic acid

Chemical shifts of iduronic acid protons are reported in Table 1 and can be compared with values obtained under the same conditions (pH, temperature) with the synthetic disaccharide methyl 2-acetamido-2-deoxy-3-*O*-(α-L-idopyranuronosyl)-4-*O*-sulpho-β-D-galactopyranoside (Jacquinet & Sinaÿ, 1987; Marra *et al.*, 1990).

In the region of anomeric signals, two minor signals (1 and 2 in Fig. 2) are observed at 5.18 and 5.16 p.p.m. All the signals belonging to the residue with H-1 at 5.16 p.p.m. (signal 2 in Fig. 2) are assigned through a single two-dimensional double-quantum-correlation spectroscopy experiment (Fig. 3), and chemical shifts are readily determined (Table 1). Comparison of the observed values with data reported for non-sulphated iduronic acid (Bossennec *et al.*, 1988; Sanderson *et al.*, 1989) suggests that the signals correspond to the presence of a 2-*O*-sulphated iduronic acid unit. Thus H-2 is shifted 0.64 p.p.m. downfield by sulphation of the geminal hydroxy group, and H-1 and H-3 are also shifted downfield, by about 0.30 p.p.m. H-5 is shifted 0.13 p.p.m. downfield and H-4 is shifted upfield by 0.04 p.p.m.

In the same way, the proton at 5.18 p.p.m. has identical correlation peaks with the same chemical shifts, suggesting that different units of 2-*O*-sulphated iduronic acid occur in two different types of sequences in dermatan sulphate. For this reason there are two anomeric signals, the chemical shifts of the remaining protons being practically unaffected by the sequence effect, except for H-5 [two very close H-5 signals, clearly seen in Fig. 2 (signals 3 and 4), are correlated with H-4 of iduronic acid 2-sulphate in Fig. 3].

Quantitative analysis indicates that iduronic acid 2-sulphate units are present to similar extents in pig skin dermatan sulphate and pig intestine dermatan sulphate, where they represent 10% and 6% respectively of the total uronic acid residues.

The sulphation pattern of the *N*-acetylgalactosamine units linked to 2-*O*-sulphated iduronic acid could explain the presence of the two anomeric signals observed for the latter. Indeed, previous studies on the structure of dermatan sulphate revealed the presence of non-sulphated *N*-acetylgalactosamine units (pig skin dermatan sulphate; Fransson *et al.*, 1974) as well as 6-sulphated *N*-acetylgalactosamine units (Población & Michelacci, 1986; Munakata *et al.*, 1987) and 4,6-di-*O*-sulphated *N*-acetylgalactosamine units (spleen dermatan sulphate; Munakata *et al.*, 1987). Fransson *et al.* (1974) proposed that in pig skin dermatan sulphate iduronic acid 2-sulphate units are next to non-sulphated

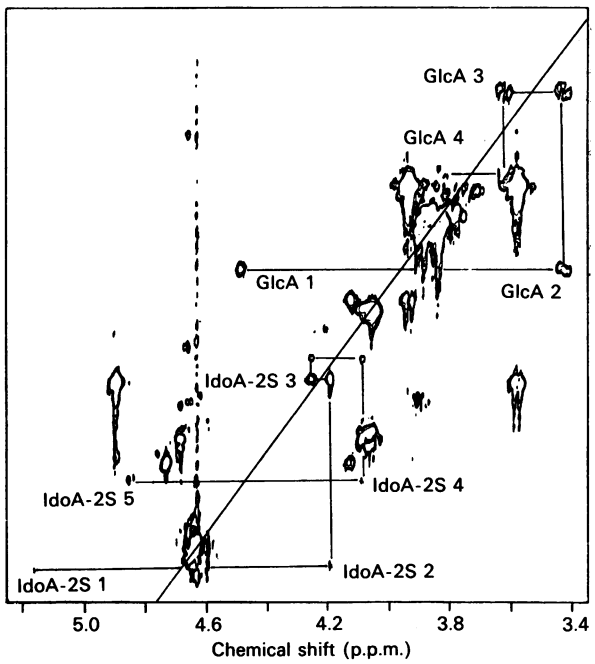

Fig. 3. Two-dimensional proton–proton double-quantum-correlation spectrum of pig skin dermatan sulphate

Lines indicate correlations for signals of iduronic acid 2-sulphate and glucuronic acid.

