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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 chloride[6-10], sodium acetate[11-14], sodium hydroxide[15], or sodium iodide[16]. Na^+ ions have been used as a cationization agent in the concentration range of 1 mM[6, 11-13, 15] to 20 mM[14].

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.

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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

MALDI-TOF MS (Applied Biosystems, Forster City, CA, USA) with a 337-nm N_2 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,

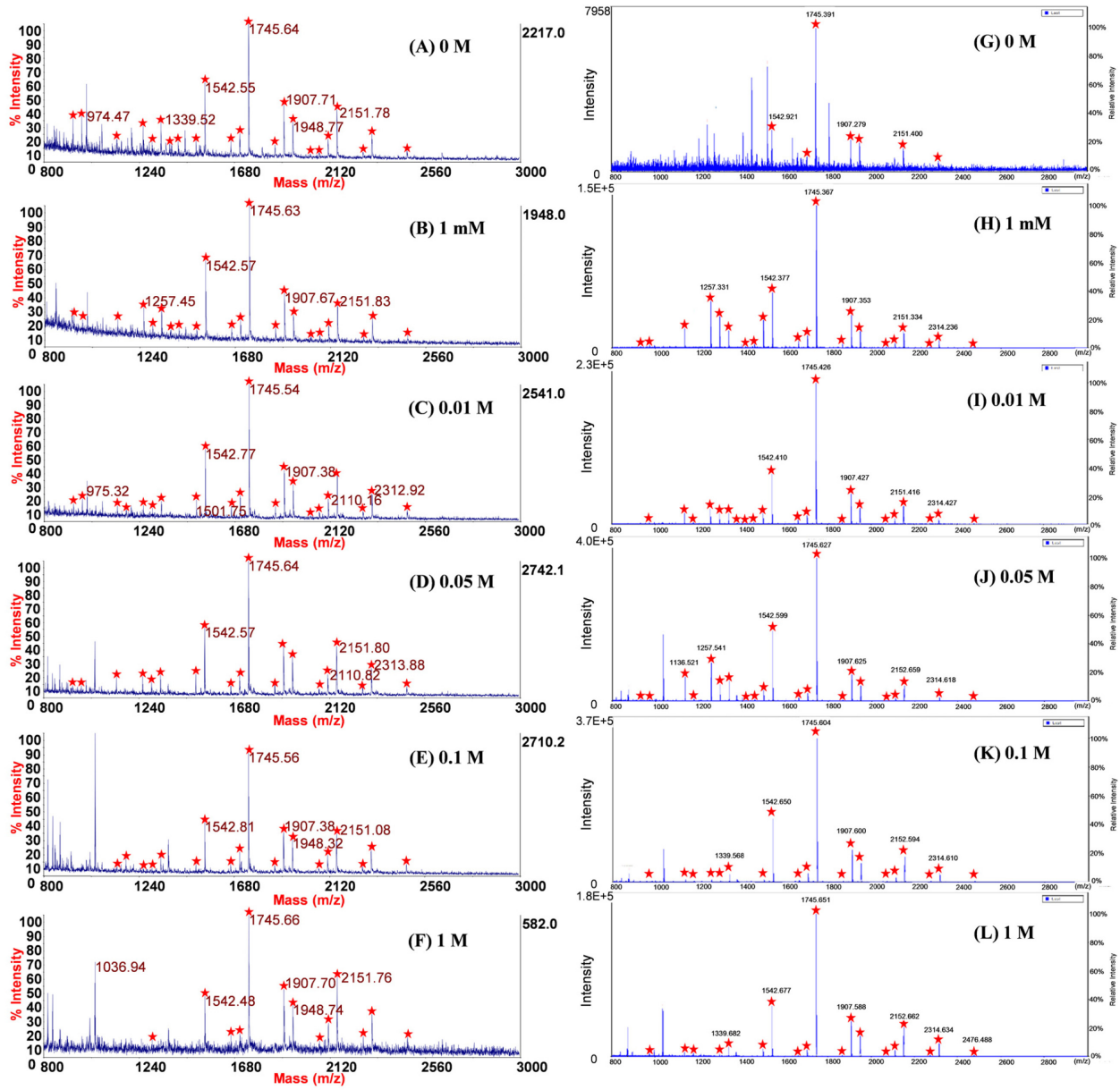

Figure 1. MALDI mass spectra of glycans released from ovalbumin acquired using a Voyager DE-STR MALDI-TOF MS (left, A-F) and a Tinkerbell RT MALDI-TOF MS (right, G-L) for various NaCl concentrations.

Table 1. Summary of the identified glycans from deglycosylation of ovalbumin for various NaCl concentrations using a Voyager DE-STR MALDI-TOF MS and a Tinkerbell RT MALDI-TOF MS.

