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Convenient and rapid analysis of linkage isomers of fucose-containing oligosaccharides by matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry

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Matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF MS) was used to analyze three pyridylamino (PA)-fucosyloligosaccharides isolated from human milk: lacto-N-fucopentaose (LNFP) I [Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-PA], LNFP II [Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc-PA], and LNFP III [Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-PA]. These oligosaccharides are linkage isomers. MALDI-QIT-TOF MS provides MS^n spectra, which we used to characterize these PA-oligosaccharides. MS/MS/MS analysis of the non-reducing end trisaccharide ions generated by MS/MS was able to distinguish these oligosaccharide isomers. The MALDI-QIT-TOF MS is a very convenient and rapid method, therefore, it would be useful for high throughput structural analyses of various types of pyridylaminated oligosaccharide isomers.
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Introduction

During the past decade, mass spectrometry (MS) has become a key method for elucidating the carbohydrate structures of glycoconjugates. Nevertheless, the structural elucidation of oligosaccharides is still challenging, because the structures involve sequence, branching, and linkage differences. Previously, we analyzed the structures of fucose-containing oligosaccharides using HPLC and the post-source decay (PSD) mode of MALDI-TOF MS [1]. The structures of oligosaccharides were analyzed successfully by comparing the intensities of PSD fragment ions. Other investigators have discussed the relationship between the types of glycosidic bonds and the fragment ion intensities obtained in the PSD mode of MALDI-TOF MS for several cyclodextrin derivatives and two sialyl lactoses [2,3]. Recently, Pfenninger and Karas and other investigators reported the analysis of intact oligosaccharides using nano-electrospray ion-trap MS [4–8].

Fucose-containing oligosaccharides are widely distributed in mammalian tissues [9] and play important roles in blood transfusion, numerous ontogenic events, and cell-to-cell recognition [10–15]. Moreover, fucosylated oligosaccharides have been implicated in the pathogenesis of several human diseases. In cancer, two prominent examples of altered glycosylation involve fucose-containing oligosaccharides. The expression of the A and B blood group antigens is lost in many tumors with concomitant increases in H and Lewis^y expression, and the changes correlate with a poor clinical prognosis [16,17]. In addition, the up-regulation of sialyl Lewis^x and Lewis^a has been demonstrated in numerous cancers.

Fucopentaoses are ideal compounds for evaluating MS methods because they vary only in the position and linkage of the fucose (Fuc) and galactose (Gal) [18,19]. In LNFP I, Fuc links to the Gal of lactotetraose via an α1–2 linkage; in LNFP II, Fuc links to the GlcNAc of lactotetraose via an

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α1–4linkage; and in LNFP III, Fuc links to the GlcNAc of lactotetraose via an α1–3 linkage.

The derivatization of N- and O-linked oligosaccharides of glycoproteins with pyridylamine (PA) and 2-aminobenzamide (2AB) is widely used for structural analysis [20–22]. We can separate and detect these fluorescence-labeled oligosaccharides using HPLC with high sensitivity, and we can then differentiate ion series in the MS/MS spectra of related ions in terms of ions with or without PA or 2AB tags at the reducing ends.

Our present work explores the use of MALDI-QIT-TOF MS to analyze the structure of fucopentaose positional isomers. MALDI is high-throughput/high-sensitivity ionization, and MALDI-QIT-TOF MS has the capability to perform MS^n analyses in which ions are stored in a trap, selected for fragmentation, and fragmented in a cycle that can be repeated several times. Consequently, MS^n mass spectra are useful for the structural analysis of PA-oligosaccharides and give sequence, branching, and linkage information. In this work, we performed MS^n analyses of [M + Na] ions, because PA-oligosaccharides appear to easily adduct alkali metal ions. Significant structural information was obtained from the MS^3 spectra of the nonreducing terminal trisaccharide residues, Fuc-Gal-GlcNAc and Gal-(Fuc)-GlcNAc. Therefore, this method is a convenient and high-throughput method for analyzing oligosaccharide structures.

Experimental procedures

Materials

Three pyridylamino (PA) derivatives of fucose-containing oligosaccharides were purchased from Takara Biomedicals (Kusatsu, Japan): LNFP I (A), LNFP II (B), and LNFP III (C), as listed in Figure 1. These oligosaccharides were prepared as 10 pmol/μl solutions in water. All the solvents used for the MS analyses were of HPLC grade. Bradykinin and angiotensin II were obtained from Sigma-Aldrich (Steinheim, Germany).