N-acetylgalactosamine units. Unfortunately it was impossible to check this hypothesis (for instance by an n.O.e. spectroscopy experiment) on natural compounds, where the relevant minor *N*-acetylgalactosamine signals cannot be observed in the presence of the major ones. The influence of the sulphation pattern of *N*-acetylgalactosamine units, however, appears in partially oversulphated dermatan sulphate (see below and Table 2).

Variously *O*-sulphated iduronic acid units were also studied in chemically oversulphated dermatan sulphate preparations obtained through sulphation with SO_3–pyridine complex of an alkylammonium salt of pig intestine dermatan sulphate. From what is known of the relative reactivity of the different hydroxy groups, one expected preferential sulphation at position 6 of *N*-acetylgalactosamine, followed by sulphation at positions 2 and 3 of iduronic acid residues. In fact, under smooth conditions, where only slightly more than one extra sulphate group per disaccharide unit is introduced on the molecule (OSO_3^-/CO_2^-

Table 2. Chemical shifts of uronic acid signals in chemically oversulphated dermatan sulphate derivatives

Measurements were made at pH 7 in 0.15 M-NaCl at 310 K. Abbreviation: N.D., not detected.

	Chemical shift (p.p.m.)				
	H-1	H-2	H-3	H-4	H-5
IdoA in natural pig intestine dermatan sulphate	4.88	3.53	3.90	4.10	4.72
IdoA in partially oversulphated pig intestine dermatan sulphate	4.90 (+0.02*)	3.56 (+0.03*)	3.97 (+0.07*)	4.11 (+0.01*)	4.74 (+0.02*)
IdoA-(2-OSO_3) in natural pig intestine dermatan sulphate	5.16	4.17	4.23	4.06	4.85
IdoA-(2-OSO_3) in partially oversulphated pig intestine dermatan sulphate	5.22 (+0.06†) (+0.32†)	4.19 (+0.02†) (+0.63†)	4.23 (−0.00†) (+0.26†)	N.D.	N.D.
IdoA-(3-OSO_3) in partially oversulphated pig intestine dermatan sulphate	4.99 (+0.09†)	3.75 (0.19‡)	4.77 (+0.80‡)	4.35 (+0.24‡)	~4.78 (av.)
IdoA-(2,3-di-OSO_3) in extensively oversulphated pig intestine dermatan sulphate	5.23 (+0.33‡)	4.37 (+0.81‡)	4.96 (+0.99‡)	4.29 (+0.18‡)	4.89

* Difference from chemical shift with IdoA in natural pig intestine dermatan sulphate.
† Difference from chemical shift with IdoA-(2-OSO_3) in natural pig intestine dermatan sulphate.
‡ Difference from chemical shift with IdoA in partially oversulphated pig intestine dermatan sulphate.

ratio 2:1), we obtained practically complete sulphation at position 6 of *N*-acetylgalactosamine and a mixture of iduronic acid, iduronic acid 2-sulphate and iduronic acid 3-sulphate units. Under more drastic conditions we obtained extensively oversulphated dermatan sulphate (OSO_3^-/CO_2^- ratio 3.66:1) in which iduronic acid residues were practically quantitatively converted into iduronic acid 2,3-disulphate.

The proton chemical shifts of these variously substituted units could be determined by using two-dimensional double-quantum-correlation spectroscopy experiments; they are reported in Table 2. The comparison of chemical shifts for iduronic acid and iduronic acid 2-sulphate units in natural and partially oversulphated dermatan sulphate indicated that 6-*O*-sulphation of *N*-acetylgalactosamine, i.e. the presence of 4,6-disulphated *N*-acetylgalactosamine units in partially oversulphated dermatan sulphate, does not dramatically affect proton chemical shifts of iduronic acid, either sulphated or not. The largest differences are observed for H-1 of iduronic acid 2-sulphate and H-3 of iduronic acid (0.06 and 0.07 p.p.m. respectively). Thus if iduronic acid 3-sulphate were present in natural dermatan sulphate preparations its anomeric signal would most probably appear at approx. 5 p.p.m. and the occurrence of iduronic acid 2,3-disulphate would give rise to another signal at approx. 5.2 p.p.m. On the basis of the two-dimensional double-quantum-correlation spectroscopy experiment the presence of such residues can be excluded in the preparations that we have analysed.

