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Purification and characterization of dermatan sulfate from the skin of the eel, *Anguilla japonica*

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Abstract

Glycosaminoglycans were isolated from the eel skin (*Anguilla japonica*) by actinase and endonuclease digestions, followed by a β-elimination reaction and DEAE-Sephacel chromatography. Dermatan sulfate was the major glycosaminoglycan in the eel skin with 88% of the total uronic acid. The content of the IdoA2Sα1→4GalNAc4S sequence in eel skin, which shows anticoagulant activity through binding to heparin cofactor II, was two times higher than that of dermatan sulfate from porcine skin. The anti-IIa activity of eel skin dermatan sulfate was determined to be 2.4 units/mg, whereas dermatan sulfate from porcine skin shows 23.2 units/mg. The average molecular weight of dermatan sulfate was determined by gel chromatography on a TSKgel G3000SWXL column as 14 kDa. Based on ^{1}H NMR spectroscopy, the presence of 3-sulfated and/or 2,3-sulfated IdoA residues was suggested. The reason why highly sulfated dermatan sulfate does not show anticoagulant activity is discussed. In addition to dermatan sulfate, the eel skin contained a small amount of keratan sulfate, which was identified by keratanase treatment.

Keywords: Delmatan sulfate; Eel skin; *Anguilla japonica*; ^{1}H NMR; Anti-factor; IIa activity

1. Introduction

Dermatan sulfate (DS) is a member of a family of structurally complex, sulfated (except hyaluronan), linear polysaccharides called glycosaminoglycans (GAGs).[1–3] The other members of this family of molecules are heparin, heparan sulfate, chondroitin sulfate, keratan sulfate and hyaluronan. DS and chondroitin sulfate are structurally similar and make up a subfamily of GAGs called galactosaminoglycans.[2] GAGs are often found attached to a protein core, resulting in a macromolecule called a proteoglycan.[4,5] These proteoglycans localize on cell surfaces, inside the cells, but are mainly found in the extracellular matrix.[6] Their biological functions are supposed to be the important role of cell–cell interaction, binding a variety of biologically important proteins and localizing these at cell surface.[6,7] Glycosaminoglycan DS has been used as an experimental therapeutic agent to modulate a variety of these biological processes, and now DS is one of the most important candidates for antithrombotic agent

Abbreviations: DS, dermatan sulfate; DQF, double quantum filtered; COSY, chemical shift correlated spectroscopy; ΔDi-0S, 2-acetamido-2-deoxy-3-*O*-(β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; ΔDi-4S, 2-acetamido-2-deoxy-4-*O*-sulfo-3-*O*-(β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; ΔDi-6S, 2-acetamido-2-deoxy-6-*O*-sulfo-3-*O*-(β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; ΔDi-UA2S, 2-acetamido-2-deoxy-3-*O*-(2-*O*-sulfo-β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; ΔDi-diS$_B$, 2-acetamido-2-deoxy-4-*O*-sulfo-3-*O*-(2-*O*-sulfo-β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; ΔDi-diS$_D$, 2-acetamido-2-deoxy-6-*O*-sulfo-3-*O*-(2-*O*-sulfo-β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; ΔDi-diS$_E$, 2-acetamido-2-deoxy-4,6-di-*O*-sulfo-3-*O*-(β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; ΔDi-triS, 2-acetamido-2-deoxy-4,6-di-*O*-sulfo-3-*O*-(2-*O*-sulfo-β-D-*xylo*-hex-4-enopyranosyluronic acid)-D-galactose; GAG, glycosaminoglycan; GalNAc, 2-acetamido-2-deoxy-D-galactose (*N*-acetylgalactosamine); GlcA, glucuronate; IdoA, iduronate; NHP, normal human plasma; NMR, nuclear magnetic resonance.