Theoretical *m/z* values (average)	Structures	Voyager						Tinkerbell					
		0 M	1 mM	0.01 M	0.05 M	0.1 M	1 M	0 M	1 mM	0.01 M	0.05 M	0.1 M	1 M
933.82	$Hex_3(HexNAc)_2Na$	○	○	○	○				○		○		
974.87	$Hex_2(HexNAc)_3Na$	○	○	○	○				○	○	○	○	○
1137.01	$Hex_3(HexNAc)_3Na$	○	○	○	○	○			○	○	○	○	○
1178.07	$Hex_2(HexNAc)_4Na$			○		○				○	○	○	○
1258.09	$Hex_5(HexNAc)_2Na$	○	○	○	○	○			○	○	○	○	
1299.14	$Hex_4(HexNAc)_3Na$	○	○	○	○	○	○		○	○	○	○	○
1340.20	$Hex_3(HexNAc)_4Na$	○	○	○	○	○			○	○	○	○	○
1381.26	$Hex_2(HexNAc)_5Na$	○	○							○			
1420.24	$Hex_6(HexNAc)_2Na$	○	○						○	○	○		
1461.29	$Hex_5(HexNAc)_3Na$								○	○	○		
1502.34	$Hex_4(HexNAc)_4Na$	○	○	○	○	○			○	○	○	○	○
1543.39	$Hex_3(HexNAc)_5Na$	○	○	○	○	○	○	○	○	○	○	○	○
1664.48	$Hex_5(HexNAc)_4Na$	○	○	○	○	○	○		○	○	○	○	○
1705.53	$Hex_4(HexNAc)_5Na$	○	○	○	○	○	○	○	○	○	○	○	○
1746.58	$Hex_3(HexNAc)_6Na$	○	○	○	○	○	○	○	○	○	○	○	○
1867.67	$Hex_5(HexNAc)_5Na$	○	○	○	○	○			○	○	○	○	○
1908.73	$Hex_4(HexNAc)_6Na$	○	○	○	○	○	○	○	○	○	○	○	○
1949.78	$Hex_3(HexNAc)_7Na$	○	○	○	○	○	○	○	○	○	○	○	○
2029.81	$Hex_6(HexNAc)_5Na$	○	○	○	○								
2070.86	$Hex_5(HexNAc)_6Na$	○	○	○	○	○	○		○	○	○	○	○
2111.92	$Hex_4(HexNAc)_7Na$	○	○	○	○	○	○		○	○	○	○	○
2152.97	$Hex_3(HexNAc)_8Na$	○	○	○	○	○	○	○	○	○	○	○	○
2274.06	$Hex_5(HexNAc)_7Na$	○	○	○	○	○	○		○	○		○	○
2315.11	$Hex_4(HexNAc)_8Na$	○	○	○	○	○	○	○	○	○	○	○	○
2477.25	$Hex_5(HexNAc)_8Na$	○	○	○	○	○	○		○	○	○	○	○
Number of identified glycans		23	23	22	21	19	13	7	22	23	22	20	19

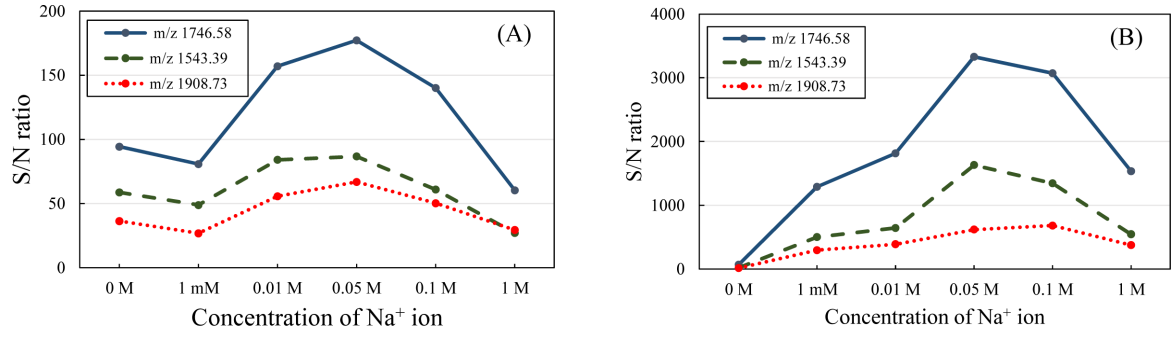

Figure 2. The effect of NaCl concentration for representative glycan peaks using (A) the Voyager DE-STR MALDI-TOF MS and (B) the Tinkerbell RT MALDI-TOF MS.

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 mM to 1 M.

Figure 2 shows the change in the signal-to-noise (S/N) ratio for the three most abundant glycan peaks: $Hex_3(HexNAc)_6Na$ at *m/z* 1,746.58, $Hex_3(HexNAc)_5Na$ at *m/z* 1,543.39, and $Hex_4(HexNAc)_6Na$ at *m/z* 1,908.73. For both MS instruments, 0.05 M NaCl provided the best S/N ratio for the $Hex_3(HexNAc)_6Na$ 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.

Acknowledgements

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