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CHARACTERIZATION OF THE PRODUCTS GENERATED FROM OVERSULPHATED DERMATAN SULPHATE ISOMERS WITH CHONDROITINASE-B BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Characterization with specific chondroitinase-B of the constituents of the unsaturated sulphated disaccharides generated from variously sulphated dermatan sulphate (DS) isomers in human kidney tissues was carried out by high-performance liquid chromatography, using a sulphonized styrene–divinylbenzene copolymer. The products derived from the variously sulphated DS isomers with the chondroitinase-B at specific unsaturated disaccharides were characterized as unsaturated mono-, di- and trisulphated disaccharides. The results suggest that chondroitinase-B converts DS isomers into unsaturated 4-sulphated disaccharide, unsaturated disulphated disaccharides types B and H (ΔDi-diS_B and ΔDi-diS_H) and unsaturated trisulphated disaccharide (ΔDi-triS), which possess sulphate linkage(s) at position 4 of the galactosamine residues and, in addition, at position 6 of galactosamine and/or position 2 of iduronic acid. The ΔDi-diS_B, ΔDi-diS_H and ΔDi-triS were found for the first time in human kidney tissue.

INTRODUCTION

Dermatan sulphate (DS) isomers, among other glycosaminoglycans (GAGs), exhibit some heterogeneity as to their sulphate content, the positions of sulphates and their repeated chain structures [1–5]. In the past decade, it has become evident that DS and oversulphated DS isomers play important roles in fibrotic and repair processes, in mucopolysaccharidoses and in certain physiological functions [6–10]. The highly heterogeneous structures of DS isomers make it difficult to identify variously sulphated DS isomers by means of conventional electrophoretic procedures [8–10].

Chondroitinase-B is an enzyme that exclusively degrades DS into its constitutional saccharide units but does not attack other chondroitin sulphate (Ch-S)

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isomers [11,12]. However, it remains to be determined whether chondroitinase-B specifically attacks DS only or if it also digests related DS isomers.

High-performance liquid chromatography (HPLC) has been used for the characterization and quantification of unsaturated disaccharides (ΔDi-S) generated from both DS and Ch-S isomers with chondroitinases-ABC and -AC [13–19]. Satisfactory characterization and separation of unsaturated monosulphated, disulphated and trisulphated disaccharides (ΔDi-monoS, ΔDi-diS and ΔDi-triS) generated from variously sulphated DS and Ch-S isomers were performed by HPLC with a sulphonized styrene–divinylbenzene copolymer [17,18]: DS was indirectly estimated by subtraction of the amount of ΔDi-4S after digestion with chondroitinase-AC from that with the ABC-lyase.

This paper reports the direct enzymatic analysis, after chondroitinase-B digestion, of human kidney DS isomers by the above-mentioned HPLC method. The results for the products generated from DS isomers on digestion with chondroitinase-B show that the ΔDi-S generated with the B-lyase, in comparison with AC- and ABC-lyases, were ΔDi-4S, ΔDi-diS_H, ΔDi-diS_B and ΔDi-triS. Thus, the characterization of heterogenous DS isomers could be achieved at the constitutional disaccharide level.

