Structure Elucidation of Hexasaccharide Derivatives Obtained from Keratan Sulfates by Ion Trap Mass Spectrometry

Masayuki Kubota, Keiichi Yoshida, Akira Tawada, and Mamoru Ohashi


REGULAR PAPER

1. Introduction

Glycoconjugates such as chondroitin sulfates, heparan sulfates, and keratan sulfates play important roles in the recognition between biomolecules on the cell surface and function as part of various interactions. These functions are controlled by repeated sugar species and specific modifications. Numerous papers on the characterization of carbohydrates using mass spectrometry have been published. For example, fast atom bombardment ionization followed by collision-induced dissociation tandem mass spectrometry (FAB-CID-(MS)2) has been applied to the characterization of glycosaminoglycan-derived oligosaccharides. We have demonstrated that FAB-CID-(MS)2 can be successfully applied to the characterization of several oligosaccharides originating from chondroitin sulfates, hyaluronan, heparin and heparan sulfates, tetrasaccharide tri- and tetra-sulfates from immunoreactive chondroitin sulfate fragments, and oligosaccharides of the lactosamine series derived from keratan sulfates. In this paper we would like to demonstrate the structure elucidation of a modified hexasaccharide obtained from keratan sulfates by the ESI-ion trap (MS)2 technique.

2. Experimental

2.1 Materials

All samples were supplied from Seikagaku Co. As with the notations of saccharides described in the previous paper, we use L1 for N-acetyllactosamine and 3L1 for a lactosamine trimer. The structures of L1 and 3L1 are shown in Scheme 1.

3L1P1, 3L1P2, and 3L1P3: A mixture of the three compounds was prepared from shark keratan sulfate by partial desulfation with methanolic hydrochloride prepared from anhydrous methanol and acetyl chloride followed by digestion with keratanase II as described by Brown et al. The resulting mixtures of desulfated oligosaccharides were then separated into fractions by repeated gel-permeation chromatography and anion-exchange chromatography.

2.2 Mass spectrometry

FAB mass spectra were recorded on a ThermoFinnigan TSQ700 triple stage quadrupole mass spectrometer equipped with an IonTech FAB gun. A Xenon beam with an energy of 8 kV was used. Samples were dissolved in water to a concentration of 20 μg/mL and 0.5 μL of the sample was loaded onto the probe with 0.5 μL of matrix solution [glycerol-thioglycerol = 1 : 1 (v/v)]. FAB-MS/MS spectra were obtained using argon collision gas at typically 0.133 Pa with collision energy of 30 eV.

ESI-CID-(MS)2 spectra were recorded on a ThermoFinnigan LCQ Deca Ion-trap mass spectrometer. Samples were dissolved in methanol-water [1 : 1 (v/v)] to a concentration of 10 ng/μL and introduced into the mass spectrometer using a syringe pump operated at
10 μL/min. The spray voltage and heated capillary temperature was set to 5 kV and 300°C, respectively. CID-(MS)n were performed using collision energies which produced the maximum number of desired product ions.

3. Results and Discussion

3.1 FAB-CID-(MS)n spectra of 3L1

The positive and negative FAB-CID-(MS)n spectra of sodiated 3L1, [M+Na]+ (m/z 1,136), and deprotonated 3L1, [M−H]− (m/z 1,112), are shown in Figs. 1a and 1b, respectively. In the positive ion spectrum, the glycosidic bond cleavage ions, Y1 (m/z 244), Y2 (m/z 406), Y3 (m/z 609), Y4 (m/z 771), B5 (m/z 915), B4 (m/z 753), B3 (m/z 550), and B2 (m/z 388) according to the notation proposed by domon and Costello22) are clearly seen. In the negative ion mode, in addition to the glycosidic bond cleavage ions at C5 (m/z 909), Y4 or C4 (m/z 747), C3 (m/z 544), Y2 or C2 (m/z 382), B5 (m/z 891), B4 (m/z 729), B3 (m/z 526), and B2 (m/z 364), the ring cleavage ions at 0,2A6 (m/z 1,011), 2,4A6 (m/z 951), 0,2A4 (m/z 646), and 0,2A2 (m/z 281) are observed. Table 1 summarizes the fragment ions of 3L1 and its derivatives.