(A) Lacto-N-fucopentaose Ⅰ

Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-3Glc-PA

(B) Lacto-N-fucopentaose Ⅱ

Galβ1-3GlcNAcβ1-3Galβ1-4Glc-PA
4
|
Fucα1

(C) Lacto-N-fucopentaose Ⅲ

Galβ1-4GlcNAcβ1-3Galβ1-4Glc-PA
3
|
Fucα1

Figure 1. Structures of the pyridylamino (PA-) derivatives of the fucose-containing oligosaccharides used in this study.

2,5-Dihydroxy- benzoic acid (Wako Pure Chemicals Industries, Osaka, Japan) was used as the matrix.

Matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight (MALDI-QIT-TOF) mass spectrometry

MALDI experiments were performed on an AXIMA-QIT MALDI quadrupole ion trap time-of-flight mass spectrometer (SHIMADZU, Kyoto, Japan). The instrument consists of four main sections: an ion source, an introduction region, an ion trap, and a reflectron time-of-flight mass analyzer. The pressure in the ion source was 6×10^{-6} Torr. Matrix-assisted laser desorption of samples was carried out using pulses of light (337 nm, 3-ns pulse width) generated by a nitrogen laser with a maximum pulse rate of 10 Hz. The mass spectra were displayed with 100 accumulations of the profile obtained from two laser shots. The ions are directed into the ion trap by an electrostatic lens assembly, which is designed to ensure high transmission of the ion beam into the ion trap and to minimize the field strength directly above the sample surface. The ion trap chamber can be supplied with two separate and independent gases. A continuous flow of He is typically used for collisional cooling. The pulsed gas, Ar, was used to enhance ion cooling or to impose collision-induced fragmentation [23]. The selection-excitation-collision fragmentation cycle can be repeated to induce multiple fragmentation for MS^n. A floating chevron multi-channel plate detector records ion arrivals.

The MS and MS^n spectra were calibrated externally using a standard commercial peptide calibration mixture containing 1 pmol each of bradykinin (fragments 1-7; $[M + H]^+$ 757.40) and angiotensin II ($[M + H]^+$ 1046.54) (Sigma, St. Louis, MO, USA) per μl. 2,5-Dihydroxybenzoic acid (DHB; MW 154.1 Da) was used as the matrix at a concentration of 10 mg/ml in 10% aqueous ethanol. All of the milk oligosaccharides were prepared as 10 pmol/μl solutions in water. Two microliters of each sample solution was dissolved in the same volume of the matrix solution. Aliquots of the resulting mixtures were placed on a target plate. The crystallization process was accelerated using a gentle stream of cold air. Then, the co-crystallized matrix and analyte were introduced into the mass spectrometer.

Results and discussion

Figure 1 shows the structures of the three PA-fucopentaoses used in this study, and Figure 2 shows the MALDI-QIT-TOF MS spectra of the oligosaccharide derivatives in positive ion mode. The [M + Na] ions form the major peak at m/z 954, and the mass spectra of these isomeric oligosaccharides are the same, as shown in Figure 2. The MS^2 spectra of LNFP I, II, and III, shown in Figure 3(A)–(C), respectively, were obtained using [M + Na] ions at m/z 954 as the precursor ion. The fragmentation based on the nomenclature established by Domon and Costello [23] and their assignments are summarized in Figure 4 and Table 1. Under these conditions, m/z 792 ion was not detected in LNFP

Table 1. Summary of the observed fragment ions from the MS^2 spectra of the PA-oligosaccharides. The selected first precursor ion was m/z 954

m/z	*LNFP I*	*LNFP II*	*LNFP III*
808	Y4 + Na	Y3β+ Na	Y3β + Na
792		Y3α+ Na	Y3α + Na
696	B4 + Na	B3 + Na	B3 + Na
646	Y3 + Na	Y3α/3β + Na	Y3α/3β + Na
552	C3 + Na	C2 + Na	C2 + Na
550	B4/Y4 + Na	B3/Y3β + Na	B3/Y3β + Na
534	B3 + Na	B2 + Na	B2 + Na
443	Y2 + Na	Y2 + Na	Y2 + Na
406	C3/Y4 + Na	C2/Y3β + Na	C2/Y3β + Na
388	B3/Y4 + Na	B2/Y3β + Na	B2/Y3β + Na

