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SHORT COMMUNICATION

A new technique distinguishing α2-3 sialyl linkage from α2-6 linkage in sialyllactoses and sialyl-*N*-acetyllactosamines by post-source decay fragmentation method of MALDI-TOF mass spectrometry

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α2-3 and α2-6 sialyl linkage types of sialyllactoses and sialyl-*N*-acetyllactosamines were analyzed by post-source decay (PSD) fragmentation method using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. A new matrix of norharmane was suited for the MALDI-TOF measurements of sialyl oligosaccharides. The fragment ions B_1 produced by the cleavage of α2-3 sialyl linkages indicate much higher intensity than those produced by the cleavage of α2-6 sialyl linkages in sialyllactoses and sialyl-*N*-acetyllactosamines. Thus, α2-3 sialyl linkages cleave much easier than α2-6 sialyl linkages in MALDI-PSD fragmentation method. These results suggest that the new techniques using PSD fragmentation of MALDI-TOF mass spectrometry enables us to distinguish α2-3 sialyl linkage from α2-6 linkage in sialyl oligosaccharides.

Keywords: **sialyl oligosaccharides, α2-3/α2-6 sialyl linkage analysis, MALDI-TOFMS, PSD fragmentation, new matrix**

Introduction

Sialyl oligosaccharides play important roles in biological activity such as cell-cell interaction, cell-substance adhesion, and virus-host interaction [1]. In many cases, the expression of the biological activities depends on the structures of sialyl oligosaccharides. Therefore, it is very important to develop a new method to analyze in detail sialyl linkage types. Until now the linkage analyses by mass spectrometry for methylated oligosaccharides have been reported using GC/MS and collisional-activation tandem mass spectrometry [2,3].

In this study, we analyze non-derived sialyl oligosaccharides by post-source decay (PSD) fragment method using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. NeuNAcα2→3Galβ1→4Glc/GlcNAc (3′-sialyllactose/3′-sialyl-*N*- acetyllactosamine) and NeuNAcα2→6Galβ1→4Glc/GlcNAc (6′-sialyllactose/6′-sialyl-*N*-acetyllactosamine) are structural isomers (Figure 1). The structural difference of these compounds is only at sialyl linkage. It is very difficult to distinguish these structural isomers by NMR, MS, and other spectroscopic methods until now.

A new type reflector of curved field reflection in TOF mass detector enables a simultaneous focusing of a wide mass range of metastable fragment ions and observation of the entire PSD spectrum in a single experiment [4]. Thus, the relative intensity of the PSD fragment ions can be discussed in more accurate level. We previously reported the exploitation of the relative ion intensities in the MALDI-PSD fragment spectra of oligosaccharides [5–9].

The matrix of 2,5-Dihydroxybenzoic acid (DHBA) was generally used for oligosaccharides. 2′,4′,6′-trihydroxyacetophenone (THAP) was used for sialyl oligosaccharides by D. I. Papac et al. [10]. We try to use a new matrix of norharmane [11,12] for the measurements of sialyllactoses and sialyl-*N*-acetyllactosamines in our experiments.

