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Application News

Sialic Acid Stabilizing Kit for Linkage Isomer Discrimination: SialoCapper™-ID Kit
MALDI-TOF Mass Spectrometer: MALDI-7090

Analysis of Serum *N*-Glycans of Gene-KO Mouse Using MALDI-7090 and SialoCapper-ID Kit

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User Benefits

- Sialic acid linkage isomer can be discriminated only by mass spectrometry without LC separation or sialidase enzyme treatment.
- By combining with glycan purification beads "BlotGlyco®," glycan purification and sialic acid derivatization can be performed as a one-pot reaction.
- By using the MS^2 analysis, the sialic acid linkage can be determined more reliably, omitting the verification by other biochemical experiments.

■ Introduction

Protein glycosylation is involved in various biological events. In particular, sialic acid at the non-reducing end of the glycan is important because its presence and linkage type have been implicated in viral infections and cancer.

Usually, liquid chromatography (LC) separation and sialidase treatment are used to discriminate sialic acid linkage isomers; however, there are technical problems such as the need for structurally unambiguous standard samples and the difficulty in discriminating complicated glycans.

In recent years, sensitive and high-throughput mass spectrometry (MS) has been widely used for glycan analysis. However, there have been some intrinsic problems, such as the loss of sialic acid residues during analysis and the inability to distinguish linkage isomers with the same mass.

In this article, we introduce an analysis example of *N*-glycans released from serum glycoproteins of sialyltransferase knock out (KO) mice. Samples were derivatized using the sialic acid stabilizing kit for linkage isomer discrimination "SialoCapper-ID Kit" followed by analysis using a MALDI-TOF-MS.

■ Sialic Acid Linkage-Specific Derivatization

Our patented sialic acid linkage-specific alkylamidation (SALSA method) prevents the loss of sialic acid residues during both glycan pretreatment and MS analysis by neutralizing the residues. In addition, it allows the MS-based discrimination of sialic acid linkage isomer by derivatizing the residues in a linkage-specific manner.[2,3] The SialoCapper-ID Kit is a novel reagent kit for glycan pretreatment that simplifies the SALSA procedure.

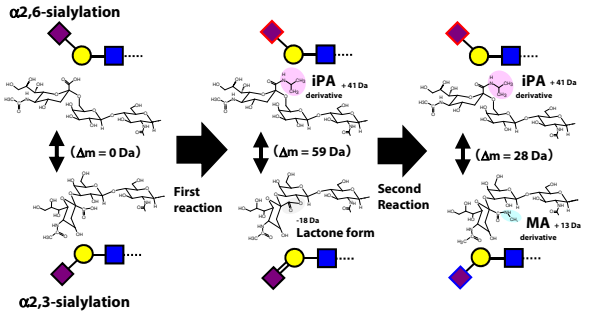

Fig. 1 Derivatization scheme of SALSA method
α2,6- and α2,3-linked sialic acids are amidated by isopropylamine (iPA) and methylamine (MA), respectively, by sequential two-step reactions.

■ Sialyltransferase-KO Mouse

Representative α2,6-sialyltransferases, ST6-Gal1 and ST6-Gal2 were knocked out. For comparison, following four types of mice were prepared; without KO (WT), ST6Gal1-KO, ST6Gal2-KO, and double KO (DKO).

■ Glycan Release from Glycoproteins

First, serum glycoproteins derived from gene-KO mice were denatured and reduced with SDS and DTT. After adding NP-40, PNGaseF was added, and *N*-linked glycans (*N*-glycans) were released from the glycoprotein by reacting the solution at 37 °C for o/n (ca. 18 h).

■ *N*-Glycan Purification and Solid-phase Sialic Acid Linkage-Specific Derivatization

Released *N*-glycans were purified using "BloGlyco®" (Sumitomo Bakelite Co., Ltd.). BlotGlyco® is a glycan purification bead that can specifically capture the reducing end of glycans. By using the beads in combination with the SialoCapper-ID Kit, glycan purification and sialic acid derivatization can be performed as a one-pot reaction (Fig. 2).

