Accurate localization of labile tyrosine sulfation in peptides using electron activation dissociation (EAD)

Featuring the ZenoTOF 7600 system from SCIEX

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This technical note highlights the unique ability to optimize electron kinetic energy (KE) to achieve accurate localization of labile tyrosine sulfation in low-charged peptides using EAD.

Tyrosine sulfation is an important post-translational modification (PTM) responsible for strengthening protein-protein interactions. Although not extremely common in biotherapeutic proteins, its presence in monoclonal antibodies (mAbs) has been reported previously[1] and can affect target binding. Tyrosine sulfation is very labile, making the localization of this PTM in a peptide sequence challenging for traditional MS/MS techniques, such as collision-induced dissociation (CID) and low-energy electron capture dissociation (ECD).[2] Recently, it was demonstrated that an EAD platform method is a powerful tool for confident peptide mapping, differentiation of isomers and accurate localization of glycosylation in a single injection using hot ECD.[3–6]

In this technical note, the electron KE of EAD was fine-tuned to generate sulfate-containing fragments from 2 sulfated peptides, thereby allowing accurate localization of this labile PTM in the peptide sequence (see EIEIO in Figure 1).

Key features of EAD with tunable electron KE

- **Characterization of a wide range of peptides**: The ability to fine-tune electron KE enables characterization of various peptides including singly charged[6] and sulfated peptides that are challenging for traditional ExD approaches
- **Ease-of-use**: Optimization of electron KE can be easily performed in a data-dependent acquisition or a targeted method
- **Reproducible and highly sensitive**: EAD provides consistent fragmentation patterns and high MS/MS sensitivity, enhanced by the Zeno trap
- **Optimized single injection method**: One EAD method provides best-in-class single injection analysis for biopharmaceutical peptide mapping

ZenoTOF 7600 system EAD cell

Peptide type	ECD (1 eV)	Hot ECD (7 eV)	EIEIO (>9 eV)
Common, multiply charged	+++	+++	+
Singly charged	-	++	+++
Amino acid isomers	-	+++	++
Glycosylated	+++	+++	+
Sulfated, low charge	-	+	+++

Figure 1. In-depth characterization of peptides using EAD. The table explains the best KE for different peptide analyses. A platform EAD method[4–6] using an electron KE=7 eV via hot electron capture dissociation (ECD) within the ZenoTOF 7600 system (left) is the best option for characterization of a wide range of peptides in a single injection. The low-energy EAD (KE=1 eV) via ECD mechanism is suitable for the characterization of common peptides and glycopeptides, while EAD with KE>9 eV via electron-impact excitation of ions from organics (EIEIO) is particularly powerful for the analysis of singly charged species and labile sulfated peptides present in low charge states.

Methods

Sample preparation: Sulfated leucine enkephalin (LES) and cholecystokinin fragment 26–33 (CLKS) were purchased from Sigma Aldrich with the following sequences:

LES: Tyr (SO_3H)-Gly-Gly-Phe-Leu-OH
CLKS: Asp-Tyr (SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

Chromatography: Peptides were separated using an ACQUITY CSH C18 column (2.1 x 150 mm, 1.7 µm, 130 Å, Waters), which was kept at 60°C in the column oven of an ExionLC system (SCIEX). Table 1 shows the LC gradient used for peptide separation at a flow rate of 0.25 mL/min with mobile phases A and B consisting of 1% formic acid (FA) in water and 0.1% FA in acetonitrile, respectively.

Table 1. LC gradients for separation of sulfated peptides.

Time [min]	A [%]	B [%]
Initial	*95*	*5*
2	*95*	*5*
3	*80*	*20*
21	*40*	*60*
22	*10*	*90*
25	*10*	*90*
26	*95*	*5*
30	*95*	*5*

Mass spectrometry: MS data were acquired using a ZenoTOF 7600 system. The charge state 1+ for LES and 2+ for CLKS were targeted for EAD in MRM^{HR} experiments in which electron KE was varied between 1 eV and 25 eV. The key MS parameters are listed in Tables 2 and 3.

Table 2. TOF MS parameters.