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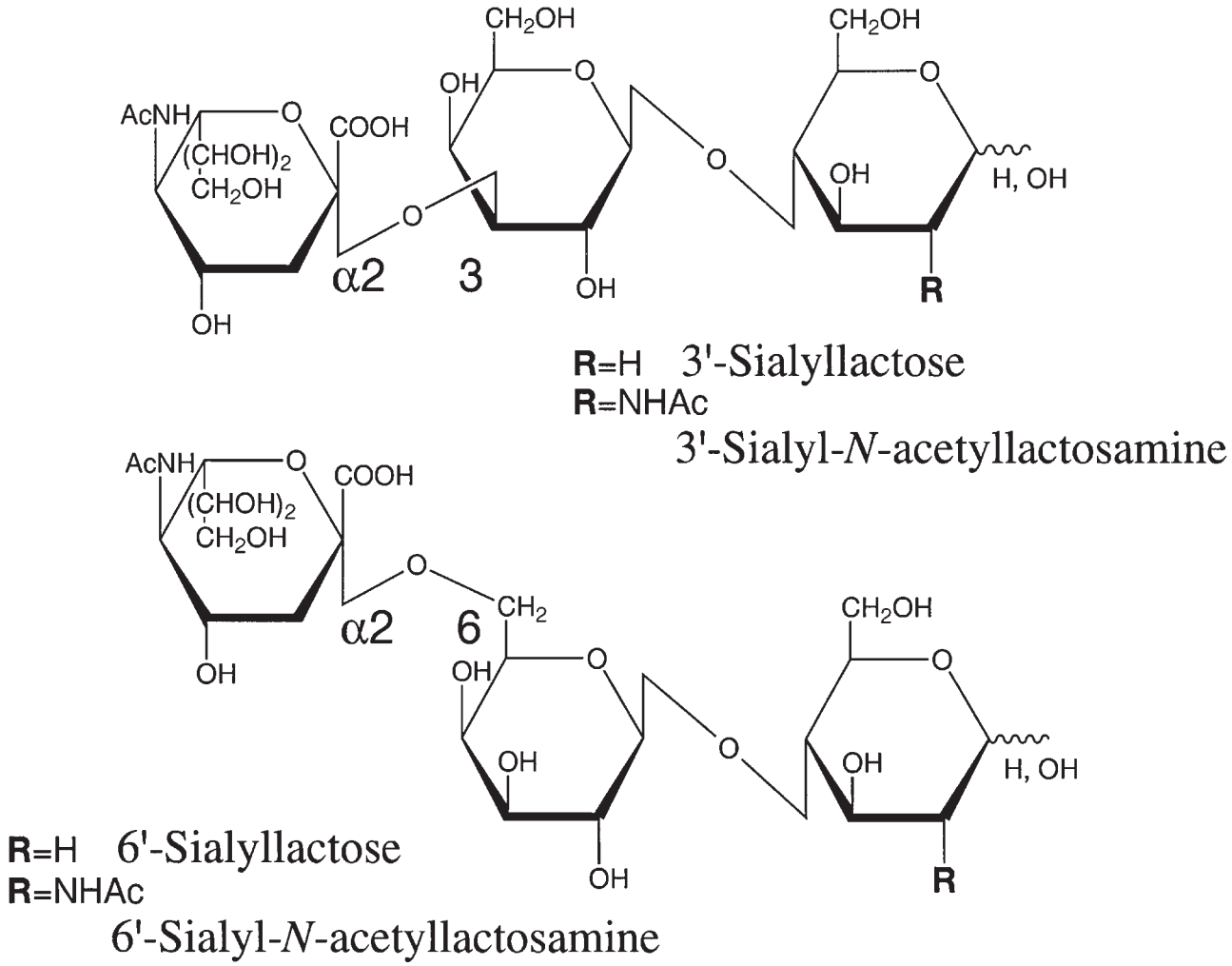
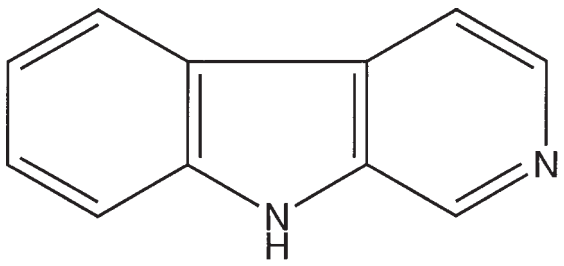


Figure 1. Structures of 3′- and 6′-sialyllactoses and sialyl-*N*-acetyllactosamines.