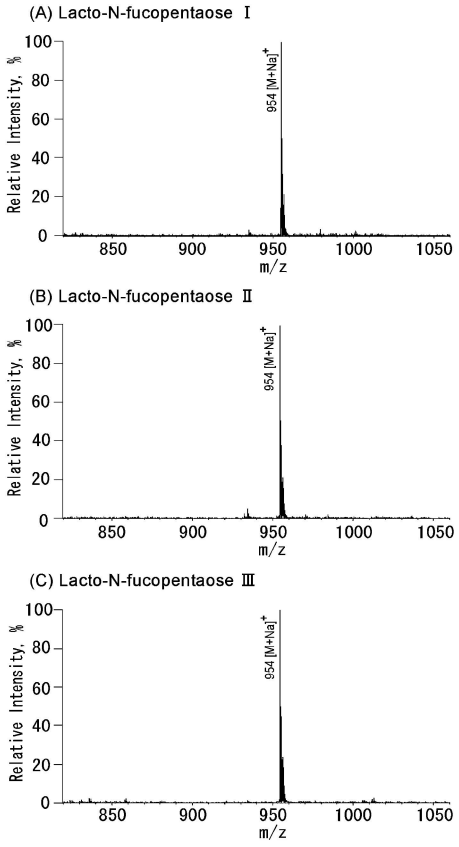

Figure 2. MS spectra of LNFP I (A), II (B), and III (C).

I and the relative intensities of m/z 388 and 696 ions in LNFP I was different from those of LNFP II and III. The difference of relative intensities of m/z 406 and 550 ions was observed between LNFP II and III. However, these relative intensities of the fragment ions were not always reproducible. Therefore, in order to reach to definitive structures, we performed MS^3 analysis which provides characteristic ions derived from trisaccharides at the non-reducing end.

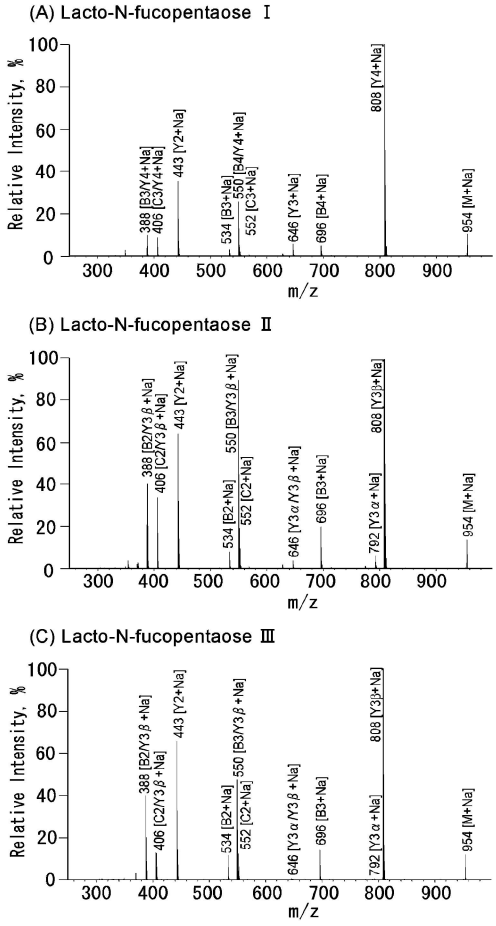

Figure 3. MS^2 spectra of LNFP I (A), II (B), and III (C) derived from precursor ions $[M + Na]^+$ at m/z 954. The structures are inserted with the fragmentation annotated.

We performed MS^3 measurements that focused on the ions of the non-reducing ends. Figure 5(A)–(C) show the MS^3 spectra derived from the second precursor ion at m/z 534 of LNFP I, II, and III, respectively. The fragment ions obtained from the second precursor ion of LNFP I at m/z 534 (B3 + Na) were detected at m/z 388 (B3/Y4 + Na), 349 (C2 + Na), and 331 (B2 + Na). By contrast, the fragment ions at m/z 388 (B2/Y3β + Na), 372 (B2/Y3α + Na), and 354 (B2/Z3α + Na) were detected from the second precursor ion of LNFP II at 534 (B2 + Na), and those at 388 (B2/Y3β + Na) and 370 (B2/Z3β + Na) were obtained from the precursor ion of LNFP III at 534 (B2 + Na), as shown in Table 2. The specific fragment ions are m/z 349 (C2 + Na) for LNFP I, 372 (B2/Y3α + Na) and 354 (B2/Z3α + Na) for LNFP II, and 370 (B2/Z3β + Na) for LNFP III. Furthermore, we obtained similar results on measuring the MS^3 spectra of the second precursor ion at m/z 552 for LNFP I, II, and III (data not shown). These results indicate that the structures of these oligosaccharides could be characterized using the MS^3 analysis of related ions.

