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Journal of Chromatography B, 670 (1995) 3–10

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

High-performance liquid chromatographic identification of eight constitutional disaccharides from heparan sulfate isomers digested with heparitinases

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Abstract

Identification with specific heparan sulfate-lyases, heparitinase I and heparinase of the constitutional unsaturated disaccharide (ΔDi-S_{HS}) derived from heparan sulfate (HS) isomers and heparin was achieved using high-performance liquid chromatography (HPLC) with a sulfonated styrene–divinylbenzene copolymer. Eight ΔDi-S_{HS} products derived from HS isomers were identified. Enzymatic digestion with heparitinase I and heparinase converts heterogeneous sulfated HS isomers and heparin into different ΔDi-S_{HS}. The practical application of these enzymes was examined using specific enzymes and HPLC. In a patient with Hurler syndrome, eight individual Δi-S_{HS} were identified in urinary HS isomers.

1. Introduction

Among glycosaminoglycans (GAGs), heparan sulfate (HS) compounds have a highly heterogeneous structure with regard to sulfate contents, positions of the sulfates and their repeated chain formations [1–3]. These HS isomers are present in the extracellular matrix and on the cell surface, particularly in the case of the mucopolysaccharidosis seen in Hurler syndrome [4]. HS isomers and other sulfated GAGs also exhibit physiological activities such as anti-coagulant effects, anti-thrombogenicity and acceleration of cell growth [5–7]. Hurler syndrome, an autosomally linked disorder of connective tissue, is associated with the storage of GAGs and elevated urinary GAGs, HS and dermatan sulfate isomer [4]. The highly heterogeneous structure of HS isomers, including heparin, has been determined using conventional chemical analysis and electrophoresis [7–10].

Heparitinase I and heparinase extensively digest HS isomers and heparin into constitutional unsaturated disaccharides (ΔDi-S_{HS}), but do not influence other GAGs [11–13]. Whether or not heparitinases degrade HS isomers to the constitutional ΔDi-S_{HS} has not been entirely elucidated.

High-performance liquid chromatography (HPLC) is beneficial for identifying unsaturated

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0378-4347/95/$09.50
SSDI 0378-4347(95)00150-6

disaccharides (ΔDi-S) degraded from chondroitin sulfate (CS) and dermatan sulfate (DS) isomers with chondroitinases [14–17]. HS isomers plus heparin have been resolved to reduced ΔDi-S_{HS}, either on HPLC [17] or on capillary zone electrophoresis [18]. Practical application with heparitinase and heparinase is needed to separate individual ΔDi-S_{HS} generated from natural HS sources [19,20].

Using HPLC with a sulfonated styrene–divinylbenzene copolymer, we characterized ΔDi-S digested with chondroitinases from variously sulfated CS and DS isomers [21–25]. We separated six ΔDi-S_{HS} forms originating from HS isomers after digestion with heparitinase I and heparinase [26,27].

We have now extended our enzymatic work on HS isomers and heparin. Individual ΔDi-S_{HS} have a greater variety than reported earlier [1]. We report here that ΔDi-S_{HS} generated from HS isomers, using urine from a patient with Hurler syndrome, can be separated into eight individual ΔDi-S_{HS}; i.e., with enzymatic digestion, HS isomers were degraded to unsaturated, one non-sulfated, three monosulfated, three disulfated and one trisulfated disaccharide units (ΔDi-nonS_{HS}, ΔDi-monoS_{HS}, ΔDi-diS_{HS} and ΔDi-triS_{HS}). This method is precise and reproducible; the relative standard deviations of retention times are 1% within 1 week.