Parameter	Values
Ion source gas 1	*40 psi*
Ion source gas 2	*40 psi*
Curtain gas	*35 psi*
CAD gas	*7*
Source temperature	*400°C*
Spray voltage	*5500 V*
TOF start mass	*200 m/z*
TOF stop mass	*2000 m/z*
Accumulation time	*0.1 s*
Declustering potential	*20 V*
Collision energy	*10 V*

Table 3. EAD MRM^{HR} parameters.

Parameter	Values
TOF start mass	*100 m/z*
TOF stop mass	** m/z*
Accumulation time	*0.1 s*
Q1 resolution	*Unit*
Zeno trap	*ON*
Electron beam current	*5000 nA*
ETC	*100%*
Electron KE	*1 to 20 eV*

** Stop mass were 1000 m/z for LES and 1500 m/z for CLKS.*

Data processing: The EAD MRM^{HR} data were analyzed using the Explorer module and the Bio Tool Kit within SCIEX OS software.

Optimization of electron KE for EAD

The power of a generic EAD platform method using an electron KE of 7 eV for in-depth peptide characterization was highlighted previously.[3–6] In this technical note, the unique capability of fine-tuning electron KE to achieve optimal EAD data for 2 sulfate-containing peptides will be demonstrated. The result showcases the versatility of EAD with tunable electron KE for characterization of challenging peptides in unique cases.

The electron KE, one of the key parameters for EAD, can be adjusted in an information-dependent acquisition (IDA) or a MRM^{HR} method. Depending on the electron KE applied, the EAD mechanism may be altered, from the classic low-energy ExD to hot ECD or EIEIO.[3] The effect of electron KE on peptide fragmentation, differentiation of isomers and localization of glycopeptides has been described in a previous technical note.[6] In this work, the singly charged LES- and doubly charged CLKS-containing labile tyrosine sulfation were targeted in EAD MRM^{HR} experiments in which electron KE was varied from 1 eV to 20 eV.

Localization of tyrosine sulfation in LES

The EAD spectra of the singly charged LES acquired at electron KEs < 7 eV contained very few fragments (data not shown). These results were expected as fragmentation of singly charged species with EAD requires hot electrons with high KE, such as in EIEIO.[3] Figure 2 displays the EAD spectra of LES at electron KEs of 7-20 eV. All these spectra contain a series of abundant *a*/*b* ions without the sulfate group (see peaks labeled with * in Figure 2), indicating the labile nature of tyrosine sulfation. EAD of LES at KE=7 eV generated 2 low-abundant sulfate-containing *a*/*b* ions (b_3 and a_4+1, see Figure 2A), which were insufficient to accurately localize the sulfate group. Increasing the electron KE to 11 eV or higher (Figure 2B–2D) resulted in the detection of a nearly complete series of sulfate-containing *a*/*b* ions, thereby allowing unambiguous localization of the sulfate group. The effect of the electron KE on the formation of 4 sulfate-containing fragments (a_1, a_2, b_2 and b_3) is summarized in Figure 3. An electron KE of 15 eV resulted in the overall strongest signal for these fragments.

