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Application News

No. B101

MALDI-TOF Mass Spectrometry

Analysis of *N*-Linked Glycan using MALDImini™-1 Compact MALDI Digital Ion Trap Mass Spectrometer: Structural Analysis and Identification of Sialyl Linkage Isomers

N-linked glycosylation to proteins plays an important role in various biological phenomena. In particular, sialic acids existing at sugar chain terminals and their linkage types are known to be key factors related to numerous diseases, such as antigenicity and viral infections.

In recent years, mass spectroscopy (MS) has been widely used in glycan analysis. However, there were various problems related to sialic acid residues, in that they are unstable and thus easily lost while analysing, and furthermore, it is not possible to differentiate linkage isomers by MS. On the other hand, HPLC analysis is mainly used to identify the linkage type of sialic acids, but this method also has technical problems, for example, identification is difficult in the case of glycans with numerous sialic acids.

Focusing on a blood serum-derived *N*-linked glycan, this article introduces an example in which sialic acid residue was stabilized by using the sialic acid linkage specific alkylamidation (SALSA) method, which was developed by Shimadzu Corporation, and detection and analysis were performed with a Shimadzu MALDImini-1 compact MALDI digital ion trap (MALDI-DIT) mass spectrometer.

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Release of *N*-Linked Glycans from Glycoprotein

The blood serum used in this study is a commercial product for research purposes. First, 5 µL of the commercial serum was denatured and reduced with SDS (sodium dodecyl sulfate) and DTT (dithiothreitol). After adding NP-40 (Nonidet P-40), PNGaseF (peptide-*N*-glycosidase F) was added, and *N*-linked glycans were released from the glycoprotein by reacting the solution at 37 °C for 18 h.

Sialic Acid Linkage Specific Alkylamidation (SALSA)

Sialic acid linkage specific alkylamidation (SALSA *1), which was developed by our company, is a technology that not only chemically neutralizes sialic acids and prevents their preferential loss, but also makes it possible to differentiate sialyl linkage isomers by MS, which inherently have the same mass, by causing changes in the mass corresponding to the linkage types (Fig. 1).[(1), (2)]

Of the *N*-linked glycans extracted from the glycoprotein, 4 µL was mixed directly with 20 µL of the SALSA reaction solution, and this mixture then was reacted at room temperature for 1 h. Following this, a stabilizing reagent for a lactone structure was added and lightly mixed, and the excess reagent was removed by using GL-Tip Amide (GL Sciences Inc.).

*1 Patent No. WO/2016/159291

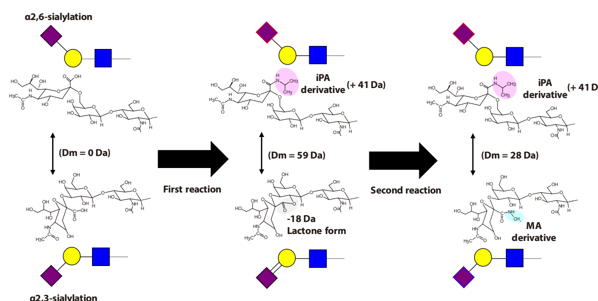

Fig. 1 Schematic Diagram of Sialic Acid Linkage Specific Alkylamidation (SALSA) Method
In a two-step reaction, α2,6 sialic acid is amidated with isopropylamine (iPA) while α2,3 sialic acid is amidated with methylamine (MA), producing a mass difference which allows to differentiate the two forms of sialic acid by MS.

2AB Labeling of Glycans and Placement on MALDI Plate

After sialic acid linkage specific modification, the reducing terminal of the glycans was labeled with 2-aminobenzamide (2AB), and the excess reagent was removed with GL-Tip Amide.

The sample solution (0.5 µL) was placed on the MALDI target plate, and was dried by overlaying the matrix solution (0.5 µL). MS^n analysis was carried out with a MALDImini-1 compact MALDI-DIT mass spectrometer (Fig. 2).

The matrix used here was CHCA (α-cyano-4-hydroxycinnamic acid) with added sodium chloride.

* For details concerning MALDImini-1, refer to Application News No. B100.

Fig. 2 Appearance of MALDImini™-1 Compact MALDI-DIT Mass Spectrometer

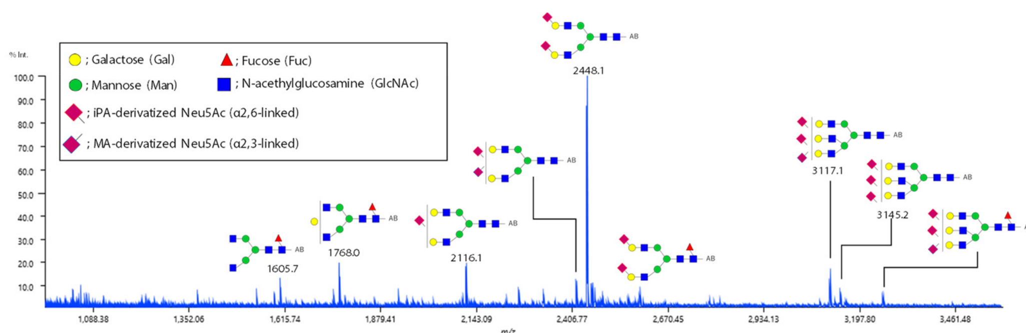

Fig. 3 Mass Spectrum (MS) of Blood Serum Glycoprotein-Derived *N*-Linked Glycan Obtained with MALDImini-1