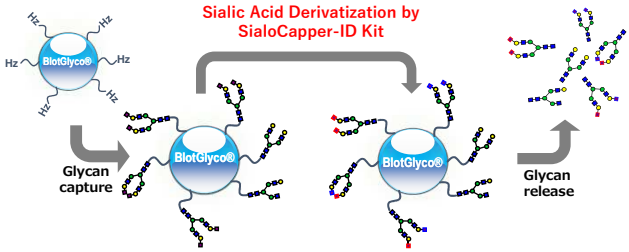

Fig. 2 Glycan purification and solid-phase sialic acid derivatization using BlotGlyco® and SialoCapper-ID Kit

■ Glycan Labeling and Mass Spectrometry

The reducing end of the *N*-glycan released from BlotGlyco® was labeled with 2-aminobenzoic acid (2AA), and excess reagents were removed using a commercially available HILIC-SPE chip.

The sample solution and matrix solution were deposited on a MALDI plate and left to dry. Mass spectra were obtained using MALDI-7090 (Fig. 3) in the negative ion reflectron mode. Super-DHB was used for the matrix.

The ASDF (Axial Spatial Distribution Focusing) function was used for MS^2 measurement. This allows high-resolution MS^2 spectra to be obtained even under high-energy CID conditions, enabling more accurate analysis.

Fig. 3 MALDI-TOF mass spectrometer "MALDI-7090"

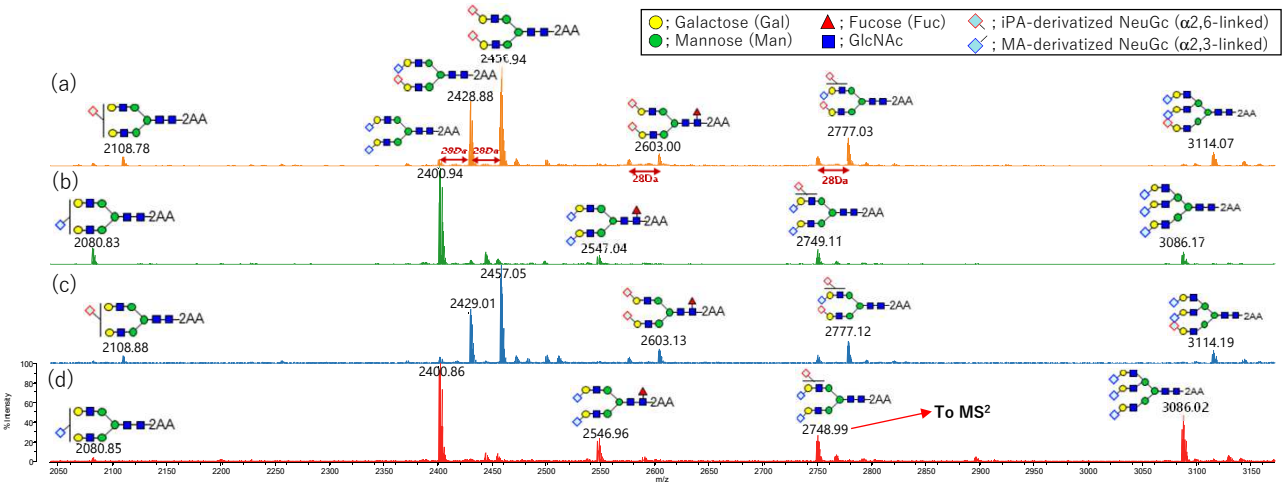

Fig. 4 MS¹ mass spectra of *N*-glycans derived from the glycoproteins of gene-KO mouse sera.
(a) WT, (b) ST6Gal1-KO, (c) ST6Gal2-KO, (d) DKO

■ MS¹ Analysis of *N*-Glycan from Mouse Serum

Various glycan peaks were detected with a difference of 28 Da corresponding to the difference of α2,3-/α2,6-linkages (Fig. 4). These *m/z* values were analyzed with the Software "Supporting Tool for SialoCapper-ID Kit" to calculate the monosaccharide composition (Table 1). Based on these results, we estimated the structure of *N*-glycans by considering past literatures and the known glycan biosynthetic pathways. By focusing on the di-antennary *N*-glycans, the disappearance of α2,6-sialylation in ST6Gal1-KO and DKO indicates that the enzyme responsible for the addition of α2,6-sialic acid is ST6Gal1.