Glucuronic acid

The occurrence of glucuronic acid in dermatan sulphate preparations has been known for a long time, and Munakata *et al.* (1987) have suggested that 2- or 3-*O*-sulphated glucuronic acid residues could be involved in sequences able to activate the plasma protein heparin cofactor II.

The two-dimensional double-quantum-correlation spectroscopy experiment allowed us easily to localize proton signals of H-1, H-2 and H-3 of glucuronic acid residues (Fig. 3). H-4 and H-5 could be precisely located by using an n.O.e. spectroscopy experiment. The chemical shifts for this unit are reported in Table 1. The values (Table 1) compare well with data obtained under identical conditions (pH, temperature) with the synthetic disaccharide methyl 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranuronosyl)-4-*O*-sulpho-β-D-galactopyranoside (Marra *et al.*, 1990) and differ slightly from the ones usually observed for glucuronic acid protons in heparin sequences (Petitou *et al.*, 1988). The residues in the preparations that we have analysed are non-sulphated, since we found that sulphation at position 2 of methyl glucuronate induces a 0.81 p.p.m. downfield shift on H-2 and 0.16 and 0.21 p.p.m. downfield shifts at H-1 and H-3 respectively (P. Duchaussoy & M. Petitou, unpublished work). The relative intensities of signals 6, 11 and 12 (Fig. 2) are also in favour of a single kind of glucuronic acid residue.

Quantitative analysis of the signals of glucuronic acid revealed the presence of about 17% of such residues (related to total uronic acids) in pig skin dermatan sulphate, compared with 1% in pig intestine dermatan sulphate.

Galactosamine

The ^{1}H-n.m.r. spectrum of *N*-acetylgalactosamine 4-sulphate shows very strongly coupled signals, which can be assigned at high field (Sanderson *et al.*, 1989) and also on the two-dimensional double-quantum-correlation spectrum (see Fig. 4 and Table 3). As shown in Table 3, the values are in excellent agreement with those obtained for the *N*-acetylgalactosamine 4-sulphate unit in the synthetic disaccharides methyl 2-acetamido-2-deoxy-3-*O*-(α-L-idopyranuronosyl)-4-*O*-sulpho-β-D-galactopyranoside (Jacquinet & Sinaÿ, 1987; A. Marra, unpublished work) and methyl 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranuronosyl)-4-*O*-sulpho-β-D-galactopyranoside (Marra *et al.*, 1990).

Chemical sulphation at position 6 allowed us to determine the chemical shifts of H-6,6′ in 4,6-di-*O*-sulphated *N*-acetylgalactosamine units, and thus to confirm the presence of such units in pig intestine dermatan sulphate, where minor signals are observable at 4.29 and 4.25 p.p.m., coupled with a signal at 4.07 p.p.m. (Fig. 4). Welti *et al.* (1979) and more recently Holme & Perlin (1989) reported similar values for 6-*O*-sulphated *N*-acetylgalactosamine units in chondroitin sulphate. Such signals could not be seen on the two-dimensional contour plot of pig skin dermatan sulphate, suggesting that this unit is less represented in products of this origin. This observation is in agreement with data of Munakata *et al.* (1987) and Población & Michelacci (1986), who found 6-sulphated and 4,6-disulphated *N*-acetylgalactosamine units in dermatan sulphates of different origins except pig skin dermatan sulphate. Therefore, as mentioned above, the two anomeric signals of 2-sulphated iduronic acid that are observed in both pig skin dermatan sulphate and pig intestine

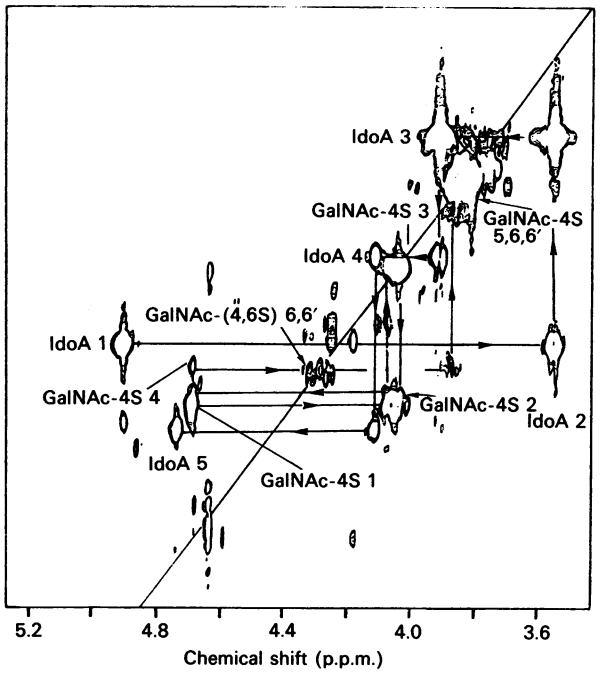

Fig. 4. Two-dimensional proton–proton double-quantum-correlation spectrum of pig intestine dermatan sulphate