Confirmation of Sialic Acid Linkage Type by MS² Analysis

Mainly complex glycans, including biantennary and triantennary species, were detected from the blood serum glycoprotein-derived *N*-linked glycans (Fig. 3).

Fig. 4 shows a comparison of the MS^2 spectra of triantennary glycans obtained by the MALDImini-1. As these two glycans were detected by the 28Da difference, it is conjectured that they are α2,3-/α2,6- isomers. Because the neutral loss masses equivalent to the modified sialic acids in MS^2 were confirmed, data backing the linkage types of the sialic acids were obtained. It was found that *m/z* 3117.1 was a mixture of α2,3- and α2,6-, whereas *m/z* 3145.2 comprised only α2,6-.

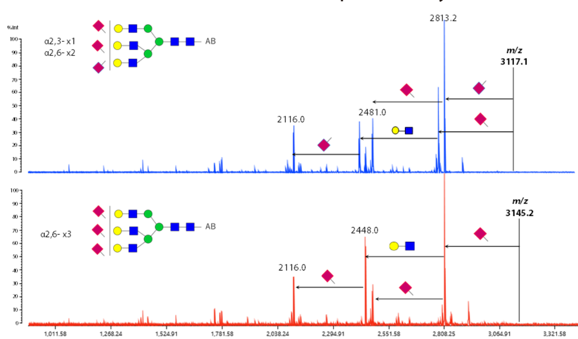

Fig. 4 Comparison of MS² Mass Spectra of Triantennary Glycans *m/z* 3117.1 and *m/z* 3145.2 Detected by MS
The linkage types can be differentiated based on the mass of the neutral loss of modified sialic acid residues from the nonreducing ends.

Glycan Structural Analysis by MSⁿ Analysis

In the MS^2 glycan analysis, fragment ions caused by the cleavage of glycosyl bonds was mainly detected. However, when an analyte contains many residues of the same mass, as in the case of glycans, it is sometimes difficult to determine the origin of the fragment ions from the glycan.

The origin of fragment ions can be clarified by conducting an MS^3 analysis (Fig. 5). Fragment ion *m/z* 720.0 in the MS^2 of biantennary glycan *m/z* 2448.1 cannot be explained by sequential glycan elimination from the nonreducing terminal side. Therefore, if the MS^3 of the fragment ion (*m/z* 2107.0) containing sialic acid and the fragment ion not containing sialic acid (*m/z* 1783.9) are compared, fragment ion *m/z* 720.0 cannot be detected from the latter. Based on this fact, it was understood that fragment ion *m/z* 720.0 originated from the trisaccharide on the nonreducing terminal side, which includes sialic acid. Furthermore, analysis of the core structure of the *N*-linked glycan was possible from the MS^3 of fragment ion *m/z* 1053.4.

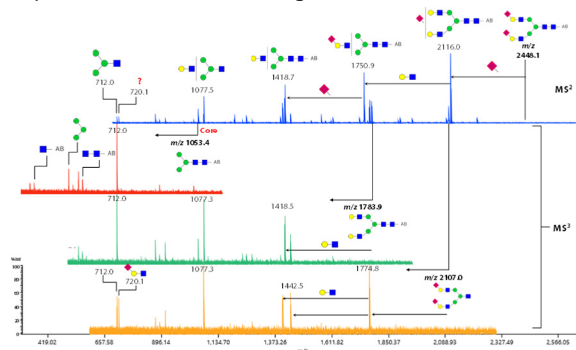

Fig. 5 MSⁿ Mass Spectrum of Biantennary Glycan *m/z* 2448.1 Detected by MS

Conclusion

As shown in this experiment, the combination of stabilization of sialic acid by the SALSA method and MS^3 analysis by the MALDImini-1 compact MALDI-DIT mass spectrometer can be considered an effective technique for structural analysis of glycans, including the linkage type of sialic acids.

References

(1) Nishikaze T, et al. (2017) Differentiation of Sialyl Linkage Isomers by One-Pot Sialic Acid Derivatization for Mass Spectrometry-Based Glycan Profiling. *Anal Chem* 89:2353–2360.

(2) Hanamatsu H, et al. (2018) Sialic Acid Linkage Specific Derivatization of Glycosphingolipid Glycans by Ring-Opening Aminolysis of Lactones. *Anal Chem* 90(22):13193–13199.

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