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approved for the prophylaxis of postoperative deep-vein thrombosis, which may lead to pulmonary embolism in patients undergoing elective hip replacement surgery.[8]

Glycosaminoglycan DS has important anticoagulant[9–11] and antithrombotic activities.[12–16] The mechanisms for these activities are complex involving soluble plasma proteins, the endothelial cell lining the vessel wall,[17] subendothelial smooth muscle cells,[18], platelets[19] and the fibrinolytic pathway.[20] The glycosaminoglycan chains of decorin, biglycan and thrombomodulin can act as anticoagulants by inhibiting thrombin, either directly through heparin cofactor II or antithrombin III, or indirectly through protein C activation.[9,21] Since these proteoglycans are found on both the luminal surface and subluminal surface, they provide a localized anticoagulant affect affording thromboresistant surfaces at sites in both the intact and damaged vessel.[22,23] Dermatan sulfate also shows antithrombotic activity by inhibiting the thrombin-induced aggregation of platelets and may activate the fibrinolytic pathway by causing the release of tissue plasminogen activator.[12,24]

The most thoroughly investigated activity associated with DS is its acceleration of heparin cofactor II mediated inhibition of thrombin.[2,13,25] The discovery of this second circulating thrombin inhibitor has focused attention from heparin to endogenous glycosaminoglycans such as DS and heparan sulfate.[26–28] Based on these findings, there are a number of future prospects for DS in clinical use. One of the interesting applications for DS is in the preparation of medical devices and artificial tissues.[29,30] New applications of DS in implantation, tissue culturing and transplantation are now under consideration.[2]

DS is routinely prepared from porcine and bovine intestinal mucosa or porcine skin.[31] However, based on the recent disaster of bovine spongeform encephalopathy (BSE), it becomes more important to find safe, natural resources for glycosaminoglycans. Because the presence of dermatan sulfate in eel skin has been reported by histochemical techniques,[31–33] this paper describes the isolation and partial characterization of dermatan sulfate from the skin of the eel (or eel skin), *Anguilla japonica*.

2. Materials

Eels were collected from a local fish market in Seoul, South Korea. The following enzymes were obtained from the commercial sources indicated: pronase (type XXV from *Streptomyces griseus*, Sigma Chemical Co., St. Louis, MO, USA), alcalase (proteolytic enzyme from *Bacillus subtilis*, 2.4L, NovoNordisk, Bagsvaerd, Denmark), endonuclease (Benzonase EC 3.1.30.2, Sigma), chondroitin ABC lyase (chondroitinase ABC from *Proteus vulgaris*, EC 4.2.2.4, Sigma), chondroitin ACII lyase (chondroitinase ACII from *Arthrobacter aurescens*, EC 4.2.2.5, Seikagaku Corporation, Tokyo, Japan), chondroitin B lyase (chondroitinase B from *Flavobacterium heparinum*, EC 4.2.2) and heparinases I, II and III from *Flavobacterium heparinum* (Seikagaku Corporation). Alcian blue were obtained from Sigma. Heparin/dermatan sulfate disaccharide standards were from Seikagaku Corporation. Dialysis membrane for desalting was from Spectrum Medical. DS and heparin from porcine intestinal mucosa were from Celsus Laboratory, Cincinnati, OH, USA. DS from porcine skin was kindly gifted from Taiho Pharmaceutical Co. Ltd., Tokushima, Japan. All other reagents used were analytical grade. UV spectroscopy was performed on a Shimadzu model UV 160 spectrometer equipped with a thermostated cell.

3. Methods

3.1. Isolation of GAGs

3.1.1. Extraction. Eel skin (500 g) was cut into small pieces that were homogenized in a homogenizer in 3 L of ice-cold acetone, and after decantation overnight at 4 °C, the resulting supernatant was discarded. The pellet was suspended in 3 L of ice-cold 2:1 chloroform–methanol and was then re-homogenized and decanted. The extraction with chloroform–methanol was performed three times. The defatted material was finally washed with cold ethyl ether and dried overnight. The residue was weighed to obtain dry weight of defatted material (120 g).

