ASMS2011 ThP583

Highly sensitive MALDI analyses of glycopeptides using liquid matrices 3-AQ/CHCA and 3-AQ/CA

Yuko Fukuyama, Natsumi Funakoshi, Shinichi Iwamoto, Koichi Tanaka
[1] SHIMADZU CORPORATION, Nishinokyo-kuwabaracho, Nakagyo-ku, Kyoto 604-8511, Japan

SHIMADZU

1: Introduction

There is significant demand in the improvement of mass analytical methods for posttranslational modification (PTM), especially for disease-associated carbohydrates. Our group has previously reported a highly sensitive MALDI method for carbohydrate analysis using liquid matrices [*ASMS2010*, WP301; *Anal. Chem.*, **2008**, *80*, 2171]. However, such methods for glycopeptides have hardly been reported. We report here a highly sensitive MALDI method for glycopeptide analysis using conventional liquid matrix 3-aminoquinoline (3-AQ)/ *α*-cyano-4-hydroxycinamic acid (CHCA) and a novel matrix 3-AQ/*p*-coumaric acid (CA). The detection limit of glycopeptides was 10 amol with sufficient sensitivity for analysis. Additionally, MS^n measurement at 100 amol was successfully performed.

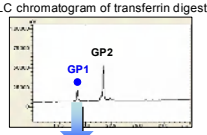
2: Experimental

2-1: Glycopeptide analyte

- Commercial glycoprotein transferrin (human, SIGMA T3309) was digested by trypsin, and glycopeptides were enriched by *Sepharose*™ CL-4B (GE Healthcare) using methods reported by Wada, Y. *et. al.* [1].
- Disialylated biantennary N-linked glycopeptides (GP1) of transferrin was isolated by HPLC (*Prominence*™; Shimadzu Corporation, Japan) as shown in Figure 1. Quantity of the analyte was determined by a calibration curve method using angiotensin II as external standard.

[1] Wada, Y.; Tajiri, M.; Yoshida, S. *Anal. Chem.* **2004**, *76*, 6560-6565.

- LC chromatogram of transferrin digests



402-CGLVPVLAENYNK-414
monoisotopic mass: 3680.5
(◆: NeuAc, ○: Gal, ■:GlcNAc, ●: Man)

Figure 1. Isolated transferrin glycopeptide: GP1

2-2: Matrices

Eight species of commercial and/or previously-reported liquid matrices were evaluated [2-6]. Results demonstrated that GP1 was detected with highest sensitivity using optimized 3-aminoquinoline (3-AQ)/ *α*-cyano-4-hydroxycinamic acid (CHCA) (Table 1). On the other hand, a novel liquid matrix 3-AQ/*p*-coumaric acid (CA) was also successful in ionizing GP1 with sensitivity comparable to 3-AQ/CHCA. 3-AQ/CHCA and 3-AQ/CA solutions were prepared as follows (Figure 2):

- **3-AQ/CHCA:** 10 mg of CHCA (recrystallized, LaserBio labs) was dissolved in 600 μL of 20 mM ammonium phosphate in 50/50 acetonitrile (ACN)/water. 20 mg of 3-AQ (≥99.0%, Fluka) was dissolved in 150 μL of the CHCA solution. The obtained solution was diluted ten-fold and used as 3-AQ/CHCA solution.
- **3-AQ/CA:** 3-AQ was mixed with CA (>98%, SIGMA-Aldrich) at 9:1 (mol/mol) ratio and dissolved in 2 mM ammonium phosphate in 50/50 ACN/water. The obtained solution was used as 3-AQ/CA solution.
- **3-AQ/*p*CA:** 3-AQ was mixed with the custom-ordered CA purified by Dojinkagaku, Japan or NARD institute, Ltd., Japan at 9:1 (mol/mol) ratio and dissolved in 2 mM ammonium phosphate in 50/50 ACN/water. The obtained solution was used as 3-AQ/*p*CA solution.
- **2,5-dihydroxybenzoic acid (DHB):** 1 mg of DHB (recrystallized, LaserBio labs) was dissolved in 1 mL of 50/50 ACN/water and obtained solution was used for a 600μm *μFocus MALDI plate*™ (Hudson Surface Technology, Inc. USA). On the other hand,10 mg of DHB was dissolved in 1 mL of 50/50 ACN/water and the obtained solution was used for *sample plate 2.8 mm ring X 384 well*™ (SUS plate) (Shimadzu corporation, Japan).