Lines indicate correlations for signals of iduronic acid and *N*-acetylgalactosamine 4-sulphate. Protons H-5, H-6 and H-6′ of *N*-acetylgalactosamine 4-sulphate (denoted as GalNAc-4s 5,6,6′) and H-6,H-6′ of *N*-acetylgalactosamine 4,6-disulphate [denoted as GalNAc(4,6)S 6,6′] are not isolated on the contour plot.

Table 3. Chemical shifts of *N*-acetylgalactosamine protons in dermatan sulphate

Measurements were made at pH 7 in 0.15 M-NaCl at 310 K.

	Chemical shift (p.p.m.)						
	H-1	H-2	H-3	H-4	H-5	H-6,6′	Ac
GalNAc-(4-OSO_3) in dermatan sulphate	4.70	4.05	4.03	4.69	3.80	3.79–3.82	2.08
GalNAc-(4-OSO_3) in synthetic GlcA1→3GalNAc-(4-OSO_3)	4.49	4.05	4.10	4.80	3.84	3.82	2.03
GalNAc-(4-OSO_3) in synthetic IdoA1→3GalNAc-(4-OSO_3)	4.53	4.06	4.04	4.73	3.86	3.80–3.82	2.05

dermatan sulphate are probably not related to the presence of 6-sulphated *N*-acetylgalactosamine units.

A careful examination of the two-dimensional double-quantum-correlation spectroscopy map indicates that signal 5 (4.74 p.p.m.) may belong to H-1 of an *N*-acetylgalactosamine unit. The intensity of this signal, compared with that of glucuronic acid residues, suggests some links between them. More precisely, this signal could be induced by the sequence GalNAc→GlcA or GlcA→GalNAc.

Paper-chromatography experiments established the existence of non-sulphated *N*-acetylgalactosamine residues in pig skin dermatan sulphate (Fransson *et al.*, 1974). The present study does not allow us to draw conclusions concerning this unit.

Acetamido signals in the 2 p.p.m. region are probably influenced by the sequence. At first sight the major signal is surrounded by a downfield signal, tentatively connected to the presence of iduronic acid 2-sulphate and two or three upfield signals tentatively linked to the presence of glucuronic acid.

Conformation of the iduronic acid unit

Along with the fine structure of the polymer, local conformation might affect the biological properties of the macromolecule. The conformation of the iduronic acid unit has been extensively studied (Casu *et al.*, 1988), and molecular-mechanics computation data indicate that this unit may adopt three major conformations with comparable energies, namely $^4C_1$, $^1C_4$ and $^2S_0$ (Ragazzi *et al.*, 1986).

As a first step towards conformational analysis we report here on qualitative data of the n.O.e. spectroscopy experiments on pig intestine dermatan sulphate, partially oversulphated dermatan sulphate and extensively oversulphated dermatan sulphate that were used to analyse iduronic acid units with various patterns of sulphate substitution in different environments.

Fig. 5 shows the n.O.e. spectroscopy map for pig intestine dermatan sulphate. As expected, correlation peaks are observed for several protons (see the assignments on the Figure). Most noticeable is the strong effect between IdoA 2 and IdoA 5, characteristic of the presence of the $^2S_0$ conformer.

In contrast, analysis of the n.O.e. spectroscopy map obtained for oversulphated dermatan sulphate (Fig. 6) clearly indicates that this IdoA 2–IdoA 5 interaction is completely abolished when the iduronic acid unit is sulphated at position 2 and the only n.O.e. observed with H-2 is the H-1–H-2 interaction, in accordance with a $^1C_4$ conformation. The same observation can be made on a natural preparation of dermatan sulphate particularly rich in 2-sulphated iduronic acid. Concerning 3-sulphated iduronic acid residues, H-2 interacts with H-1 and with another proton. However, the very close chemical shifts of protons 3 and 5 in iduronic acid 3-sulphate preclude any conclusion regarding the presence of an H-2–H-5 interaction n.O.e. in this unit. Nevertheless, the absence of any n.O.e. between H-1 and H-3 is again in favour of a $^1C_4$ conformation, and the n.O.e. observed with H-2 most probably results from an H-2–H-3 interaction. Examination of molecular models and

