Fragmentation Behavior and Stability of Sulfated Peptides in Positive Ion Mode

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Overview

The goal is to study sequence effects of model sulfopeptides to identify tyrosine sulfation by mass spectrometry in positive ion mode without the use of adduct ions. Here, we examined the influence of basic amino acid residues on sulfopeptide stability. We compared several tandem mass spectrometry (MS/MS) activation methods in positive ion mode. Electron transfer dissociation (ETD), electron capture dissociation (ECD) and free racial initiated peptide sequencing (FRIPS) all retained sulfate in a charge state-dependent manner in the presence of arginine.

Introduction

Importance of tyrosine sulfation:

- Roles in inflammation, hemostasis, immunity, and protein-protein interactions.

Challenges in tyrosine sulfation identification:

- Predominant SO_3 loss during ionization and MS/MS fragmentation in positive ion mode.
- Negative ion mode and alternative MS/MS activation methods allow improved sulfate retention [1, 2]; however, such techniques have low efficiency and bioinformatics tools are optimized for positive ion mode [3].

Ion-ion interaction to form stable complex:

- Our group previously showed that metal or alkylamine adducts can stabilize sulfate groups, in positive ion mode.
- Recent work also showed that guanidinium adducts afford higher sulfopeptide stability [4].

Sequence effect in sulfation identification:

- We recently found that tryptic sulfopeptides appear more stable than standard, acidic sulfopeptides in positive ion mode [5]. However, as expected, complete sulfonate loss was observed upon higher energy collision induced dissociation (HCD) (Fig. 1.).

Here, we begin to systematically examine sequence effects on sulfate stability in positive ion mode and explore alternative MS/MS activation methods for sulfate retention.

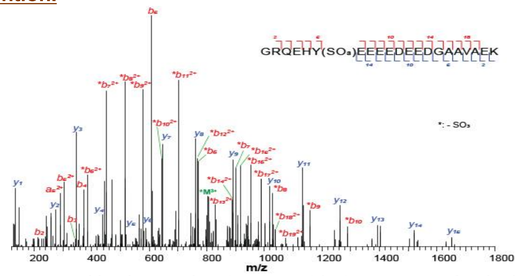

Figure 1. Positive ion mode LC-HCD MS/MS spectrum of a doubly protonated tryptic peptide from rat Golgi membrane proteins. Complete neutral sulfonate (SO_3) loss was observed upon HCD [5].

Methods

- SolariX FT-ICR and Orbitrap Fusion Lumos instruments were used for MS^2 and MS^3 experiments.
- Acidic, native sulfated peptides and synthetic sulfopeptides (from Genscript) were at 1 µM in methanol:water (50:50, v/v) with 0.1% formic acid for positive ion mode mass spectrometry analysis.
- Sulfopeptides were analyzed by four MS/MS techniques in positive ion mode: collision induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), and free radical initiated peptide sequencing (FRIPS).
- For FRIPS experiments, sulfopeptides were conjugated with *o*-TEMPO-Bz-NHS tag in DMSO and incubated overnight at room temperature. DMSO was removed with a C18 cartridge.

Results

Positive ion Mode Stability of Sulfated Peptides

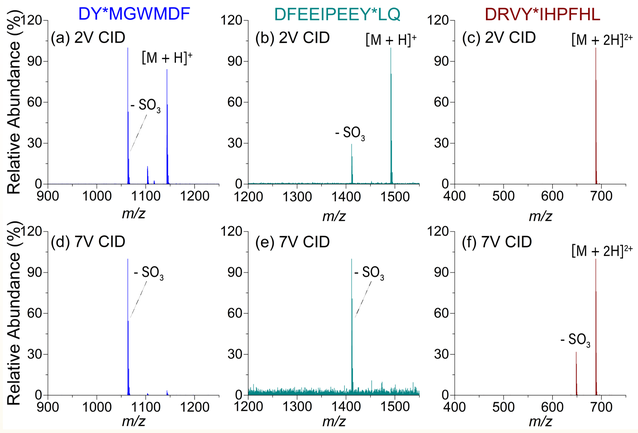

Figure 2. Positive ion mode mass spectra of sulfated peptides: singly charged CCKS 26-33 (a, d), singly charged hirudin 54-65 (b, e), and doubly charged synthetic sulfoangiotensin I (c, f) at two collision voltages. Asterisks (*) denote sulfated tyrosines. At 2 V collision energy peptide fragmentation is typically not observed; however, the native, acidic sulfopeptides showed significant sulfonate (SO_3) loss (a, b). By contrast, the more basic sulfoangiotensin I peptide did not dissociate at this low voltage (c). At 7 V, the native sulfopeptides were completely desulfated (d, e) and the synthetic, more basic sulfopeptide began to undergo sulfonate loss (f).