EXPERIMENTAL

Enzymes and unsaturated disaccharides

Reference unsaturated non-sulphated, 4-sulphated and 6-sulphated disaccharides (ΔDi-0S, ΔDi-4S and ΔDi-6S) were prepared from chondroitin, chondroitin 4-sulphate (Ch-4S) and chondroitin 6-sulphate (Ch-6S), using chondroitinase-ABC in Tris buffer, pH 7.8 [2,5]. The reference unsaturated non-sulphated disaccharide (ΔDi-$0S_{HA}$) from hyaluronic acid (HA) was obtained by digestion with chondroitinase-AC in phosphate buffer, pH 6.0 [2,5]. Chondroitinase-B, which was prepared from *Flavobacterium heparinum* by the method reported previously [12], specifically degrades DS isomers [12]. Chondroitinase-ABC (EC 4.2.2.4), chondro-4-sulphatase (EC 3.1.6.9) and chondro-6-sulphatase (EC 3.1.6.10) were obtained from *Proteus vulgaris* [2,5], and chondroitinase-AC (EC 4.2.2.5) from *Fl. heparinum* [2,5]. DS, chondroitin, Ch-4S and Ch-6S, and the corresponding enzymes were prepared at the Tokyo Institute of Seikagaku Kogyo (Tokyo, Japan). Chondro-2-sulphatase was prepared from *Fl. heparinum* [19]. Chondroitin sulphate B (Ch-S_B) [20] was prepared from cockscombs. ΔDi-triS was synthetically produced through the sulphation of ΔDi-diS_B. Chondroitin sulphate D (Ch-S_D) was prepared from shark cartilage [21]. ΔDi-diS_B and ΔDi-diS_D, generated, respectively, from Ch-S_B and Ch-S_D, and ΔDi-triS were provided by Seikagaku Kogyo. Chondroitin sulphate E (Ch-S_E) from squid cartilage was a kind gift from Professor N. Seno [22]. Chondroitin sulphate G (Ch-S_G) was obtained from human aorta; its main constituent is ΔDi-diS_G [23]. An oversulphated DS, which was prepared from kidney tissue [7], comprises chondroitin sulphate H (Ch-S_H) [24].

The oversulphated DS and Ch-S isomers were degraded completely with chondroitinase-ABC or -AC to the corresponding ΔDi-diS. These ΔDi-monoS, ΔDi-

diS and ⊿Di-triS were purified by column chromatography and by high-voltage electrophoresis as reported previously [23]. The ⊿Di-S family thus prepared gave single spots on paper chromatography. Such ⊿Di-triS, ⊿Di-diS and ⊿Di-monoS were desulphated by digestion with chondro-4- and chondro-6-sulphatases, as well as chondro-2-sulphatase, to generate the corresponding lower sulphated ⊿Di-S. Chondroitinase-B exclusively digested DS and its isomers (ca. 100 μg) with 0.1 U in 0.024 *M* phosphate buffer (pH 7.5) at 37°C for 4 h. DS (or Ch-S) compounds (ca. 100 μg) were extensively digested with 0.2 U of either chondroitinase-ABC in 0.1 *M* Tris buffer (pH 7.8) or the AC-lyase in Tris buffer (pH 6.0) at 37°C for 120 min [2,5,8–10]. Extensive digestion of ⊿Di-S (20 μg) with sulphates at position 4 and/or 6 of galactosamine residues with 0.1 U of chondro-4- or -6-sulphatase was carried out in 0.1 *M* Tris buffer (pH 8.0) at 37°C for 120 min [2,5,8–10]. Chondro-2-sulphatase digested the substrates in 0.05 *M* phosphate buffer, pH 6.7, at 37°C for 60 min [19]. Methanol, acetonitrile, formic acid and other chemicals of HPLC grade were purchased from Wako Fine Chemicals (Tokyo, Japan).

Electrophoresis of macromolecular GAGs

Electrophoretic separation of the GAG compounds was carried out before and after chondroitinase digestion on cellulose acetate strips in three buffers, as reported previously [2,8–10]: 0.1 *M* pyridine–formic acid (pH 3.0) at 0.5 mA/cm for 50 min; 0.1 *M* calcium acetate at 0.5 mA/cm for 180 min; and 0.1 *M* barium acetate at 5 V/cm for 180 min. The GAGs thus separated were characterized by staining with Alcian Blue followed by destaining with 0.2 *M* acetic acid.

Dowex column chromatography

Kidney GAGs were fractionated by Dowex 1-X2 column (45×1 cm I.D.) chromatography with increasing sodium chloride molarity, in 0.25 *M* increments, to separate the DS isomers together with other GAGs [8–10]. After desalting by passage through Sephadex G-10, the GAGs were analysed.

