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ASMS2013 ThP19_329

MALDI mass spectrometry analysis of sialylated glycoprotein by in-gel derivatization for sialic acids

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ms3d FIRST Program

1. Introduction

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) is an useful tool for the structural analysis of glycoproteins. MS^1 and MS^2 analyses of *N*-glycans released from glycoproteins are performed to obtain both glycan profiles and detailed structure of glycans. We previously reported the on-target AQ-labeling technique using a liquid matrix as a highly sensitive method for *N*-glycan profiling.[1, 2] Recently we also reported that AQ-labeled *N*-glycans exhibit simple and informative negative-ion CID spectra with product ions due to cross-ring cleavages of the chitobiose core and ions specific to two antennae (D and E ions).[3]

The major limitation of current *N*-glycan profiling methods is the partial loss of sialic acid residues in MS^1 experiment. So far, to suppress the dissociation, many derivatization methods for sialic acid have been proposed such as methylesterification, methylamidation, and amidation. However, most of these methods require complicated purification procedures prior to MS.

Here, we developed an in-gel derivatization method for sialylated glycoprotein following SDS-PAGE, a common method of electrophoresis applied to isolate a purposive protein from complex biological samples. Among tested, the combination of the novel in-gel methylamidation method and the AQ-labeling method was determined to be best for the structural analysis of sialylated *N*-glycans.

(1) Kaneshiro, K. et al. *Anal. Chem.* **2011**, 10, 3663-7.
(2) Kaneshiro, K. et al. *Anal.Chem.* **2012**, 16, 7146-51.
(3) Nishikaze, T. et al. *Anal. Chem.* **2012**, 21, 9453-61.

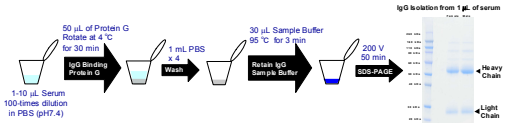
2. Materials and Methods

2-1. Materials

- Transferrin Human / Sigma-Aldrich
- Serum – Normal human – Male (single donor) / Tokyo Future Style, Inc.
- Serum – Normal human – Female (single donor) / Tokyo Future Style, Inc.
- Protein G Sepharose™4 Fast Flow / GW Healthcare Life Sciences
- NuPAGE® LDS Sample Buffer (x4) / Invitrogen
- NuPAGE® Novex 10% Bis-Tris Gel / Invitrogen
- Methylamine hydrochloride / Sigma-Aldrich
- 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride n-Hydrate (DMT-MM) / Wako

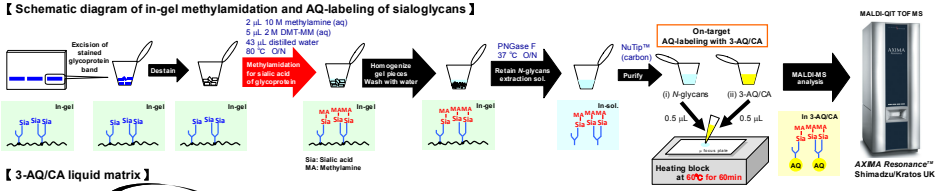
2-2. Isolation of IgG from serum

【 Schematic diagram of IgG Isolation from serum 】

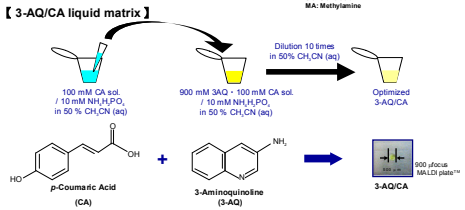


2-3. MALDI-MS analysis of sialoglycans by in-gel methylamidation followed by AQ-labeling method

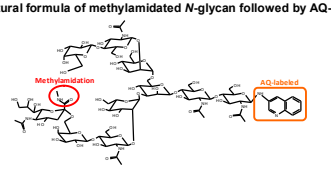
【 Schematic diagram of in-gel methylamidation and AQ-labeling of sialoglycans 】



【 3-AQ/CA liquid matrix 】



【 Structural formula of methylamidated *N*-glycan followed by AQ-labeling 】



3. Results and Discussion

3-1. In-gel derivatization efficiency of sialic acids on glycoprotein

Conventional in-solution derivatization methods for sialoglycan were applied to in-gel derivatization for sialylated glycoprotein.

◆ The choice of solvent was crucial. In-gel derivatization reaction could only be performed in water (not in MeOH or DMSO).

