


Localization of labile tyrosine sulfation using an alternative electron-based MS/MS approach in positive and negative ion modes

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ABSTRACT

This poster highlights the unique ability of electron-activated dissociation (EAD) to achieve accurate localization of labile tyrosine sulfation in both positive and negative ion modes. This was the first application of EAD in the negative ion mode for the characterization of a protein modification on a commercial instrument. The result showcases negative EAD as a powerful tool for accurate localization of tyrosine sulfation, which is a labile biopharma modality that is challenging to analyze in the positive ion mode.

INTRODUCTION

Tyrosine sulfation can affect antigen binding and the biological activity of monoclonal antibodies (mAbs).[1] A recent study revealed that tyrosine sulfation proteoforms affected the potency of a potential drug candidate for HIV-1 prevention, indicating that this modification is a potential critical quality attribute (CQA).[2] The labile nature of tyrosine sulfation poses an analytical challenge to collision-based MS/MS approaches and low-energy electron capture dissociation (ECD), which leads to the cleavage of the sulfate group in positive mode.[3] This study applied an alternative EAD approach with tunable electron kinetic energy (KE) to tyrosine sulfated peptides in both positive and negative ion modes. The data were compared to assess the capability of each approach for the localization of tyrosine sulfation.

MATERIALS AND METHODS

Sample:
The powders of sulfated leucine enkephalin (LES) and sulfated cholecystokinin fragment 26–33 amide (CLKS) (Sigma-Aldrich) were dissolved in water and then diluted to a 10µM solution. A 2 µL sample of each solution was injected for LC-MS analysis using EAD.

HPLC:
The peptides were chromatographically separated with the LC gradients described previously.[4,5] The separation was achieved using a Waters ACQUITY CSH C18 column (2.1 × 150 mm, 1.7 µm, 130 Å) at a flow rate of 0.25 mL/min. The column was kept at 60°C in the column oven of an ExionLC AD system from SCIEX. The mobile phases A and B were 0.1% formic acid (FA) in water and 0.1% FA in acetonitrile, respectively.

MS/MS:
LC-MS data were acquired in SCIEX OS software using the ZenoTOF 7600 system from SCIEX (Figure 1). The singly and doubly protonated or deprotonated species of LES and CLKS were targeted for positive or negative EAD in MRM^{HR} experiments, in which electron KE was varied between 0 eV and 25 eV. The MRM^{HR} data were analyzed using the Explorer module within SCIEX OS software.


ZenoTOF 7600 system

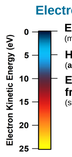
EAD cell

Peptide type	EAD (7 eV)	EAD (>10 eV)
Common, multiply charged	+++	
Long (>5 kDa)	+++	
Singly charged	+	+++
Amino acid isomers	+++	
N- or O-glycosylated	+++	
Glycated	+++	
Disulfide linked	+++	
Sulfated	+	+++

Figure 1. The ZenoTOF 7600 system equipped with an EAD cell offers powerful capabilities for biopharma characterization. The EAD platform method using an electron KE of 7 eV provides an excellent fragmentation of a wide range of peptides. The tunability of electron KE further enables an in-depth characterization of challenging molecules, such as singly charged peptides and sulfated peptides.

RESULTS

Electron activated dissociation (EAD)



ECD – Electron capture dissociation
(multiply charged peptides and proteins)

Hot ECD – Hot electron capture dissociation
(**amino acid isomers**, glycopeptides, disulfide-bonded peptides, etc.)

EIEIO – Electron impact excitation of ions from organics
(singly charged molecules)

Figure 2. EAD offers 3 fragmentation mechanisms in 1 technique. EAD enables the access to 3 different fragmentation mechanisms, including the classic low-energy ECD (KE of 0-5 eV), hot ECD (KE of 5-10 eV) and EIEIO (KE of >10 eV). EIEIO mechanism was leveraged in this study for the characterization of sulfated peptides in positive and negative ion modes.

Positive EAD

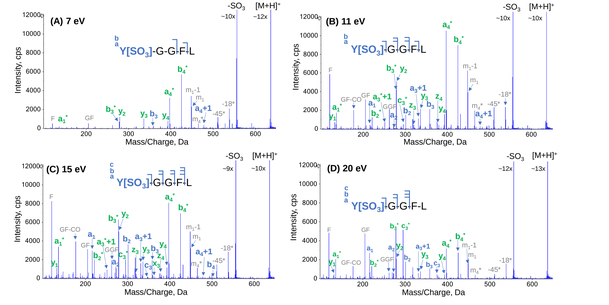


Figure 3. Positive EAD spectra of singly charged LES at various electron KEs. An electron KE of 7 eV generated a limited number of sequence ions containing the sulfate group (A). By contrast, higher electron KEs produced a series of sulfate-containing a/b ions for confident localization of this labile modification (B–D), despite the increasing level of internal fragmentation. The fragments labeled with * do not contain the sulfate group. The peaks with gray labels correspond to the products from internal fragmentation or side chain losses. Only sulfate-containing sequence ions were displayed in the bond cleavage scheme.