2. Experimental

2.1. Unsaturated disaccharides and enzymes

Reference ΔDi-nonS_{HS} (ΔDi-0S_{HS}), ΔDi-monoS_{HS} (ΔDi-2S_{HS}, ΔDi-6S_{HS} and ΔDi-NS_{HS}) and ΔDi-diS_{HS} [ΔDi-(2,6)S_{HS}, ΔDi-(2,N)S_{HS} and ΔDi-(6,N)S_{HS}] were prepared from bovine kidney HS isomers (Table 1) [11–13], and two newly identified ΔDi-S_{HS}, i.e., ΔDi-2S_{HS} and ΔDi-(2,6)S_{HS}, were included. ΔDi-triS_{HS} was generated from heparin [26]. Heparitinase I (EC 4.2.2.8) and heparinase (EC 4.2.2.7), prepared from *Flavobacterium heparinum*, as described elsewhere [26,27], specifically degrade HS isomers and heparin but not other GAGs. Heparitinase I digests HS isomers with relatively low-sulfated HS compounds whereas heparinase degrades compounds with higher sulfated HS and heparin. The heparinase and heparitinase I that we used show similar activities to heparinase I and heparinase III (Sigma, St. Louis, MO, USA), respectively. Concomitant treatment with both enzymes generates over 96% of the HS compounds. 2-O-Sulfatase was prepared from *Flavobacterium heparinum* [28]. These enzymes and ΔDi-S_{HS} were all prepared at the Tokyo Institute of Seikagaku Kogyo (Tokyo, Japan). ΔDi-2S_{HS}, ΔDi-(2,6)S_{HS}, ΔDi-(2,N)S_{HS} and ΔDi-triS_{HS}, which have sulfate at the 2-O-posi-

Table 1
Abbreviations of unsaturated disaccharides derived from heparan sulfate isomers and heparin and their structures

Abbreviation	Structure
ΔDi-nonS_{HS}	Unsaturated non-sulfated disaccharide
ΔDi-0S_{HS}	4-Deoxy-2-O-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-amino-deoxy-D-glucose
ΔDi-monoS_{HS}	Unsaturated monosulfated disaccharides from HS
ΔDi-NS_{HS}	4-Deoxy-2-O-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-deoxy-2-sulfamido-D-glucose
ΔDi-2S_{HS}	4-Deoxy-2-O-sulfo-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-amino-deoxy-D-glucose
ΔDi-6S_{HS}	4-Deoxy-2-O-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-amino-deoxy-6-O-sulfo-D-glucose
ΔDi-diS_{HS}	Unsaturated disulfated disaccharides
ΔDi-(2,6)S_{HS}	4-Deoxy-2-O-sulfo-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-amino-deoxy-6-O-sulfo-D-glucose
ΔDi-(6,N)S_{HS}	4-Deoxy-2-O-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-deoxy-2-sulfamido-6-O-sulfo-D-glucose
ΔDi-(2,N)S_{HS}	4-Deoxy-2-O-sulfo-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-deoxy-2-sulfamido-D-glucose
ΔDi-triS_{HS}	Unsaturated trisulfated disaccharides
ΔDi-triS_{HS}	4-Deoxy-2-O-sulfo-α-L-*threo*-hex-4-enopyranosyluronic acid(1–4)-2-deoxy-2-sulfamido-6-O-sulfo-D-glucose

tion of the uronic acid units, were digested with the 2-O-sulfatase to generate the corresponding lower sulfated ΔDi-0S_{HS}, ΔDi-6S_{HS}, ΔDi-NS_{HS} and ΔDi-(6,N)S_{HS} [25].

Heparitinase I and heparinase (10 mU each) exclusively digested HS isomers (ca. 50 μg in 25 μl of solution) in 10 m*M* acetate buffer in 1.0 μ*M* calcium acetate solution, final pH 7.0, at 37°C for 120 min. 2-O-Sulfatase (0.1 U) digested ΔDi-S_{HS} (20 μg) in 0.05 *M* phosphate buffer (pH 6.7) at 37°C for 60 min [25]. Methanol, acetonitrile and distilled water of HPLC grade were purchased from Wako (Osaka, Japan). Other chemicals, such as formic acid and ammonia, of specially purified grade, were used after filtration.

2.2. Electrophoresis of macromolecular GAGs

Electrophoretic separation of GAGs was carried out, before and after the enzymatic digestion, on cellulose acetate strips in three buffers [8,9]: 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 2.5% alcian blue solution, and then destained with 0.2 *M* acetic acid.