3.1.2. Digestion with pronase. Defatted tissue (100 g) was digested with 1 g of pronase in 1 L of 0.1 M Tris-HCl buffer containing 2 mM $CaCl_2$, 3% ethanol (pH 7). After 48 h of incubation at 50 °C, the digest was heated at 100 °C for 5 min. The sample was cooled and centrifuged at $1,500 \times g$ for 20 min, and the resulting pellet was discarded. The supernatant was diluted to 1 L with the same buffer and applied on a DEAE Sephacel anion-exchange column (4×30 cm) eluted with 3 column volumes of water, 3 column volumes of 3% and 16% aq sodium chloride solution. Fractions obtained in the 3% and 16% sodium chloride washes were precipitated with 80% of ethanol, centrifuged at $2,000 \times g$ for 20 min, combined and freeze-dried overnight to afford 2.2 g of crude peptidoGAG/GAG mixture.[34,35]

3.1.3. Digestion with endonuclease and β-elimination. For degradation of nucleic acids, a sample (2.2 g crude peptidoGAG/GAG mixture) was dissolved in 300 mL

of 0.05 M Tris-HCl buffer, pH 7, 1 mM $MgCl_2$, 3 mM sodium acetate containing 25,000 units of endonuclease. The mixture was incubated at 37 °C for 15 h, and the reaction was terminated by heating at 100 °C for 1 h.[35] The mixture was then treated with 0.5 M NaOH containing 0.3 M sodium borohydride (20 mL/g) at 4 °C for 12 h. The mixture was then neutralized with 2 M HCl and concentrated to one-tenth volume by evaporation. The protein/peptide residues were removed by addition of perchloric acid (final concentration, 5%), followed by centrifugation at $10,000 \times g$ for 60 min at 4 °C. The supernatant was dialyzed against water at 4 °C for 12 h, and the GAGs were precipitated by addition of cetylpyridinium chloride (CPC, final concentration, 0.1%) containing 0.03 M NaCl for 3 h at 4 °C. The GAG–CPC complex was collected by centrifugation at $2,300 \times g$ for 15 min. The precipitate was washed twice with 0.1% CPC. GAGs were extracted from the GAG–CPC complex by addition of 2.5 M NaCl, and the mixture was centrifuged at $2,300 \times g$ for 15 min. GAGs were precipitated from the supernatant by addition of 11 vol. of 85% ethanol for 16 h at 4 °C and were collected by centrifugation at $2,300 \times g$ for 15 min. Finally, the collected GAGs were dissolved in water (25 mL), dialyzed against water, and freeze-dried to afford 400 mg GAG mixture.[35,36]

The GAG-containing mixture (200 mg in 150 mL of water) was applied to anion-exchange chromatography on a DEAE Sephacel column (2×20 cm, obtained from Pharmacia, Sweden) and eluted with 2 column-volumes of 0.5 M NaCl, 1 M, 1.2 M, 1.4 M and 1.6 M NaCl. Fractions obtained in each of these washes were precipitated with 85% of ethanol, and then centrifuged at $1,500 \times g$ for 20 min and freeze-dried. Four fractions affording ethanol precipitates were obtained: 1. 0.5 M NaCl fraction, 22 mg; 2. 1.0 M NaCl fraction, 64 mg; 3. 1.2 M NaCl fraction, 12 mg; and 4. 1.4 M NaCl fraction, 8 mg.[36]

Each fraction was analyzed by electrophoresis on cellulose acetate membrane using 1 M pyridine–acetic acid buffer (pH 3.5) at 0.5 mA/cm for 20 min. GAGs were stained by 0.5% Alcian Blue in 0.3% acetic acid.[37]

3.2. Characterization of GAGs

3.2.1. Enzymatic depolymerization of GAGs. A solution containing 20 μg of each carbohydrate (as weight) containing fraction in 10 μL of 50 mM sodium phosphate buffer at pH 7.6 for chondroitin B lyase treatment.[38,39] Chondroitin B lyase (1 mU) in 1 μL of the same buffer was added to the DS substrate solution, and the reactions were incubated in polyethylene vials at 37 °C in a water bath that was gently shaken. After 12 h of incubation, the reaction was stopped and analyzed by PAGE.