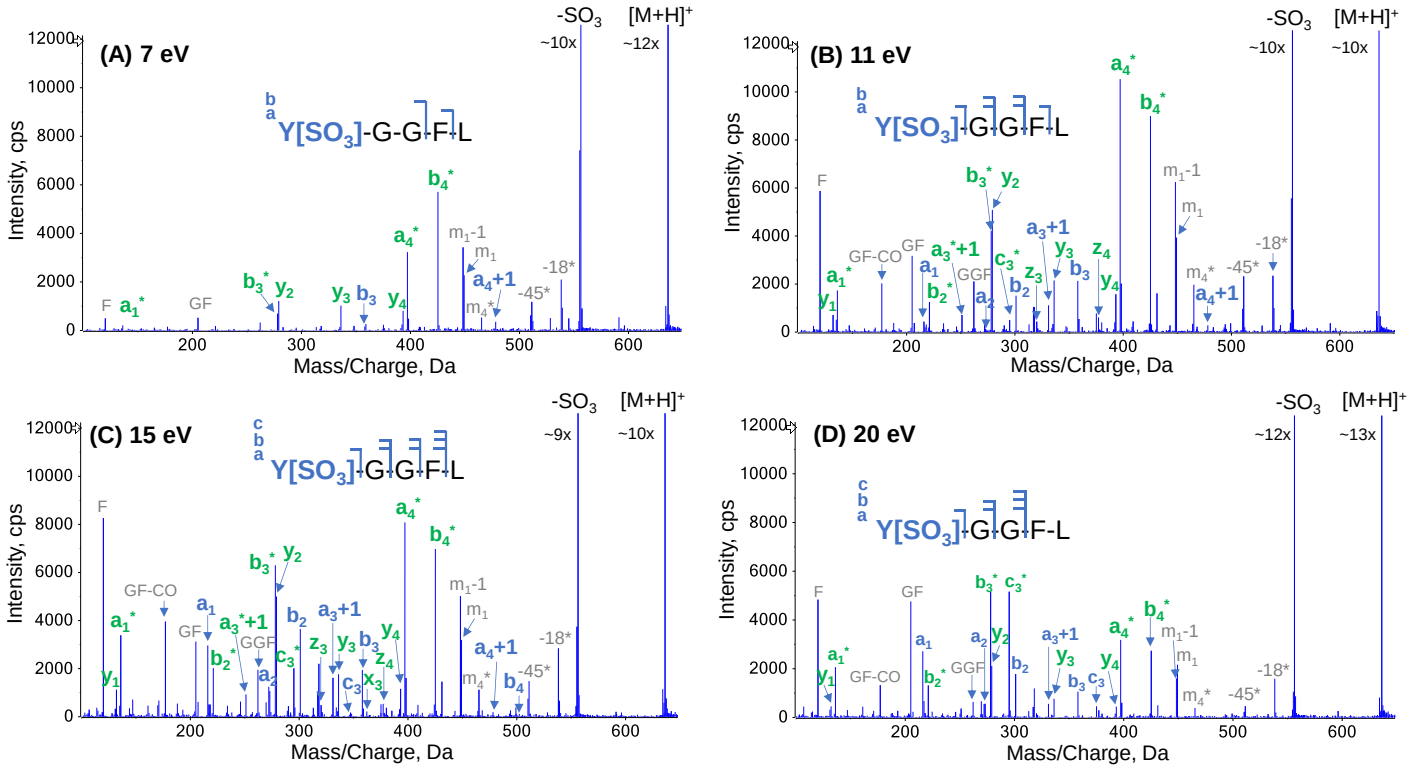

Figure 2. EAD spectra of singly charged LES at various electron KEs. An electron KE of 7 eV used in the platform method[4] generated a limited number of sequence ions containing the sulfate group (A). By contrast, higher electron KEs produced a series of sulfate-containing *a*/*b* ions for confident 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.

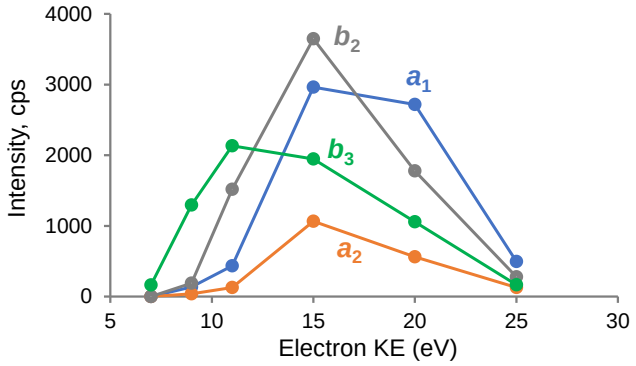

Figure 3. Effect of electron KE on the signal intensity of selected sulfate-containing *a*/*b* fragments from EAD of LES. The optimal electron KE for generating these sulfate-containing fragments was 15 eV.

Localization of sulfation in CLKS

Tyrosine sulfation in CLKS is known to be very labile. A previous study revealed that low-energy electron capture dissociation (ECD) of doubly protonated CLKS yielded no sequence ions containing the sulfate group.[2] A similar result was observed for EAD under the ECD-like conditions (KE=1 eV, Figure 4A), except that the sulfate group was retained on 2 low-abundant sequence ions (b_5/c_5). However, this result alone was not sufficient to pinpoint the location of the sulfate group in CLKS. Increasing the electron KE to 15 eV produced more sulfate-containing *a*/*b* fragments, including the a_2/b_2 ions that correspond to cleavages adjacent to the tyrosine residue (Figure 4B). The detection of c_1 without the sulfate group, which was absent in the spectrum with KE=1 eV (Figure 4A), further supported the localization of sulfation in CLKS at the second residue.

