Preparation and LC-MS Analysis of Procainamide-Labeled O-Glycans Using EZGlyco® O-Glycan Prep Kit

Introduction

Glycosylation of proteins has been of a great interest since researches in immunology and cellular biology have revealed pivotal roles of glycans in various biological activities. In biopharmaceuticals, thus, the glycan structure analysis has a fundamental importance to provide an expected function for recombinant glycoprotein drugs.

S-BIO's **EZGlyco® O-Glycan Prep Kit** allows quick, simple, and reproducible O-glycan preparations from glycoproteins in the form of 2-aminobenzamide (2-AB) label. A straightforward operation of the Kit utilizes a combination of proprietary chemical reagent for the O-glycan release and Glycan Capturing Bead to efficiently recover small O-glycans (Figure 1). The 2-AB fluorescent tag has been commonly used in an HPLC measurement of glycan molecules due to its high fluorescent sensitivity and the availability of a wide range of 2-AB-labeled standards regardless of N- or O-glycans.

Recently, techniques involving mass spectrometry (MS) such as LC-MS has been commonly utilized for glycan characterization. For such an application combining hydrophilic interaction chromatography (HILIC), fluorescent detection and MS, a demand for higher MS sensitivity has been a growing interest. One of such options is procainamide as demonstrated its effectiveness in N-glycan analyses elsewhere, which uses the same chemistry as 2-AB labels the reducing end of glycans via reductive amination.

Here, to provide another option for labeling the O-glycans with **EZGlyco O-Glycan Prep Kit**, we adapted procainamide in place of 2-AB in the operational workflow. The results show that procainamide label would be one of the choices to allow sensitive fluorescent and MS detections of O-glycans in LC-MS.

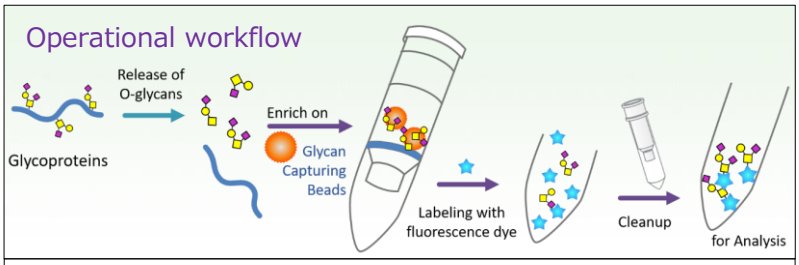

Figure 1. Operation workflow of labeled O-glycan preparation with EZGlyco O-Glycan Prep Kit.
The Kit readily allows flawless preparation of O-glycans including release from glycoproteins, enrichment, labeling, cleanup for glycan characterizations within 5-6 hours. Previously, those tedious operations take 2 to 3days with other methods, whereas the Kit provides a rapid and efficient recovery of O-glycans.

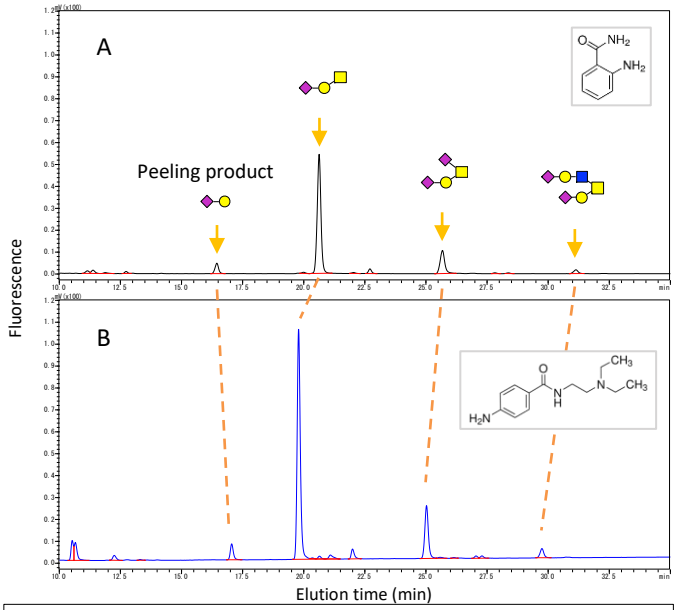

Figure 2. Fluorescent chromatograms of O-glycans derived from bovine fetuin.
A) 2-AB-labeled O-glycans, B) procainamide-labeled O-glycans.