2.3. GAG preparation and Dowex column chromatography

Urinary GAGs from a patient with Hurler syndrome and also human artery and kidney GAGs were used as specimens rich in both HS isomers and DS isomers. The procedures used were as described previously [29,30]. In brief, the GAG specimens were lyophilized and digested with pronase at the rate of 50 mg/g defatted dry mass weight (pH 7.0) at 45°C for 12 h. Cold trichloroacetic acid was added to the solution at a concentration of 10%. The supernatants were dialysed to cut off under M_r 3000, using a seamless dialysis tube against distilled water. The non-dialysable GAGs were concentrated and mixed with four volumes of ethanol to precipitate GAGs, followed by fractionation on a Dowex 1-X2 (Cl^-) column using NaCl of increasing molarity, in 0.25 *M* increments, to separate the HS isomers, together with other GAGs [8,9,31]. After desalting by passage through Sephadex G-10, the GAGs were digested with heparitinase and heparinase to the constitutional ΔDi-S_{HS} and analysed by HPLC [26,27].

2.4. HPLC apparatus and procedures

A Model 803D HPLC apparatus (Toyo Soda, Tokyo, Japan) was used as the solvent-delivery system, as described [21–23]. Identification of the ΔDi-S_{HS} was carried out at 232 nm with a Model SS 5600 detector (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 used to measure retention times and peak heights or areas. To identify individual ΔDi-S_{HS}, a Shodex RS (Type DC-613) ion-exchange chromatographic column, packed with a fully porous ion-exchange resin (Na^+ form) composed of a sulfonated styrene–divinylbenzene copolymer [21–23], was purchased from Showa Denko (Tokyo, Japan). This resin column is available from American Showa Denko (New York, USA) and Europe Showa Denko (Düsseldorf, Germany). The resin, with a particle size of 6 μm, was packed into two stainless-steel columns (150 × 6 mm I.D.), connected to a guard column (46 × 6 mm I.D.).

The determination of ΔDi-S_{HS} by HPLC was carried out according to previously reported methods [26,27], but with some modification. Each degraded product, i.e., ΔDi-S_{HS} (0.2–10-μg aliquot in 5–100 μl), was injected on to the Shodex RS column and chromatographed at a flow-rate of 1.0 ml/min with acetonitrile–methanol–0.8 *M* ammonium formate (pH 4.5) (68.5:10:21.5, v/v/v) as the mobile phase, as described below. The composition of the mobile phase was kept constant with an ERC-3110 degasser (Erma Optical Works, Tokyo, Japan). The HPLC separation of ΔDi-S_{HS} from specimens and standards was carried out at 70°C at 0.5 MPa. The eluates were monitored by measur-

ing the UV absorbance at 232 nm and signals were automatically recorded at a chart speed of 2.5 mm/min to measure retention times and peak areas. Conventional HPLC on a silica gel column was sometimes performed, as described [21–24].

3. Results

3.1. Electrophoresis

By means of electrophoretic characterization, the enzymatic activity of urinary HS isomers from a patient with Hurler syndrome, and other specimens with heparitinase I and heparinase, was determined and a comparison was made with intact GAGs [29–31]. Urinary GAGs from the patient were prepared at 0.75 and 1.25 *M* NaCl on a Dowex 1-X2 column and there was one major band corresponding to the HS isomer.

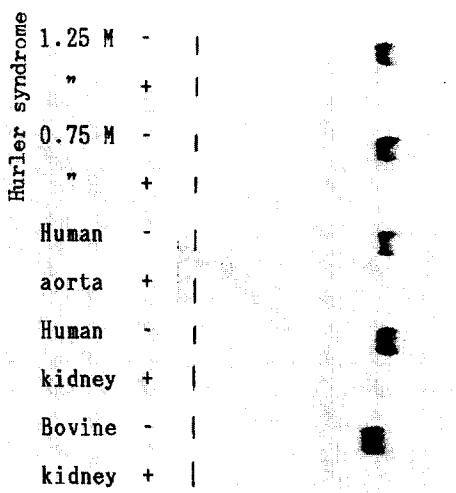

Fig. 1. Electrophoretic pattern of heparan sulfate isomers in the 0.75 and 1.25 *M* NaCl fractions obtained from a patient's urine with Hurler syndrome before (−) and after treatment (+) with heparitinase I and heparinase. Heparan sulfate specimens from human artery and bovine and human kidney were tested for digestibility against simultaneous treatment with heparitinase I and heparinase. Electrophoresis was carried out at 0.5 mA/cm in pyridine–formic acid buffer for 50 min.