3.2 FAB-MS spectra of a mixture of 3L1P1, 3L1P2, and 3L1P3, and FAB-CID MS spectra of positive [M+Na]+ and negative [M−H]− ion of 3L1P1

The positive and negative FAB-MS spectra of the mixture of 3L1P1, 3L1P2, and 3L1P3 are shown in Figs. 2a and 2b, respectively. The peaks at m/z 1,128, 1,142, and 1,160 in the positive spectrum correspond to those of m/z 1,126, 1,140, and 1,158 in the negative spectrum, respectively, indicating that the sample is a mixture of three compounds whose molecular weights are 1,127, 1,141, and 1,159. The characteristic spectral features of negative FAB-MS are remarkable. Four

![Scheme 1. Structures of L1 and 3L1](image)

Figs. 1a and 1b. The positive (1a) and the negative (1b) ion mode FAB-CID-(MS)n spectra of 3L1.
groups of peaks are easily assigned to $Y_2$ ($m/z$ 396, 410, 428), $Y_3$ ($m/z$ 599, 613, 631), $Y_4$ ($m/z$ 761, 775, 793), $Y_5$ ($m/z$ 964, 978, 996), and $[M-8H]^-$ ($m/z$ 1,126, 1,140, 1,158). From the spectra of the mixture, peaks corresponding to 3L1P1 were chosen and the positive $[M+Na]^+$ and negative $[M-H]^-$ FAB-CID-(MS)² spectra of this compound were obtained as shown in Figs. 3a and 3b, respectively. The glycosidic bond cleavage ions (Y series) are clearly observed in the negative spectrum.

### 3.3 The ESI MS spectrum of the mixture

The positive and negative ESI-MS spectra of the mixture are shown in Figs. 4a and 4b, respectively. Again the $[M+Na]^+$ ion group in the positive ion mode and the $[M-H]^-$ ion group in the negative ion mode are clearly shown as the mixture of three compounds in these spectra.

### 3.4 Structure of 3L1P1

The CID-(MS)² of the $[M+Na]^+$ ion, $m/z$ 1,150, is shown in Fig. 5 together with the peak assignment. The major peaks are labeled as $Y_2$ ($m/z$ 420), $Y_3$ ($m/z$ 623), $Y_4$ ($m/z$ 785), $Y_5$ ($m/z$ 988), and $B_2$ ($m/z$ 388), $B_3$ ($m/z$ 550), $B_4$ ($m/z$ 753), and $B_5$ ($m/z$ 915). The positive (MS)³ spectrum of 3L1P1 ($m/z$ 1,150>785>420) is superimposed on the (MS)² spectrum of the standard N-acetyllactosamine methyl glycoside (L1-CH₃) as shown in Figs. 6a and 6b. Similarly, the negative (MS)³ spectrum of 3L1P1 exhibits nicely the glycosidic bond cleavage ions, $B_2$ ($m/z$ 891), $B_3$ ($m/z$ 729), $B_5$ ($m/z$ 526), $B_2$ ($m/z$ 364), $Y_2$ ($m/z$ 396), $Y_4$ ($m/z$ 599), $Y_4$ ($m/z$ 761), and $Y_5$ ($m/z$ 964), as shown in Fig. 7. The negative (MS)³ spectrum ($m/z$ 1,126>396) almost overlap again with the negative (MS)² spectrum of L1-CH₃ as shown in Figs. 8a and 8b. On the basis of these results it
became clear that the structure of 3L1P1 is the methyl glycoside of 3L1, whose structure and fragment ions in the negative ion mode are shown in Fig. 7.