3: Results

3-1: Highly sensitive (known) liquid matrix

100 amol GP1 was detected using a liquid matrix 3-AQ/CHCA

Table 1. Detection limit of GP1 using previously-reported liquid matrices[a]

Matrices	GP1 (mol/well) positive	GP1 (mol/well) negative
3-AQ/CHCA [2]	**0.1 f**	**0.1 f**
DHBB [3]	10 f	10 f
CHCAB [3]	100 f	10 f
G_2CHCA [4]	10 f	1 f
G_2CA [5]	1 f	1 f
GCA [5]	1 f	10 f
IMTBA/DHB [6]	1 f	1 f
DIEA/DHB [6]	10 f	1 f
DHB	10 f	1 f

- DHBB: *n*-butylamine/DHB
- CHCAB (Fluka, 67336): *n*-butylamine/CHCA
- G_2CHCA: 1,1,3,3-tetramethyl guanidine (TMG)/CHCA
- G_2CA, GCA: TMG/*p*-coumaric acid
- IMTBA/DHB: N-isopropyl-N-methyl-*tert*-butylamine/DHB
- DIEA/DHB: N,N-diisopropylethylamine/DHB

[a] All matrices were prepared as shown in 2-2 and 2-3 by using *μFocus MALDI plate*™ to be analyzed by MALDI-QIT-TOFMS.

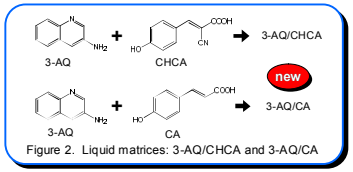


Figure 2. Liquid matrices: 3-AQ/CHCA and 3-AQ/CA

2-3: Mass spectrometry

The analyte solution and the matrix solution were mixed (1:1, v/v) and 1μL of the mixture was applied to a 600μm *μFocus MALDI plate*™ (Hudson Surface Technology, Inc. USA) for analysis by MALDI-QIT-TOFMS (*AXIMA Resonance*™, Shimadzu/Kratos, UK) in positive and negative ion modes.

[2] Kolli,.V.S.K.; Orlando, R. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 923-926.
[3] Mank, M.; Stahl, B.; Boehm, G. *Anal. Chem.* **2004**, *76*, 2938–2950.
[4] Laremore, T. N.; Murugesan, S.; Park, T.-J.; Avci, F. Y.; Zagorevski, D. V.; Linhardt, R. J. *Anal. Chem.* **2006**, *78*, 1774-1779.
[5] Fukuyama, Y.; Nakaya, S.; Yamazaki, Y.; Tanaka, K. *Anal. Chem.* **2008**, *80*, 2171-2179.
[6] Crank, J. A.; Armstrong, D. W. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1790-1800.

3-2: Highly sensitive (novel) liquid matrix

Twenty-three liquid matrices were newly designed based on the result from Table 1 and were evaluated. Results demonstrated that GP1 was detected using 3-AQ/CA with high sensitivity comparable to 3-AQ/CHCA (Table 2).

Table 2. Detection limit of GP1 using 3-AQ/CHCA and 3-AQ/CA[a]

	GP1 (mol/well) positive	GP1 (mol/well) negative
3-AQ/CA	**0.1 f**	**0.1 f**
3-AQ/CHCA	**0.1 f**	**0.1 f**
DHB	10 f	1 f

[a] All matrices was prepared as shown in 2-2 and 2-3 by using *μFocus MALDI plate*™ to be analyzed by MALDI-QIT-TOFMS.