Simultaneous digestion with specific heparitinase I and heparinase results in the disappearance of this single HS band in electrophoresis (Fig. 1). The presence of HS isomers was confirmed by separation in three buffer solutions for electrophoresis, indicating that these enzymes digested HS isomers.

3.2. HPLC of standard $\Delta Di\text{-}S_{HS}$

Using this HPLC system, standard $\Delta Di\text{-}nonS_{HS}$, $\Delta Di\text{-}monoS_{HS}$, $\Delta Di\text{-}diS_{HS}$ and $\Delta Di\text{-}triS_{HS}$ (Table 1) and the possible appearance of additional peaks were examined. The sulfated $\Delta Di\text{-}S_{HS}$ all possess sulfate(s) at different 2-O-positions of the uronic acid unit and NH_2 and 6-O-positions of the glucosamine residue units. The best mobile phase composition of the $\Delta Di\text{-}S_{HS}$ peak was established, with slight modification, as described [26,27]. The eight standard $\Delta Di\text{-}S_{HS}$ gave individually a single peak (Fig. 2). The retention times of the eight $\Delta Di\text{-}S_{HS}$ were characteristic, increasing in the order $\Delta Di\text{-}2S_{HS}$, $\Delta Di\text{-}0S_{HS}$, $\Delta Di\text{-}6S_{HS}$, $\Delta Di\text{-}(2,6)S_{HS}$, $\Delta Di\text{-}(2,N)S_{HS}$, $\Delta Di\text{-}NS_{HS}$, $\Delta Di\text{-}(6,N)S_{HS}$ and $\Delta Di\text{-}triS_{HS}$ (Table 2). The appearance of $\Delta Di\text{-}S_{HS}$ with the sulfate(s) at NH_2 of the glucosamine units, $\Delta Di\text{-}(2,N)S_{HS}$, $\Delta Di\text{-}NS_{HS}$, $\Delta Di(6,N)_{HS}$ and $\Delta Di\text{-}triS_{HS}$, was delayed in comparison with the desulfated $\Delta Di\text{-}2S_{HS}$, $\Delta Di\text{-}0S_{HS}$, $\Delta Di\text{-}6S_{HS}$ and $\Delta Di\text{-}(6,N)S_{HS}$, respectively. The $\Delta Di\text{-}S_{HS}$ which contained the sulfate(s) at the 2-O-position of the uronic acid unit [$\Delta Di\text{-}2S_{HS}$, $\Delta Di\text{-}(2,N)S_{HS}$] appeared at relatively earlier retention times compared with $\Delta Di\text{-}0S_{HS}$ and $\Delta Di\text{-}NS_{HS}$, respectively.

$\Delta Di\text{-}triS_{HS}$ derived from HS isomers appeared as the last peak. 2-O-Sulfatase digested the 2-O-sulfate(s) of variously sulfated $\Delta Di\text{-}2S_{HS}$ compounds to the correspond low-sulfated $\Delta Di\text{-}S_{HS}$. An increasing proportion of acetonitrile in the solvents results in longer intervals among neighbouring $\Delta Di\text{-}S_{HS}$ peaks, i.e., $\Delta Di\text{-}2S_{HS}$, $\Delta Di\text{-}0S_{HS}$ and $\Delta Di\text{-}6S_{HS}$. Eight individual $\Delta Di\text{-}S_{HS}$ were clearly separated and their retention times were reproducible, even when HPLC analysis was carried out on different days; the relative standard deviation was less than 1% within 1 week.

