Direct determination of tyrosine sulfation sites in proteomic analysis

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Introduction

- Tyrosine sulfation is an understudied posttranslational modification (PTM) of crucial biological significance.
- Conventional liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have limited ability to directly identify sulfation sites due to the extreme sulfate lability and low sulfopeptide ionization efficiency in positive mode.
- Sulfated and phosphorylated tyrosines can be misassigned due to their isobaric nature; however, high-resolution mass spectrometers can differentiate between these two PTMs at the MS1 level. Furthermore, PTM-containing fragments are observed for phosphopeptides but not sulfopeptides in collision-induced dissociation (CID)/beam-type CID (HCD).
- Complete sulfonate elimination can also occur upon electron capture/transfer dissociation (ECD/ETD) depending on sulfopeptide sequence/basicity and charge state.
- Here, we report detection of sulfate-containing fragments from bottom-up LC-ECD MS/MS, thus allowing direct determination of tyrosine sulfation sites.

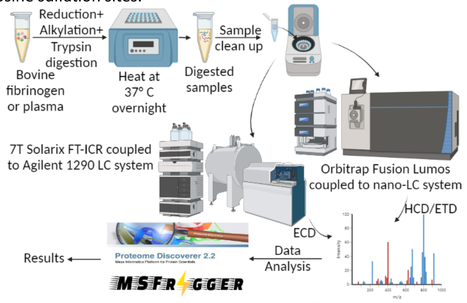

Figure 1. Bovine fibrinogen and plasma samples were reduced with dithiothreitol, alkylated with iodoacetamide and digested with trypsin. Tryptic peptides were then cleaned up with C18 spin columns before LC-MS/MS analysis. The resulting mass spectra were analyzed with Proteome discoverer 2.2. and MSFragger 3.7.

Materials and Methods

- Bovine fibrinogen and bovine plasma (Sigma-Aldrich) were digested overnight with sequencing grade modified trypsin (Promega) at 1:50 enzyme/protein ratio and 37 °C.
- Nanoflow LC (nLC)-HCD MS/MS was performed with an Orbitrap Fusion Lumos using a C18 75 µm x 50 cm Acclaim PepMap™ 100 column. The autosampler, column, and source gas were set to 4, 46, and 285 °C, respectively. The HCD collision energy was 32 %.
- ETD was triggered upon neutral loss of either 79.9663 (phosphate) or 79.9568 (sulfate).
- Sulfopeptide candidates from HCD data were added to an inclusion list or time-segmented method for LC-ECD MS/MS with an Agilent 1290 HPLC coupled to a 7 Tesla Solarix Fourier transform ion cyclotron resonance (FT-ICR) instrument (Bruker) via an Agilent InfinityLab Poroshell 120 EC-C18 column. The autosampler, column, and source gas temperatures were set to 6, 40, and 250 °C, respectively. The ECD heater was at 1.5 A.
- ETD with targeted inclusion list was also performed with supplemental HCD (EThcD) at collision energy 15 %.