HPLC apparatus and procedures

HPLC analyses were carried out as previously reported [17,18]. A Model 803D HPLC apparatus (Toyosoda, Tokyo, Japan) was used as the solvent-delivery system. Detection of ⊿Di-S peaks was carried out at 232 nm with a detector (Model SS 5600; Senshu Science, Tokyo, Japan). An automatic chromatographic analyser (Model CC 11; System Instruments, Tokyo, Japan) and a recorder (Type SS 250F; Sekonic, Tokyo, Japan) were employed for measurement of retention times, peak heights and peak areas. For the separation of individual ⊿Di-S, a Shodex RS (Type DC-613) ion-exchange chromatography column, packed with a fully porous ion-exchange resin (Na^+) composed of a sulphonized styrene–divinylbenzene copolymer [17,18], was purchased from Showa Denko (Tokyo, Japan). The resin, with a particle size of 6 μm, was packed into a stainless-steel column (150×6 mm I.D.), which was connected to a guard column (46×6 mm I.D.).

The analysis of ⊿Di-S by HPLC was carried out by a modification [18] of the

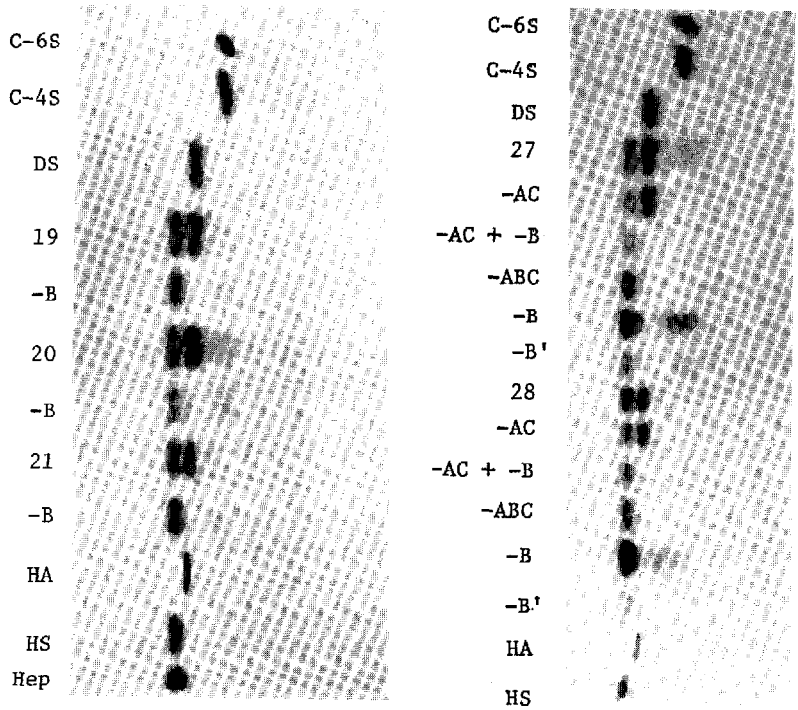

Fig. 1. Electrophoretic pattern of GAGs prepared from human kidney tissue on cellulose acetate membranes in barium acetate buffer before and after digestion with chondroitinase-B, in comparison with that with the ABC- and AC-lyases. Note that the band corresponding to reference DS was completely eliminated by digestion with chondroitinase-B. The main bands remaining undigested with the B-lyase were those of HS, followed by a thin band of HA and diffuse bands of Ch-S isomers. On digestion with chondroitinase-AC, the bands corresponding to reference Ch-S isomers were degraded whereas two bands corresponding to standard HS and DS remained undigested. On digestion with both chondroitinase-AC plus -B or the ABC-lyases, all bands corresponding to reference Ch-S and DS were degraded and a single band corresponding to standard HS remained undigested. 19, 21, 27 and 28 are the numbers of GAGs in the kidney samples, the data for the "sample 27" being presented in the following figures and tables. -B, -AC and -ABC are abbreviations for chondroitinase-B, -AC and -ABC, respectively. -B′ indicates the same treatment as -B, but the amount is less than that in the case of -B.

