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Application News

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

N-Glycan Analysis of ACE2 as SARS-CoV-2 Receptor

~ Discrimination of Sialic Acid Linkage Isomer using SialoCapper-ID Kit ~

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User Benefits

- Sialic acid linkage type, which is closely related to the infectivity of the virus, can be easily evaluated.
- Sialic acid linkage isomer can be easily discriminated by mass spectrometry without LC separation or sialidase treatment.
- Because sialic acid residues can be stabilized by a simple procedure using SialoCapper-ID Kit, neutral and acidic glycans can be detected simultaneously.

■ Introduction

Sialic acids mainly exist at the non-reducing end of the glycans *via* α2,3-/α2,6-linkages. Some examples are showing the association of the difference in linkage types and viral infectivity. For example, human influenza viruses recognize (attach to) α2,6-linked sialic acids, invading the body and causing infection, whereas avian influenza viruses recognize α2,3-linked sialic acids. Because the sialic acid usually presents in the epithelial cells of the upper respiratory tract is in the α2,6-linked form, avian influenza viruses have difficulty invading human bodies. Glycan analysis is of high importance to clarify the mechanism of viral infection process and develop therapeutic medicines.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the recent pandemic of coronavirus disease 2019 (COVID-19), uses angiotensin-converting enzyme II (ACE2) as a host cell receptor. As with other viruses, the specific linkage isomer of sialic acid is likely to be involved in the infection process, and the glycan analysis of ACE2 is in progress.[1)]

In this article, we show an example of MALDI-TOF-MS analysis of *N*-linked glycans (*N*-glycans) released from ACE2. The sialic acid linkage isomer was also differentiated by using "SialoCapper-ID Kit," the sialic acid stabilizing kit for linkage isomer discrimination.

■ 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.

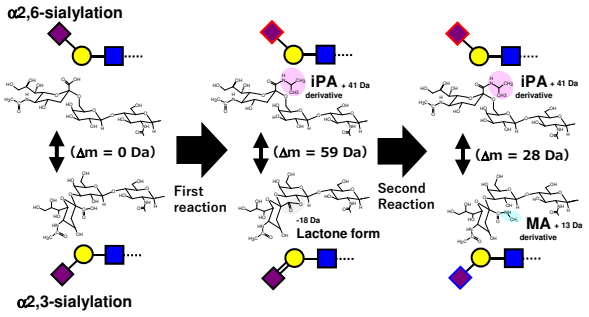

Fig. 1 Derivatization scheme of SALSA method
α2,6- and α2,3-linked sialic acids are amidated by isopropylamine (iPA) and methylamine (MA), respectively, by sequential two-step reactions.

■ *N*-Glycan Release from Human ACE2

Commercially available recombinant human ACE2 protein, ab 151852 (Abcam), was used as a sample. ACE2 was denatured and reduced with SDS and DTT. After adding NP-40, PNGaseF was added, and *N*-glycans were released by reacting the solution at 37 °C for o/n (ca. 18 h).

■ In-Solution Sialic Acid Derivatization

Five µL of *N*-glycan solution released from ACE2 was derivatized by SialoCapper-ID Kit. Excess reagents were then removed by using GL-Tip Amide (GL Science, Inc.).

■ AB Glycan Labeling

The reducing end of the *N*-glycan derivatized by SialoCapper-ID Kit was labeled with 2-aminobenzamide (AB), and excess reagents were removed using GL-Tip Amide.

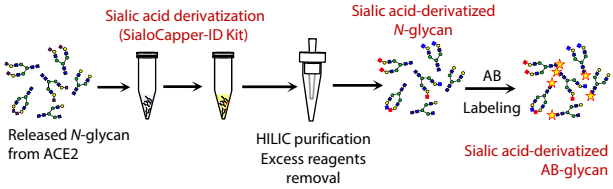

Fig. 2 ACE2 *N*-glycan derivatization/labeling workflow using SialoCapper-ID Kit

■ Mass Spectrometry

A 0.5 µL sample solution was deposited on a MALDI target plate, and then a 0.5 µL matrix solution was mixed on the plate and left to dry. Mass spectra were obtained using MALDI-8020 (Fig. 3). 10 mg/mL Super-DHB* (50% acetonitrile, 0.5 mM NaCl) was used as the matrix solution. The monosaccharide composition of detected *N*-glycan peaks was estimated using the Supporting Tool for SialoCapper-ID Kit.