Results

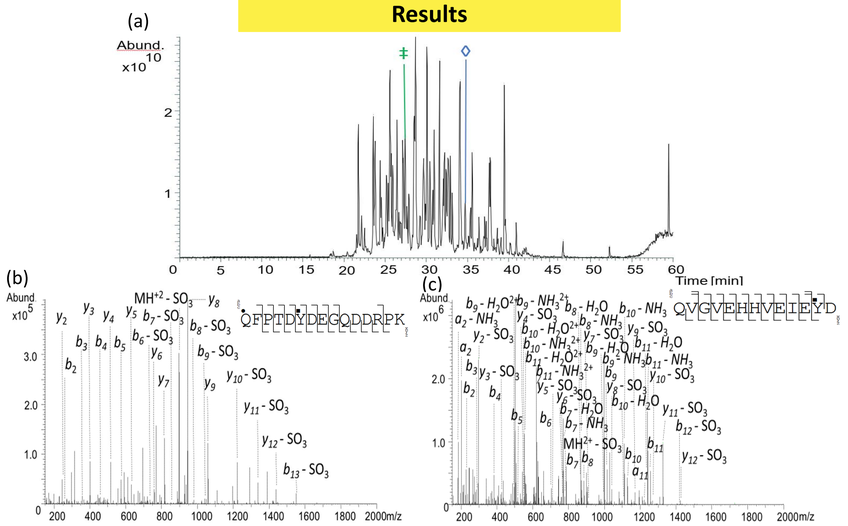

Figure 2. nLC-HCD MS/MS analysis of tryptically digested bovine fibrinogen on an Orbitrap Fusion Lumos resulted in detection of the known sulfopeptide QFPTD*YDEGQDDRPK (*Y=sulfotyrosine) from the N-terminus of the beta chain (residues 1-15) [1], eluting at 34.6 min (blue diamond)-(a). The HCD spectrum of the doubly charged peptide showed no sulfated fragments-(b). Proteome Discoverer also identified the doubly charged peptide QVGVEHHVEIEYD from the C-terminus of fibrinogen gamma-B chain (residues 432-444) as sulfated or phosphorylated, eluting at 27.3 min (green double dagger)-(a). Therefore, manual spectral interpretation was required. No PTM-containing fragments were observed in the HCD spectrum-(c), supporting a sulfopeptide assignment. With the goal of generating sulfate-containing fragment ions, an alternative nLC method involving loss-triggered ETD was used. However, neither sulfopeptide was detected.

Results cont.

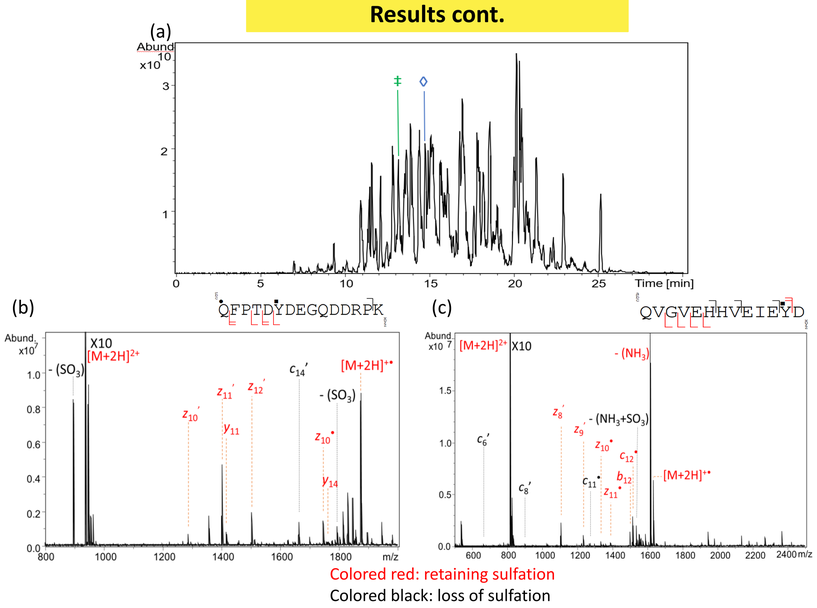

Figure 3. LC-ECD MS/MS on a SolariX FT-ICR instrument with the two sulfopeptides (Fig. 2) added to the preferred list for fragmentation. This approach allowed these relatively low abundance peptides to be selected for ECD. Gamma-B and beta chain tryptic peptides eluted at 13.2 (green double dagger) and 14.8 min (blue diamond), respectively-(a). Both intact and desulfated precursor ions were observed, however, sulfate-retaining fragment ions were detected-(b,c).

Next, we used the open database search tool MSFragger to search the nLC-HCD MS/MS data from the tryptically digested fibrinogen and plasma samples acquired on the Orbitrap Fusion Lumos. MSFragger has newly implemented features referred to as "labile mode" search [2]. By using labile mode searches, it is possible to filter spectra for diagnostic ions that are characteristic of a specific labile modification, allowing for more accurate identification of modified peptides. Labile mode can also incorporate information about the remainder masses of modified peptide fragments, improving the accuracy of spectral identification and modification localization. We ran the already available workflow template for labile phosphorylation and also customized it to search for sulfation. MSFragger reports identified peptide sequences, assigned modifications and the mass shift from the base peptide sequence. This search resulted in additional sulfopeptide candidates.