previously reported method [17]. Each ΔDi-S (0.5–10 μg aliquot per 10–20 μl) was injected into the Shodex RS column and chromatographed at a flow-rate of 1.0 ml/min with a mobile phase of acetonitrile–methanol–0.8 *M* ammonium formate (pH 4.5) (65:15:20, v/v/v). The composition of the mobile phase was kept constant with the use of a Degasser ERC-3110 (Erma Optical Works, Tokyo, Japan). The present HPLC separation was carried out at 70°C at 15 kg/cm^2 for 30 min. The eluate was monitored as to the UV absorbance at 232 nm, and the signals were automatically recorded with a recorder at a chart speed of 2.5 or 5 mm/min for determination of the retention times, peak heights and peak areas. On occasions, conventional HPLC on a silica gel column was also carried out as reported previously [17,18].

RESULTS

Electrophoresis

The products generated from kidney DS isomers with chondroitinase-B were compared with those obtained with other chondroitinases by means of electrophoretic characterization (Fig. 1). GAGs from human kidney showed two major bands corresponding to HS and DS, with a diffuse band corresponding to reference Ch-S isomers (Fig. 1). HS accounted for approximately one third of the total GAGs, but was not investigated further in this study. On treatment with the specific chondroitinase-B, the single DS band disappeared completely. This digestibility of the GAGs with the enzyme was also confirmed by the separation in different buffer solutions for electrophoretic characterization (not shown in the figure). The degradation of DS isomers with chondroitinase-ABC, and the B-plus AC-lyases, was identical, as judged on electrophoretic comparison. Thus, the electrophoretic findings indicate that the chondroitinase-B digests exclusively DS isomers.

High-performance liquid chromatography

With the described HPLC system, reference ⊿Di-monoS, ⊿Di-diS and ⊿Di-triS, and the possible occurrence of additional peaks due to the presence of impurities were examined. The results showed that each reference ⊿Di-S gave a single peak without any contaminants. The retention times of the individual ⊿Di-S generated from DS and Ch-S isomers of human kidney cortex were similar to those reported previously [18]. The optimal mobile phase composition was found to be 65:15:20 (v/v/v) acetonitrile–methanol–0.8 *M* ammonium formate (pH 4.5) [18], when ⊿Di-$0S_{HA}$ derived from HA was present among DS and Ch-S isomers of human kidney tissue. The individual ⊿Di-S were sufficiently resolved and their retention times were reproducible even when the HPLC assay was performed on different days: the difference was less than 0.5%.

The present HPLC results showed that a few peaks appeared following the generation of DS isomers with chondroitinase-B: the main peak corresponded to ⊿Di-4S, and the intermediate peaks to ⊿Di-diS_B and ⊿Di-diS_H (chromatogram not shown in the figure). The proportions of ⊿Di-4S, ⊿Di-diS_B and ⊿Di-diS_H derived from DS isomers were ca. 10:2.5:1. The ⊿Di-S generated from the kidney DS and Ch-S isomers with chondroitinase-B were digested further with chondroitinase-ABC, which digested all the DS and Ch-S isomers into their constituent disaccharide units. The observation that the ⊿Di-diS_B and ⊿Di-diS_H remained undigested on additional treatment with chondroitinase-ABC confirmed the presence of the ⊿Di-diS_B and ⊿Di-diS_H without any contaminants.

On digestion with chondroitinase-AC, the majority of ⊿Di-6S followed by moderate amounts of ⊿Di-4S and ⊿Di-$0S_{HA}$ and appreciable amounts of ⊿Di-0S and ⊿Di-diS_E were detected with the present HPLC method (Fig. 2). Further digestion with chondroitinase-B of the kidney DS isomers that had been treated with the AC-lyase resulted in new peaks and/or enhancement of peaks on the initial peaks with the AC-lyase. The new peaks generated on treatment of the DS isomers with the B-lyase were ⊿Di-diS_B and ⊿Di-triS. The enhanced peaks seen on