Experimental

Analyte

Fetuin from fetal bovine serum, 25 µg/assay
Cat No. F2379, Sigma-Aldrich

Procainamide labeling

- Reagent: Procainamide hydrochloride
  MATRIX SCIENTIFC, product No. 149481
- Preparation of labeling solution
  1. Dissolve at 171.2 mg/mL in MeOH-acetic acid-pure water (45:10:45).
  2. Add 20 µL of component **7** reconstituted as in the Kit instruction to one mL of the above solution (Step 11-2). Mix well.
- Reaction conditions: Follow the instruction in the Kit as for the 2-AB label.

MS measurements

MS system:	LCMS-IT-TOF, Shimadzu
Ionization:	ESI
Voltage:	2.05 kV
Detection range:	m/z 250—2,500

LC conditions

LC system:	Nexera, Shimadzu
Column:	ACQUITY UPLC® Glycan BEH Amide (130 Å, 1.7 µm, 2.1 x 150 mm, Waters)
Column temp.:	40°C
Injection vol.:	1 µL (equiv. for 0.5 µg of fetuin)
Fluorescent detection:	
RF-20Axs, Shimadzu	
Gain: 4, Sensitivity: med, responses: 1.0 sec	
2-AB:	ex 330 nm/em 420 nm
Procainamide:	ex 308 nm/em 359 nm
Flow rate:	0.2 mL/min

Mobile phase A: 40% acetonitrile, 0.1% formic acid
Mobile phase B: 90% acetonitrile, 0.1% formic acid

Time (min)	%A	%B
0	0	100
50	100	0
70	100	0
80	0	100

Results and Discussion

It has been shown that fetuin from fetal bovine serum possesses both N- and O-glycans. The O-glycans found are all sialylated and three structures are demonstrated as we detected in Figures 2A and 2B for 2-AB and procainamide labeled recoveries, respectively. Besides, NeuAc-Gal, an artificial degraded O-glycan, *aka* peeling product, was detected even though the abundance was limited at quite low amounts (ca. 5%) due to an advantage of the reagent applied in the **EZGlyco O-Glycan Prep Kit**, demonstrating that procainamide fluorescent tag gave virtually the same O-glycan profile for fetuin O-glycans with twice as high as the signals of 2-AB label under the conditions in this study.

In the ESI-MS analysis, 2-AB label gave preferable results in the negative ion mode over positive ion mode in terms of simplicity of MS spectra and sensitivity over the ions detected. On the other hand, the procainamide label showed readily detectable ions with higher sensitivity in especially positive ion mode due to its superior ionization efficiency (Figure 3).

Conclusions

Procainamide labeling was readily adapted in the protocol of **EZGlyco O-Glycan Prep Kit** in the same way as 2-AB labeling with a simple modification where reagent **6** (2-AB) was replaced with commercially available procainamide hydrochloride. Both labeling tags provided practically the same glycan profile.

Fetuin O-glycans labeled with procainamide gave improved fluorescence and positive ion mode MS sensitivities over those of 2-AB label.

Besides 2-AB tag which has been commonly used and a wide range of labeled glycan standards are available for N- and O-glycans, an option with an improved sensitivity was successfully incorporated by replacing 2-AB reagent with procainamide in **EZGlyco O-Glycan Prep Kit**.