Materials and methods

All mass spectra were measured by KOMPACT MALDI IV instrument (Shimadzu Corp., Japan). Operation conditions were as follows; nitrogen laser (337 nm), acceleration energy of 20 kV, reflection mode. DHBA and norharmane were used as matrix at a concentration of 10 mg/mL (DHBA in 40% acetonitrile, norharmane in 50% acetonitrile aqueous solution). After 0.5 μL sample solution was added to 0.5 μL matrix solution on a plate, the sample plate was dried completely. 500 pmol of samples were used on a spot (50 pmol of samples also showed the same spectral results). In the positive measurements using DHBA, an aliquot of 0.5% NaCl solution was added to accerelate ionization. Each spectrum was the average of one hundred



Structure of norharmane

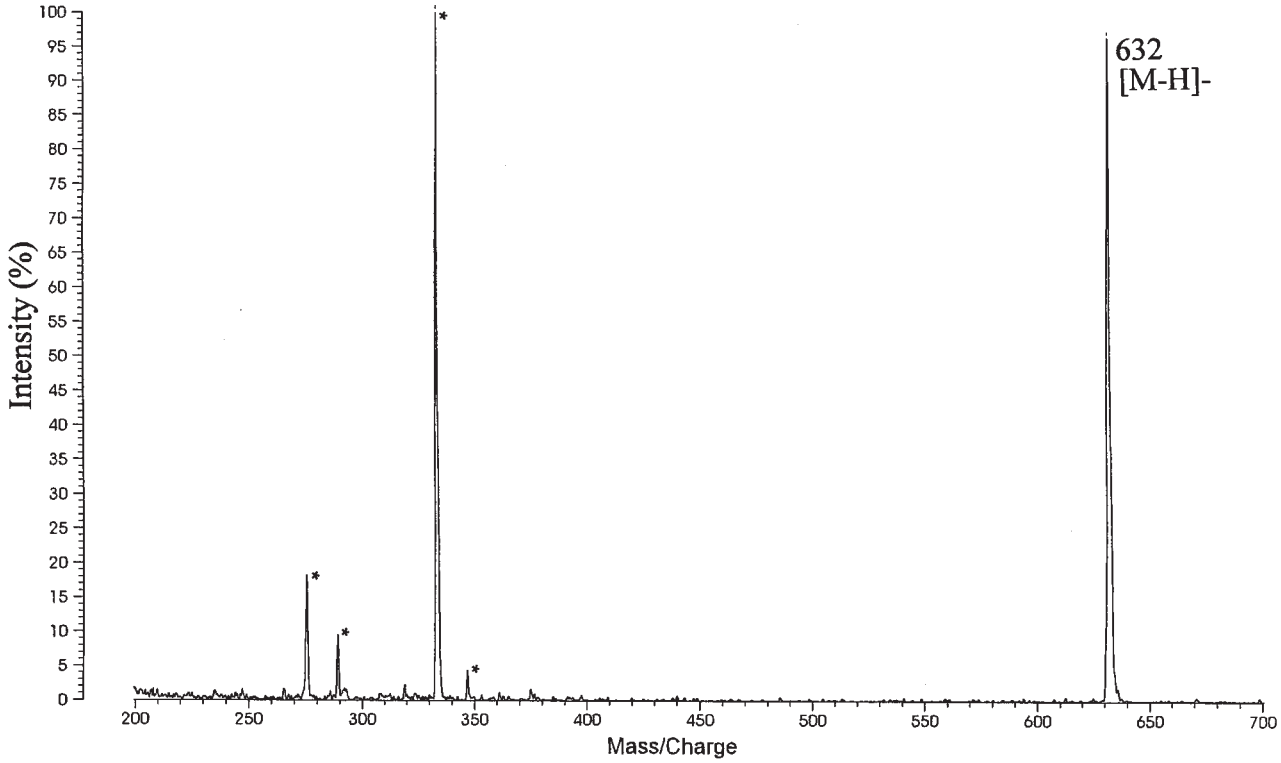


Figure 2. The negative mode MALDI-TOF mass spectrum of 3′-sialyllactose using a matrix of norharmane. *The ion peaks marked by asterisk come from the matrix.

shots. To detect the PSD fragment ions, the laser power was adjusted to about 40 μJ. The fragment ions were consistently observed in ten times measurements under the same conditions. The reproducibility of the PSD fragment ion intensity was satisfactorily high [5–9].

In the mass spectra shown in Figures 2 to 4, ions are labelled with the nominal mass of the isotopomer containing only ^{12}C, ^{1}H, and ^{16}O.

Sialyllactoses and sialyl-*N*-acetyllactosamines are commercially available (Funakoshi, Co., Japan).