* Super-DHB: A 9:1 mixture of DHB (2,5-dihydroxybenzoic acid) and MSA (5-methoxysalicylic acid)

Fig. 3 MALDI-TOF mass spectrometer "MALDI-8020"

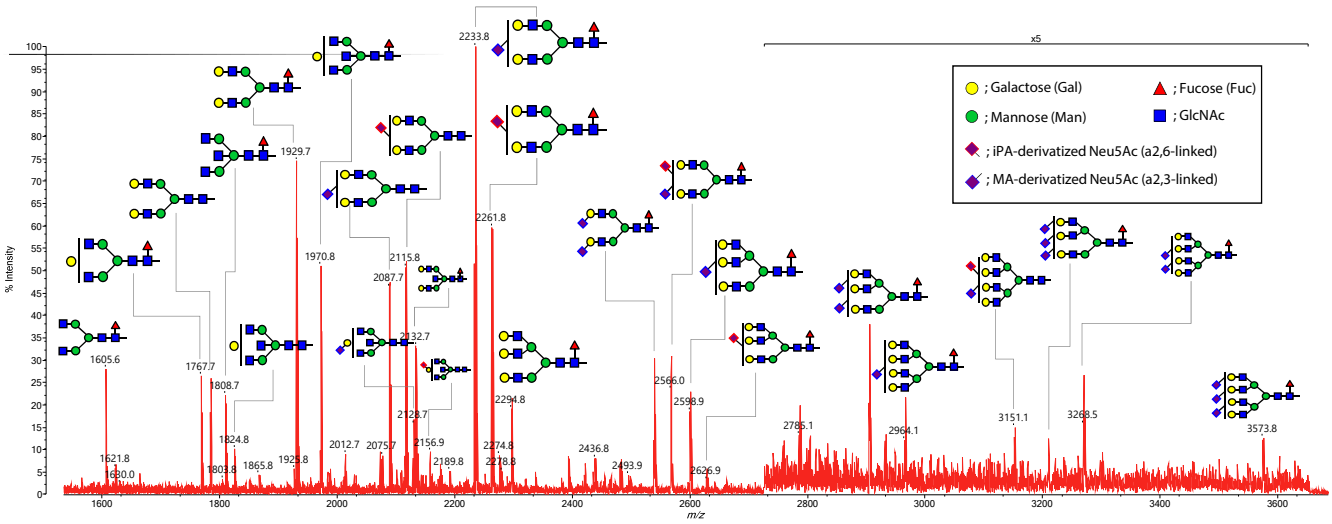

Fig. 4 Mass spectrum of human ACE2 *N*-glycans obtained by MALDI-8020

■ MS¹ Analysis of *N*-Glycans from Human ACE2

MALDI-8020 measurement detected various glycan peaks consisting mainly of complex-type *N*-glycans from human ACE2 (Fig. 4). The monosaccharide composition of the detected glycan peaks was calculated using the software "Supporting Tool for SialoCapper-ID Kit" (Table 1). The glycan structures estimated from the software calculation results, the past literature, and known biosynthetic pathways of *N*-glycans are shown in the spectrum.

The SialoCapper-ID Kit neutralizes sialic acids, ionization efficiencies of sialylated glycans result is comparable to that of neutral glycans. Thus, the peak intensities can be directly compared, and the relative amounts of glycans can be evaluated more accurately. From the peak intensity of *N*-glycans obtained, the ratio of neutral glycans to sialylated glycans and the ratio of α2,3-/α2,6-linkage types in sialylated glycans were calculated (Fig. 5). This recombinant human ACE2 was found to have more α2,3-sialic acid than α2,6-sialic acid.