We were able to obtain structural information for the pentasaccharides (LNFP I, II, and III) in terms of sequence, linkage,

Table 2. Summary of the observed fragment ions from the MS^3 spectra of the PA-oligosaccharides. The selected second precursor ion was m/z 534

m/z	*LNFP I*	*LNFP II*	*LNFP III*
388	B3/Y4 + Na	B2/Y3β+ Na	B2/Y3β+ Na
372		B2/Y3α+ Na	
370			B2/Z3β + Na
354		B2/Z3α + Na	
349	C2 + Na		
331	B2 + Na		

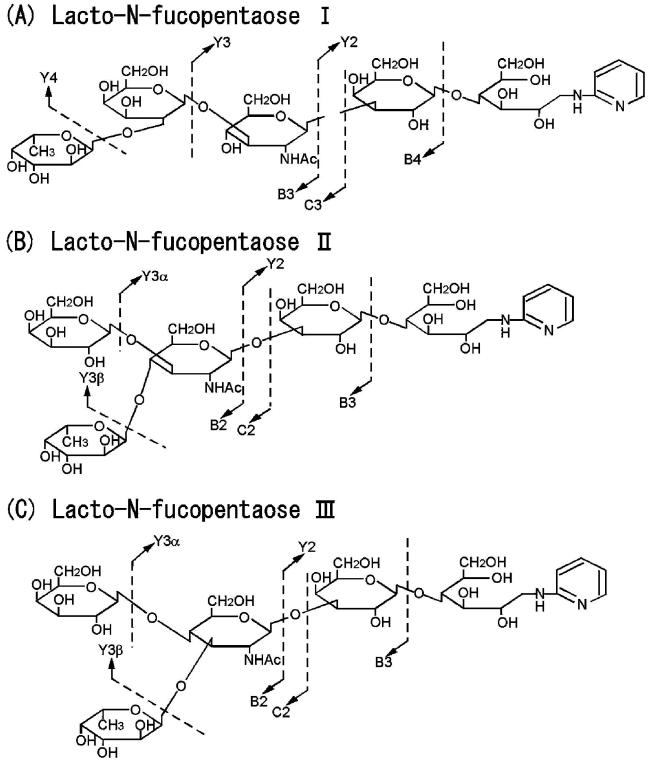

Figure 4. Glycosidic cleavage found in the MS^2 spectra of the LNFP I (A), II (B), and III (C).

and branching using MS^2 and MS^3 analyses performed with MALDI-QIT-TOF MS, which has the advantage of simplicity and short acquisition time. This study showed that MALDI-QIT-TOF MS is a convenient and rapid tool for the structural characterization of oligosaccharide linkage isomers.

Ojima *et al.* [25] has been published a paper on the analysis of N-linked oligosaccharides and they reached to a conclusion that MS^n of [M + Na] ions is useful for the analysis of complicated oligosaccharide structures rather than MS^2 analysis of [M + Na] ions because of more abundant structural information. Furthermore, two kinds of model N-glycans with different branched structures can be distinguished by comparing the relative peak intensities of fragment ions in MS^3 spectra. This paper supports our results.

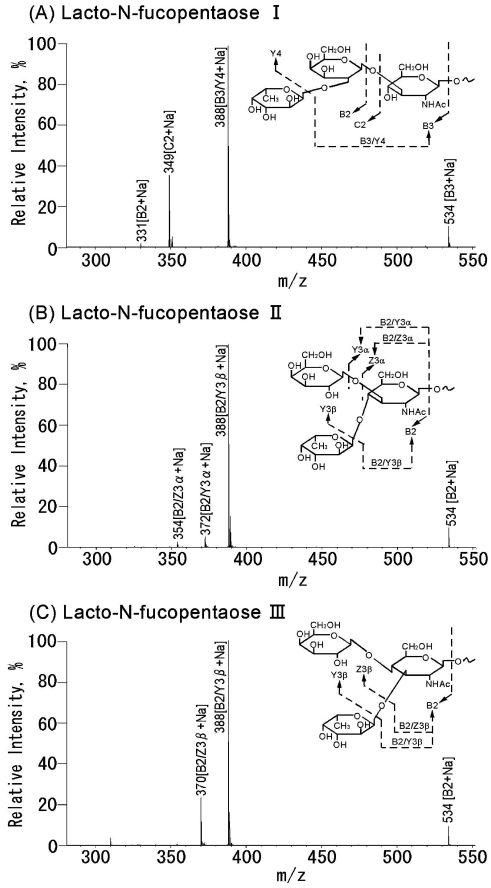

Figure 5. MS^3 spectra of LNFP I (A), II (B), and III (C). m/z 954 was selected as the first precursor ion and m/z 534 as the second precursor ion. The samples were doped with NaCl. The structures are inserted with the fragmentation annotated.

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