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Application News

A Low Peeling, Fast and Easy Approach for Protein O-linked Glycan Analysis

Derrick Tan, Yonghai Lu, Tian Hua Wang

User Benefits

- A simple and straightforward sample preparation workflow for O-glycan analysis
- Lower peeling products in O-glycan sample preparation

■ Introduction

Protein glycosylation has been shown to be related to protein activity. It is therefore of great interest to study glycan structures of glycoproteins in immunology and cellular biology. On top of that, it is also essential to analyze glycans in recombinant glycoprotein drugs to ensure consistent glycosylation profile. Glycan can be linked to protein either via asparagine (N-glycan) or via serine/threonine (O-glycan). Previously, we have published an application news on N-glycan analysis.[1] Typical analysis of glycan involves cleavage of the glycan from the protein and subsequently derivatization with fluorescent labels (e.g., 2-aminobenzamide, 2-AB) followed by analysis using HPLC. Unlike N-glycans which have well-established glycan releasing strategy, the O-glycans lack practical method for releasing of intact O-glycans. O-glycan releasing method such as hydrazinolysis often results in large amount of side reaction peeling products and requires long preparation time. This study demonstrates a simplified workflow using the S-Bio EZGlyco™ O-Glycan Prep kit, which exhibits low peeling and a reduced sample preparation time for O-glycan characterization. The solution incorporates the Shimadzu Nexera-i MT system and highly sensitive fluorescence detector (RF-20A).

■ Experimental

Fetuin Sample:

Fetuin from fetal bovine serum was used in this study. It was diluted with Milli-Q water to 5 mg/mL prior to sample preparation.

Fast Sample Preparation (less than 5h):

S-Bio EZGlyco™ O-Glycan Prep kit provides all the necessary reagents required for sample preparation, except certain common reagents such as acetic acid, acetonitrile and methanol. The kit allows efficient O-glycan release, enrichment, 2-AB labeling, and final labeled O-glycan cleanup in less than 5h. A detailed procedure for sample preparation is described in the kit instruction manual (S-Bio Cat. No. BS-41601Z). After the final cleanup step, the eluted labeled O-glycans were diluted with equal volume of acetonitrile for analysis by HPLC.

LC-Fluorescence and LC-MS Detection:

The sample analyses were conducted on a Shimadzu Nexera-i MT system equipped with a highly sensitive fluorescence detector, RF-20A. Identity of glycan peaks was confirmed using Shimadzu LCMS-9030 (Q-TOF). Table 1 lists the LC-fluorescence and LC-MS conditions used in detail.

Table 1. LC-Fluorescence and LC-MS conditions

LC-Fluorescence conditions	
LC system:	Shimadzu Nexera-i MT
Column:	Shim-pack GIST-HP Amide 1.9 µm, 150 × 2.1 mm *1
Column Temp.:	45 °C
Flow rate:	0.25 mL/min
Mobile phase A:	100 mM Ammonium formate
Mobile phase B:	Acetonitrile
Gradient program:	0 min, 80% B, 2 min, 80% B, 13 min, 50% B, 18 min, 50% B, 18.01 min, 80% B, 28 min, 80% B
Injection volume:	2 µL
Fluorescence detector:	Shimadzu RF-20A
Excitation:	330 nm
Emission:	420 nm
LC-MS conditions	
LC system:	Shimadzu Nexera-X2
Column:	Shim-pack GIST-HP Amide 1.9 µm, 150 × 2.1 mm *1
Column temp.:	45 °C
Flow rate:	0.25 mL/min
Mobile phase A:	100 mM Ammonium formate
Mobile phase B:	Acetonitrile
Gradient program:	0 min, 80% B, 2 min, 80% B, 13 min, 50% B, 18 min, 50% B, 18.01 min, 80% B, 28 min, 80% B
Injection volume:	20 µL
MS system:	Shimadzu LCMS-9030 (Q-TOF)
Interface:	Heated ESI (Positive or Negative)
Interface voltage:	4 kV
Interface temp:	300 °C
Nebulizing gas:	N2, 3 L/min
Heating gas flow:	Zero air, 10 L/min
DL temperature:	250 °C
Drying gas flow:	N2, 10 L/min
Heat block temp:	400 °C
MS mode:	MS scan
Mass range:	200 – 1500 *m/z*
MS mode:	MS/MS scan
Mass range:	50 – 1500 *m/z*

*1 P/N: 227-30947-05

■ Results and Discussion

LC-fluorescence analysis of released, 2-AB labeled O-glycans is one of the popular approach for determining protein glycosylation. Figure 1 shows the separation and florescence detection of 2-AB labeled O-glycans from fetuin. The separated glycans were identified by LC-MS/MS as shown in Figure 2, which agree with that reported in S-bio application notes.[2] Low level of peeling product (Peak 1) was observed, which ensures more accurate quantification of the released O-glycans. Additionally, injection-to-injection variability of the LC-fluorescence system was evaluated. Variations in peak area and retention time were less than 2% and 0.2% RSD respectively for all peaks (Table 2).

■ Conclusion

This study demonstrated an easy and fast solution for protein O-linked glycan analysis. Taking less than 5h, the sample prepared using the EZGlyco™ O-Glycan Prep kit produces low amount of peeling product, allowing more accurate analysis of O-linked glycans.