100 amol GP1 was detected using a new liquid matrix 3-AQ/CA

3-3: Highly sensitive MS^n analysis

Highly sensitive MS^n analysis using 3-AQ/CHCA

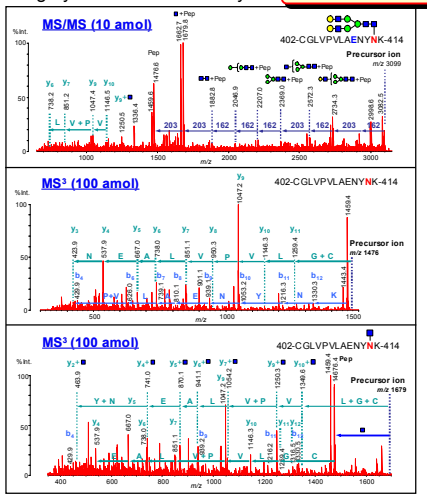


Figure 3. Positive MS^n spectra of 10 amol or 100 amol GP1 using 3-AQ/CHCA with MALDI-QIT-TOFMS.

3-4: Effect of purified CA (*p*CA)

Sensitivity of GP1 using 3-AQ/CA was improved by purification of CA (*p*-coumaric acid) (Table 3, Figure 4).

Table 3. Detection limit of GP1 using 3-AQ/CA and 3-AQ/*p*CA[a]

	GP1 (mol/well) positive	GP1 (mol/well) negative
3-AQ/*p*CA (*purified*)	**(0.01 f)**	**0.01 f**
3-AQ/CA	**0.1 f**	**0.1 f**
DHB	10 f (1 f)	1 f

[a] 3-AQ/CA was prepared as shown in 2-2 and 2-3 by using *μFocus MALDI plate*™ , and DHB was using by *μFocus MALDI plate*™ or SUS plate to be analyzed by MALDI-QIT-TOFMS.

10 amol GP1 was detected using 3-AQ/*p*CA

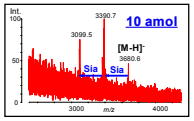
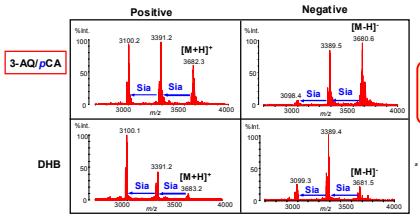


Figure 4. Negative mass spectrum of 10 amol GP1 using 3-AQ/*p*CA with MALDI-QIT-TOFMS.

3-5: Suppression of sialic acids dissociation

Dissociation of GP1's sialic acids was suppressed using 3-AQ/CA and/or 3-AQ/*p*CA compared with DHB and 3-AQ/CHCA (Figure 5).



402-CGLVPVLAENYNK-414

Dissociation of sialic acids was suppressed using 3-AQ/*p*CA

[a] 3-AQ/CA and DHB was prepared as shown in 2-2 and 2-3 by using *μFocus MALDI plate*™ to be analyzed by MALDI-QIT-TOFMS.

Figure 5. Mass spectra of 10 fmol GP1 using DHB and 3-AQ/*p*CA with MALDI-QIT-TOFMS[a].

4: Conclusion

- Gycopeptide: GP1 was detected with high sensitivity (10 -100 amol detection limit) using 3-AQ/CHCA and a novel liquid matrix 3-AQ/CA.
- Highly sensitive MS and MS^n analyses were confirmed using these matrices.
- 3-AQ/pCA (purified 3-AQ/CA) showed highest sensitivity.
- Dissociation of sialic acids was suppressed using 3-AQ/CA (or 3-AQ/*p*CA).

5: Acknowledgement

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).