Results and discussion

When DHBA was used as a matrix for the positive measurements of sialyllactoses, the molecular related ions $[M+Na]^+$ and $[M-H+Na2]^+$ at *m/z* 656 and 678 were observed in the MALDI-TOF mass spectra. In addition to that, the product ions $[NeuNAc-H+2Na]^+$ and $[M-NeuNAc+Na]^+$ at *m/z* 336 and 365 were also observed, which were caused by the cleavage of sialyl linkages. One of the reasons of the detection of the product ion is that the sialyl linkage cleaves much easier than other glycosidic linkages. Another reason is that the matrix DHBA can promote loss of sialic acid [10].

A new matrix of norharmane was used in the negative measurements. In the negative mode MALDI-TOF mass spectra of sialyllactoses, only one molecular ion at *m/z* 632 was observed (Figure 2). The chemical species is the deprotonated ion $[M-H]^-$. The product ion produced by the cleavage of the sialyl linkage was not observed. Thus, the negative measurements using norharmane as a matrix is suite for the molecular weight measurements of sialyl oligosaccharides.

In the negative mode MALDI-PSD fragment spectra of sialyllactoses using norharmane, the precursor ion $[M-H]^-$ at *m/z* 632 and the fragment ion at *m/z* 290 were observed clearly (Figures 3 and 4). The fragment ion was produced by the cleavage of the sialyl linkage. The chemical species is $[M-Gal-Glc-H]^-$ (or $[NeuNAc-H]^-$), which was caused by B type fragmentation (B_1; Figure 5) [13]. The relative intensity of the fragment ion B_1 of 3′-sialyllactose (*ca.* 30% of the precursor ion) was much higher than that of 6′-sialyllactose (*ca.* 5% of the precursor ion) in the MALDI-PSD fragment spectra (Figures 3 and 4). Thus, α2-3 sialyl linkage cleaves much easier than α2-6 sialyl linkage. These results indicate that α2-3 and α2-6 sialyl linkages are able to be distinguished by the analysis of the relative ion intensity in MALDI-PSD fragmentation.

In cases of sialyl-*N*-acetyllactosamines, the precursor ions at *m/z* 673 and the fragment ions B_1 at m/z 290 were observed clearly in their negative mode MALDI-PSD frag-