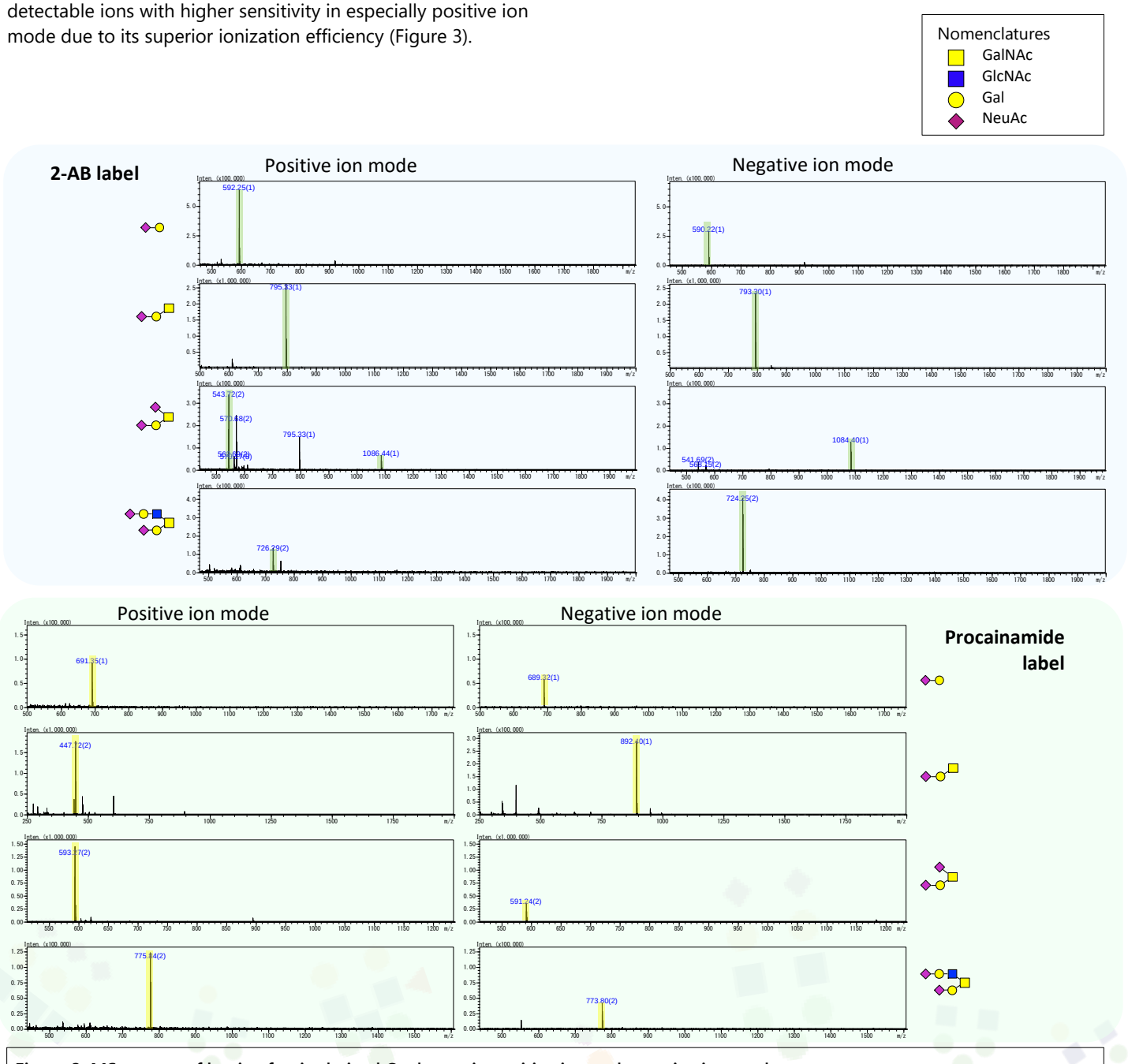

Figure 3. MS spectra of bovine fetuin derived O-glycans in positive ion and negative ion modes.
Upper pane) 2-AB-labeled O-glycans, **Lower pane)** procainamide-labeled O-glycans.