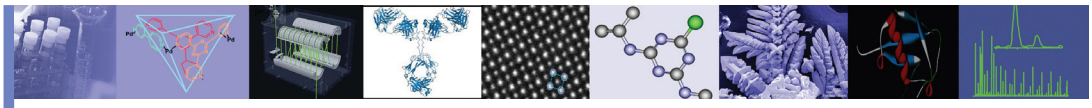

Applications Note

SpiralTOF-TOF

High-energy CID Mass Spectrometry of Oligosaccharides

Introduction:

Matrix assisted laser desorption ionization (MALDI) is a powerful and useful ionization technique that is commonly used for the analysis of biomolecules such as oligosaccharides. There are many applications of oligosaccharides in which various ionization techniques and mass spectrometers were used for their analysis [1]. In particular, tandem mass spectrometry techniques are often used to sequence these molecules.

Recently, JEOL developed a new tandem TOF-TOF instrument coupled with MALDI that is called the SpiralTOF. The 1st TOF consists of 4 toroidal electric sectors that fold a 17 meter flight path into a one meter box. This design provides several unique advantages for TOF-TOF analysis. The 2nd TOF has (a) 20 kV high-energy CID, (b) monoisotopic precursor ion selection, and (c) no PSD ions in the product ion mass spectrum.

In this study, we analyzed several oligosaccharides by using the JMS-S3000 SpiralTOF-TOF tandem mass spectrometer system.

Experimental:

All oligosaccharides (Laminaritetraose, Stachyose, α-Cyclodextrin, β-Cyclodextrin, γ-Cyclodextrin) were commercially available items that were used without further purification. Each oligosaccharide standard solution was dissolved in water. 2,5-Dihydroxybeozoic acid (DHB) was dissolved in 40% ethanol at a concentration of 10 mg/mL. Next, the oligosaccharides standard solution and matrix solution were mixed together 1:1 by volume. Afterwards, 0.5 µL of this mixture was placed on the MALDI target plate. Finally, the dried sample was measured using the JMS-S3000 SpiralTOF-TOF.

Results:

The A, B and C fragment ions for oligosaccharides are labeled as the non-reducing terminal ends while the X, Y and Z fragment ions are labeled as the reducing terminal ends. The nomenclature fragmentation pathway of oligosaccharides by tandem MS is shown in Figure 1[modified from Reference 2].

In this study, we did not do further purification for all oligosaccharide standards, matrix and solvents. As a result, the sodiated molecules were the most intense peak in each positive-ion MALDI mass spectrum (see Figure 2). Therefore, these sodiated molecules were selected as the precursor ions for the SpiralTOF-TOF analysis. MALDI TOF-TOF spectra of Laminaritetraose and Stachyose are shown in Figure 3. Laminaritetraose is a tetrasaccharide that consists of four β-D-glucose units that are linked as Glc(β1→3)Glc(β1→3)Glc(β1→3)Glc. Stachyose is also a tetrasccharide that consists of two α-D-galactose units, one α-D-glucose unit, and one β-D-fructose unit that are linked as Gal(α1→6)Gal(α1→6)Glc(α1→2β)Fru. These isomers showed significantly different mass spectral patterns. B and Y fragment ions were generated from the glycosidic bond cleavage were observed as the dominant components in each MALDI TOF-TOF spectrum. The fragmentation scheme for Laminaritetraose is shown in Figure 4. High-energy CID fragmentation occurs within a shorter time-scale than low-energy CID, which means that it provides a cross-ring cleavage that gives useful structural information about the oligosaccharides and their glycoconjugates. This cross-ring cleavage was also observed for both oligosaccharides, as indicated by the presence of the fragment ions m/z 599, 569, 555, etc. (see Figure 3). All of this information is essential for determining the structure of each oligosaccharide and differentiating structural isomers from each other.