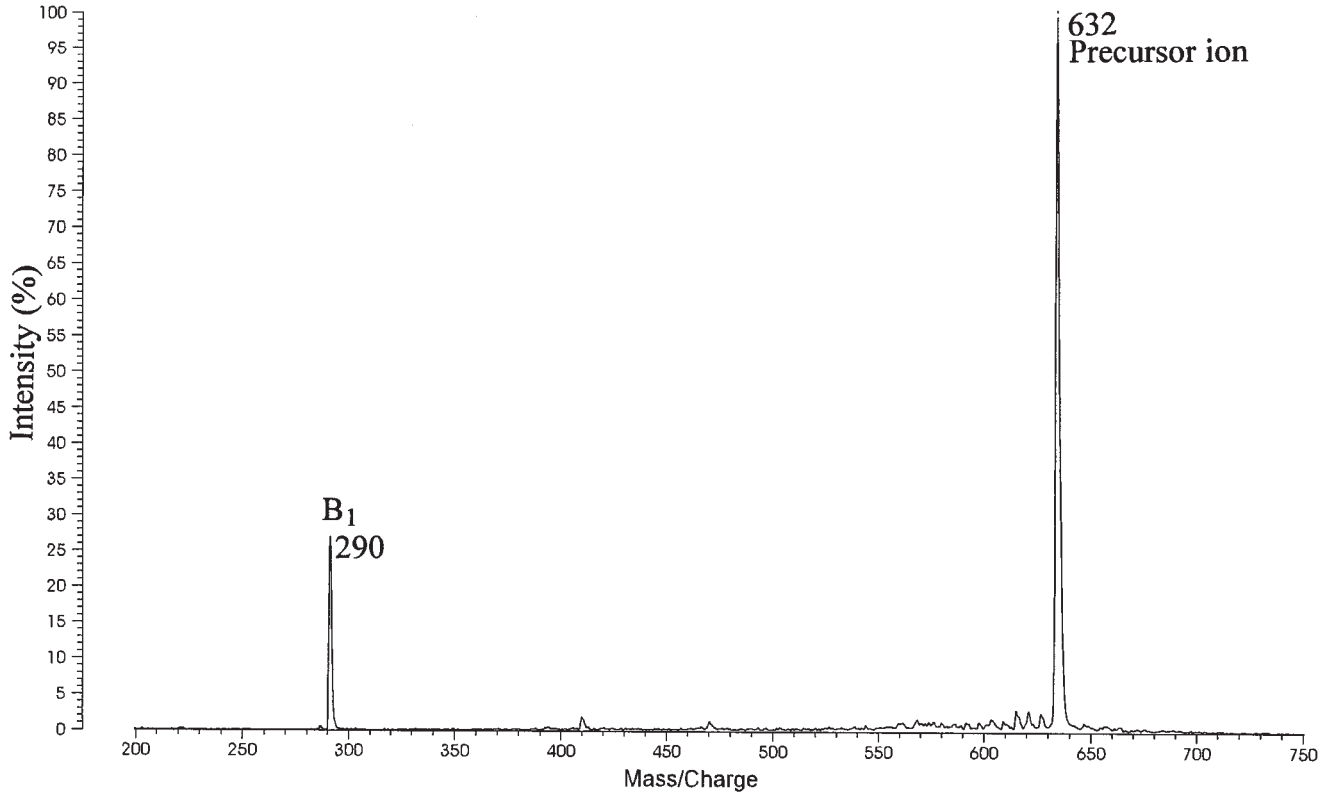


Figure 3. The negative mode MALDI-PSD fragment spectrum of 3′-sialyllactose.