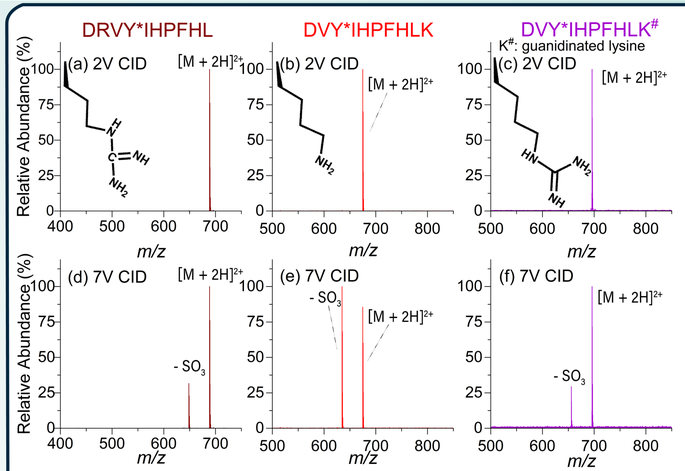

Figure 3. Positive ion mode mass spectra of doubly charged sulfoangiotensin I (a, d), modified (lysine-containing, tryptic-like) sulfoangiotensin I (b, e), and guanidinated modified sulfoangiotensin I (c, f). Asterisks (*) denote sulfation, and pound (#) denotes guanidination. At 2 V collision energy, all three basic residue-containing peptides showed sulfate retention (a-c). At 7 V, the arginine (d) and guanidinium (f) containing peptides showed similar stability (~30% SO_3 loss) whereas the lysine-containing peptide showed a higher degree of SO_3 loss (e). However, the presence of lysine appears to impart higher stability compared with the absence of basic sites (Fig. 2d, e).

ECD of Sulfated Angiotensin I

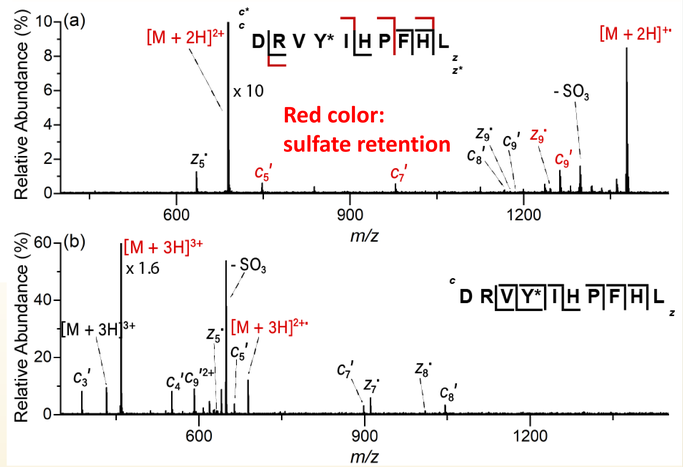

Figure 4. ECD spectra of doubly- (a) and triply- (b) protonated sulfoangiotensin I. Interestingly, in contrast to previous work on native, acidic sulfopeptides [6], significant sulfate retention was observed for the 2+ precursor ion. Fragment ions retaining sulfation are highlighted in red. By contrast, sulfate retention was not observed for the 3+ precursor ion.

ETD and FRIPS of Sulfated Angiotensin I

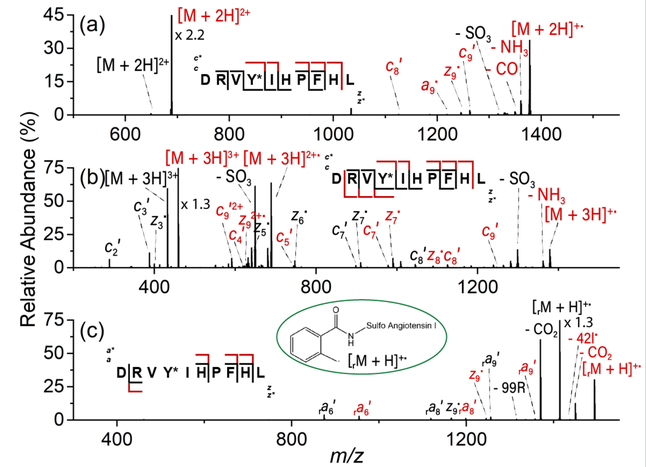

Figure 5. ETD of doubly- (a) and triply- (b) protonated sulfoangiotensin I as well as FRIPS MS^3 of the singly-protonated peptide (c). In contrast to ECD (Fig. 4), sulfated fragment ions were observed for both the 2+ and 3+ charge state in ETD. Interestingly, in contrast to previous work with native, acidic sulfopeptides [6], sulfate retention was also seen for the 1+ charge state in FRIPS. Lower case 'r' denotes truncated FRIPS tag [7]. Fragment ions retaining sulfation are highlighted in red.

Conclusions

- Sulfopeptides containing arginine or guanidinated lysine show significantly higher stability in positive ion mode compared with acidic, native sulfopeptides. The presence of lysine also improves stability to a lesser extent than arginine/guanidinium. This higher stability may be due to salt bridge interactions with a deprotonated sulfate.
- Fragment ions retaining sulfation were observed for an arginine-containing sulfopeptide in ETD (3+ and 2+ charge state), ECD (2+ charge state), and FRIPS (1+ charge state), consistent with the increasing levels of energy input in these three MS/MS techniques.
- Among these fragmentation methods, ETD appears most suitable to identify tyrosine sulfation in tryptic peptides.

References & Acknowledgement

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