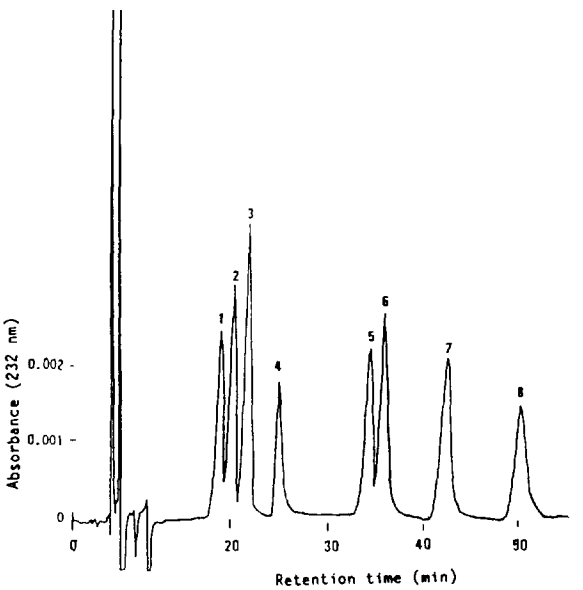

Fig. 2. Representative chromatograph of standard ΔDi-S_{HS} prepared from HS isomers by digestion with heparitinase I and heparinase. HS isomers were dissolved (10 μg in 5 μl) and applied to the HPLC column in amounts of 3–6 μg each. The chromatographic parameters in the system are as follows: theoretical plate height (N) = 5000 maximum; pressure stability, 0.5 MPa; pH, 2.6; temperature, 70°C; reproducibility, 99%. Some peaks are slightly tailing on the baseline. Peaks: 1 = ΔDi-2S_{HS}; 2 = ΔDi-0S_{HS}; 3 = ΔDi-6S_{HS}; 4 = ΔDi-(2,6)S_{HS}; 5 = ΔDi-(2,N)S_{HS}; 6 = ΔDi-NS_{HS}; 7 = ΔDi-(6,N)S_{HS}; 8 = ΔDi-triS_{HS}.

3.3. HPLC for ΔDi-S_{HS} of urinary HS

The retention times and the peak areas of the individual ΔDi-S_{HS} generated from urinary HS isomers prepared from a patient with Hurler syndrome and various HS compounds were also examined. When the urinary GAGs were fractionated on a Dowex 1-X2 column by increasing the NaCl molarity, the major components of HS isomers appeared in the 0.75 and 1.25 M NaCl eluates. Most GAGs fractionated had been simultaneously digested with both enzymes and degraded HS isomers were no longer detectable by electrophoresis (Fig. 1).

The analytical results for ΔDi-S_{HS} of urinary GAGs in the case of Hurler syndrome obtained by HPLC are summarized in Table 2. The HPLC pattern showed seven or eight peaks of ΔDi-S_{HS} in which a major peak of ΔDi-0S_{HS} plus intermediates of ΔDi-6S_{HS} and ΔDi-NS_{HS} appeared (Fig. 3). There were minor peaks as ΔDi-diS_{HS} and ΔDi-triS_{HS}, i.e., ΔDi-(2,6)S_{HS}, ΔDi-(2,N)S_{HS}, ΔDi-(6,N)S_{HS} and ΔDi-TriS_{HS}. Characteristic peaks of ΔDi-2S_{HS} and ΔDi-(2,6)S_{HS} were detected in the 0.75 M NaCl fraction. The new peak of ΔDi-(2,6)S_{HS} also appeared in the 1.25 M fraction while ΔDi-2S_{HS} was not detectable.

Thus eight peaks of the ΔDi-S_{HS} derived from urinary HS isomers from a patient with Hurler syndrome were detected after simultaneous digestion with heparitinase I and heparinase, indicating that the HS isomers and heparin are heterogeneous. The yield of ΔDi-S_{HS} generated from the HS isomers with heparitinase I and heparinase was estimated to be ca. 96%. The main peak of ΔDi-0S_{HS} appeared in the 0.75 and 1.25 M NaCl eluates, with a smaller amount of ΔDi-6S_{HS} at the shoulder. The appearance of a small peak of ΔDi-TriS_{HS} in the 1.25 M NaCl eluate indicates the presence of heparin. When increasing molarity of NaCl in Dowex column chromatography, the peak of ΔDi-0S_{HS} decreased, whereas increased proportions of sulfated ΔDi-S_{HS}, such as ΔDi-6S_{HS}, ΔDi-(2,N)S_{HS}, ΔDi-(6,N)S_{HS} and ΔDi-triS_{HS}, became evident. Presumably the sulfate content of HS isomers increases with increase in the molarity of the NaCl solution with which the HS isomers were eluted.

