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Tandem mass spectrometry for characterization of unsaturated disaccharides from chondroitin sulfate, dermatan sulfate and hyaluronan

TADASHI II[1], SATOSHI OKUDA[2], TAKASHI HIRANO[3] and MAMORU OHASHI[3]*

[1] *Research and Development Laboratories, Soda Aromatic Company, Ltd, Noda-shi, Chiba 270-02, Japan*
[2] *Department of Chemistry, College of General Education, Nagoya University, Chikusaku, Nagoya 464-01, Japan*
[3] *Department of Applied Physics and Chemistry, The University of Electro-Communications, Chofu, Tokyo 182, Japan*

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Fast atom bombardment tandem mass spectrometry has been used in the characterization of non-, mono-, di- and trisulfated disaccharides from chondroitin sulfate, dermatan sulfate and hyaluronan. The positional isomers of the sulfate group of mono- and disulfated disaccharides were distinguished from each other by both positive- and negative-ion fast atom bombardment tandem mass spectra, which gave sufficient information characteristic of the isomers. The anomeric isomers of nonsulfated disaccharides were characterized by the technique in the positive-ion mode. This fast atom bombardment collision induced dissociation mass spectrometry/mass spectrometry technique was also applied successfully to the characterization of trisulfated disaccharide.

Keywords: Unsaturated disaccharide; glycosaminoglycan, chondroitin sulfate; hyaluronan; FABMS, CID, MS/MS.

Abbreviations: FABMS, fast atom bombardment mass spectrometry; MI, metastable ion; CID, collision induced dissociation; MIKE, mass analysed ion kinetic energy; SIMS, secondary ion mass spectrometry; MS/MS, mass spectrometry/mass spectrometry; HPLC, high performance liquid chromatography; ΔGlcA, D-gluco-4-enepyranosyluronic acid; CS, chondroitin sulfate; DS, dermatan sulfate; HA, hyaluronan; ΔUA-GalNAc, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose; ΔUA-GalNAc4S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulfo-D-galactose; ΔUA-GalNAc6S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulfo-D-galactose; ΔUA2S-GalNAc, 2-acetamido-2-deoxy-3-*O*-(2-*O*-sulfo-β-D-gluco-4-enepyranosyluronic acid)-D-galactose; ΔUA2S-GalNAc4S, 2-acetamido-2-deoxy-3-*O*-(2-*O*-sulfo-β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulfo-D-galactose; ΔUA2S-GalNAc6S, 2-acetamido-2-deoxy-3-*O*-(2-*O*-sulfo-β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulfo-D-galactose; ΔUA-GalNAcDiS, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4,6-di-*O*-sulfo-D-galactose; ΔUA2S-GalNAcDiS, 2-acetamido-2-deoxy-3-*O*-(2-*O*-sulfo-β-D-gluco-4-enepyranosyluronic acid)-4,6-di-*O*-sulfo-D-galactose; ΔUA-GlcNAc, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-glucose.

Introduction

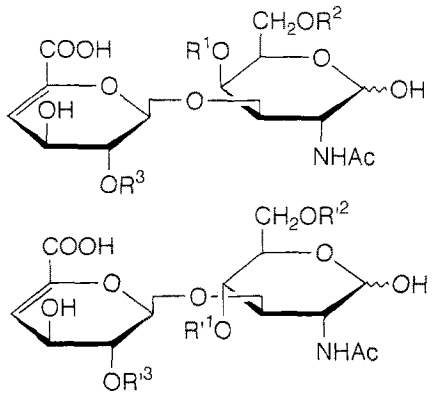
In vertebrates, both chondroitin sulfate (CS) and dermatan sulfate (DS) are covalently linked to core proteins to form proteoglycans, which are distributed in a variety of tissues and cells and play important roles in a wide range of biological processes [1–3]. Hyaluronan (HA) is also present in various tissues and organs and plays numerous important roles including formation of aggregates with other proteoglycan monomers [4–6].

CS is a linear polymer containing D-glucuronic acid (β1-3)*N*-acetyl-D-galactosamine(β1-4) repeating units with microvariability of *O*-sulfation patterns [7, 8]. Exhaustive treatment of CS with chondroitin ABC or AC lyase [9] affords a mixture of eight Δ4,5-unsaturated disaccharided, one of which is nonsulfated, three are isomeric monosulfates, another three are isomeric disulfated, and the remaining one is trisulfated disaccharide [10–13] (for structures, see Table 1). DS is an isomeric polymer of CS. It contains L-iduronic acid, the C-5 epimer of D-glucuronic acid, in place of D-glucuronic acid in repeating units of CS. Using chondroitin ABC or B lyase [9], DS also can be depolymerized to the mixture of the same eight unsaturated disaccharides as that

* To whom correspondence should be addressed.

Table 1. Compound investigated.

Unsaturated disaccharides from chondroitin/dermatan sulfate				
Compound	*RMM*[a]	R^1	R^2	R^3
ΔUA-GalNAc	379	H	H	H
ΔUA-GalNAc4S	459	SO_3H	H	H
ΔUA-GalNAc6S	459	H	SO_3H	H
ΔUA2S-GalNAc	459	H	H	SO_3H
ΔUA2S-GalNAc4S	539	SO_3H	H	SO_3H
ΔUA2S-GalNAc6S	539	H	SO_3H	SO_3H
ΔUA-GalNAcDiS	539	SO_3H	SO_3H	H
ΔUA2S-GalNAcDiS	619	SO_3H	SO_3H	SO_3H
Unsaturated disaccharides from hyaluronan				
Compound	*RMM*[a]	R'^1	R'^2	R'^3
ΔUA-GlcNAc	379	H	H	H



[a] RMM represents the relative molecular mass of the free acid.

produced from CS. Finally, HA is a nonsulfated polymer consisting of D-glucuronic acid(β1-3)*N*-acetyl-D-glucosamine(β1-4) repeating units. HA can be dissociated into single unsaturated disaccharide on treatment with chondroitin ABC lyase or HA lyase (from *Streptococcus*) [9] (for structure, see Table 1).

Analyses of disaccharides from CS, DS and HA are very important for the structure elucidation of CS and DS, and are helpful in the clinical diagnosis of some disorders [14, 15]. At present, nine authentic disaccharides mentioned above are commercially available. Using these compounds as standards, analysis of unsaturated disaccharides can be performed by high performance liquid chromatography (HPLC) after enzymatic depolymerization of a sample [16–18]. However, it is very difficult to identify all of these disaccharides by their retention time only, because sufficient separation of the respective isomers of non-, mono- and disulfated disaccharides by a single chromatography is impossible [16–18]. Therefore, tedious enzymatic analyses with appropriate sulfatases are required for the definitive identification of these isomeric sulfated disaccharides [16–18].