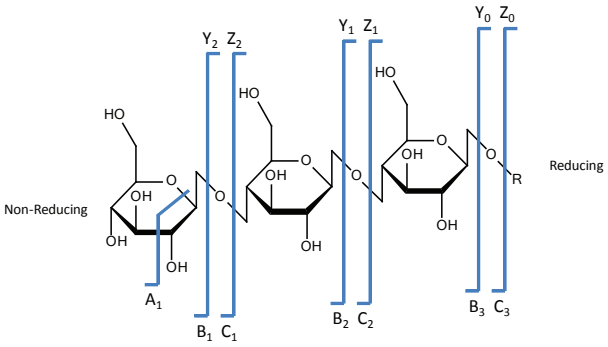

Figure 1. Fragmentation pathways.

Cyclodextrins are cyclic oligosaccharides consisting of five or more α-D-glucose units that are linked as Glc(α1→4)Glc. The MALDI TOF-TOF spectrum for each Cyclodextrin is shown in Figure 5. In each case, the B ion series from glycosidic bond cleavage were the dominant ions observed in the TOF-TOF spectra. Additionally, a number of fragment ions were observed that were the result of cross-ring cleavage.

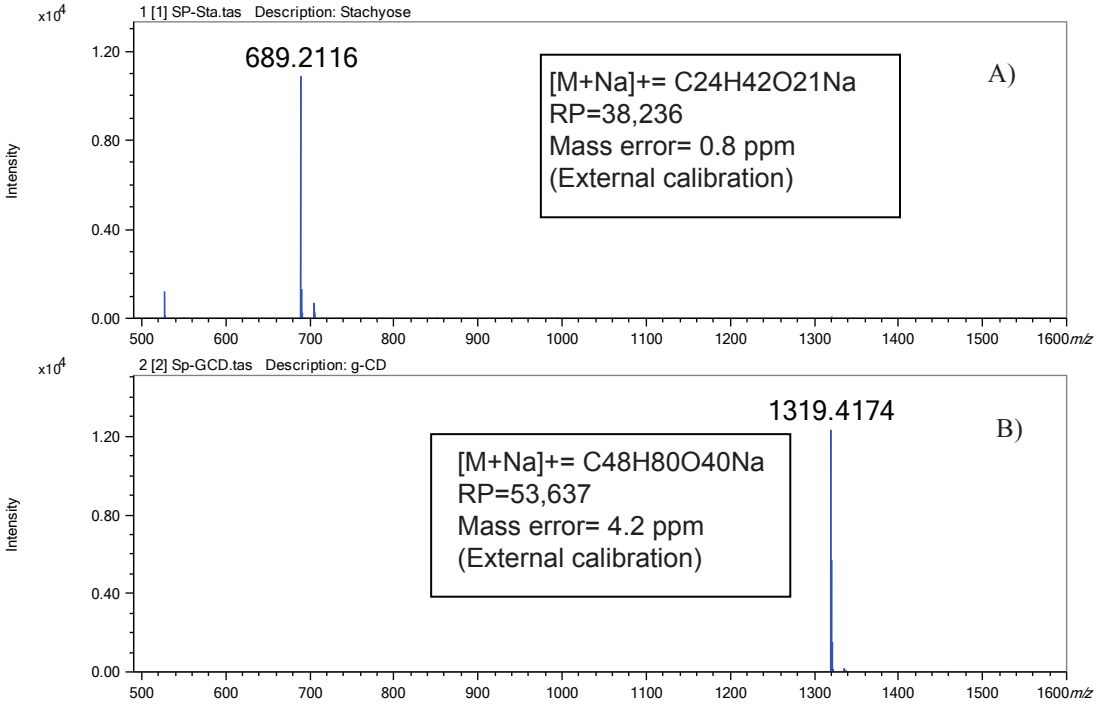

Figure 2. MALDI TOF spectra. A) Stachyose, B) γ-Cyclodextrin

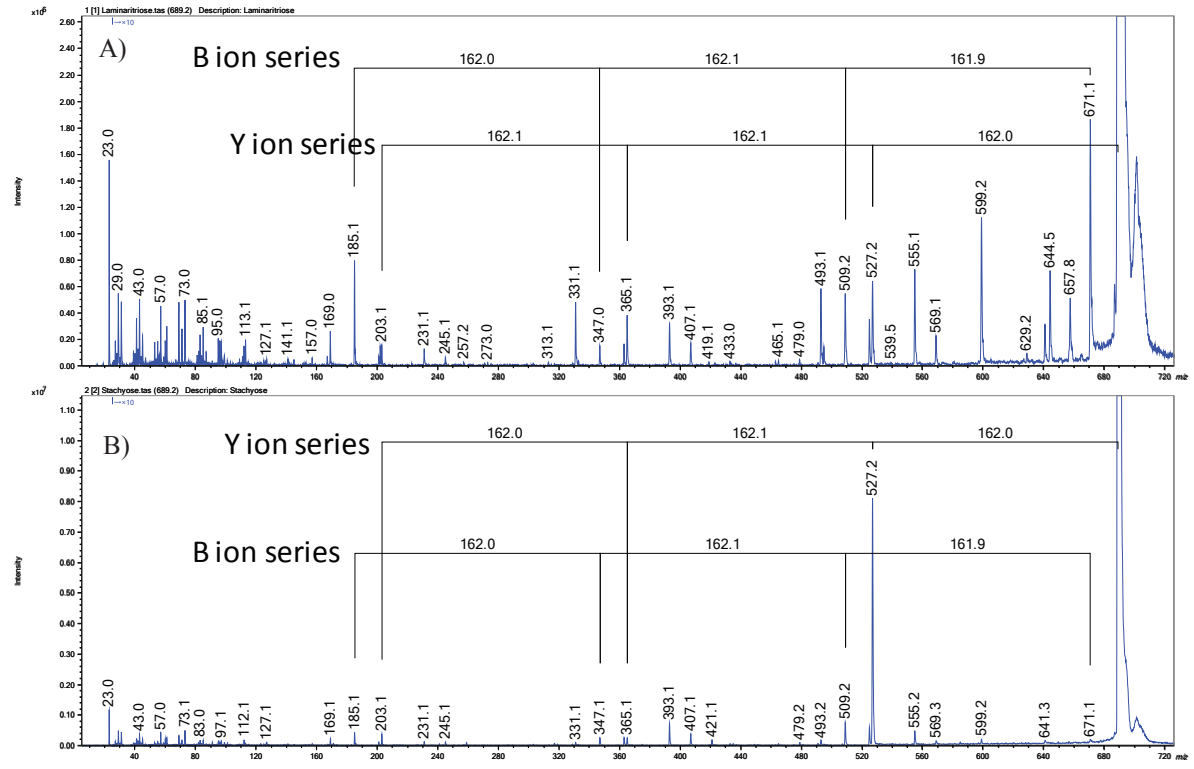

Figure 3. MALDI TOF-TOF spectra. A) Laminaritetraose, B) Stachyose

MS-012313

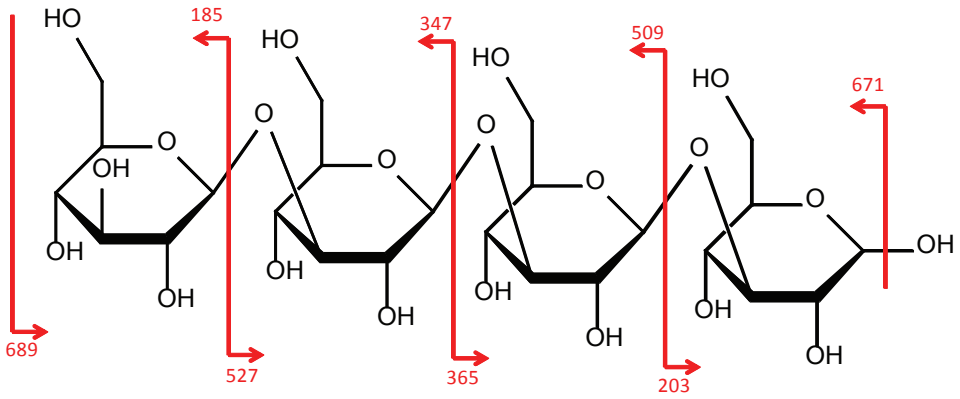

Figure 4. The glycosidic bond cleavage to generate B and Y ion series for Laminaritetraose