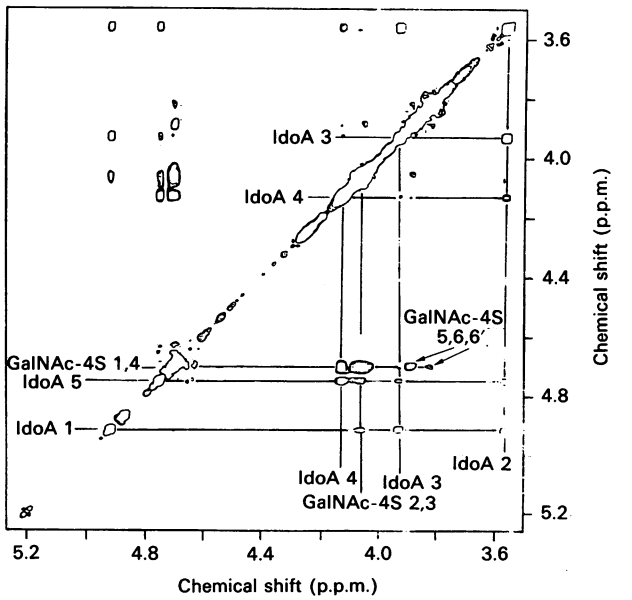

Fig. 5. N.O.e. spectrum of pig intestine dermatan sulphate

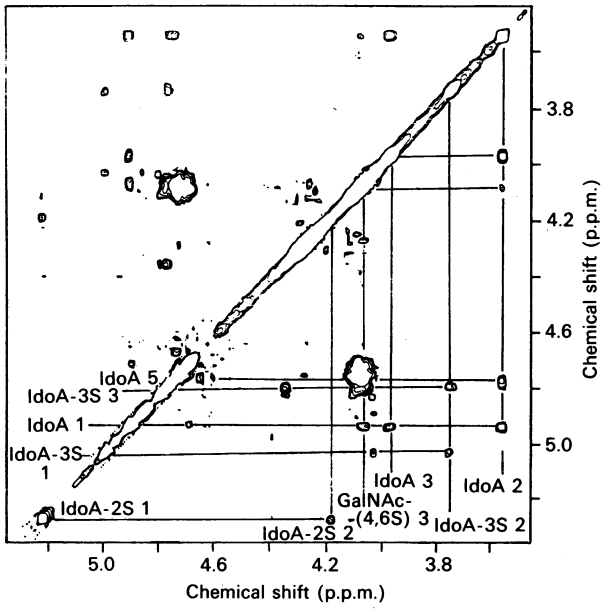

Fig. 6. N.O.e. spectrum of partially oversulphated pig intestine dermatan sulphate

n.m.r. data on persulphated oligosaccharides containing iduronic acid also support the presence of the $^1C_4$ conformer.

It also appears from Fig. 6 that in non-sulphated iduronic acid units an H-2–H-5 interaction n.O.e. is present. Thus sulphation at position 6 of *N*-acetylgalactosamine residues does not seem to influence dramatically the conformation of the neighbouring iduronic acid residues.

In extensively sulphated dermatan sulphate no n.O.e. signal is observed between protons 2 and 5, again suggesting that the iduronic acid unit precludes the $^2S_0$ conformation.

One might expect to get some sequence information involving minor (non-sulphated or 6-sulphated) *N*-acetylgalactosamine residues from the n.O.e. spectroscopy experiments. In fact, the very close chemical shifts of protons of the *N*-acetylgalactosamine units preclude the assignment of signals to uniquely substituted units of this sugar.

In conclusion, the two preparations of natural dermatan sulphate that we have analysed contain only 2-*O*-sulphated iduronic acid residues and glucuronic acid as minor uronic acid residues. *N*-Acetylgalactosamine units are more difficult to analyse and we could only detect sulphation at position 6 of some units, but we were unable to establish whether they were sulphated at position 4 or not. The striking difference in overall composition and particularly in glucuronic acid content between the two preparations clearly demonstrates that the term 'dermatan sulphate' designates a family of related molecules, the functions of which remains to be established and correlated to the above structural features.

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