3.2.2. Gradient PAGE analysis. Gradient polyacrylamide gel electrophoresis (PAGE) was performed on a 10-cm vertical slab gel unit, from Atto Scientific Instruments (Tokyo, Japan), equipped with a Model 1420B power source from Bio-Rad (Richmond, CA).[40] Polyacrylamide linear gradient resolving gel plates (10×12 cm, 5–10% acrylamide) were prepared and run as previously described.[40] The molecular sizes of the purified carbohydrates were determined by comparing with commercially available DS standards.

3.2.3. ^{1}H NMR analysis. For ^{1}H NMR spectroscopy, approximately 5 mg of each sample was freeze-dried from 0.5-mL portions of ^2H_2O (99.6%, Sigma, St. Louis, MO), to exchange the labile protons with deuterium. The thoroughly dried sample was redissolved in 0.7 mL of ^2H_2O (99.6%), and transferred to an NMR tube. All spectra were determined on a JEOL ECP-600 spectrometer equipped with a 5-mm triple resonance tunable probe with standard JEOL software at 298 K on 700-μL samples at 0.1–0.5 mM. The HOD signal was suppressed by presaturation during 3 s.[41]

3.2.4. Disaccharide composition analysis by HPLC. Samples were each dissolved in 20 μL of 50 mM sodium acetate buffer, pH 7.6, and chondroitin ACII and ABC lyases (1 munit (mU) each) were added together for determination of the unsaturated disaccharide composition of DS. On the other hand, samples were dissolved in 10 μL of 50 mM sodium phosphate buffer (pH 7.1) containing 100 mM NaCl, and treated with the mixture of heparin lyases I, II and III (0.01 unit each). The digestion mixtures were kept in a 37 °C water bath overnight. Unsaturated disaccharides produced enzymatically from DS and heparin–heparan sulfate were determined by a reversed-phase ion-pair chromatography with sensitive and specific post-column detection. A gradient was applied at a flow rate of 1.5 mL/min on a Senshu Pak Docosil (4.6×150 mm) at 60 °C.[42,43] The eluents used were as follows: A, H_2O; B, 0.2 M sodium chloride; C, 10 mM tetra-*n*-butylammonium hydrogen sulfate; D, 50% acetonitrile. The gradient program was as follows: 0–10 min, 1–4% eluent B; 10–11 min, 4–15% eluent B; 11–20 min, 15–25% eluent B; 20–22 min, 25–53% eluent B; 22–29 min, 53% eluent B; equilibration with 1% B for 20 min. The proportions of eluent C and D were constant at 12 and 17%, respectively. To the effluent were added aqueous 1.0% (w/v) 2-cyanoacetamide solution and 0.30 M sodium hydroxide at the same flow rate of 0.35 mL/min by using a double plunger pump. The mixture passed through a reaction coil (internal diameter, 0.5 mm; length, 10 m) set in a dry reaction temperature controlled bath at 125 °C and a cooling coil (internal diameter, 0.25 mm; length, 3 m) that followed. The effluent was monitored fluorometrically (excitation, 346 nm; emission, 410 nm).

3.2.5. High-performance size-exclusion chromatography (HPSEC) for molecular weight of DS[44]. The HPSEC system consisted of a Jasco 980-PU pump (Nihon-bunko, Co., Tokyo) and Rheodyne 7725i loop injector (USA), and a conductivity detector (CM-8, TOSOH Co., Tokyo), equipped with platinum needle electrodes (0.1 mm diameter × 1 mm long) spaced 1 mm with a cell volume of 20 μL. A TSK-GEL G3000SWXL (6 μm, 7.8 mm i.d. × 300 mm, TOSOH Co., Japan) column connected with a Dowex 50W-X8 suppressor column (200–400 mesh, H^+ form, 5.0 mm i.d. × 200 mm) was used at 30 °C. The mobile phase, comprised of 5 mM borate (pH 7.0 adjusted by 10 mM NaOH), was delivered at a flow rate of 0.5 mL/min. A Hitachi integrator D-2500 was used to acquire and analyze the data.

