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Application News

Sialic Acid Stabilizing Kit for Linkage Isomer Discrimination: SialoCapper™-ID Kit
MALDI-TOF Mass Spectrometer: MALDI-8020

Sialic Acid Linkage Isomer Discrimination of *N*-glycans derived from Rat Cochlea using SialoCapper-ID Kit

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User Benefits

- The sialic acid linkage types of complicated glycans can be identified without comparing to the analysis results of standard glycan samples.
- SialoCapper-ID Kit can be applied to labeled glycans, and therefore sialic acid linkage analysis can be performed without changing the conventional analysis workflow.
- Because LC separation is unnecessary, the sialic acid linkage types can be determined only from the MS^1 measurement of MALDI-MS.

■ Introduction

Sialic acid is a family name of acidic monosaccharides, including *N*-acetylneuraminic acid (NeuAc) and *N*-glycolylneuraminic acid (NeuGc), mainly present on non-reducing terminal of glycans. Sialic acids can exist with α2,3- or α2,6-linkage types. Such linkage isomer is biologically important, because the difference in linkage types can be associated with various diseases such as virus infection and cancer.

In recent years, mass spectrometry (MS) has become widely used to analyze glycans. In particular, the technique of estimating the structure of the glycan from the retention time information in liquid chromatography (LC) and the analysis result of MS is used as a powerful tool. However, it was still challenging to determine the sialic acid linkage types of complicated glycans containing multiple sialic acid residues.

In this article, we introduce an example of discriminating sialic acid linage types on PA-labeled glycans by unique derivatization using "SialoCapper-ID Kit" followed by MALDI-TOF MS analysis. Prior to MALDI-MS analysis, the main structure of the glycans was determined by two dimensional LC and LC/MS analysis.[1)]

■ 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.

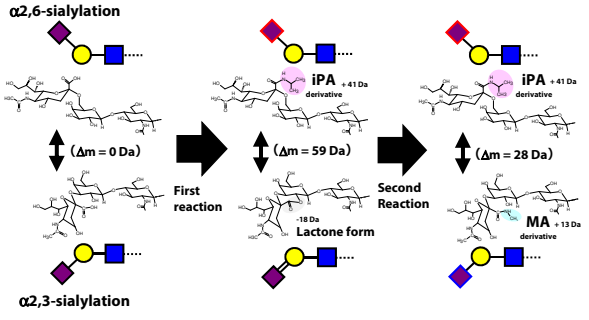


Fig. 1 Derivatization scheme of SALSA method
α2,6- and α2,3-linked sialic acids are amidated by isopropylamine (iPA) and methylamine (MA), respectively, by sequential two-step reactions.

■ Analysis Workflow

N-linked glycans (*N*-glycans) were released by hydrazinolysis from glycoproteins derived from the stria vascularis of the rat cochlea. The reducing end of the *N*-glycans was labeled with 2-aminopyridine (PA).

PA-labeled glycans were then fractionated according to the number of sialic acids by DEAE anion exchange HPLC and further fractionated using reversed-phase (RP) HPLC on an ODS column. Fractionated *N*-glycans were analyzed by normal-phase (NP) HPLC using an amide column and LC-MS, and the structure of the *N*-glycans was determined from the results of two-dimensional (2-D) HPLC analysis (RP/NP) and LC/MS analysis.

Finally, the sialic acid linkage-specific derivatization was carried out using the SialoCapper-ID Kit for the fractionation in which the linkage types of the sialic acid were not determined.

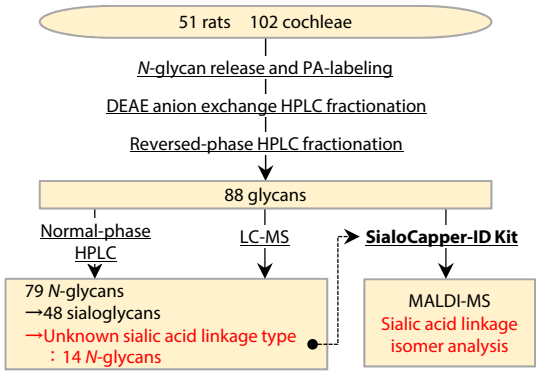


Fig. 2 Analysis workflow of *N*-glycans derived from rat cochlea[1)]

■ In-Solution Sialic Acid Derivatization

After desalting 14 fractions of PA-labeled glycans with a carbon chip, sialic acid linkage-specific derivatization was carried out as a liquid-phase reaction in a tube using the SialoCapper-ID Kit. Excess reagents were removed using a commercially available HILIC-SPE chip, and the derivatives were dried under vacuum (Fig. 3). The maximum amount of fractionated PA-sugars was about 100 fmol to 1 pmol/sample.