The proportions of ΔDi-monoS_{HS}, ΔDi-diS_{HS} and ΔDi-triS_{HS} to ΔDi-0S_{HS} increased with increasing NaCl concentration. The HS isomers in the 0.75 and 1.25 M NaCl fractions consisted of non-sulfated, monosulfated, disulfated and trisulfated HS, in proportions of *ca.* 84:8.5:6:0.5 and 62:27:8:1, respectively.

In general, the retention time of ΔDi-S_{HS} is sufficiently reproducible using the present HPLC method. An alternative determination of ΔDi-S_{HS} is made by HPLC on silica gel, which is not always reproducible but with which separation is feasible regarding sulfate content(s): ΔDi-nonS_{HS}, ΔDi-monoS_{HS} and ΔDi-diS_{HS} [32]. This method is efficient when peaks of the generated

Table 2
Distribution of constitutional unsaturated disaccharides originating from heparan sulfate isomers of a patient with Hurler syndrome after digestion with heparitinase I and heparinase

ΔDi-S_{HS}	ΔDi-2S_{HS}	ΔDi-0S_{HS}	ΔDi-6S_{HS}	ΔDi-(2,6)S_{HS}	ΔDi-(2,N)S_{HS}	ΔDi-NS_{HS}	ΔDi-(6,N)S_{HS}	ΔDi-triS_{HS}	Total ΔDi-S_{HS}
Retention time (min)	18.18	20.06	21.67	25.22	35.13	37.47	43.66	51.56	–
0.75 *M* NaCl fraction	1.46	84.41	3.12	3.65	1.44	3.87	0.96	0.50	99.41
1.25 *M* NaCl fraction	0.06	61.54	13.48	2.32	3.08	13.84	2.30	1.69	98.31
Total fraction	0.43	72.97	8.66	2.98	2.26	7.86	1.63	1.10	97.89

The values indicate with respect to total HS isomers on HPLC using a resin made from sulfonated styrene–divinylbenzene copolymer.

