SHIMADZU ASMS2012 TP200

Sensitive Analysis of Neutral *N*-Glycan using Anion Doped Liquid Matrix by Negative-Ion Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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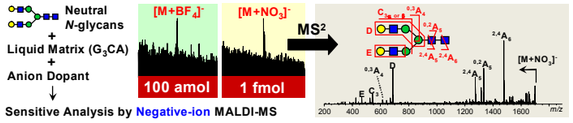
ms3d FIRST Program

1. Overview

- ✓ Combinations of five liquid matrices and nine anions were evaluated for sensitive analyses of underivatized neutral *N*-glycans in negative-ion MALDI-MS.
- ✓ Among tested, G_3CA exhibited outstanding performances for all the anions.
- ✓ Low-femtomole or sub-femtomole detection limits could be achieved using NO_3^- or BF_4^- doped G_3CA.
- ✓ A combination of G_3CA and NO_3^- is the most suitable for sensitive analyses, especially for MS^2 measurement.
- ✓ A combination of G_3CA and BF_4^- is a possible alternative for superior sensitivity in MS^1 detection and medium sensitivity in MS^2 measurement.



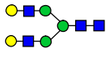
2. Introduction

Negative-ion fragmentation of *N*-glycans has been proven to be more informative than that of positive-ion. It defines structural features such as the specific composition of the two antennae, the location of fucose, and the presence or absence of bisecting GlcNAc. In general, negative ionization of neutral *N*-glycans is much more difficult than positive ionization because of lack of acidic groups. The most promising approach is to ionize them as anionic adducts. However, anionic adduct formation of neutral *N*-glycans by MALDI-MS remains a challenging task, and the detection limit in negative-ion mode is merely at the sub-picomole level. Here, we demonstrate that G_3CA acts as an effective matrix for sensitive MALDI analysis of neutral *N*-Glycan in negative-ion mode.

3. Methods

3.1. Analyte

NA2 glycan

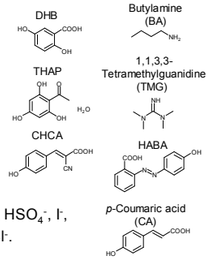


M.W. 1641.5

○ ; Gal
○ ; Man
■ ; GlcNAc

3.2. Matrices and anions

- ✓ DHBB (BA + DHB, 1:1)
- ✓ GTHAP (TMG + THAP, 1:1)
- ✓ G_2CHCA (TMG + CHCA, 2:1)
- ✓ G_2HABA (TMG + HABA, 2:1)
- ✓ G_3CA (TMG + CA, 3:1)



- ✓ Ammonium salts of PF_6^-, BF_4^-, HSO_4^-, I^-, NO_3^-, Br^-, SCN^-, $H_2PO_4^-$ and Cl^-.

3.3. Preparation for liquid matrices

Matrix (DHB, THAP, HABA, or CA) + counter cation (BA or TMG) in MeOH
↓ SpeedVac (overnight)
↓ Dissolve in 50% ACN at a concentration of 100mg/mL
Dilute fivefold with 50% ACN containing 2.5mM anions

Matrix : Anion 20~50 : 1 (mol/mol)

3.4. Sample preparation and mass spectrometry

- ✓ On target mix (Sample solution 0.5μL + matrix solution 0.5μL)
- ✓ μFocus MALDI plate™ 700μm (Hudson Surface Technology)
- ✓ MALDI-QIT-TOF-MS (AXIMA Resonance,™ Shimadzu/Kratos)

4. Results and Discussion

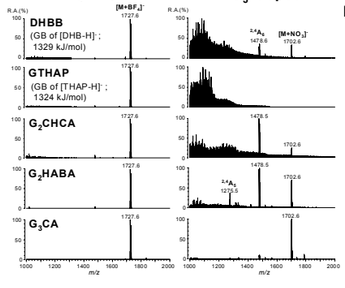
4.1 Evaluation of anion-doped liquid matrices

Combinations of five liquid matrices and nine anions were used to ionize *N*-glycans as anionic adducts, and their performances for sensitive analyses were evaluated. Representative spectra were shown in Figure 1 (below).

Anion	Nominal mass	GB (kJ/mol)	
PF_6^-	145	1157	Anions with Low GB
BF_4^-	87	1204	
HSO_4^-	97	1265	
I^-	127	1294	
NO_3^-	62	1330	Anions with High GB
Br^-	79, 81	1332	
SCN^-	58	1343	
$H_2PO_4^-$	97	1351	
Cl^-	35, 37	1373	

- ✓ Strong signals of anion-adducted NA2 glycans in combinations with any liquid matrices used.
- ✓ No or negligible fragment ions
- ✓ DHBB, GTHAP, and G_2CHCA…Little or no signals of adducts
- ✓ G_2HABA…Weak signals of adducts and extensive fragment ions
- ✓ **G_3CA… Strong signals of adducts**

The results obtained in this study could be interpreted based on the **gas-phase basicity (GB)** of anions.



GB was important parameter. Because…

- ✓ Anionic adducts of glycans can be considered as proton-bound mixed dimers of anions, $[M-H]^-\cdots H^+\cdots[A]^-$.
- ✓ Anions with high GB are easily neutralized by matrix molecules.