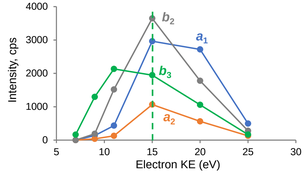


Figure 4. Effect of electron KE on the signal intensity of selected sulfate-containing a/b fragments from positive EAD of LES (1+). The optimal electron KE for generating these sulfate-containing fragments was 15 eV in the positive ion mode.

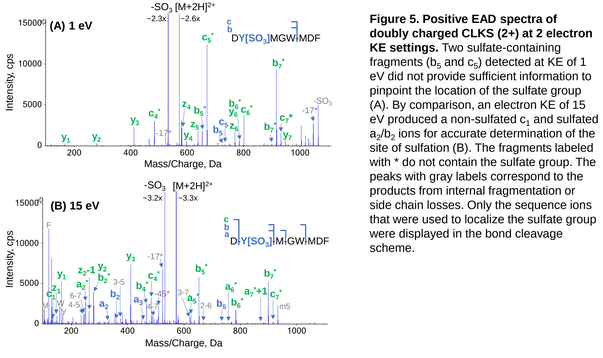


Figure 5. Positive EAD spectra of doubly charged CLKS (2+) at 2 electron KE settings. Two sulfate-containing fragments (b_5 and c_5) detected at KE of 1 eV did not provide sufficient information to pinpoint the location of the sulfate group (A). By comparison, an electron KE of 15 eV produced a non-sulfated c_1 and sulfated a_2/b_2 ions for accurate determination of the site of sulfation (B). The fragments labeled with * do not contain the sulfate group. The peaks with gray labels correspond to the products from internal fragmentation or side chain losses. Only the sequence ions that were used to localize the sulfate group were displayed in the bond cleavage scheme.

Negative EAD

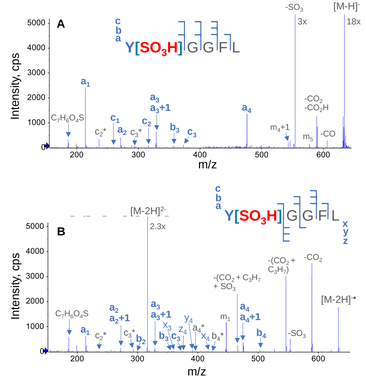


Figure 6. Negative EAD (24 eV) MS/MS spectra of LES. The EAD spectra of the 1-(A) and 2- (B) species of LES were acquired using an electron KE of 24 eV. Negative EAD resulted in a complete series of a-type ions and several b/c fragments containing the sulfate group, allowing confident determination of the sulfation site. The peak assigned as $C_3H_6O_4S$ corresponds to the sulfated side chain of the tyrosine residue (same in Figure 7). The m_n (n=1-6) ions correspond to the neutral-loss peaks of the charged reduced species in which the side chain of the amino acid residue in position *n* (from the N-terminus) was cleaved (same in Figure 7). The sequence fragments labeled with * do not contain the sulfate group (same in Figure 7).

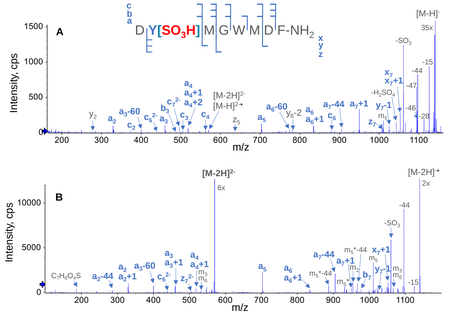


Figure 7. Negative EAD (24 eV) MS/MS spectra of CLKS. Negative EAD of 1- (A) and 2- (B) of CLKS using an electron KE of 24 eV led to a nearly complete series of a-type ions and other sequence ions containing the sulfate group, allowing confident determination of the sulfation site. The neutral losses of 44 and 60 from a-type ions correspond to cleavages of CO_2 and C_2H_4S from the aspartic acid and methionine residues, respectively.

CONCLUSIONS

- Accurate localization of labile tyrosine sulfation on a singly and doubly charged peptide was achieved using positive EAD with an optimal KE of 15 eV. However, positive EAD spectra of sulfated peptides are complex and difficult to interpret.
- In contrast with positive EAD, the loss of sulfation from the sequence fragments was absent or occurred at a low rate in negative EAD.
- The low complexity of the negative EAD spectra facilitated data interpretation and peak assignment
- Negative EAD provided extra information about peptide sequence by generating neutral-loss peaks diagnostic for specific amino acid residues.

REFERENCES

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TRADEMARKS/LICENSING