Secondary ion mass spectrometry (SIMS) and fast atom bombardment mass spectrometry (FABMS) can provide one of the best methods for obtaining structural information on *O*-sulfated sugar compounds even with small amounts of samples. For example, we have shown that metastable ion mass spectrometry on both positive- and negative-ion SIMS is very useful for the characterization of positional and anomeric isomers of monosulfated D-glucuronic acid derivatives [19]. On the other hand, Lamb *et al.* successfully used collision induced dissociation (CID)-mass analysed ion kinetic energy (MIKE) spectra on the negative-ion FABMS to distinguish isomeric monosulfated disaccharides from glycosaminoglycans [20].

It is known that in the postive-ion FABMS, CID-MS/MS of lithiated disaccharide give useful information on the differentiation of the linkage position in the five isomeric sugars [21]. Our previous study also showed that MI spectra of sodiated molecules on SIMS gave sufficient information characteristic of the sulfated saccharide isomers not only in the negative-ion mode but also in the positive-ion mode [19].

Here we report the characterization of a series of non-, mono-, di- and trisulfated unsaturated disaccharides from CS, DS and HA based on the positive- and negative-ion FAB CID-MS/MS.

Materials and methods

Materials

The unsaturated disaccharides investigated are listed in Table 1. All compounds (sodium salts) were standard kits for HPLC obtained from Seikagaku Kogyo Co. (Tokyo, Japan).

Mass spectrometry

Mass spectra were recorded on a Finnigan MAT TSQ 700 triple stage quadruple mass spectrometer equipped with an Ion Tech FAB gun. A xenon beam with an energy of 8 keV was used. About a 1 µl aliquot of an aqueous sample solution (*ca.* 1.0 mg in 50 µl) was mixed with 1 µl of matrix and then placed on the FAB target. Glycerol and triethanolamine were used as the matrices in positive- and negative-ion modes respectively.

CID MS/MS spectra were taken using argon as the collision gas at typically 1.0 mTorr to reduce the beam of parent ions by approximately 30%. Collision energies were used at 5 eV for nonsulfated disaccharides, and 30 eV for

Table 2. Molecule related and major fragment ions (m/z) and relative peak intensities (%) in the positive ion FABMS of non-, mono-, di- and trisulfated unsaturated disaccharides.

Compound[a]	*Molecule related ions*[b,c]						*Major fragment ions*
	$[M+H]^+$	$[M+Na]^+$	$[M-H+2Na]^+$	$[M-2H+3Na]^+$	$[M-3H+4Na]^+$	$[M-4H+5\mathrm{Na}]^+$	
ΔUA-GalNAc	380 (34)	402 (100)	424 (24)				
ΔUA-GlcNAc	380 (38)	402 (100)	424 (32)				
ΔUA-GalNAc4S		482 (51)	504 (100)	526 (21)			402 (72) $[(M-H+2Na)-NaSO_3+H]^+$
ΔUA-GalNAc6S		482 (39)	504 (100)	526 (74)			402 (61) $[(M-H+2Na)-NaSO_3+H]^+$
ΔUA2S-GalNAc		482 (79)	504 (100)	526 (14)			402 (60) $[(M-H+2Na)-NaSO_3+H]^+$
ΔUA2S-GalNAc4S			584 (40)	606 (80)	628 (20)		504 (100) $[(M-2H+3Na)-NaSO_3+H]^+$
ΔUA2S-GalNAc6S			584 (25)	606 (58)	628 (16)		504 (100) $[(M-2H+3Na)-NaSO_3+H]^+$
ΔUA-GalNAcDiS			584 (53)	606 (84)	628 (27)		504 (100) $[(M-2H+3Na)-NaSO_3+H]^+$
ΔUA2S-GalNAcDiS				686 (25)	708 (50)	730 (13)	606 (100) $[(M-3H+4Na)-NaSO_3+H]^+$

[a] See Table 1 for structures.
[b] M represents the fully protonated acid.
[c] Peaks are normalized to the base peak in the molecule related region.

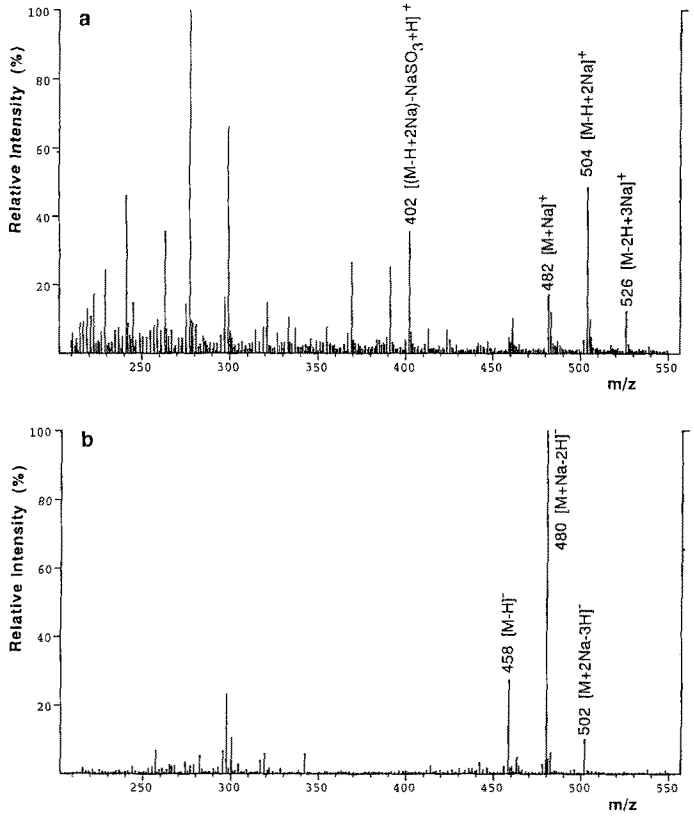


Figure 1. FAB mass spectra of monosulfated unsaturated disaccharide ΔUA-GalNAc4S. (a) positive-ion mode, (b) negative-ion mode.

sulfated disaccharides. At least 10 scans were averaged to obtain each MS/MS spectrum.