■ Acknowledgement

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Table 2. Injection-to-injection repeatability peak area and retention time (n = 6) of O-glycans

Peak	Peak area	RSD (%)	RT (min)	RSD (%)
Peak 1	7.45%	1.69	7.100	0.15
Peak 2	67.56%	0.30	8.193	0.18
Peak 3	22.39%	0.40	10.496	0.12
Peak 4	2.60%	0.77	11.349	0.10

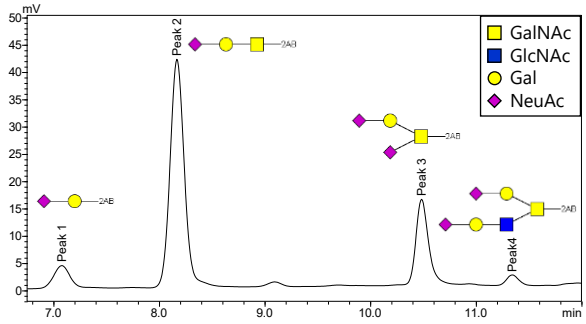

Figure 1. Chromatogram of O-glycans from fetuin.

■ References

1. Application News No. AD-0234A, Shimadzu Corporation.
2. Preparation and LC-MS Analysis of Procainamide-Labeled O-Glycans Using EZGlyco™ O-Glycan Prep Kit, S-bio.

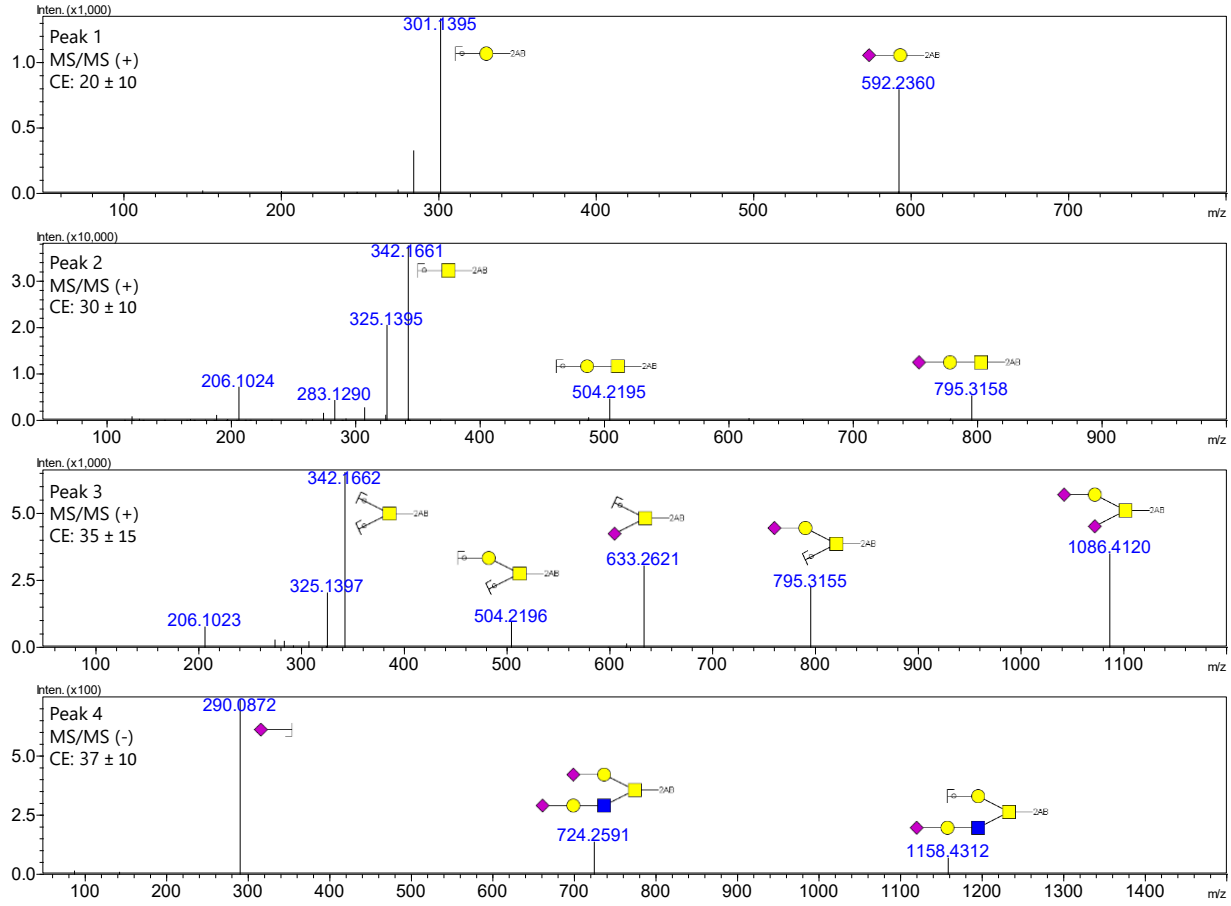

Figure 2. MS/MS spectra of 2-AB labeled O-glycans from fetuin.

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