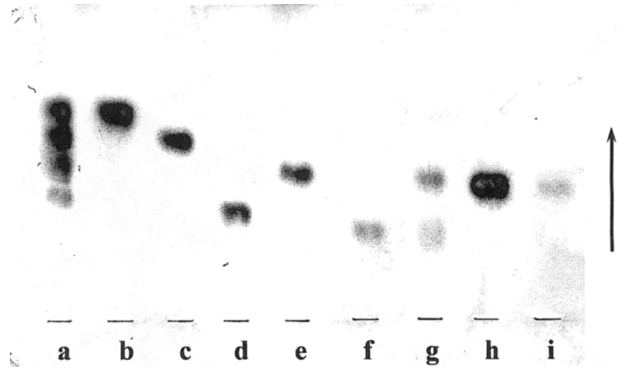

Fig. 1. Electropherogram of partially purified glycosaminoglycans from eel skin on cellulose acetate membrane. *Lanes from left to right*: a, a mixture of heparin (HP), chondroitin sulfate (CS), dermatan sulfate (DS) and hyaluronan (HA) (from top to bottom); b, HP; c, CS; d, HA; e, DS; f–i, fractions 1–4 separated on DEAE Sephacel column chromatography. The arrow indicates the direction of electrophoretic migration.

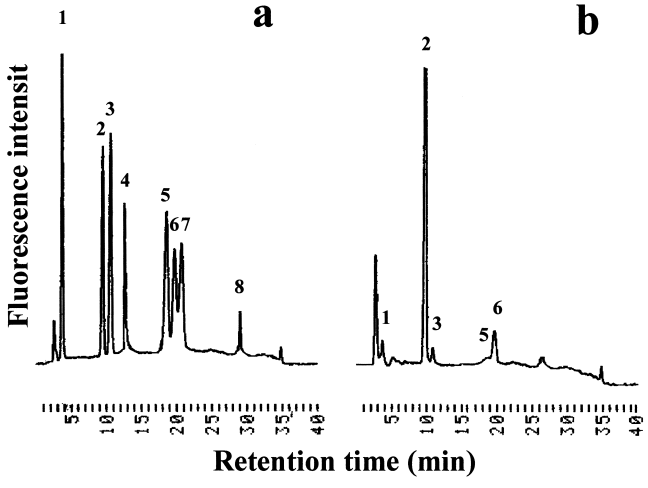

Fig. 2. Disaccharide analyses of dermatan sulfate *Panels:* a, the unsaturated disaccharide standards b, Fraction 3 treated with chondroitin ABC lyase. *Peaks:* 1, ΔDi-0S; 2, ΔDi-4S; 3, ΔDi-6S; 4, ΔDi-UA2S; 5, ΔDi-S_E; 6, ΔDi-S_B; 7, ΔDi-S_D, 8, ΔDi-triS.

3.2.6. Anti-factor IIa activity. Normal human plasma (NHP) was collected from healthy volunteers. Anti-factor IIa activity was measured by incubating 50 μL of eel skin DS (1 ∼ 100 μg/mL) in 50 mM Tris-HCl buffer containing 227 mM NaCl, pH 8.3 with 30 μL of NHP and 20 μL of human thrombin (1.2 NIH units/mL) at 25 °C for 30 s. Chromozym TH (tosyl-glycyl-prolyl-arginine-4-nitanilide acetate) 50 μL (1.9 μmol/mL) was added, and the amidolytic activity of thrombin was determined at 405 nm. A Shimadzu UV–VIS spectrophotometer model 1200 (Shimadzu Co., Japan) was used, and activity was calculated in comparison with heparin (172 units/mg) purchased from Sigma.[45,46]