Results and discussion

POSITIVE- AND NEGATIVE-ION FAB SPECTRA OF NON-, MONO-, DI- AND TRISULFATED UNSATURATED DISACCHARIDES

As summarized in Table 2, the positive-ion FAB spectra of non-(ΔUA-GalNAc and ΔUA-GlcNAc), mono-(ΔUA-GalNAc4S, ΔUA-GalNAc6S and ΔUA2S-GalNAc), di-(ΔUA2S - GalNAc4S, ΔUA2S - GalNAc6S and ΔUA-GalNAcDiS) and trisulfated (ΔUA2S-GalNAcDiS) unsaturated disaccharides exhibited unambiguously the peaks of molecule related ions, $[M+H]^+$ and/or $[M\text{-}nH+(n+1)Na]^+$ (where $n = 0$–4), as reported earlier [22, 23]. M represents the fully protonated molecule throughout this article. As a typical example, the spectrum of ΔUA-GalNAc4S is shown in Fig. 1a. In the positive-ion FAB spectra of the sulfated disaccharides, the most abundant peaks in the molecular ion region correspond to the cations having dissociated sulfate groups, The losses of sodium sulfite with hydrogen transfer (i.e. $-NaSO_3$, $+H$) from these cations were also commonly observed, resulting in intense peaks 102 units lower in m/z (Table 2, Fig. 1a) [22–24]. The sensitivities of the positive-ion FABMS are almost the same as those of the negative-ion.

Also in the negative-ion FABMS, the above-mentioned nine disaccharides give distinct peaks corresponding to molecule related ions $[M+n\text{Na-}(n+1)H]^-$ (where $n = 0$–4), as found earlier [20, 22–23, 25–26] (data not shown). Figure 1b shows the negative-ion FAB mass spectrum of ΔUA-GalNAc4S as a typical example.

However, it is impossible to distinguish the nonsulfated epimeric isomers or the mono- or disulfated positional isomers using only these positive- or negative-ion FABMS.

MS/MS SPECTRA OF THE POSITIVE ION FAB OF NON-, MONO-, DI- AND TRISULFATED UNSATURATED DISACCHARIDES

(*I*) *Nonsulfated disaccharides*

In contrast to the normal FABMS, the positive-ion CID-MS/MS spectra of $[M+H]^+$ ions provide sufficient information to distinguish the epimeric isomers of nonsulfated disaccharides. MS/MS spectra of $[M+H]^+$ ions (m/z380) of ΔUA-GalNAc and ΔUA-GlcNAc are shown in Fig. 2. To describe the fragment ion drawn in each Figure, we use the nomenclature, shown in Scheme 1, proposed by Domon and Costello [27]. The most prominent peaks at m/z362 corresponding to $[(M+H)\text{-}H_2O]^+$ ions were commonly observed in both spectra. The spectrum of ΔUA-GlcNAc (Fig. 2b) exhibits an intense peak at m/z222 and a weak one

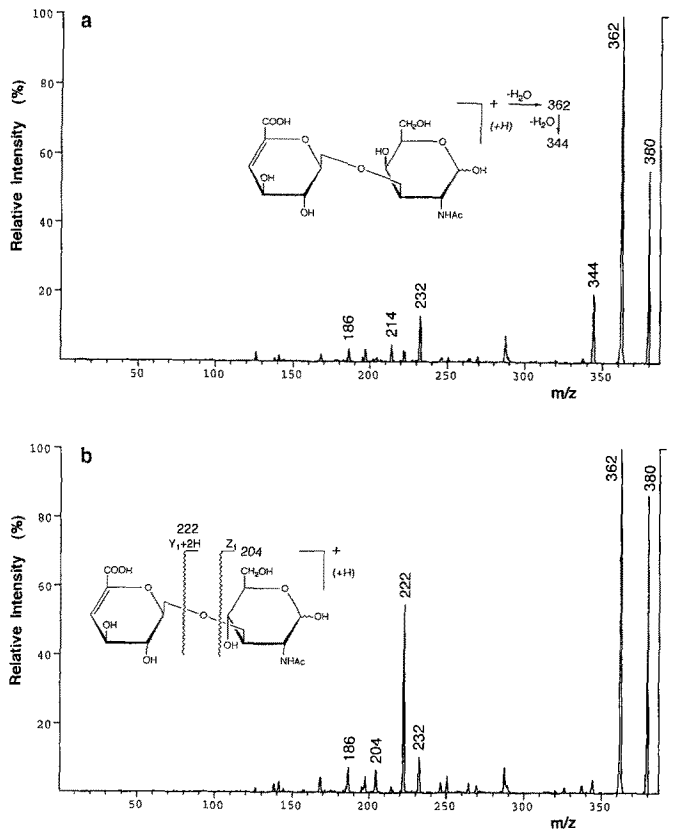
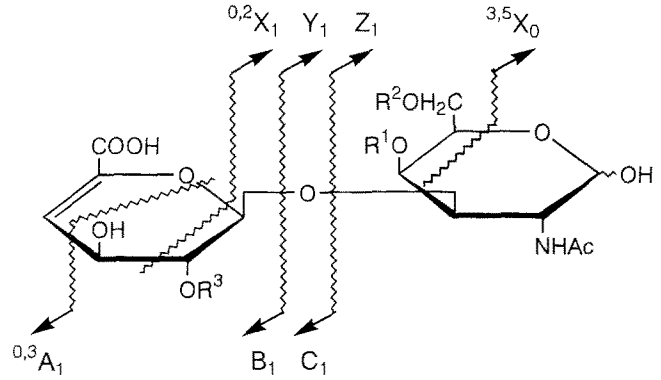


Figure 2. MS/MS spectra of nonsulfated unsaturated disaccharides having $[M+H]^+$ ions (m/z380), as the parent ion. (a) ΔUA-GalNAc, (b) ΔUA-GlcNAc.



Scheme 1. The nomenclature for disaccharide fragmentation proposed by Domon and Costello [27].

at *m/z*204 corresponding to $[Y_1+2H]^+$ and $[Z_1{}^+]$ ions, respectively, both of which are produced by the cleavage of the glycosidic bond. While the spectrum of ΔUA-GalNAc (Fig. 2a) shows the characteristic peak at *m/z*344 corresponding to the $[(M+H)\text{-}2H_2O]^+$ ion derived from the $[(M+H)\text{-}H_2O]^+$ ion (*m/z*362). On the basis of this different fragmentation behaviour of $[M+H]^+$ ions, the epimeric isomers, ΔUA-GalNAc and ΔUA-GlcNAc can be easily distinguished from each other. The hydroxy group at C-4 in the *N*-acetyl-D-galactosamine (GalNAc) residue of ΔUA-GalNAc may inhibit proton transfer which causes the formation of $[Y_1+2H]^+$ and $[Z_1]^+$ ions.

In the CID-MS/MS spectra of $[M+Na]^+$ and $[M-H+2Na]^+$ ions of ΔUA-GalNAc and ΔUA-GlcNAc, no significant differences were observed (data not shown).