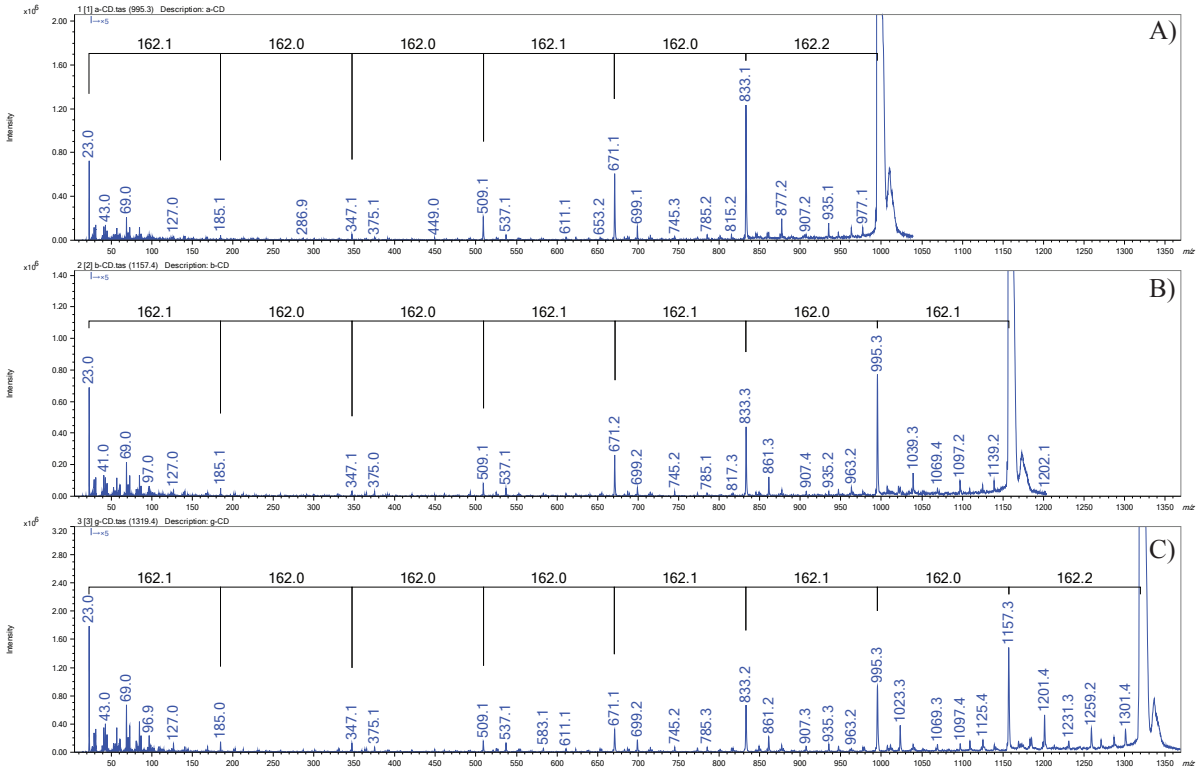

Figure 5. MALDI TOF-TOF spectra. A) α-Cyclodextrin, B) β-Cyclodextrin, C) γ-Cyclodextrin

Conclusion:

In this work, we showed a brief study in which the high-energy CID measurements for several oligosaccharides were measured by using the SpiralTOF-TOF. The B and Y ion series were observed as the main components in each TOF-TOF spectrum. Furthermore, we could also see a number of fragment ions that were generated from cross-ring cleavage, which was helpful in differentiating structural isomers. These results show that high-energy CID coupled with MALDI provides a good platform for determining oligosaccharide structural information that will not generally be available when analyzing these compounds by other mass spectrometry techniques.

Reference:

[1] Joseph Zaia, Mass Spectrometry of Oligosaccharides, ***Mass Spectrometry Reviews***, Volume 23, Issue 3, pages 161–227, May/June 2004

[2] Domon B, Costello CE. 1988b. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra ofglycoconjugates. ***Glycoconjugate J*** 5:397–409.