Effect of Cationization Agent Concentration on Glycan Detection Using MALDI TOF-MS

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Abstract: The effect of cationization agent concentration on glycan detection via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was investigated using Na⁺ ions in the form of NaCl as the cationization agent. NaCl solution concentrations ranging from 1 mM to 1 M were investigated. Glycans from ovalbumin were mixed with the cationization agent solution and the 2,5-dihydroxybenzoic acid (2,5-DHB) matrix solution in a volume ratio of 1:1:1. The resulting mixture was loaded onto the MALDI plate. Two MALDI-TOF MS instruments (Voyager DE-STR MALDI-TOF MS and Tinkerbell RT MALDI-TOF MS) were used for detection of glycans. The best detection, in terms of the number of identified glycans, the peak intensity, and the signal-to-noise (S/N) ratio, was obtained with NaCl concentrations of 0.01–0.1 M for both MALDI-TOF MS instruments.

Keywords: MALDI-TOF MS, glycans, cationization agent, PNGase F, 2,5-dihydroxybenzoic acid

Introduction

Glycosylation is a common post-translational protein modification.1 Glycans are products of glycosylation that play key roles in biological processes.2,3 N-glycans are commonly released through deglycosylation of glycoproteins using peptide N-glycosidase F (PNGase F)4 and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).5 A cationization agent is commonly added to glycan samples to improve the ionization of glycans during positive-ion-mode MALDI-TOF MS analysis. Among cations, Na⁺ is the most commonly used as a cationization agent in the form of sodium chloride6-10, sodium acetate11-14, sodium hydroxide15, or sodium iodide16. Na⁺ ions have been used as a cationization agent in the concentration range of 1 mM6, 11-13, 15 to 20 mM14.

In this study, we investigated the effect of cationization agent concentration on the detection of glycans using MALDI-TOF MS. NaCl was selected as the cationization agent, and 2,5-dihydroxybenzoic acid (2,5-DHB) was used for the matrix solution. NaCl concentrations ranging from 1 mM to 1 M were prepared for the current investigation, and glycans were obtained via deglycosylation of ovalbumin. Mixtures of the matrix solution, glycan sample, and NaCl (1:1:1, v/v/v) were loaded onto a MALDI plate and analyzed using MALDI-TOF MS.

Experimental

Ovalbumin (albumin from chick egg whites, cat. num. A5503), PNGase F (cat. num. P7367), ammonium bicarbonate (ABC, cat. num. A6141), 2,5-DHB (cat. num. 149357), and sodium chloride (NaCl, cat. num. S6191) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN, HPLC grade, cat. num. 100029) was purchased from Merck (Whitehouse Station, NJ, USA), and phosphoric acid (PA, cat. num. P1836, 85%) was purchased from DUKSAN (South Korea). Ovalbumin (10 mg) was dissolved in 1 mL of 50 mM ABC buffer to prepare the ovalbumin stock solution. To release N-glycans from ovalbumin, PNGase F was added in a ratio of 1 unit per 200 µL of ovalbumin stock solution and mixed gently for 2 h at 37°C (500 rpm). To prepare the matrix solution, 2,5-DHB (10 mg) was dissolved in 1 mL of 50% ACN/1% PA aqueous solution.17 Na⁺ ions were used as the cationization agent, and NaCl solutions were prepared at concentrations ranging from 1 mM to 1 M (1 mM, 0.01 M, 0.05 M, 0.1 M, and 1 M).

A MALDI plate target sample was prepared by loading 1.5 µL of a mixture of the matrix, glycan sample, and NaCl (1:1:1, v/v/v). MALDI mass spectra were recorded in positive-ion reflectron mode using a Voyager DE-STR

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Cationization Agent Concentrations on Glycan Analysis in MALDI TOF-MS

MALDI-TOF MS (Applied Biosystems, Forster City, CA, USA) with a 337-nm N₂ laser source and a Tinkerbell RT MALDI-TOF MS (ASTA, Suwon, South Korea) with a 349-nm Nd:YLF UV laser source.

Assignment of glycan peaks was performed based on the results of the previous study.17

Results and discussion

To investigate the effect of cationization agent concentration on the detection of glycans, various concentrations of Na⁺ were prepared and mixed with the glycan sample solution and the matrix solution in a ratio of 1:1:1 (v/v/v). A small amount of the mixture (1.5 µL) was then loaded onto the MALDI plate and analyzed using two different mass spectrometers (Voyager MALDI-TOF MS and Tinkerbell MALDI-TOF MS).

Figure 1 shows the MALDI mass spectra of glycans from ovalbumin for various concentrations of Na⁺. The two MALDI-TOF MS instruments provided very similar glycan profiles, in which the most and second-most abundant peaks were observed at m/z 1,745.5 and 1,542.5,
respectively. The maximum intensity was observed with 0.05 M Na$^+$ for the two instruments. Table 1 summarizes the glycans identified in the current study for the mass spectra shown in Fig. 1. The Voyager MALDI-TOF MS exhibited similar high numbers of identified glycans using Na$^+$ concentrations of 0-0.1 M, while the Tinkerbell MALDI-TOF MS provided similar high numbers of identified glycans using Na$^+$ concentrations of 1 M to 1 M.
Figure 2 shows the change in the signal-to-noise (S/N) ratio for the three most abundant glycan peaks: Hex$_3$(HexNAc)$_6$Na at $m/z$ 1,746.58, Hex$_4$(HexNAc)$_5$Na at $m/z$ 1,543.39, and Hex$_4$(HexNAc)$_6$Na at $m/z$ 1,908.73. For both MS instruments, 0.05 M NaCl provided the best S/N ratio for the Hex$_3$(HexNAc)$_6$Na peak at $m/z$ 1,746.58 among the NaCl concentrations used in this study. For Na$^+$ concentrations that were too low (e.g., 0 M) or too high (e.g., 1 M), the S/N ratio and the number of identified glycan peaks decreased (Table 1).

**Conclusion**

The effect of cationization agent concentration on glycan detection using MALDI-TOF MS was investigated. The dried-droplet deposition method was used to load a mixture of equal volumes of cationization agent, glycan sample, and matrix solution. Based on the number of identified glycans and the S/N ratio, Na$^+$ concentrations ranging from 0.01 to 0.1 M were shown to optimize the detection of glycans.

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