4. Results and discussion

4.1. Purification

Homogenization of 500 g of a skin of eel in acetone and subsequent extraction in 2:1 chloroform–methanol resulted in 120 g (24%) of defatted tissue. Defatted tissue was next subjected to proteolytic digestion, followed by a reductive β-elimination reaction. The supernatant obtained from on protease digestion was diluted and adsorbed on to a DEAE Sephacel column. Elution with three column volumes of water and one column volume of NaCl solutions, followed by precipitation with 80% ethanol, afforded a total of 2.2 g of GAG-containing fractions. Nucleic acids were removed through exhaustive digestion with endonuclease. Anion-exchange chromatography of this enzyme-treated sample afforded uronic acid-containing fractions eluting with 0.5 M, 1 M, 1.2 M and 1.4 M sodium chloride. Analysis of the fractions by cellulose acetate membrane visualized with Alcian Blue revealed the presence of negatively charged species migrating in each of the samples, including two bands corresponding to the migration positions of hyaluronan/keratan sulfate and dermatan sulfate standards (Fig. 1). A band, which was negative against the carbazole assay as determined by absorbance at 205 nm, was eluted in the void volume of the 0.5 M fraction. On the other hand, peaks eluted in the 1.0, 1.2 and 1.4 M fractions were positive against carbazole assay. In total, four negatively charged fractions in which one was negative and the others were positive against carbazole assay, were obtained (fractions 1–4), and each was dialyzed to remove salt and then freeze dried. Fraction 1, which was supposed to be keratan sulfate, was treated with keratanase,[47] and fractions 2, 3 and 4, which were supposed to be DS, were treated with chondroitin B lyase, and these samples were analyzed by the PAGE (data not shown). Fig. 2 shows disaccharide analysis with chondroitin ABC lyase digestion of the standard DS from porcine skin and the major DS fraction (fraction 3) from eel skin in this study.

Table 1
Disaccharide composition of dermatan sulfate from eel skin

	MW	anti-IIa	Unsaturated disaccharide (%)							
	Da	unit/mg	ΔDi-0S	ΔDi-4S	ΔDi-6S	ΔDi-S_E	ΔDi-S_B	ΔDi-S_D	ΔDi-triS	SO_3 − /disaccharide
porcine skin	19,000	23.2	4.8	91.1	n.d.	n.d.	4.2	n.d.	n.d.	0.995
porcine intestine	21,000	8.6	6.2	87.1	12.1	4.4	2.3	n.d.	n.d.	1.126
eel skin	14,000	2.4	6.0	81.3	n.d.	0.2	12.4	n.d.	n.d.	1.065

n.d.: not detected.

DS can be degraded enzymatically by using bacterial chondroitin lyases. Chondroitin ACII lyase acts primarily on chondroitin sulfate, and chondroitin ABC lyase cleaves both chondroitin sulfate and DS and chondroitin B lyase acts only on DS. In routine analysis, chondroitin B lyase is used to confirm the presence of DS and distinguish it from chondroitin sulfate. All the fractions were analyzed by PAGE before and after treatment with chondroitin B lyase. Three of the fractions were very sensitive to chondroitin B lyase fractions 2, 3 and 4. On the other hand, the GAG found in fraction 1, was significantly sensitive to keratanase; however, the amount of recovered GAG from fraction 1 was not enough for further characterization. The complete characterization of this GAG will be reported in the near future.

4.2. Characterization

4.2.1. Disaccharide Compositional Analysis. A common approach for structural analysis of DS has been to characterize the disaccharide structures following its complete enzymatic depolymerization.[39,48] The digestion of DS with the mixture of chondroitin ABC and ACII lyases typically leads to complete conversion of DS to disaccharides. Table 1 summarizes disaccharide composition, molecular weight and anti-IIa activity of DS from eel skin and from porcine skin and intestinal mucosa. The amount of the disaccharide unit IdoA2Sα1→3GalNac4Sβ, which is known to be the binding site to heparin cofactor II,[9–12] in DS from eel skin, is much higher than those of porcine skin and intestinal mucosa.