(*II*) *Monosulfated disaccharides*

The results of the positive-ion CID-MS/MS of $[M-H+2Na]^+$ ions (*m/z*504) of isomeric monosulfated disaccharides, ΔUA-GalNAc4S, ΔUA-GalNAc6S and ΔUA2S-GalNAc, indicate that it is possible to distinguish between monosulfated disaccharides only when the sulfate groups reside at a different saccharide unit (i.e. ΔUA2S-GalNAc from ΔUA-GalNAc4S and ΔUA-GalNAc6S). The CID-MS/MS spectra of $[M-H+2Na]^+$ (*m/z*504) of the monosulfated disaccharides are shown in Fig. 3. The spectra of ΔUA-GalNAc4S and ΔUA-GalNAc6S (Fig. 3a, b) closely resemble each other. In both spectra, the most predominant peak at *m/z*424 corresponding to the $[(M-H+2Na)-SO_3]^+$ ion and two intense peaks at *m/z*221 and 203 corresponding to the $[C_1+2Na]^+$ and $[B_1-2H+2Na]^+$ ions, respectively, produced by the glycosidic bond cleavage are observed. The glycosidic bond cleavage also yielded the weak peaks at *m/z*346 ($[Y_1+2Na]^+$), 226 ($[(Y_1+2Na)-NaHSO_4]^+$) and 328 ($[Z_1-2H+2Na]^+$). In the low mass region, the ions arising from sodiated sulfate groups are observed at *m/z*104 ($[NaHSO_3]^+$) and 143 ($[Na_2HSO_4]^+$).

Although three peaks with similar intensities to that of ΔUA-GalNAc4S and ΔUA-GalNAc6S were also observed at *m/z*226, 221 and 203 in the CID-MS/MS spectra of

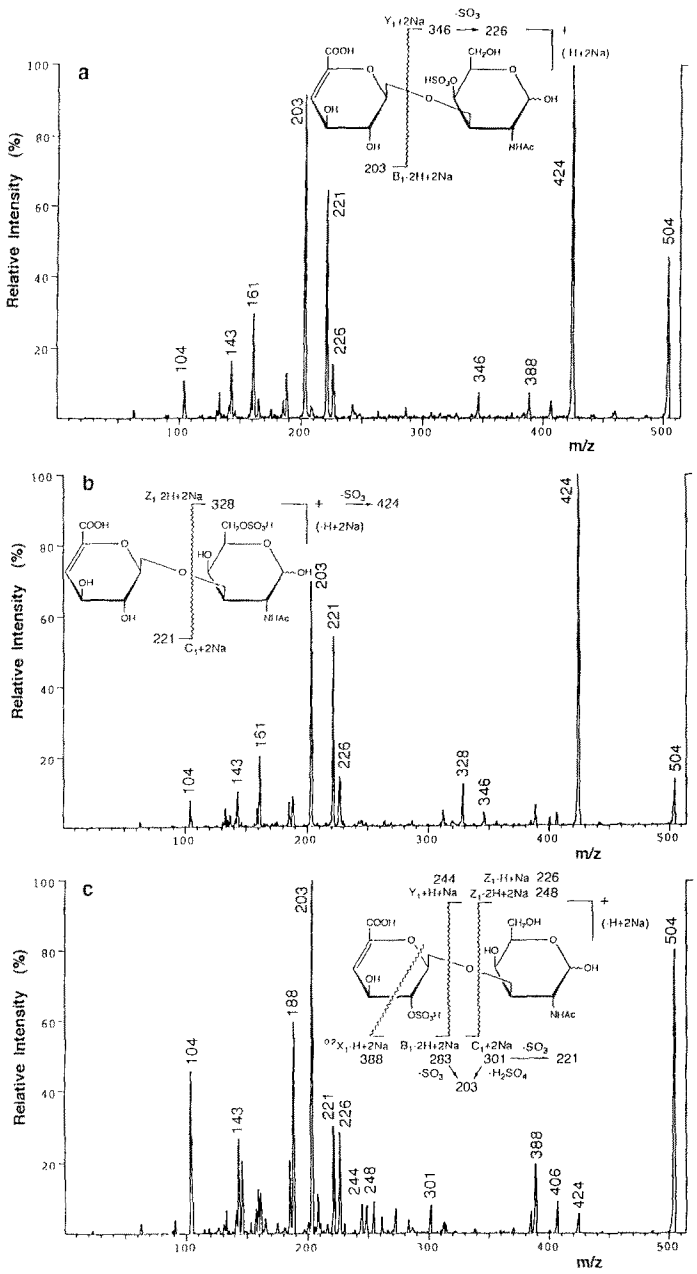


Figure 3. MS/MS spectra of monosulfated unsaturated disaccharides having $[M-H+2Na]^+$ ions (*m/z*504), as the parent ion. (a) ΔUA-GalNAc4S, (b) ΔUA-GalNAc6S, (c) ΔUA2S-GalNAc.

ΔUA2S-GalNAc (Fig. 3c), it is possible to distinguish ΔUA2S-GalNAc from ΔUA-GalNAc4S and ΔUA-GalNAc6S by dramatic differences in intensities of the peaks at *m/z*424 ($[(M-H+2Na)-SO_3]^+$) and 188. However the origin of the ion at *m/z* 188 is not yet clear. The origins of the fragment ions at *m/z* 388, 301, 283, 248, 244, 226, 221 and 203 were rationalized as illustrated in Fig. 3c, respectively.

The positive-ion CID-MS/MS spectra of $[M-2H+3Na]^+$ ions (*m/z*526) of ΔUA-GalNAc4S, ΔUA-GalNAc6S and ΔUA2S-GalNAc are shown in Fig. 4, where more pronounced differences among these isomers are observed. In the spectrum of ΔUA-GalNAc6S (Fig. 4b), the