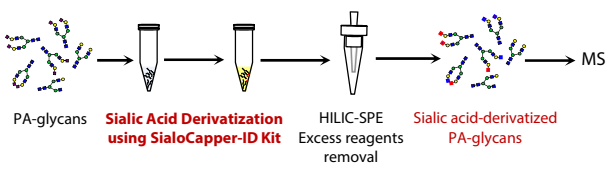


Fig. 3 In-solution derivatization procedure using the SialoCapper-ID Kit

■ Mass Spectrometry

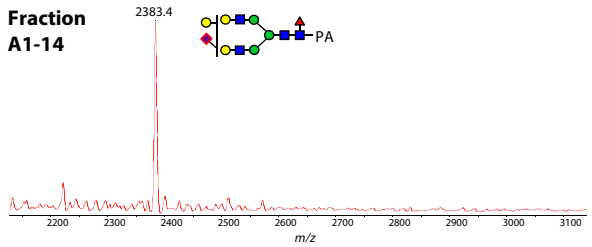
The resulting sample was re-dissolved in 10 µL of water; 0.5 µL was deposited on a MALDI plate. Then, 0.5 µL of the matrix solution was mixed on the plate and left to dry. Mass spectra were obtained by a MALDI-TOF mass spectrometer "MALDI-8020" (Fig. 4). CHCA (α-cyano-4-hydroxycinnamic acid) containing sodium chloride was used as the matrix.

Fig.4 MALDI-TOF mass spectrometer "MALDI-8020"

■ MS¹ Analysis of *N*-Glycans from Rat Cochlea

By using MALDI-8020, sialic acid-derivatized PA-glycan peaks can be successfully detected. In addition to the structural determination by 2-D HPLC and LC/MS, the sialic acid linkage type can be discriminated based on the mass change by the sialic acid linkage-specific derivatization using the SialoCapper-ID Kit.

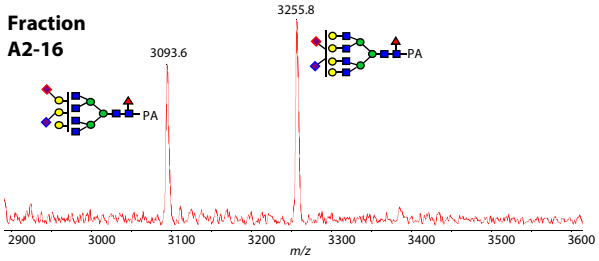
Fig. 5 shows the results of A1-14 fraction containing a singly sialylated PA-glycan. The monosaccharide composition was assumed to be [PA-Hex6HexNAc4dHex1NeuAc1]; however, the linkage type of NeuAc was unknown. When measured by MALDI -8020 after the sialic acid derivatization, a peak was observed at *m/z* 2383.4. The sialic acid linkage type was determined to be α2,6- by comparing the theoretical value after the sialic acid modification of the sugar chain with the estimated structure.



Original	After sialic acid derivatization by SialoCapper-ID Kit		
m/z (calc, Ave.)	Sialic Acid Linkage Type	*m/z* (calc, Ave.)	*m/z* (obs.)
Glycan composition: PA-Hex6HexNAc4dHex1NeuAc1			
2342.13	**NeuAc(α2,6-)1**	2383.22	**2383.40**
	NeuAc(α2,3-)1	2355.17	N.D.

Fig. 5 Mass spectrum of the A1-14 fraction and the result of sialic acid linkage type discrimination

Fig. 6 shows an example of discriminating sialic acid linkage type of tetra-antennary glycans with two sialic acid residues. Because these complicated glycans are challenging to synthesize, it is difficult to determine the linkage type by matching the LC results (retention time) of synthesized glycan standard and biological sample. However, by using the SialoCapper-ID Kit based on chemical modification, the sialic acid linkage type can be directly determined from the results of mass spectrometry.



Original	After sialic acid derivatization by SialoCapper-ID Kit		
m/z (calc, Ave.)	Sialic Acid Linkage Type	*m/z* (calc, Ave.)	*m/z* (obs.)
Glycan composition: PA-Hex6HexNAc6dHex1NeuAc2			
3039.77	NeuAc(α2,6-)2	3121.96	N.D.
	NeuAc(α2,6-)1 NeuAc(α2,3-)1	3093.90	**3093.60**
	NeuAc(α2,3-)2	3065.85	N.D.
Glycan composition: PA-Hex7HexNAc6dHex1NeuAc2			
3201.91	NeuAc(α2,6-)2	3284.10	N.D.
	NeuAc(α2,6-)1 NeuAc(α2,3-)1	3256.04	**3255.80**
	NeuAc(α2,3-)2	3227.99	N.D.

Fig. 6 Mass spectrum of the A2-16 fraction and the results of sialic acid linkage type discrimination

The peak after the sialic acid derivatization was also detected from all other fractions, and the sialic acid linkage type was able to be distinguished. Although LC/MS can also be used to analyze derivatized glycans by SialoCapper-ID Kit, MALDI-MS is advantageous for easy and quick peak confirmation. This is especially useful when analyzing multi-samples, such as when analyzing many fractions of *N*-glycans.

■ Conclusion

Using the SialoCapper-ID Kit, the sialic acid residues on PA-glycans can be easily derivatized in linkage-specific manner, and the sialic acid linkage type can be quickly discriminated from the MS¹ measurement by MALDI-MS. By performing the derivatization by the SialoCapper-ID Kit followed by MALDI-MS analysis in addition to 2-D HPLC and LC/MS analysis, PA-glycan structures can be easily determined.

■ Acknowledgments

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<References>
1) Nonomura Y, et al. (2019) Characterisation of N-glycans in the epithelial-like tissue of the rat cochlea. *Sci Rep.* 9: 1551
2) 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.
3) Hanamatsu H, et al. (2018) Sialic Acid Linkage Specific Derivatization of Glycosphingolipid Glycans by Ring-Opening Aminolysis of Lactones. *Anal. Chem.* 90: 13193-13199.

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