It is very interesting that even though DS with high content of ΔDi-S_B disaccharide units from eel skin shows less anti-IIa activity compared to those of porcine DS samples (Table 1). It is well understood that the binding and active site of dermatan sulfate through heparin cofactor II contains the contiguous IdoA2Sα1→3GalNac4Sβ sequence,[25] and at least four repeating units of this sequence are required for anti-IIa activity. Furthermore, the importance of IdoAα1→3GalNac4S,6S for the binding to heparin cofactor II is speculated.[25] The result shown in Table 1 suggests that (i) the IdoA2Sα1→3GalNac4Sβ units in DS from eel skin may be delocalized, and there is no contiguous sequence of the disaccharide unit, or (ii) a lack of the IdoAα1→3GalNac4S,6S unit in dermatan sulfate from eel skin may cause the low affinity of the DS chain for heparin cofactor II. A detailed study of this point on the DS chain from eel skin will be published in the near future. On the other hand, no unsaturated disaccharides obtained by heparin I, II and III lyases could be detected for fractions 2, 3 and 4 (data not shown), as had been previously suggested.[33]

4.2.2. NMR spectroscopy. ^{1}H NMR analysis of dermatan sulfate fractions provided a rapid, semi-qualitative and quantitative assay of each monosaccharide in the sample. The spectra also yielded information on the sulfate ester substitutions of the intact DS. The 600-MHz 1D ^{1}H NMR spectra of the purified DS and commercially available DS standards were acquired to analyze their structure (Fig. 3).

Detailed structural analysis has been previously reported of different DS species showing various features of chain organization and composition.[49] Knowledge of the variations in composition and organization of DS from different sources is becoming increasingly important to elucidate the relationships between the structure of dermatan sulfate and its biological function. The DS sample from eel skin shows a 1D ^{1}H NMR spectrum that is DS, and also suggests that eel skin DS might be slightly different from commercially available DS (Fig. 3). The monosaccharide composition of eel skin DS was estimated by the integration of the reporter signals of sulfated and unsulfated uronates and *N*-acetylgalactosamine (GalNAc) residues, which were observed at the isolated region in the spectra. The spectrum of eel skin DS shows that the *N*-acetylgalactosamine residues

in DS are 4-*O*-sulfonated based on the H-4 signal at 4.65 ppm.[41] The integration of the H-2 signals of unsulfated IdoA and GlcA at 3.53 and 3.40 ppm, respectively, shows the major uronate residue of DS is IdoA.[39,41] The signal at 5.22 ppm is assigned to the anomeric proton of the IdoA2S residue in eel skin DS. Interestingly, the presence of the H-1 signals at 5.2–5.4 ppm may suggest that eel skin DS may contain unusual, highly sulfated IdoA residues such as IdoA3S and/or IdoA2S3S. Based on both NMR spectra and the disaccharide analyses, eel skin contains a highly sulfated region. Because highly sulfated sequences found in eel skin are known to be inactive with chondroitin lyase ABC or B treatment, 2D NMR experiments were performed for eel skin DS to assign unusual signals. The eel skin DS was independently treated with chondroitinases ABC and B, and the molar ratio of the disaccharide amount produced by each enzyme was 1.0:0.88. However, no other oligosaccharides were detected by PAGE (data not shown). The spectrum obtained by the 2D double quantum filtered chemical shift correlation spectroscopy (DQF-COSY) in Fig. 4 clearly shows that the spin network from each H-1 signal at 5.2–5.4 ppm can be attributed to H-1 of 3-sulfated and 2,3-disulfated IdoA as expected. To our knowledge, this is the first report on dermatan sulfate containing 3-sulfated IdoA residues, whereas the presence of 2- and 2,3-disulfated IdoA residues have been reported previously.[50–52] Eel skin might be useful, not only as a source of DS for clinical use, but also for studies of biological synthesis and enzymatic action on DS.