Matrix + $[A]^-$ ⟶ $[Matrix-H]^-$ + HA

Figure 1. Negative-ion mass spectra of NA2 glycan at 100fmol using various liquid matrices and anions as dopants.

4.2 Detection limits (see section 4.4 regarding anion selection)

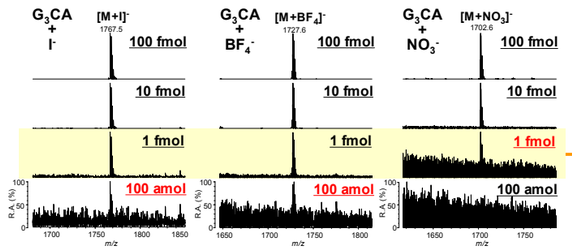


Figure 2. Negative-ion mass spectra of NA2 glycans at various concentrations.

4.3. Mass spectra of neutral *N*-glycan mixtures (Ovalbumin)

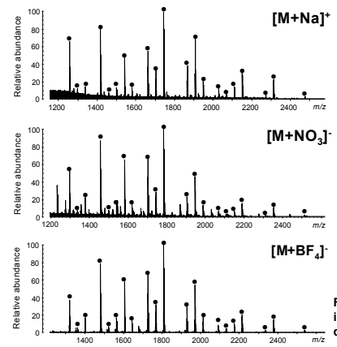
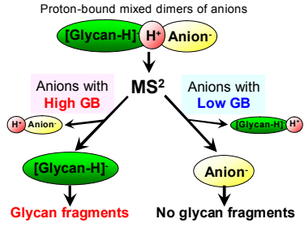


Figure 3 compares the mass spectra of *N*-glycans released from ovalbumin in both positive- and negative-ion modes using G_3CA matrix with and without anion dopants (see next section regarding anion selection). Negative-ion mode analyses of the *N*-glycans using NO_3^- or BF_4^- doped G_3CA indicated spectral patterns similar to those obtained in positive-ion mode.

Figure 3. Positive- and negative-ion mass spectra of *N*-glycans derived from Ovalbumin.

4.4. Evaluation of anionic species for MS^2 experiments



Since fragmentation of anionic adducts was initiated by proton abstraction from *N*-glycan to anions, the GB of anions is a crucial parameter for successful MS^2 analysis. Anions with low GB are unable to abstract hydroxylic protons from *N*-glycans, resulting in poor or no glycan fragment ions in the spectra.

100fmol NA2 + low GB anions (PF_6^-, HSO_4^-, and I^-)

- ✓ Poor MS^2 spectra
- ✓ Need extensive spectral accumulation
- ✓ Exception; **BF_4^-** (GB 1204 kJ/mol)
  - ✓ Glycan fragments with moderate efficiency

100fmol NA2 + high GB anions (NO_3^-, Br^-, SCN^-, $H_2PO_4^-$, and Cl^-)

- ✓ MS^2 spectra with high quality
- ✓ NO_3^- was the best
  - ✓ Strong signals of precursor ions
  - ✓ Efficient fragmentation

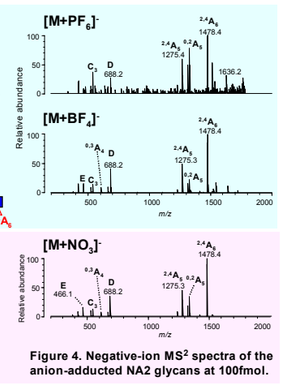


Figure 4. Negative-ion MS^2 spectra of the anion-adducted NA2 glycans at 100fmol.

MS² at 1fmol

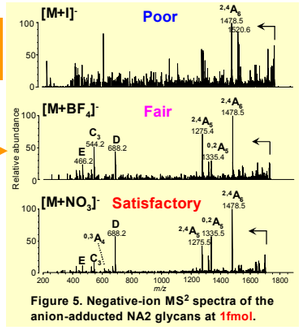


Figure 5. Negative-ion MS^2 spectra of the anion-adducted NA2 glycans at 1fmol.

The I^- adduct did not exhibit any informative signals in the MS^2 spectrum, whereas the BF_4^- adduct produced MS^2 spectra with an acceptable s/n ratio. MS^2 of the BF_4^- adduct formed product ions of glycans more efficiently than did the I^- adduct. Using NO_3^-, the MS^2 spectrum was successfully obtained with a sufficient s/n ratio. Since the NO_3^- adduct is less sensitive than the I^- or BF_4^- adduct in MS^1 analyses (Figure 2.), these results clearly indicate the effectiveness of NO_3^- adduct for sensitive MS^2 measurement.

5. Conclusions

- ✓ A combination of G_3CA and NO_3^- is the most suitable for sensitive analyses, especially for MS^2 measurement of neutral *N*-glycans in negative-ion MALDI-MS.
- ✓ A combination of G_3CA and BF_4^- is a possible alternative for superior sensitivity in MS^1 detection and medium sensitivity in MS^2 analysis.

6. Acknowledgments

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