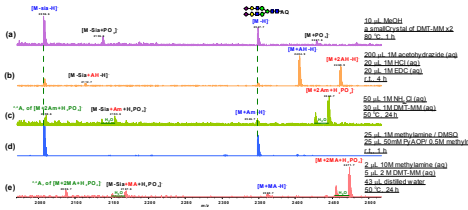
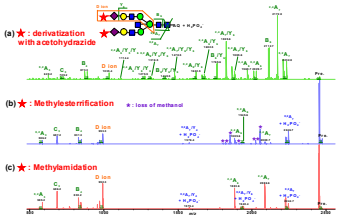


Figure 1 Negative-ion MS spectra of in-gel derivatized transferrin *N*-glycan: (a) methylesterification (Me); (b) derivatization with acetohydrazide (AH); (c) amidation (Am); (d), (e) methylamidation (MA).

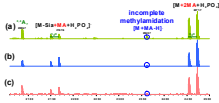
3-2. Suitability for negative-ion CID-MS



◆ Derivatization with acetohydrazide produced multiple cleavages and faint D ion in MS^2 spectra.
◆ Methyl ester form of sialoglycan showed extensive loss of MeOH molecules, interfering with correct MS^2 spectral interpretation.
◆ Methylamidated sialoglycans exhibited simple and clear MS^2 spectra.

Figure 2 Negative-ion MS^2 spectra of in-gel derivatized A2 glycans: (a) derivatization with acetohydrazide; (b)methylesterification; (c) methylamidation.

3-3. Optimum condition of in-gel methylamidation



In-gel methylamidation condition was tested at various times and temperatures to determine the optimum protocol.

◆ As the heating temperature rises, the reaction of methylamidation is promoted.
◆ The optimal results were obtained at 80 ºC for 18 h.

Figure 3 Negative-ion MS spectra of in-gel methylamidation transferrin *N*-glycans heated at different reaction temperatures: (a) 60 ℃; (b)70 ℃; (c) 80 ℃.

3-4. *N*-glycan profiles of IgG isolated from serum

Isolation of IgG from serum ⇒ in-gel methylamidation ⇒ *N*-glycan profiling

◆ IgG sialoglycans were completely methylamidated.
◆ We successfully profiled *N*-glycans of IgG isolated from 1 µL of human serum (male and female).

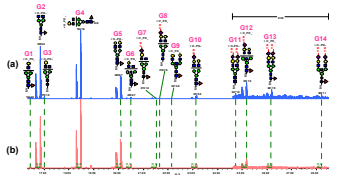


Figure 4 Negative-ion MS spectra of methylamidated *N*-glycan profiles of IgG isolated from human serum : (a) male; (b) female.

Table 1 Contents of each glycan relative to total 14 glycans ion intensities.

Glycan No.	contents (%) Male	Female
G1	1.32	1.50
G2	31.63	39.50
G3	2.54	1.71
G4	39.10	40.36
G5	15.71	17.02
G6	2.43	3.04
G7	1.19	1.26
G8	0.91	0.80
G9	0.87	0.71
G10	3.13	2.99
G11	0.24	0.24
G12	0.58	0.49
G13	0.29	0.25
G14	0.15	0.13
Total	100.00	100.00

3-5. Analysis of structure of in-gel methylamidated IgG glycan

The detail of methylamidated IgG glycan structure were analyzed by negative ion CID-MS.

◆ D ion was clearly detected in MS^2 spectra.
◆ MS^2 were successfully obtained from possible low intensity ions from MS^1 profiles.

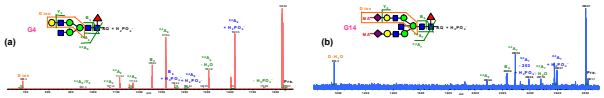


Figure 5 Negative-ion MS^2 spectra of in-gel methylamided IgG *N*-glycans isolated from male serum. The selected precusor ions were (a) *m/z* 1847.6 (G4) and (b) *m/z* 2821.1 (G14)

4. Conclusions

◆ In-gel complete derivatization for sialic acids of IgG was accomplished with an optimal condition of methylamidation using methylamine hydrochloride and DMT-MM (condensing agent).
◆ This novel method requires only washing gel pieces with water, while the conventional in-solution derivatization methods for sialylated glycans involve complicated purification processes to remove reagents and salts.
⇒Preventing loss of sample caused by time-consuming purification processes (e.g. solid-phase extraction).
◆ *N*-glycan profiling was obtained without a loss of sialic acid residues in MS^1 analysis.
◆ In-gel methylamidation followed by on-target AQ-labeling method was highly suitable for the structural characterization of IgG *N*-glycans.

5. Acknowledgements

This research is granted by the Japan Society for the Promotion of Science (JSPS) through the "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)," initiated by the Council for Science and Technology Policy (CSTP).