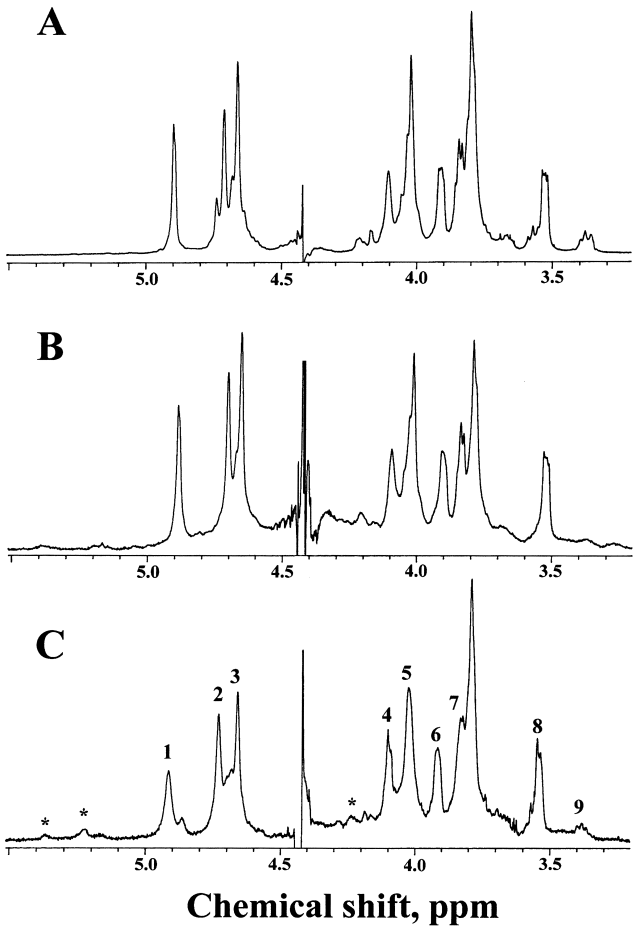

Fig. 3. One-dimensional ^{1}H NMR spectra of dermatan sulfate obtained from eel skin. A, dermatan sulfate from porcine skin; B, dermatan sulfate from porcine intestinal mucosa; C, dermatan sulfate from eel skin. *Signals:* 1, IdoA H-1; 2, IdoA H-5; 3, GalNAc4S H-1 and H-4; 4, IdoA H-4; 5, GalNAc4S H-2 and H-3; 6, IdoA H-3; 7, GalNAc4S H-5; 8, IdoA H-2; 9, GlcA H-2.

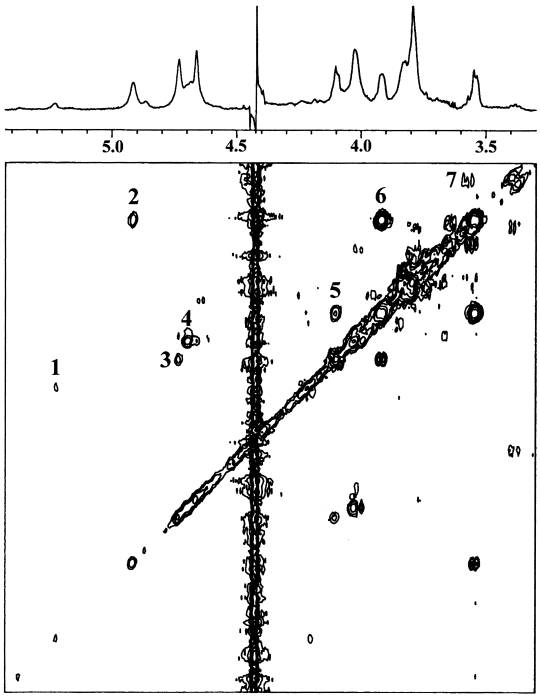

Fig. 4. Two-dimensional DQF-COSY spectrum of dermatan sulfate obtained from eel skin. *Cross peaks:* 1, IdoA2S H-1/H-2; 2, IdoA H-1/H-2; 3, IdoA H-4/H-5; 4, GalNAc4S H-1/H-2, and GalNAc4S H-3/H-4; 5, IdoA H-3/H-4; 6, IdoA H-2/H-3; 7, GlcA H-2/H-3.

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