3.5 The structure of 3L1P2

The molecular weight of this compound (3L1 + 28) together with the fragmentation pattern of the compounds shown in Fig. 2b suggests that the reducing end glycosamine moiety is dimethylated. The precise structure of this compound has not yet been determined and will be discussed elsewhere.

3.6 The structure of 3L1P3

The positive ESI-(MS)² spectrum of the sodiated 3L1
P3 molecule \([\text{M+Na}]^+\) (m/z 1,182) is shown in Fig. 9 which exhibit Y2, Y3, Y4, Y5 ions at m/z 452, 655, 817, and 1,020, respectively, and B5, B6, B5, B2 ions at m/z 915, 753, 550, and 388, respectively. In addition to these glycosidic bond cleavage ions, there is a remarkable peak at m/z 1,035 which corresponds to [MNa –
CHN\text{AcCH(OCH}_3\text{)}_2−H]^+$. The positive ESI-(MS)$^3$ spectrum of 3L1P3 (m/z 1,182 > 452) is shown in Fig. 10. In addition to [P−H$_2$O]$^-$(m/z 434), [P−CH$_3$OH]$^-$(m/z 420), [P−2CH$_3$OH]$^-$(m/z 388) [where P is the precursor ion at m/z 452] and Y$_1$ (m/z 290), there is a remarkable peak at m/z 305 which corresponds to [P−
CHNHAcCH(OCH₃)₂. The presence of a remarkable peak at m/z 305 suggests the presence of dimethyl acetal structure as shown in Fig. 9.

Similarly the negative ESI-(MS)² spectrum of 3L1P3 is shown in Fig. 11 in which the glycosidic bond cleavage ions Y₂, Y₃, Y₄, B₅, and B₆ are observed at m/z 428, 631, 793, 996, 891, and 729, respectively. In addition, as shown in Fig. 12, the negative ion ESI-(MS)³ spectrum of this compound (m/z 1,158 > 428) exhibits the glycosidic bond cleavage ions Y₁ (m/z 266 and 248) and B₁ (m/z 179). Furthermore there are remarkable ions of [P′−CH₃OH]⁻ (m/z 396), [P′−CH₂CO]⁻ (m/z 386), [P′−CHOCH₂OH]⁻ (m/z 368), and [m/z 368−2 CH₃OH]⁻ (m/z 304), where P′ means the precursor ion at m/z 428. The negative ion spectra also suggest the presence of a dimethyl acetal structure in 3L1P3 (Fig. 11).
4. Conclusions

In the course of investigation it was found that using a triple quadrupole mass spectrometer was relatively difficult for the characterization of modification sites on the sugar rings of oligosaccharides. In comparison, the (MS)$^n$ ability of the ion trap mass spectrometer allows further characterization of each fragment generated by CID-(MS)$^2$ permitting the acquisition of further structural information. The comparison of the negative FAB-MS of 3L1 with that of the mixture clarified that the unknown compounds are modified on the reducing end sugar of 3L1, but no precise structural information was obtained. However, when the ion trap (MS)$^n$ technique was applied, we were able to deduce the structure of the unknown derivatives of 3L1 as 3L1-OCH$_3$ and 3L1-dimethylacetal.

Since methanolic hydrochloride was used for the
desulfation process, the formation of methyl glycoside or dimethylacetal is conceivable during this process. Even if 3L1-\textit{OCH}_3 and 3L1-dimethylacetal are artifacts, the structure elucidation processes mentioned here demonstrate the unique ability of the ion-trap (MS)\textsuperscript{n} technique.

**Acknowledgments**

The authors gratefully acknowledge the support of the Seikagaku Corporation and the Mizutani Foundation for Glycoscience.

**References**


**Keywords**: Ion trap, (MS)\textsuperscript{n}, Glycoconjugate, FAB, ESI, Lactosamine

---

**Scheme 2.** The structure of dimethyl acetal derivative of disaccharide part and its fragmentation in the positive and negative modes.