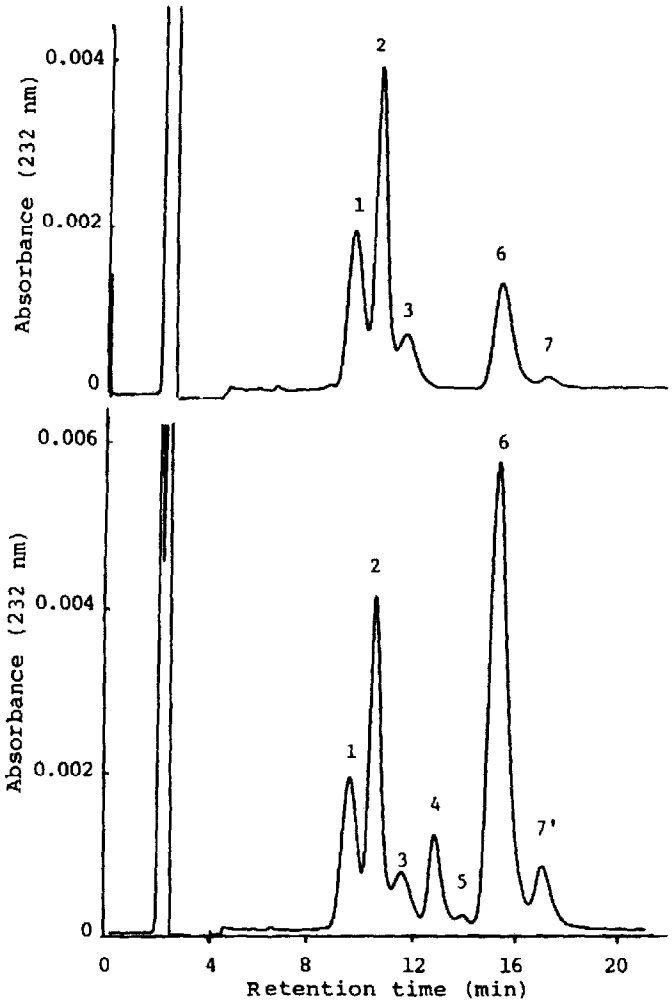

Fig. 2. Representative chromatograms of human kidney DS and Ch-S compounds (No. 27, the same as in all the following figures) by digestion with chondroitinase-AC (top) and the AC-lyase plus chondroitinase-B (bottom). Note that ΔDi-diS_B and ΔDi-triS appeared as new peaks and ΔDi-diS_H and ΔDi-4S derived from DS compounds increased as additional peaks. GAGs were applied, 10 μg per 5 μl, in equivalent amounts. Peaks: 1 = ΔDi-0S_{HA}; 2 = ΔDi-6S; 3 = ΔDi-0S; 4 = ΔDi-diS_B; 5 = ΔDi-triS; 6 = ΔDi-4S; 7 = ΔDi-diS_E; 7' = ΔDi-diS_E plus ΔDi-diS_H.

further digestion with the B-lyase were due to an increase in ΔDi-4S derived from DS and also an increase in ΔDi-diS_H. The newly elevated peaks of ΔDi-4S and ΔDi-diS_H with the B-lyase were quite obviously superimposed on those of ΔDi-4S generated from Ch-4S and that of ΔDi-diS_E, respectively.

The peaks detected after digestion with chondroitinase-ABC were the same as those generated with the B-lyase and the AC-lyase (chromatogram not shown in the figure). However, the small peaks of ΔDi-monoS_2 and ΔDi-diS_G could only be detected after digestion with chondroitinase-ABC, and not with the AC-lyase, indicating that these ΔDi-S are most probably generated from the structural chains

