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Boron released from borosilicate glass forms unusual *in situ* derivatives in MALDI-MS

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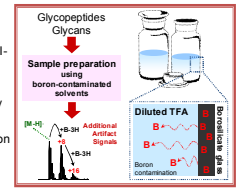
1. Overview

- ✓ Unusual artifact signals of **+8, +16, etc., Da (+8n Da)** were observed in glycopeptide MALDI-MS experiment.
- ✓ These artifact signals were identified as ***in situ* boron modifications**, which were promoted by boron contamination.
- ✓ In our case, the origin of the boron contamination appeared to be the TFA stock solution, which was stored in a **borosilicate glass bottle**.



2. Introduction

Mass spectrometry (MS), combined with a soft ionization technique such as ESI or MALDI, has been widely used for analyzing large biomolecules including glycoproteins. To ensure the reliability of the mass spectral data, artifact signals should be avoided; however, several reagents for preparing samples have been reported to introduce artifact signals by means of unwanted reactions. For example, denaturing and alkylating reagents such as urea[1] and iodoacetamide[2,3] are known to cause unexpected modification of peptides. In the analysis of the *N*-glycans, it has been mentioned before that glycerol,[4] urea,[5] and dithiothreitol[6] can cause unwanted modification on the reducing end of PNGaseF-released *N*-glycans.

Here, we demonstrate that **boron** can form unusual *in situ* derivatives of glycoconjugates such as glycans and glycopeptides in a MALDI-MS experiment. As a result, glycoconjugate signals such as $[M+H]^+$ and $[M-H]^-$ are accompanied by artifact signals of +8, +16, etc., Da (+8n Da), interfering with spectral interpretation and decreasing sensitivity. Boron contamination source is also discussed.

3. Materials and Methods

3-1. Glycopeptide Sample preparation

Tryptic digestion
↓
Desalting using carbon tip (NuTip Carbon)
Binding: H_2O
Elution: 80% ACN, 0.1% TFA
↓
Hydrophilic affinity enrichment[7]
Solid support: cellulose particle
Binding: 80% ACN, 0.1% TFA
Elution: 50% ACN, 0.1% TFA
↓
SpeedVac

3-2. Analytes

- Human serum IgG (SIGMA)
- 2-Aminobenzamide (2AB) -labeled NA2 glycan (Ludger)
- Sialylglycopeptide (SGP) (Tokyo Chemical Industry Co., Ltd.)

3-3. MS

- A 700μm μFocus MALDI plate (Hudson Surface Technology)
- DHB or 3AQ/CHCA matrices
- MALDI-QIT-TOF-MS (AXIMA-Resonance, Shimadzu/Kratos)

Refs. [1] McCarthy, J. et al. *J. Proteome Res.* **2003**, 2, 239–42. [2] Boja, E. S. et al. *Anal. Chem.* **2001**, 73, 3576–82. [3] Lapko, V. N. et al. *JMS* **2000**, 35, 572–5. [4] Trimble, R. B. et al. *J. Biol. Chem.* **1986**, 261, 12000–5. [5] Omtvedt, L. A. et al. *RCM* **2004**, 18, 2357–9. [6] Harvey, D. J. et al. *JMS* **2010**, 45, 815–9. [7] Wada, Y. et al. *Anal. Chem.* **2004**, 76, 6560–5.

4. Results & Discussion

4-1. Appearance of unusual artifact signals

Artifact signals of +8n Da were observed in the mass spectra of glycopeptide samples prepared using boron-contaminated solvents (further details such as the contamination source will be discussed later). An example is depicted in **Fig. 1**.

Compared to the reference mass spectrum (**Fig 1a**), which was prepared using boron-free solvents, the glycopeptide signals in **Fig 1b** were accompanied by **+8 and +16 Da (+8n Da) artifact signals (Fig 1b)**.

In an investigation to eliminate the artifact signals, we found that the artifact signals arise from contaminants in 50% ACN with 0.1% TFA solution, which corresponds to the eluting solution from cellulose in the glycopeptide enrichment process.