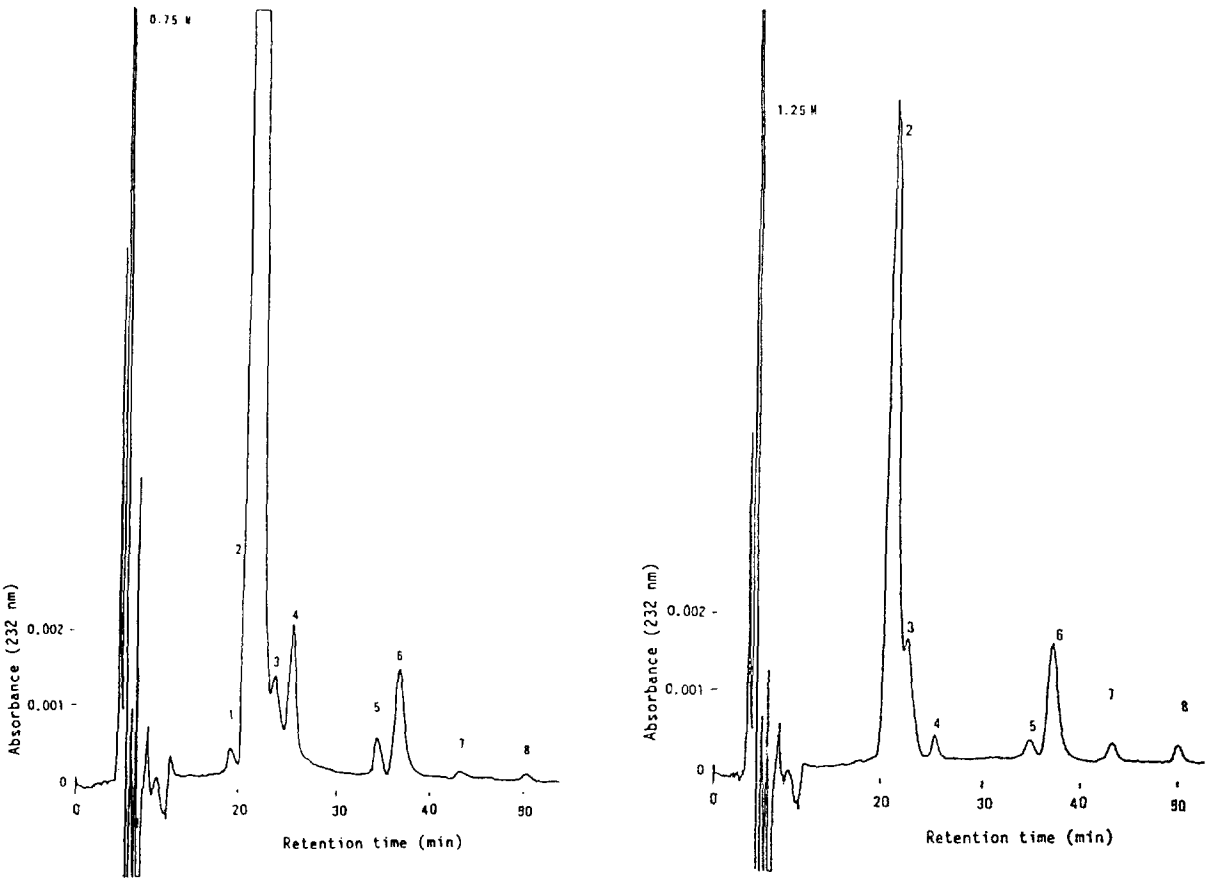

Fig. 3. HPLC patterns of ΔDi-S_{HS} digested with heparitinase I and heparinase from urinary HS isomers from a patient with Hurler syndrome. The HS isomers are major GAG eluates fractionated using 0.75 *M* NaCl (left) and 1.25 *M* NaCl (right) on a Dowex 1-X2 column. Peak numbers as in Fig. 2.

ΔDi-S_{HS} appear in close proximity, e.g., with ΔDi-$0S_{HS}$ close to ΔDi-$6S_{HS}$.

4. Discussion

The present HPLC system facilitates the partitioning and absorption of ΔDi-S_{HS} products generated from variously sulfated HS isomers on digestion with heparitinase I and heparinase. Determination of the ΔDi-S_{HS} of HS isomers with simultaneous application of the enzymes makes it feasible to differentiate eight ΔDi-S_{HS} derived from heterogeneous HS isomers and to clarify quantitative changes in individual ΔDi-S_{HS} in HS isomers. The present HPLC assay using a sulfonated styrene–divinylbenzene copolymer to identify individual ΔDi-S_{HS} generated from HS isomers yields specific, precise and reproducible results.

There are only a few methods available for the detection of structures of HS isomers in various intercellular matrices, and most are either not reproducible or are not practical. The separation of individual ΔDi-S_{HS} using heparitinase I and heparinase with the present HPLC method is efficient when amounts of the original HS isomers are minute and when little information is available on the HS isomers [27]. It is preferable to use a suitable combination of heparitinase I and heparinase to increase the yield of the degraded ΔDi-S_{HS}.

The order of retention times of ΔDi-S_{HS} obtained with this HPLC differs from that obtained by HPLC using conventional silica gel [26]. Thus, the alternative application of ΔDi-S_{HS} derived from HS isomers to the two HPLC systems can be used to confirm the compositional structure via the detection of different orders of retention times of the ΔDi-S_{HS} [26,27].

The present HPLC assay using heparitinase and heparinase is practical for characterizing variously sulfated HS isomers present in urine from patients with Hurler syndrome. The reproducibility required for the determination of eight individual ΔDi-S_{HS} is excellent. The generated ΔDi-S_{HS} appearing on digestion with the enzymes makes feasible the determination of the constitution of the original HS compounds.

Acknowledgements

We thank Dr. Y. Kariya and Mrs. Y. Yokoyama for technical assistance and Ms. M. Mizuma for preparing the manuscript. This study was supported in part by Scientific Research Grants (No. 07670529) from the Ministry of Education, Science and Culture, Japan.