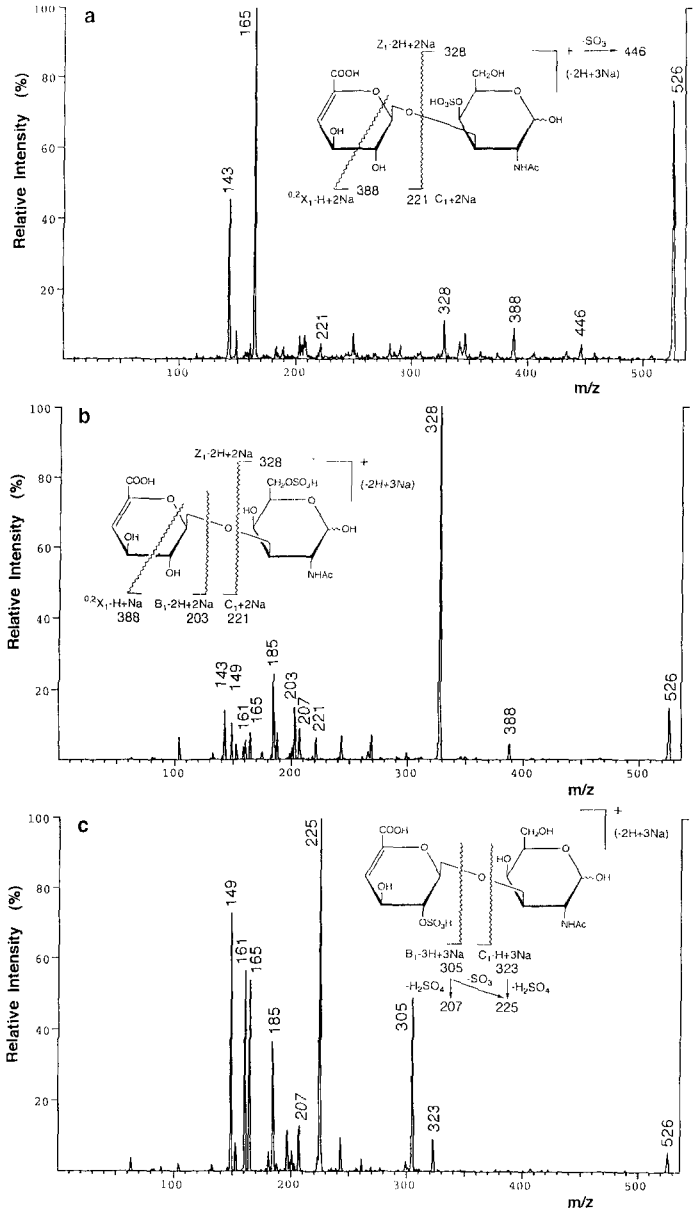


Figure 4. MS/MS spectra of monosulfated unsaturated disaccharides having $[M-2H+3Na]^+$ ions ($m/z526$), as the parent ion. (a) ΔUA-GalNAc4S, (b) ΔUA-GalNAc6S, (c) ΔUA2S-GalNAc.

$[Z_1-2H+2Na]^+$ ion at $m/z328$ produced by the elimination of sodiated unsaturated uronic acid from the parent ion gives the most predominant peak, while other fragment ions, $[^{0,2}X_1-H+Na]^+$ ($m/z388$), $[C_1+2Na]^+$ ($m/z221$) and $[B_1-2H+2Na]^+$ ($m/z203$), as well as several ions ($m/z143\sim185$) arising from the sodiated sulfate group, give weak peaks. The features of the CID-MS/MS spectrum of ΔUA-GalNAc4S (Fig. 4a) differ markedly from those of ΔUA-GalNAc6S. In the spectrum, the $[Z_1-2H+2Na]^+$ ion at $m/z328$ gives a very weak peak similar to other fragment ions, $[(M-2H+3Na)-SO_3]^+$ ($m/z446$), $[^{0,2}X-H+2Na]^+$ ($m/z388$), and $[C_1+2Na]^+$ ($m/z221$), while the $[Na_3SO_4]^+$ ion at $m/z165$ and $[Na_2HSO_4]^+$ at $m/z143$ give the most abundant and intense peaks, respectively. The CID-MS/MS spectrum of ΔUA2S-GalNAc (Fig. 4c) also exhibits the characteristic features. In the spectrum the $[B_1-3H+3Na]^+$ ($m/z305$) and $[C_1-H+3Na]^+$ ($m/z323$) ions, produced by the eliminations of *N*-acetylgalactosamine from the parent ion give intense peaks, and the ion produced by the eliminations of SO_3 from the $[B_1-3H+3Na]^+$ ion, or of H_2SO_4 from the $[C_1-H+3Na]^+$ ion gives the most intense peak at $m/z225$. A weak peak at $m/z207$ is due to the elimination of H_2SO_4 from $[B_1-3H+3Na]^+$, and four intense peaks at $m/z185$, 165, 161 and 149 corresponding to the ions derived from the sodiated sulfate group, respectively, are also observed.

These observations show that the positive-ion CID-MS/MS of $[M-2H+3Na]^+$ ions ($m/z526$) of ΔUA-GalNAc4S, ΔUA-GalNAc6S and ΔUA2S-GalNAc provide an easy way of identifying these positional isomers of monosulfates.

(*III*) *Disulfated disaccharides*

The results of the positive-ion CID-MS/MS of $[M-2H+3Na]^+$ ions ($m/z606$) of isomeric disulfated disaccharides, ΔUA2S-GalNAc4S, ΔUA2S-GalNAc6S and ΔUA-GalNAcDiS, indicate that it is possible to distinguish ΔUA-GalNAcDiS from ΔUA2S-GalNAc4S and ΔUA2S-GalNAc6S, but impossible to distinguish between ΔUA2S-GalNAc4S and ΔUA2S-GalNAc6S. The CID-MS/MS spectra of $[M-2H+3Na]^+$ ions ($m/z606$) of disulfated disaccharides are shown in Fig. 5. The spectra of ΔUA2S-GalNAc4S and ΔUA2S-GalNAc6S (Fig. 5a, b) are so similar to each other that they are almost superimposable, except for the differences in the relative intensities of the peaks at $m/z346$, 328, 165 and 143. The origin of the significant peaks at $m/z207\sim526$ were rationalized as illustrated in both Figures. It is noteworthy that, in both spectra, the characteristic fragment ions produced by the cleavage of glycosidic linkage and elimination of SO_3 commonly constitute the main peaks. As shown in Fig. 5c, ΔUA-GalNAcDiS gives the CID MS/MS spectrum markedly different from those of ΔUA2S-GalNAc4S and ΔUA2S-GalNAc6S. In this spectrum, the elimination of SO_3 from the parent ion gives the most abundant ion at $m/z526$, and two other significant peaks at $m/z448$ ($[Y_1-H+3Na]^+$) and 328 ($[(Y_1-H+3Na)\text{-}NaHSO_4]^+$). Thus, it is easy to distinguish ΔUA-GalNAcDiS from ΔUA2S-GalNAc4S and ΔUA2S-GalNAc6S by the positive-ion CID-MS/MS spectra of $[M-2H+3Na]^+$ ($m/z606$) of these isomers.