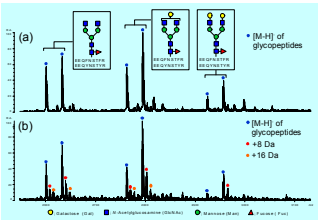


Fig. 1. Negative-ion mass spectra of glycopeptides derived from tryptic digest of IgG. (a) Reference mass spectrum. (b) Mass spectrum containing numerous artifact signals. Only major glycoforms are indicated.

Furthermore, this type of artifact signal was observed in both glycopeptides and *N*-glycan standards. The following simplified experiment was performed to clarify the cause of the artifact signals.

2AB-labeled NA2 glycan
in 200μL of 50% ACN with 0.1% TFA
↓ SpeedVac
↓ Reconstituted in 20μL H_2O
MALDI-MS

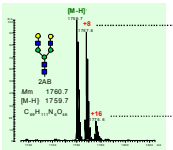


Fig. 2. Negative-ion mass spectra of 2AB-labeled NA2 glycan.

In addition to the deprotonated form $[M-H]^-$ at *m/z* 1759.7, +8 Da (*m/z* 1767.6) and +16 Da (*m/z* 1775.6) signals were clearly observed (**Fig. 2**). In fact, at this point, we could not identify these additional signals.

4-2. Identification of +8n Da signals

A few reports regarding "+8" modification can be found in the existing literature. Gronert et al. reported unique gas-phase derivatization of phosphopeptides.[8,9] With their method, the gas-phase reaction with trimethyl borate (TMB) followed by collisional activation effectively adds 8 Da to the phosphopeptide ion by incorporating a boron atom and losing three hydrogen atoms from the ion.

$$[M+H]^+ + B(OCH_3)_3 \xrightarrow[-2\ CH_3OH]{\text{TMB}\ \text{Ion/molecule Reaction}} [M-H+BOCH_3]^+ \xrightarrow[-CH_3OH]{\text{CID}} [M-2H+B]^+$$

+8 Da (three hydrogen atoms are replaced by one boron atom)

Refs. [8] Gronert, S. et al. *IJMS* **2004**, 231, 179–87. [9] Gronert, S. et al. *JASMS* **2005**, 16, 1905–14.

If the signals of +8n Da observed in our experiments correspond to the boron modification, these signals would have characteristic isotopic distributions because a boron atom has a unique isotope pattern.

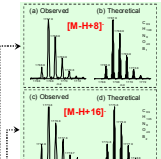


Fig. 3. Observed and theoretical isotopic distributions of (a,b) $[M-H+8]^-$ and (c,d) $[M-H+16]^-$.

As seen in **Fig. 3**, the +8 and +16 signals have characteristic isotope distributions, which agree with the theoretical distributions calculated from the assumption that one boron atom is added thereby losing three hydrogen atoms per 8 Da increment.

To study the incorporation of boron into our analytes, boron-containing compounds were intentionally added to the sample. Doping samples with boric acid increased the +8n Da signals (**Figs. 4a-c**). Using isotope-enriched boric acid, $^{11}B(OH)_3$, caused the signal corresponding to the ^{10}B isotope at *m/z* 1766.7 to disappear (**Fig. 4d**).

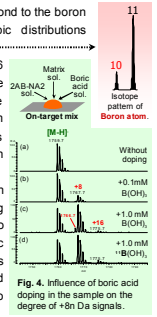


Fig. 4. Influence of boric acid doping in the sample on the degree of +8n Da signals.

+8n Da signals can be attributed to the incorporation of boron atoms.

4-3. Localization of boron modification by MS/MS

- Comparison of MS/MS spectra of a model glycopeptide (SGP); **$[M+H]^+$ vs. $[M+H+8]^+$**

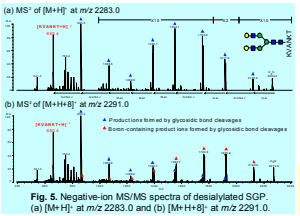


Fig. 5. Negative-ion MS/MS spectra of desialylated SGP. (a) $[M+H]^-$ at *m/z* 2283.0 and (b) $[M+H+8]^-$ at *m/z* 2291.0.