References

[1] A. Linker and P. Hovingh, Biochemistry, 11 (1972) 563.
[2] M. Yanagishita and V.C. Hascall, J. Biol. Chem., 267 (1992) 9451.
[3] U. Lindahl, in D.A. Lane and U. Lindahl (Editors), Heparin: Chemical and Biological Properties, Clinical Application, Edward Arnold, London, 1989, pp. 159–189.
[4] V.A. McKusick and E.F. Neufeld, in J.B. Stanbury and J.B. Wangaarden, (Editors), the Metabolic Basis of Inherited Disease, McGraw-Hill, New York, 1987, pp. 751–777.
[5] K. Izuka and K. Murata, Atherosclerosis, 16 (1972) 217.
[6] K. Murata, K. Izuka and K. Nakazawa, Atherosclerosis, 29 (1978) 95.
[7] D. Aviezer, E. Levy, M. Safran, C. Svahn, E. Buddecke, A. Schmidt, G. David, I. Vlodavsky and A. Yayon, J. Biol. Chem., 269 (1994) 114.
[8] K. Murata and Y. Yokoyama, Atherosclerosis, 78 (1989) 69.
[9] K. Murata, Y. Ochiai and K. Akashio, Gastroenterology, 89 (1985) 1248.
[10] A. Linker and P. Hovingh, Carbohydr. Res., 29 (1973) 41.
[11] M.E. Silva and C.P. Dietrich, Biochim. Biophys. Res. Commun., 56 (1974) 965.
[12] M.E. Silva and C.P. Dietrich, J. Biol. Chem., 250 (1975) 6841.
[13] N. Ototani, M. Kikuchi and Z. Yoshizawa, Carbohydr. Res., 88 (1981) 291.
[14] A. Hjerpe, C.A. Antonopoulos and B. Engeldt, J. Chromatogr., 171 (1979) 339.
[15] D.C. Seldin, N. Seno, K.N. Austin and R.L. Stevens, Anal. Biochem., 141 (1984) 291.
[16] G.L. Lee, D.W. Liu, J.W. Pav and H. Tieckelmann, J. Chromatogr., 212 (1981) 65.
[17] N.K. Karamanos, A. Syrokou, P. Vanky, M. Nurminen and A. Hjerpe, Anal. Biochem., 221 (1994) 189.
[18] S.A. Ampofo, H.M. Wang and R.J. Linhardt, Anal. Biochem., 199 (1991) 249.
[19] U.R. Desai, H.M. Wang and R.J. Linhardt, Biochemistry, 32 (1993) 8140.
[20] M. Lyon, J.A. Deakin and J.T. Gallagher, J. Biol. Chem., 269 (1994) 11208.
[21] K. Murata and Y. Yokoyama, Anal. Biochem., 146 (1985) 327.
[22] K. Murata and Y. Yokoyama, Anal. Biochem., 149 (1985) 261.
[23] K. Murata and Y. Yokoyama, J. Chromatogr., 374 (1986) 37.
[24] K. Murata and Y. Yokoyama, J. Chromatogr., 425 (1986) 231.
[25] K. Murata and Y. Yokoyama, J. Biochem. Biophys. Methods, 15 (1987) 23.
[26] K. Murata and Y. Yokoyama, J. Chromatogr., 496 (1989) 27.
[27] K. Murata, Y. Yokoyama and K. Yoshida, Biochem. Int., 19 (1989) 155.
[28] M.W. McLean, J.S. Bruce, W.F. Long and F.B. Williamson, Eur. J. Biochem., 145 (1984) 607.
[29] K. Murata, in J.N. BeMiller, D.J. Manners and R.J. Sturgeon (Editors), Methods of Carbohydrate Chemistry, Vol. 10, Wiley, New York, 1994, pp. 97–104.
[30] K. Murata, Connect. Tissue Res., 4 (1976) 131.
[31] K. Murata and M. Takeda, Clin. Chim. Acta, 108 (1980) 49.
[32] K. Yoshida, S. Miyauchi, M. Kikuchi, A. Tawada and K. Yoshida, Anal. Biochem., 177 (1989) 327.