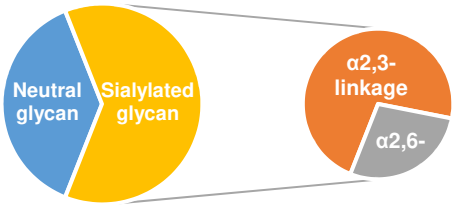

Fig. 5 Ratios of neutral glycans to sialylated glycans and α2,3-/α2,6-linkage types in sialylated glycans calculated from peak intensities

■ Conclusion

By using the SialoCapper-ID Kit, the neutral and sialylated glycans can be detected simultaneously, and the linkage types of sialic acid can be determined by MS¹ measurement only. This article shows that the *N*-glycans of ACE2, a receptor of SARS-CoV-2, and their sialic acid linkage types can be easily evaluated.

Table 1 Monosaccharide composition of *N*-glycan peaks detected from human ACE2 protein.

m/z (obs.)	*m/z* (calc.)	Glycan Composition	Intensity
1605.6	1605.60	AB-Hex3HexNAc4dHex1	40.8%
1767.7	1767.66	AB-Hex4HexNAc4dHex1	28.6%
1783.7	1783.65	AB-Hex5HexNAc4	24.5%
1808.7	1808.68	AB-Hex3HexNAc5dHex1	24.5%
1824.8	1824.68	AB-Hex4HexNAc5	10.2%
1929.7	1929.71	AB-Hex5HexNAc4dHex1	69.4%
1970.8	1970.73	AB-Hex4HexNAc5dHex1	42.9%
2087.7	2087.78	AB-Hex5HexNAc4NeuAc(α2.3-)1	42.9%
2115.8	2115.81	AB-Hex5HexNAc4NeuAc(α2.6-)1	44.9%
2128.7	2128.80	AB-Hex4HexNAc5NeuAc(α2.3-)1	12.2%
2132.7	2132.79	AB-Hex5HexNAc5dHex1	30.6%
2156.9	2156.84	AB-Hex4HexNAc5NeuAc(α2.6-)1	6.1%
2233.8	2233.84	AB-Hex5HexNAc4dHex1NeuAc(α2.3-)1	100.0%
2261.8	2261.87	AB-Hex5HexNAc4dHex1NeuAc(α2.6-)1	63.3%
2294.8	2294.84	AB-Hex6HexNAc5dHex1	20.4%
2537.9	2537.96	AB-Hex5HexNAc4dHex1NeuAc(α2.3-)2	34.7%
2566.0	2565.99	AB-Hex5HexNAc4dHex1 NeuAc(α2.6-)1NeuAc(α2.3-)1	38.8%
2598.9	2598.97	AB-Hex6HexNAc5dHex1NeuAc(α2.3-)1	30.6%
2626.9	2627.00	AB-Hex6HexNAc5dHex1NeuAc(α2.6-)1	8.2%
2903.0	2903.09	AB-Hex6HexNAc5dHex1NeuAc(α2.3-)2	20.4%
2931.0	2931.13	AB-Hex6HexNAc5dHex1 NeuAc(α2.6-)1NeuAc(α2.3-)1	8.2%
2963.7	2964.10	AB-Hex7HexNAc6dHex1NeuAc(α2.3-)1	10.2%
3149.6	3149.21	AB-Hex7HexNAc6 NeuAc(α2.6-)1NeuAc(α2.3-)1	6.1%
3207.3	3207.22	AB-Hex6HexNAc5dHex1NeuAc(α2.3-)3	6.1%
3268.1	3268.23	AB-Hex7HexNAc6dHex1NeuAc(α2.3-)2	16.3%
3572.3	3572.35	AB-Hex7HexNAc6dHex1NeuAc(α2.3-)3	10.2%

m/z indicates monoisotopic value. m/z (obs.) larger than *m/z* 3000 is the value estimated from their average mass of detected glycan peak.

<References>
1) Shajahan A, et al. (2020) Comprehensive Characterization of N- and O-Glycosylation of SARS-CoV-2 Human Receptor Angiotensin Converting Enzyme 2. *Glycobiology* in press.
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.

01-00111-EN First Edition: Apr. 2021

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