MS/MS of $[M+H+8]^+$ revealed successive losses of glycan residues. However, the intensity of the boron-containing product ions decreased with increasing glycan neutral loss (**Fig. 5b**). The signal of peptide moiety was detected at the same *m/z* value as the MS/MS of $[M+H]^+$ (*m/z* 660.4). These results indicate that **boron modification can occur anywhere in the glycan moiety**, but not in the peptide moiety.

4-4. Where does the boron come from ?

In general, no boron compounds are involved in standard preparation methods for glycopeptide samples. The origin of the boron contamination appeared to be the TFA stock solution, which was stored in a borosilicate glass bottle.

Due to its thermal stability and hardness, borosilicate glass has been adopted for laboratory glassware. In general, borosilicate glass is recognized to be chemically inert, but in fact several solutions can corrode borosilicate glass, resulting in contamination of its contents. In particular, the release of boron from borosilicate glass has been noted by many researchers as a source of contamination.[10-13]

Refs. [10] Selman, I. W. et al. *Nature* **1954**, 173, 957-8. [11] Porter, R. P. *J. Phys. Chem.* **1957**, *61*, 1260. [12] Green, G. H. et al. *J. Agric. Food Chem.* **1976**, *24*, 1245-6. [13] Kozono, S. et al. *Anal. Chim. Acta* **1998**, 368, 275–280.

We performed the simple experiment described below to demonstrate boron contamination of a TFA stock solution stored in a glass bottle.

1. 200μL of 0.1% TFA solution was prepared from various TFA stock solutions listed in **Table 1**, and the solvent was removed in vacuo. The residual impurity was then reconstituted in water (20μL).

Bottle	Material	Bottle's Color	TFA concentration	Aging
A	borosilicate glass	colorless	10%	0 day (fresh)
B	borosilicate glass	dark brown	10%	0 day (fresh)
C	PFA	milky white	10%	0 day (fresh)
D	borosilicate glass	colorless	10%	10 days
E	borosilicate glass	dark brown	10%	10 months
F	borosilicate glass	dark brown	1%	2 years
G	PFA	milky white	10%	1 month

PFA: perfluoroalkoxy copolymer resin

Table 1. Storage conditions of aqueous TFA stock solutions.

200μL 0.1%TFA
↓ SpeedVac
↓ Reconstitute in 20μL H_2O
Residual impurities from bottles A, B, C, …. G.

2. The reconstituted residual impurity was mixed with 2AB-labeled NA2 glycan and matrix solutions on the MALDI plate. After drying, MALDI mass spectra were acquired in negative-ion mode. The result is depicted in **Fig. 6**.

Residual impurity from …
- freshly prepared TFA solution in glass or PFA bottles
  ⟶ did not promote +8n Da signals
- TFA solutions "aged" in glass bottle
  ⟶ extensively promoted +8n Da signals
- TFA solutions "aged" in PFA bottle
  ⟶ did not promote +8n Da signals

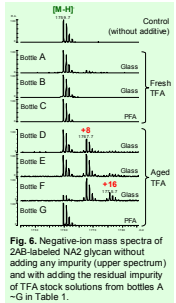


Fig. 6. Negative-ion mass spectra of 2AB-labeled NA2 glycan without adding any impurity (upper spectrum) and with adding the residual impurity of TFA stock solutions from bottles A ~G in Table 1.

These results clearly show that the initial TFA in ampules are essentially boron-free, but that dilution followed by aging in borosilicate glass bottle causes boron contamination.

5. Conclusion

We demonstrate that **boron** can form unusual *in situ* derivatives of glycoconjugates such as glycans and glycopeptides in a MALDI-MS experiment. As a result, glycoconjugate signals are accompanied by artifact signals of +8n Da, interfering with spectral interpretation. The boron contamination source appeared to be TFA aqueous solutions used for preparing glycopeptide samples, which were stored in borosilicate glassware.

It is clearly preferable to have no contact with borosilicate glass during all experiment procedures, but this is not practical. Boron can probably be released from borosilicate glassware by any solvent to a greater or lesser extent. To minimize the risk of boron contamination, we suggest using PFA bottles for storage of solvents as often as possible.

6. Acknowledgments

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