The positive-ion CID-MS/MS spectra of $[M-3H+4Na]^+$ ions ($m/z628$) of ΔUA2S-GalNAc4S, ΔUA2S-GalNAc6S and ΔUA-GalNAcDiS are shown in Fig. 6, where more pronounced differences among these three isomers are observed. In the spectrum of ΔUA2S-GalNAc4S (Fig. 6a), the most predominant peak at $m/z165$ corresponding to $[Na_3SO_4]^+$ and three intense peaks at $m/z327$, 323 and 305 corresponding to characteristic fragment ions,

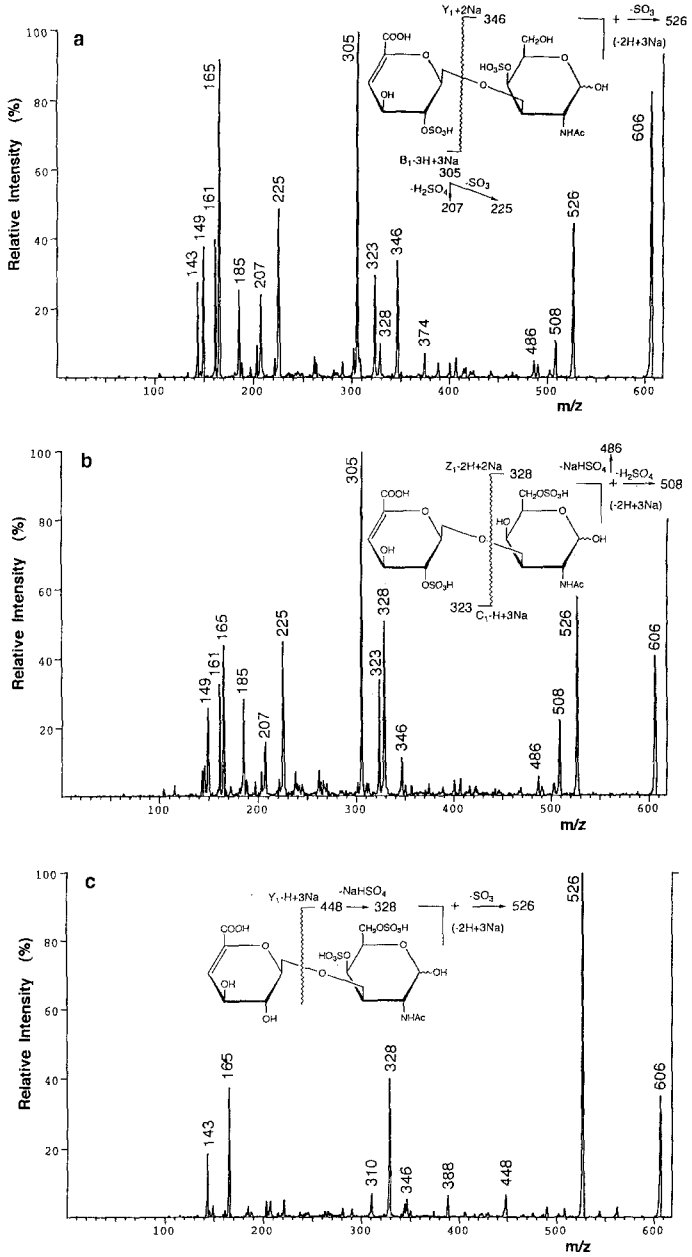


Figure 5. MS/MS spectra of disulfated unsaturated disaccharides having $[M-2H+3Na]^+$ ions (*m/z*606), as the parent ion. (a) ΔUA2S-GalNAc4S, (b) ΔUA2S-GalNAc6S, (c) ΔUA-GalNAcDiS.

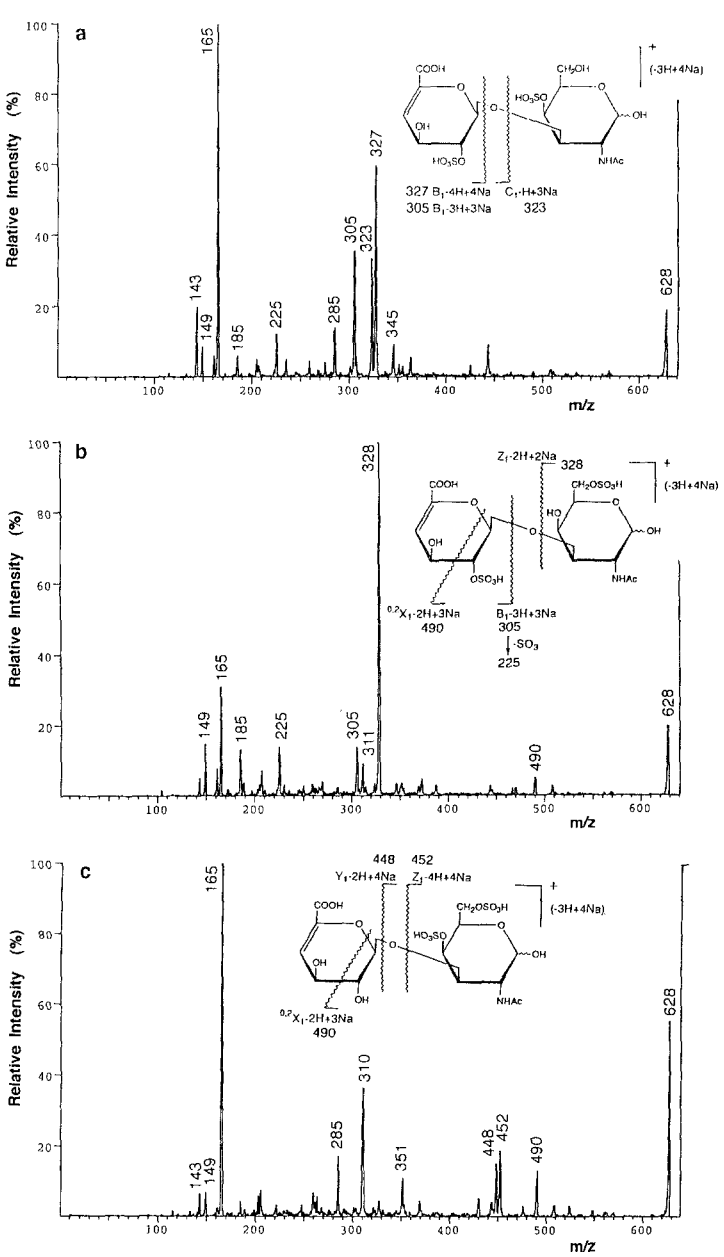


Figure 6. MS/MS spectra of disulfated unsaturated disaccharides having $[M-3H+4Na]^+$ ions (*m/z*628), as the parent ion. (a) ΔUA2S-GalNAc4S, (b) ΔUA2S-GalNAc6S, (c) ΔUA-GalNAcDiS.

$[B_1-4H+4Na]^+$, $[C_1-H+3Na]^+$ and $[B_1-3H+3Na]^+$, respectively, were observed. In the spectrum of ΔUA2S-GalNAc6S, the most abundant peak at *m/z*328 corresponding to the characteristic ion $[Z_1-2H+2Na]^+$ and two weak peaks at *m/z*490 ($[^{0,2}X_1-2H+3Na]^+$) and 305 ($[B_1-3H+3Na]^+$) are observed. Finally in the spectrum of ΔUA-GalNAcDiS, the most predominant peak at *m/z*165 ($[Na_3SO_4]^+$) and three significant peaks at *m/z*490 ($[^{0,2}X_1-2H+3Na]^+$), 452 ($[Z_1-4H+4Na]^+$) and 448 ($[Y_1-2H+4Na]^+$) were observed.