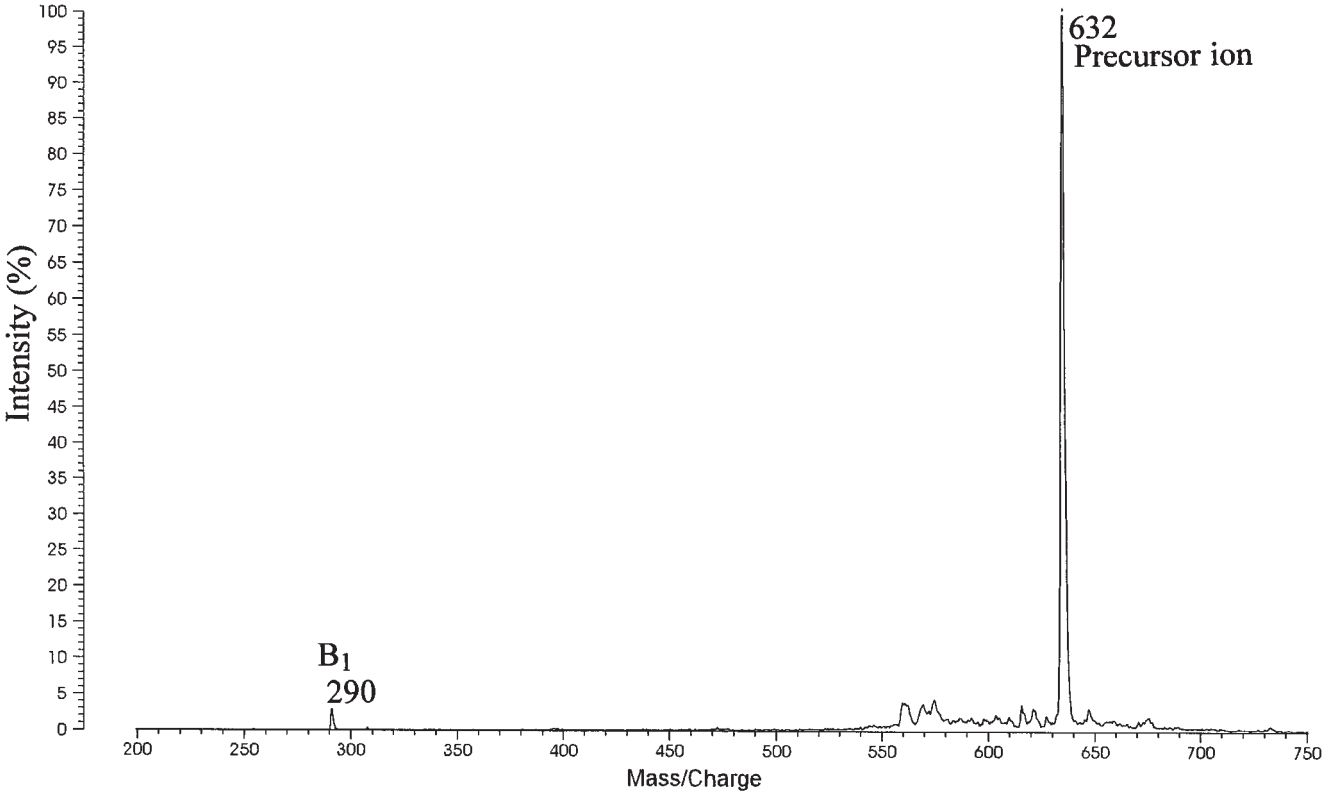


Figure 4. The negative mode MALDI-PSD fragment spectrum of 6′-sialyllactose.

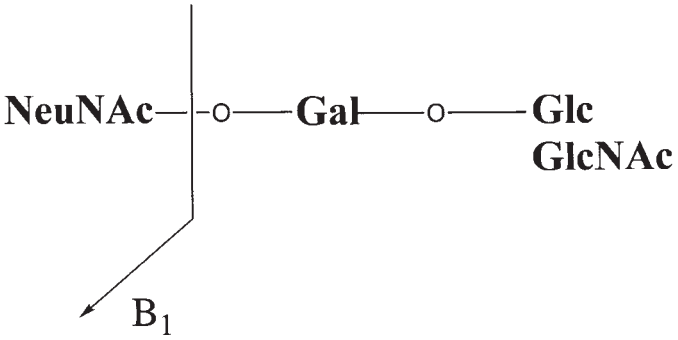


Figure 5. Fragment ion B_1 of sialyllactoses and sialyl-*N*-acetyllactosamines.

ment spectra using norharmane. The relative intensity of the fragment ion B_1 of 3′-sialyl-*N*-acetyllactosamine (*ca.* 40% of the precursor ion) was also much higher than that of 6′-sialyl-*N*-acetyllactosamine (less than 5% of the precursor ion). These two results indicate that the cleavage of α2-3 sialyl linkage occurs much easier than that of α2-6 sialyl linkage in MALDI-PSD fragmentation.

In the positive mode PSD fragment spectra of both sialyllactoses using DHBA, the precursor ions $[M-H+2Na]^+$ at *m/z* 678 and the fragment ions $[NeuNAc-H+2Na]^+$ at *m/z* 336 were observed. The fragment ions $[NeuNAc-H+2Na]^+$ were also produced by B type fragmentation (B_1). The relative intensity of the fragment ion B_1 of 3′-sialyllactose was also much higher than that of 6′-sialyllactose in this positive mode MALDI-PSD fragment spectra. The tendency of these ion intensities in sialyllactoses is the same in sialyl-*N*-acetyllactosamines. These results of the positive mode MALDI-PSD fragmentation are consistent with those of the negative mode PSD-fragmentation. Therefore, these results strongly indicate that α2-3 sialyl linkage cleaves much easier than α2-6 sialyl linkage in MALDI-PSD fragmentation.

In this study, we preliminarily reported that the relative ion intensity analysis in the MALDI-PSD fragment spectra of sialyl oligosaccharides enables us to distinguish α2-3 and α2-6 sialyl linkages. In this experiments, sialyl linkage analysis was performed by the MALDI-PSD fragmentation method without any derivatization such as permethylation. Therefore, this method will be applied to various glycoconjugates having sialic acids in near future, and it will be a potential tool for structural analysis of glycoconjugates.

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