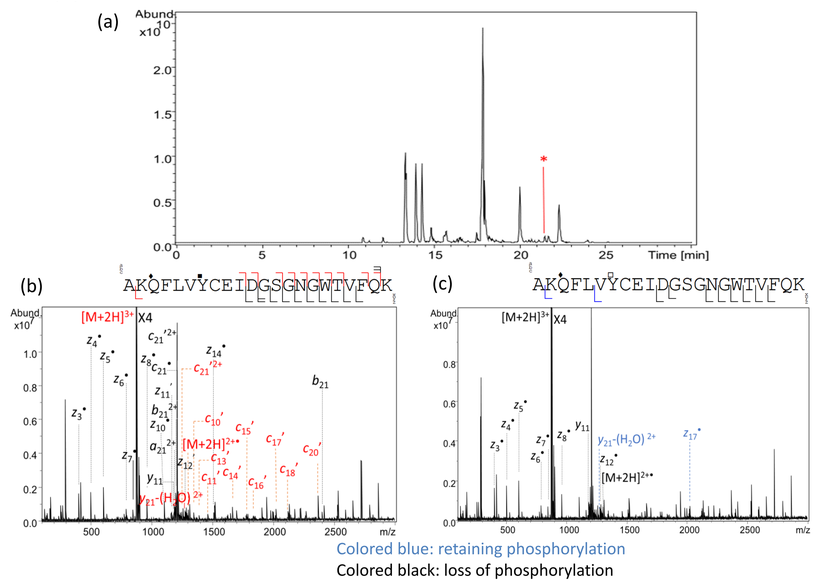

Figure 4. LC-ECD MS/MS from a time segmented method with quadrupole isolation of a targeted peptide precursor m/z value. This peptide, AKQFLVYCEIDGSGNGWTVFQK from fibrinogen gamma-B chain (residues 198-219) eluting at 21.4 min (red asterisk)-(a), was identified by MSFragger as sulfated or phosphorylated with an additional precursor mass shift of +1 Da, suggesting deamidation. The corresponding ECD spectrum was annotated as either a sulfated (b) or phosphorylated (c) peptide with a fragment ion m/z error below 10 ppm. Significantly higher sequence coverage was obtained for tyrosine sulfation (b) including several sulfate-retaining fragment ions. The ECD spectrum also localized deamidation to Gln3.

Results cont.

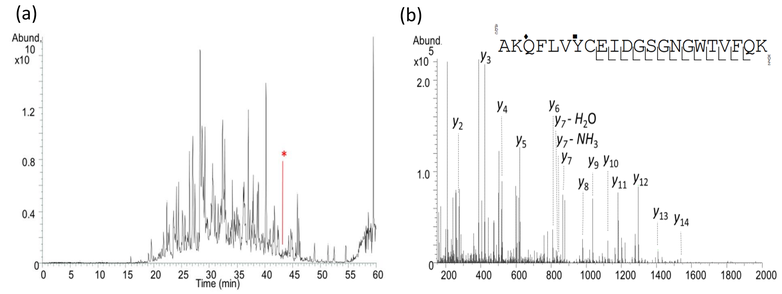

Figure 5. The MSFragger-identified sulfopeptide in Fig. 4 was also observed in nLC-HCD MS/MS analysis of tryptically digested plasma, eluting at 43.3 min (red asterisk)-(a). The corresponding HCD spectrum (b) showed confidently matched *y*-type ions, including fragments confirming deamidation at Gln3; however, no *b*-type fragments were observed. The lack of N-terminal fragments may be due to the lysine residue on the N-terminal side of the deamidated glutamine, analogous to the facile cyclization of Gln/Glu at peptide N-termini to form an imide, which has lower proton affinity. Such peptide sequences dissociate at lower energy following charge migration away from the N-terminus. [3] Consistently, complete precursor ion depletion was observed in our experiment for this peptide.

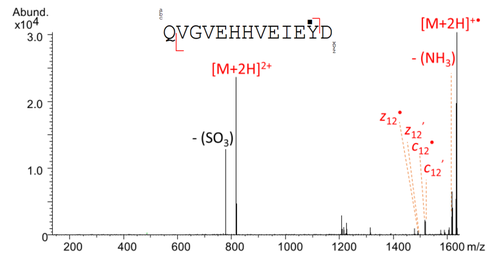

Figure 6. nLC-EThcD MS/MS analysis of the digested fibrinogen sample with a target peptide inclusion list resulted in detection of the doubly charged peptide QVGVEHHVEIE*YD. Sulfate-retaining fragments were detected, however, poor fragmentation and sequence coverage were observed.

Conclusions and Future Directions

- While higher sequence coverage is desired, ECD provided direct evidence of sulfation at tyrosine 443 of the fibrinogen gamma-B chain. This PTM is not annotated in UniProt; however, Tyr 443 sulfation has been previously reported from indirect evidence [4].
- MSFragger is a powerful search tool for mining labile modifications.
- Further work for enhancing ETD efficiency, including implementation of supercharging agents or N-terminal guanidination will be needed.
- Due to low sulfation levels, efficient sulfopeptide enrichment would enhance sulfo-Tyr detection, particularly in complex matrices such as plasma and cell lysates.

Acknowledgments

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References

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