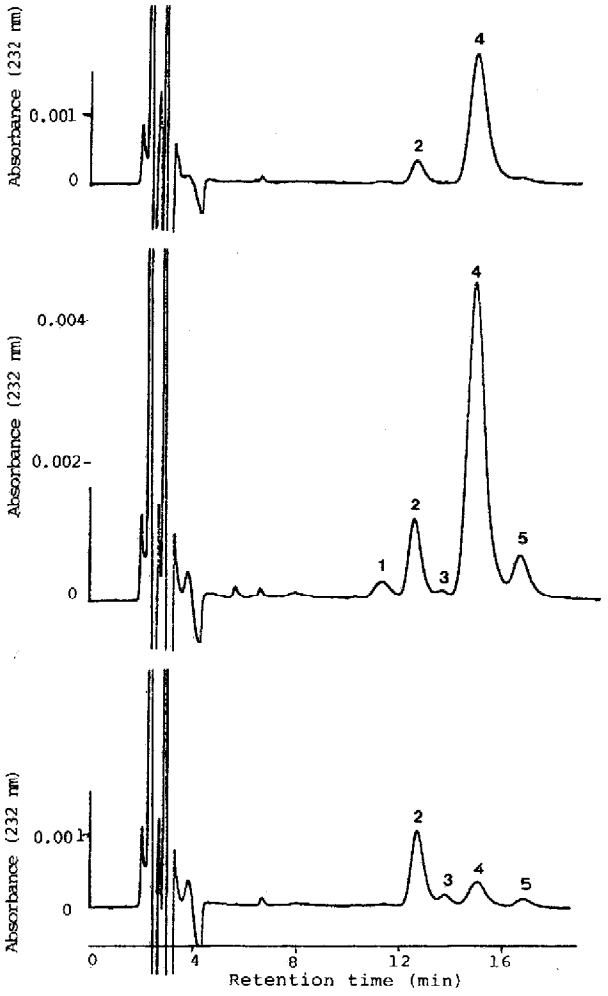

Fig. 3. HPLC patterns of ΔDi-S digested with chondroitinase-B from DS isomers, which had been fractionated by Dowex 1-X2 column with increasing sodium chloride molarity, after pre-treatment with chondroitinase-AC. (Top) 1.5 *M* sodium chloride eluate (8 μg as uronic acid); (middle) 1.75 *M* sodium chloride eluate (21 μg); (bottom) 3.0 *M* sodium chloride eluate (7 μg). Peaks: 1 = ΔDi-0S; 2 = ΔDi-diS$_B$; 3 = ΔDi-triS; 4 = ΔDi-4S; 5 = ΔDi-diS$_H$.

of DS isomers. The appearance of a small peak of ΔDi-monoS$_2$ indicates a sulphate at position 2 of iduronic acid in the DS chains. The finding that a minor peak of ΔDi-diS$_E$ appeared on digestion with chondroitinase-AC, whereas the increased peak of ΔDi-diS$_H$ was detected with the ABC-lyase or B-lyase, indicates the presence of ΔDi-diS$_H$ in kidney tissue [9].

The analytical results for ΔDi-S in the three fractions prepared from human kidney GAGs with chondroitinase-B are summarized in Table I, which shows that the characteristic ΔDi-S generated from the DS isomers are ΔDi-4S as monosulphated DS, and ΔDi-diS$_B$, ΔDi-diS$_H$ and ΔDi-triS as oversulphated DS isomers. In addition, other ΔDi-S, i.e. ΔDi-6S, ΔDi-0S, ΔDi-diS$_E$ and ΔDi-diS$_G$, are present

TABLE I

DISTRIBUTION OF THE UNSATURATED DISACCHARIDE CONSTITUENTS IN KIDNEY GAGs AFTER TREATMENT WITH CHONDROITINASE-B AND/OR OTHER CHONDROITINASES

The values are expressed as μg when ΔDi-S generated from DS and Ch-S isomers with chondroitinase-ABC are subjected to the HPLC analysis at a concentration of 22 μg per 11 μl. The numbers in parentheses are percentages when the total amounts of DS and Ch-S isomers digested with the AC-lyase plus the B-lyase or ABC-lyase are taken as 100%.