These observations show that positive-ion CID-MS/MS of $[M-3H+4Na]^+$ ions (*m/z*628) of ΔUA2S-GalNAc4S, ΔUA2S-GalNAc6S and ΔUA-GalNAcDiS provide an easy method of identifying these positional isomers of disulfated disaccharides.

(*IV*) *Trisulfated disaccharide*

As shown in Fig. 7, positive-ion CID-MS/MS spectrum of $[M-3H+4Na]^+$ ion (*m/z*708) of ΔUA2S-GalNAcDiS exhibits the dominant peaks at *m/z*628 and 610 corresponding to the ions produced by the eliminations of SO_3 and H_2SO_4, respectively. Five characteristic peaks derived by

Table 3. Major ions in MS/MS of $[M-H]^-$ and $[M+Na-2H]^-$ ions of ΔUA-GalNAc4S, ΔUA-GalNAc6S and ΔUA2S-GalNAc (*m/z*, relative peak intensities).

$[M-H]^-$			$[M+Na-2H]^-$			
ΔUA-GalNAc4S	*ΔUA-GalNAc6S*	*ΔUA2S-GalNAc*	*ΔUA-GalNAc4S*	*ΔUA-GalNAc6S*	*ΔUA2S-GalNAc*	*Ion structure*
458	458	458	480	480	480	Parent ion
342 (65)	342 (12)	342 (8)				$^{0,2}X_1-H$
			304 (3)	304 (5)		$Z_1+Na-3H$
300 (100)	300 (5)		300 (5)	300 (<1)		Y_1
282 (6)	282 (85)		282 (8)	282 (39)		Z_1-2H
					277 (82)	C_1+Na-H
			274 (9)			*m/z*304−30
					259 (45)	$B_1+Na-3H$
			244 (10)	244 (3)		*m/z*304−60
		237 (4)				B_1-2H
				220 (11)		*m/z*300−SO_3
175 (16)	175 (64)					C_1
					167 (25)	
					161 (42)	
157 (12)	157 (14)					B_1-2H
		157 (82)				*m/z*237−SO_3
			154 (100)			
	139 (18)			139 (100)		$[OHCCH_2OSO_3]^-$
139 (20)					139 (100)	
97 (35)	97 (100)	97 (100)	97 (24)	97 (19)		$[HSO_4]^-$
	87 (59)					
85 (11)	85 (14)	85 (20)				$^{0,3}A_1-H$

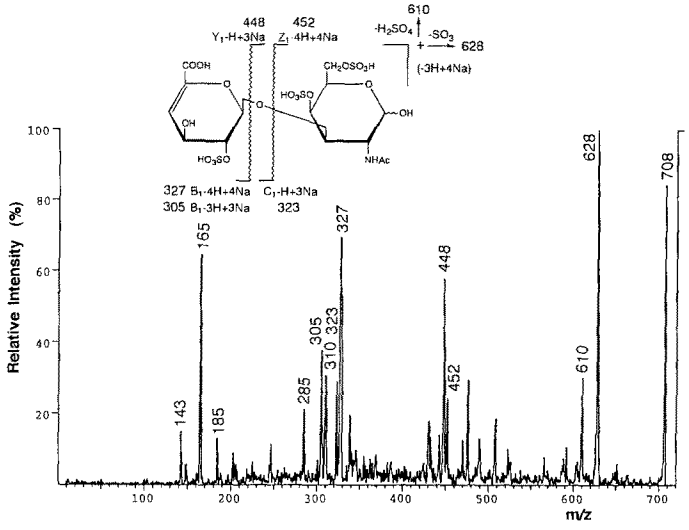


Figure 7. MS/MS spectrum of trisulfated unsaturated disaccharide ΔUA2S-GalNAcDiS having $[M-3H+4Na]^+$ ion (*m/z*708), as the parent ion.

the cleavages of the glycosidic linkage were also observed at *m/z*452 ($[Z_1-4H+4Na]^+$), 448 ($[Y_1-H+3Na]^+$), 327 ($[B_1-4H+4Na]^+$), 323 ($[C_1-H+3Na]^+$ and 305 ($[B_1-3H+3Na]^+$). These results indicate that the spectrum can be used for identification of ΔUA2S-GalNAcDiS.

MS/MS SPECTRA OF THE NEGATIVE-ION FAB OF NON-, MONO-, DI- AND TRISULFATED DISACCHARIDES

In the negative-ion CID-MS/MS of $[M-H]^-$ and $[M+Na-2H]^-$ ions, ΔUA-GalNAc and ΔUA-GlcNAc gave the spectra very similar to each other, from which it is impossible to distinguish these epimeric isomers.

The results of the negative-ion CID-MS/MS spectra of $[M-H]^-$ and $[M-2H+Na]^-$ ions of ΔUA-GalNAc4S, ΔUA-GalNAc6S and ΔUA2S-GalNAc are shown in Table 3, where pronounced differences among these isomers are observed. In the spectra of $[M-H]^-$ ions, the diagnostic peaks of ΔUA-GalNAc4S were found at *m/z*342 ($[^{0,2}X_1-H]^-$) and 300 ($[Y_1]^-$), while those of ΔUA-GalNAc6S were found at *m/z*282 ($[Z_1-2H]^-$), 175 ($[C_1]^-$), and 97 ($[HSO_4]^-$), and of ΔUA2S-GalNAc at *m/z*157 ($[(B_1-2H)-SO_3]^-$) and 97 ($[HSO_4]^-$). In the spectra of $[M+Na-2H]^-$ ions, the diagnostic peak of ΔUA-GalNAc4S was observed at *m/z*154 (not identified), while those of ΔUA-GalNAc6S were observed at *m/z*282 ($[Z_1-2H]^-$) and 139 ($[OHCCH_2OSO_3]^-$), and of ΔUA2S-GalNAc at *m/z*277 ($[C_1+Na-H]^-$) and 259 ($[B_1+Na-3H]^-$).

Lamb *et al.* reported that the negative-ion CID-MIKE spectra of $[M-H]^-$ and $[M+Na-2H]^-$ ions at high collision energy (8 KeV) were useful for distinguishing these isomeric monosulfated disaccharides [20]. Our spectra obtained

Table 4. Major ions in MS/MS of $[M+Na-2H]^-$ and $[M+2Na-3H]^-$ ions of ΔUA2S-GalNAc4S, ΔUA2S-GalNAc6S and ΔUA-GalNAcDiS (*m/z*, relative peak intensities).