Table 1 Monosaccharide Composition of *N*-glycans derived from mouse serum glycoproteins

m/z (calc.)	Glycan Composition
2080.76	AA-Hex5HexNAc4NeuGc(a2,3-)1
2108.79	AA-Hex5HexNAc4NeuGc(a2,6-)1
2400.88	AA-Hex5HexNAc4NeuGc(a2,3-)2
2428.91	AA-Hex5HexNAc4NeuGc(a2,6-)1NeuGc(a2,3-)1
2456.94	AA-Hex5HexNAc4NeuGc(a2,6-)2
2546.94	AA-Hex5HexNAc4dHex1NeuGc(a2,3-)2
2603.00	AA-Hex5HexNAc4dHex1NeuGc(a2,6-)2
2749.03	AA-Hex5HexNAc4NeuGc(a2,6-)1NeuGc(a2,3-)2
2777.07	AA-Hex5HexNAc4NeuGc(a2,6-)2NeuGc(a2,3-)1
3086.14	AA-Hex6HexNAc5NeuGc(a2,3-)3
3114.17	AA-Hex6HexNAc5NeuGc(a2,6-)1NeuGc(a2,3-)2

Supporting tool for SialoCapper-ID Kit is a software that can estimate glycan composition from given *m/z* values in the results of MS and deal with the mass change of sialic acid residues by sialic acid derivatization.

Search condition: Mass Tolerance 0.2 Da
Hex 3-10, HexNAc 2-10, dHex 0-1, Neu5Gc 0-4

■ MS² Analysis by High-Energy CID and ASDF

The DKO sample lacking two different α2,6-sialyltransferases showed a peak at *m/z* 2749, suggesting the inclusion of α2,6-sialic acid residues. MS² analysis of this peak showed the neutral loss of 348 Da corresponding to isopropylamidated NeuGc (Fig. 5). This suggests the presence of α2,6-sialic acid, indicating that the α2,6-sialic acid is added by enzymes other than ST6Gal1 and ST6Gal2.

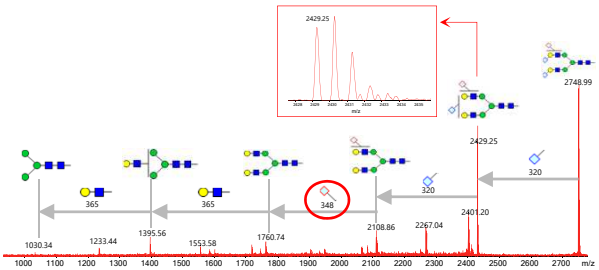

Fig. 5 MS² mass spectrum of *m/z* 2749

■ Conclusion

By using BlotGlyco® and SialoCapper-ID Kit, glycan purification and sialic acid linkage-specific derivatization can be easily performed as one-pot reaction. Although the sialic acid linkage type on the *N*-glycan treated with the kit can be discriminated by MS¹ analysis, MS² analysis is valuable for confirming unexpected results. In this study, we obtained the presence of α2,6-sialic acid, which is controlled by an enzyme other than ST6Gal1 and ST6Gal2.

■ Acknowledgments

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<References>
1) Ohmi Y. et al. (2020) Majority of alpha2,6-sialylated glycans in the adult mouse brain exist in O-glycans: SALSA-MS analysis for knockout mice of alpha2,6-sialyltransferase genes. *Glycobiology* in press
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