Chondro-itinases	ΔDi-diS_G	ΔDi-monoS_2	ΔDi-0S_{HA}	ΔDi-6S	ΔDi-0S	ΔDi-diS_B	ΔDi-triS	ΔDi-4S	ΔDi-diS_E (a) ΔDi-diS_H (b)	Total
B-lyase	0	0	0	0	0	1.47	0.17	7.78	0.92 (b)	10.34
	(0)	(0)	(0)	(0)	(0)	(6.8)	(0.8)	(35.9)	(4.2)	(47.7)
AC-lyase	0	0	2.84	4.70	1.17	0.02	0	2.34	0.23 (a)	11.30
	(0)	(0)	(13.1)	(21.7)	(5.4)	(0.1)	(0)	(10.8)	(1.1)	(52.2)
AC-lyase	0	0	2.62	4.77	1.06	1.49	0.18	10.16	1.39 (a+b)	21.67
+B-lyase	(0)	(0)	(12.1)	(22.0)	(4.9)	(6.9)	(0.8)	(46.9)	(6.4)	(100.0)
ABC-lyase	0.12	0.45	2.05	4.70	1.12	1.50	0.15	10.14	1.40 (a+b)	21.63
	(0.6)	(2.1)	(9.5)	(21.8)	(5.2)	(6.9)	(0.7)	(46.9)	(6.4)	(100.1)

as constitutional structures derived from Ch-S isomers. The present data show that the chondroitinase-B exclusively digests DS isomers to ⊿Di-4S, ⊿Di-diS_B, ⊿Di-diS_H and ⊿Di-triS, indicating that these ⊿Di-S all possess a sulphate at position 4 of galactosamine residues with or without other sulphate(s) at the position 2 of iduronic acid (⊿Di-diS_B) or at position 6 of galactosamine (⊿Di-diS_E or ⊿Di-diS_H) or both (⊿Di-triS).

When kidney GAGs were fractionated on a Dowex 1-X2 column by increasing the sodium chloride molarity, the DS isomers appeared between 1.5 and 3.0 *M* sodium chloride eluates. After the fractionated GAG compounds had been digested with the AC-lyase, the ⊿Di-4S and ⊿Di-6S generated from Ch-4S and Ch-6S were removed by centrifugation. The resulting DS isomers in the precipitates were determined by electrophoresis to insure the enzymic digestibility. They were then digested with the B-lyase. The HPLC pattern is shown in Fig. 3, which shows the three main peaks of ⊿Di-4S, ⊿Di-diS_B and ⊿Di-diS_H in each fraction. The proportions of ⊿Di-diS_B and ⊿Di-diS_H to ⊿Di-4S increased with increasing sodium chloride concentration. A small but distinct peak of ⊿Di-triS, which was frequently eluted with 3.0 *M* sodium chloride, appeared between those of ⊿Di-diS_B and ⊿Di-4S.

DISCUSSION

The determination of DS isomers with chondroitinase-B by this HPLC method makes it possible (i) to differentiate efficiently all the ⊿Di-S compounds derived from heterogenous DS isomers and (ii) to clarify the quantitative changes in the DS isomers in the fibrotic and repair processes. The application of chondroitinase-B to DS isomers, as well as other different chondroitinases, allowed the generation of the associated ⊿Di-S compounds, and it also resulted in the satisfactory separation and characterization, with the HPLC system, of individual ⊿Di-S in comparison with known reference components. The present HPLC assay, using a sulphonized styrene–divinylbenzene copolymer for the separation of individual ⊿Di-S generated from DS isomers, provides specific, precise and reproducible results.

The investigation of ⊿Di-S generated with chondroitinase-B using this HPLC method is efficient when either the amounts of the starting DS isomers are small or not much information is available on DS isomers consisting of variously sulphated DS isomers [18]. It is preferable to use an appropriate combination of chondroitinase-B with or without the AC-lyase. ⊿Di-monoS, ⊿Di-diS and ⊿Di-triS generated from sulphated DS isomers showed unique retention times and a characteristic detection order. The present high-resolution chromatography facilitates the partitioning and absorption of the products derived from variously sulphated DS isomers on digestion with the B-lyase.