Figure 5 illustrates the effect of electron KE on signal intensity of a_2, b_2 and c_1 ions from CLKS. Similar to the result obtained for LES (Figure 3), an electron KE of 15 eV was determined to be the optimal value for obtaining the most intense signal of these ions necessary for accurate localization of the sulfate group. In summary, the results described above highlight the unique capability of easily adjusting electron KE in EAD to obtain site information about labile tyrosine sulfation, which is very challenging or impossible with traditional low-energy ExD approaches.

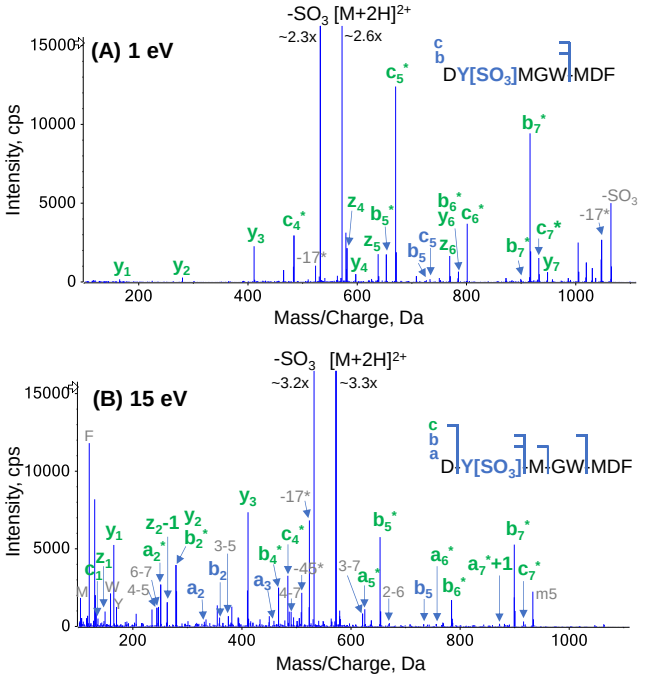

Figure 4. EAD spectra of doubly charged CLKS at 2 electron KE settings. Two sulfate-containing fragments (b_5 and c_5) detected at KE=1 eV did not provide sufficient information to pinpoint the location of the sulfate group (A). By comparison, an electron KE of 15 eV produced a non-sulfated c_1 and sulfated 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.

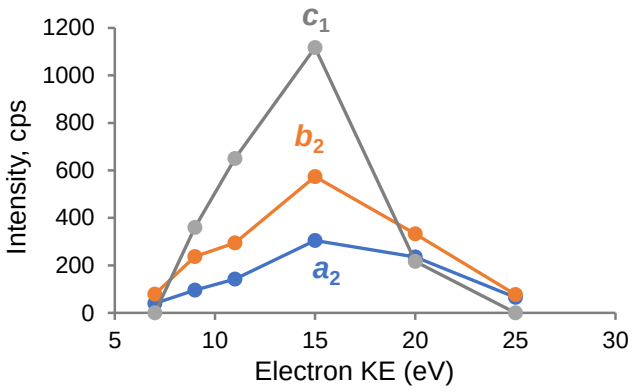

Figure 5. Effect of electron KE on the formation of selected sequence ions from EAD of CLKS. Similar to the observation made for LES, the electron KE=15 eV provided the optimal result for these CLKS fragments.

Conclusions

- Accurate localization of labile tyrosine sulfation on a singly and doubly charged peptide was achieved using EAD with tunable electron KE
- The optimal signal intensity of sulfate-containing fragments from 2 sulfated peptides analyzed in this study was accomplished with an electron KE of 15 eV
- The result described in this technical note demonstrated the power and potential of EAD for analyzing challenging molecules with fragile modifications that may be encountered in the characterization of new modalities with increasing complexity

References

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