$[M+Na-2H]^-$			$[M+2Na-3H]^-$			*Ion structure*
ΔUA2S-GalNAc4S	*ΔUA2S-GalNAc6S*	*ΔUA-GalNAcDiS*	*ΔUA2S-GalNAc4S*	*ΔUA2S-GalNAc6S*	*ΔUA-GalNAcDiS*	
560	560	560	582	582	582	Parent ion
480 (7)	480 (7)	480 (100)				Parent ion-SO_3
444 (45)	444 (25)		444 (9)	444 (8)	444 (3)	$^{0,2}X_1+Na-2H$
422 (14)						$^{0,2}X_1-H$
					402 (8)	Y_1+Na-H
			397 (17)			
					384 (100)	$Z_1+Na-3H$
			379 (21)			
			309 (22)			
304 (7)	304 (12)					$Z_1+Na-3H$
		304 (19)				$m/z384-SO_3$
300 (100)	300 (67)					Y_1
					286 (58)	$m/z384-H_2SO_4$
282 (12)	282 (58)		282 (43)	282 (100)		Z_1-2H
		282 (56)				
277 (16)	277 (22)		277 (77)	277 (79)		C_1+Na-H
					264 (58)	$m/z384-NaHSO_4$
261 (48)						
			259 (100)	259 (64)		$B_1+Na-3H$
					239 (28)	
	237 (22)					B_1-2H
			223 (72)			
	221 (45)					
199 (30)	199 (23)					
			167 (32)	167 (52)		
157 (48)	157 (89)					$m/z237-SO_3$
154 (60)		154 (46)				
139 (36)	139 (100)	139 (44)	139 (83)	139 (94)	139 (8)	$[OHCCH_2OSO_3]^-$
97 (33)	97 (30)	97 (12)	97 (26)	97 (13)	97 (27)	$[HSO_4]^-$

at low collision energy (30 eV), though different from theirs in the spectral features, also provide an easy method to differentiate the positional isomers of the monosulfates.

The results of the negative-ion CID (30 eV)-MS/MS spectra of $[M+Na-2H]^-$ and $[M+2Na-3H]^-$ ions of ΔUA2S-GalNAc4S, ΔUA2S-GalNAc6S and ΔUA-GalNAcDiS are represented in Table 4, where distinct differences among these positional isomers are also observed. In the spectra of $[M+Na-2H]^-$ ions, characteristic fragment ions of ΔUA2S-GalNAc4S are found at *m/z*444 ($[^{0,2}X_1+Na-2H]^-$), 300 ($[Y_1]^-$), 261 (not identified), 157 ($[(B_1-2H)-SO_3]^-$) and 154 (not identified), of ΔUA2S-GalNAc6S at *m/z*300 ($[Y_1]^-$), 282 ($[Z_1-2H]^-$), 221 (not identified), 157 ($[(B_1-2H)-SO_3]^-$) and 139 ($[OHCCH_2OSO_3]^-$), and of ΔUA-GalNAcDiS at *m/z*480 ($[(M+Na-2H)-SO_3]^-$), 282 (not identified), 154 (not identified) and 139 ($[OHCCH_2OSO_3]^-$). In the spectra of $[M+2Na-3H]^-$ ions, characteristic abundant ions of ΔUA2S-GalNAc4S are observed at *m/z*282 ($[Z_1-2H]^-$), 277 ($[C_1+Na-H]^-$), 259 ($[B_1+Na-3H]^-$), 223 (not identified) and 139 ($[OHCCH_2OSO_3]^-$), whereas of ΔUA2S-GalNAc6S at *m/z*282 ($[Z_1-2H]^-$), 277 ($[C_1+Na-H]^-$), 259 ($[B_1+Na-3H]^-$), 167 (not identified) and 139 ($[OHCCH_2OSO_3]^-$), and of ΔUA-GalNAcDiS at *m/z*384 ($[Z_1+Na-3H]^-$), 286 ($[Z_1+Na-3H)-H_2SO_4]^-$) and 264 ($[Z_1+Na-3H)-NaHSO_4]^-$). These observations clearly indicate that the negative-ion CID-MS/MS spectra of $[M+Na-2H]^-$ and $[M+2Na-3H]^-$ ions of the three disulfated disaccharides are also very useful to distinguish these positional isomers.

As shown in Fig. 8, negative-ion CID-MS/MS spectrum of the $[M+2Na-3H]^-$ ion (*m/z*684) of ΔUA2S-GalNAcDiS gives characteristically the B, C, Z and X-type fragment ions. The assignment of the fragment ions are illustrated in this Figure. These observations indicate that the negative-ion CID-MS/MS is also useful to identify this trisulfate.

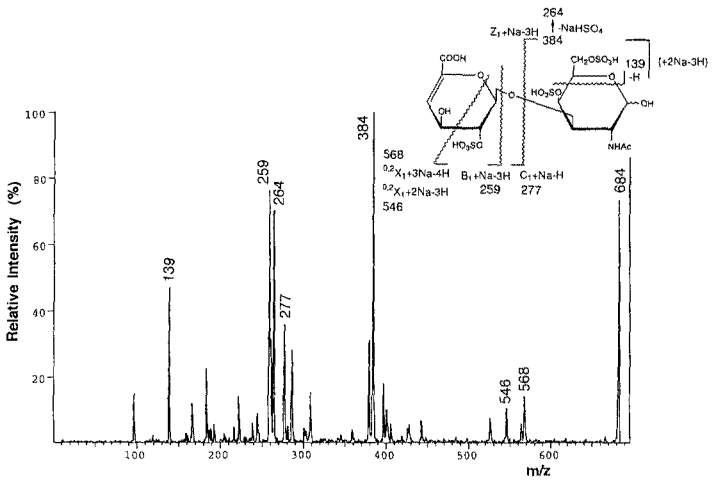


Figure 8. MS/MS spectrum of trisulfated unsaturated disaccharide ΔUA2S-GalNAcDiS having $[M+2Na-3H]^-$ ions (*m/z*684), as the parent ion.

Conclusion

Positive- and negative-ion FAB mass spectra of the sodium salts of nine disaccharides from CS, DS and HA show distinct molecule related ions, giving only molecular weight information. The CID-MS/MS spectra of the selected molecule related ions at low collision energy both in the positive- and negative-ion modes can be used to differentiate between the two epimeric isomers of nonsulfated disaccharides and the respective three positional isomers of mono- and disulfated disaccharides. The tandem FABMS in both modes also provides useful information for identification of the trisulfated disaccharide. Our results suggest that the tandem FABMS will provide a new rapid method for the analysis of disaccharides from CS and DS.

Because of the acidic nature of sulfated saccharides, the negative-ion FABMS have been widely used in the structural studies of these compounds [28]. However, our results indicate that the positive-ion tandem FABMS of sodiated molecule related ions, as well as negative-ion tandem FABMS, is useful for characterization of sulfated saccharides. We are currently extending our studies to the characterizations of unsaturated disaccharides from heparin and heparan sulfate, and unsaturated tetrasaccharides from CS.

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