The application of chondroitinase-B to the original sulphated DS isomers gave specific peaks of ⊿Di-S generated from different DS compounds, and the additional use of chondroitinases or chondrosulphatases led to the release of sulphate(s) from specific position(s) on the parent ⊿Di-S of DS compounds. Thus, the alternative application of chondroitinases to unknown parent ⊿Di-S derived

from DS isomers can be used to confirm the compositional structure through determination of the retention times of the generated ΔDi-S.

The present HPLC data for the ΔDi-S generated from kidney DS isomers indicate that not only was the major ΔDi-4S generated from DS [7] but also other ΔDi-diS and ΔDi-triS were generated from various oversulphated DS isomers. With a large amount of ΔDi-4S in the human kidney, ΔDi-diS_B and ΔDi-diS_H as well as ΔDi-triS are derived from it as oversulphated DS compounds. In addition, small amounts of ΔDi-diS_G and ΔDi-monoS_2 could be detected with the larger amounts of ΔDi-S compounds.

The sulphate group on the iduronic acid of all ΔDi-diS_G, ΔDi-diS_B and ΔDi-triS was determined to be located at position 2 on the iduronic acid residue. Our finding that identical peaks of desulphated ΔDi-diS_G, ΔDi-diS_B and ΔDi-triS appeared on digestion with chondro-2-, chondro-4- and chondro-6- sulphatases supported the view that one sulphate group of these compounds is located at an identical position 2 on the iduronic acid residue.

The retention times of ΔDi-S are more reproducible with the present HPLC assay method than those obtained from silica gel columns. However, the alternative determination of these ΔDi-S by HPLC on silica gel is also efficient, when the peaks of the original and generated ΔDi-S appear close to each other with the new HPLC method; e.g., in such cases as ΔDi-diS_D or ΔDi-diS_G close to ΔDi-monoS_2, as well as ΔDi-diS_B close to ΔDi-triS.

The present efficient HPLC assay using chondroitinase-B is useful for the characterization of variously sulphated DS isomers present in intercellular matrices. It provides the satisfactory resolution and excellent reproducibility needed for the determination for individual ΔDi-S in kidney DS isomers. The generated and/or elevated peaks appearing on digestion with chondroitinase-B make it possible to confirm the constitution of the original DS isomers.

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ABBREVIATIONS USED

GAGs, glycosaminoglycans; Ch-S, chondroitin sulphate; DS, dermatan sulphate; HA, hyaluronic acid; HPLC, high-performance liquid chromatography; ΔDi-S, unsaturated disaccharides; ΔDi-0S, unsaturated non-sulphated disaccharides; ΔDi-monoS, unsaturated monosulphated disaccharides; ΔDi-diS, unsaturated disulphated disaccharides; ΔDi-triS, unsaturated trisulphated disaccharide; ΔDi-0S_{HA}, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-glucose; ΔDi-0S, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose; ΔDi-4S, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulpho-D-galactose; ΔDi-6S, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulpho-D-galactose; ΔDi-monoS_2, 2-acetamido-2-deoxy-3-O-(2-sulpho-β-D-gluco-4-enepyranosyluronic acid)-D-

galactose; ΔDi-diS$_B$, 2-acetamido-2-deoxy-3-O-(2-sulpho-β-D-gluco-4-enepyranosyluronic acid)-4-sulpho-D-galactose; ΔDi-diS$_G$, 2-acetamido-2-deoxy-3-O-(2-sulpho-β-D-gluco-4-enepyranosyluronic acid)-6-O-sulpho-D-galactose; ΔDi-diS$_E$ or ΔDi-diS$_H$, 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4,6-bis-O-sulpho-D-galactose; ΔDi-triS, 2-acetamido-2-deoxy-3-O-(2-sulpho-β-D-gluco-4-enepyranosyluronic acid)-4,6-bis-O-sulpho-D-galactose; ΔDi-diS$_E$ and ΔDi-diS$_H$ have epimeric structures in the configuration of the hexuronic acid in the parent polymers.

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