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Application Note

Electron Capture Dissociation in Combination With High Performance Cyclic Ion Mobility Separation for Detailed Protein Studies

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Electron capture dissociation (ECD) is an alternative fragmentation technique of increasing use in the study of biomolecules.[1] Its advantages include comprehensive fragmentation of large peptides and proteins, distinction of isomeric amino acid residues, and retention of labile post-translational modifications. ECD is available as an option on the SELECT SERIES™ Cyclic™ IMS Mass Spectrometer, a unique, cyclic ion mobility-enhanced quadrupole time-of-flight mass spectrometer with high mass and mobility resolving power.

In this application brief we demonstrate the power of ECD in combination with cyclic ion mobility. Firstly, we show the separation of phosphopeptide isomers at a mobility resolving power >320 CCS/ΔCCS and their subsequent ECD-based sequencing. Secondly, we present the utility of pre-mobility ECD for topdown protein sequencing, exploiting ion mobility separation of fragment ions to increase sequence coverage. Together, these data reveal the power of ECD and cyclic ion mobility for increasing the information content for protein studies, of particular relevance for fundamental protein research and for characterizing biotherapeutics in the

biopharmaceutical industry.

Benefits

- ECD provides increased information content in peptide and protein sequencing experiments, boosting confidence in protein characterization results
- Unambiguously identify peptide isomers utilizing high resolution ion mobility separations in combination with ECD
- Increase confidence in top-down protein sequencing results by boosting the number of fragment ions detected

Introduction

Studies of proteins in the life science sector rely heavily on mass spectrometry for qualitative and quantitative assays. Most frequently, tandem mass spectrometry is used to identify and characterize proteins by virtue of amino acid sequence-informative ions, however, challenges exist that preclude detection of these ions in all cases. Firstly, post-translational modifications such as phosphorylations, acetylations, and glycosylations, must be retained in tandem MS experiments to determine their amino acid site. In many cases collision-induced dissociation provides complete coverage and retention of PTMs, but labile modifications such as phosphorylations and glycosylations may not.[2] Furthermore, for large peptides and for topdown protein sequencing, high coverage can be prevented due to incomplete fragmentation and spectrum complexity.

The SELECT SERIES Cyclic IMS Mass Spectrometer is an ion mobility-enabled instrument with a flexible geometry (Figure 1). Collision-induced dissociation is implemented in two collision cells, one before and one after the ion mobility device, offering a wide range of experimental options. The SELECT SERIES Cyclic IMS instrument is also available with optional ECD capability which can be implemented either pre- or post-IMS, offering the same flexibility in fragmentation as for CID.[3] We have previously demonstrated the use of ECD on this instrument for the sequencing of O-glycopeptides in biotherapeutics.[5] In this application brief we demonstrate the flexibility of the SELECT SERIES Cyclic IMS Mass Spectrometer with key examples which demand the high performance of cyclic ion mobility and superior Tof mass spectrometry. We show that high ion mobility resolving power in excess of 320 CCS/ΔCCS enables the separation of phosphopeptide isomers and that post-mobility ECD enables their sequencing and unequivocal identification. Furthermore, we show that pre-

mobility ECD offers advantages for topdown protein sequencing. By utilizing ion mobility to separate ECD product ions we significantly increase the coverage of the model protein ubiquitin and double the number of ions detected, increasing redundancy and therefore confidence in the assignments.

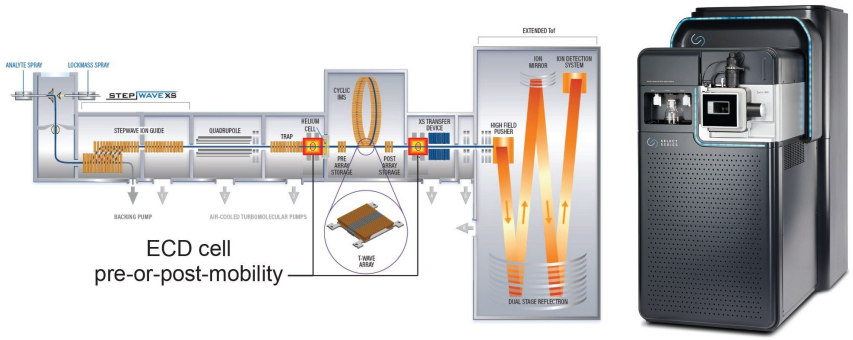

Figure 1. The SELECT SERIES Cyclic IMS instrument showing the location of the ECD cell either pre- or post-mobility.

Results and Discussion

Separation and Sequencing of Isomeric Phosphopeptides

Phosphorylations are a common post-translational modification found on proteins and are highly important in cell signalling processes. The modification itself, however, is prone to dissociation under traditional collision-based conditions. ECD is an attractive technique to employ to locate phosphosites as the process can retain phosphorylations and yield high quality peptide sequencing data.

The isomeric phosphopeptides RSpYpSRSR and RYpSpSRSR (from Sigma Phosphomix 1 and 2) were chosen for this analysis as they are structural isomers with the same positional phosphosites, the same mass, but slightly different amino acid sequences. In this experiment we subjected the $[M+H]^{2+}$ ion of the 1:1 mixture to 25 passes of mobility separation in the cyclic device, equating to a resolving power of approximately 325 (CCS/ΔCCS). Two distributions were observed (Figure 2, Top); species A at arrival time 208 and B at 210 ms. As the phosphopeptides exited the cell according to their mobilities they passed into the ECD cell for fragmentation. The position of the ECD cell post-mobility means that the product ions generated are mobility-aligned with their

precursors giving non-chimeric spectra for each peptide. Full coverage of both peptides was obtained, allowing both location of the phosphosites and confirmation of the amino acid sequences. From the data it was clear that species A was peptide RSpYpSRSR and species B was peptide RYpSpSRSR, a distinction made possible only through the combination of high resolution ion mobility and ECD fragmentation.

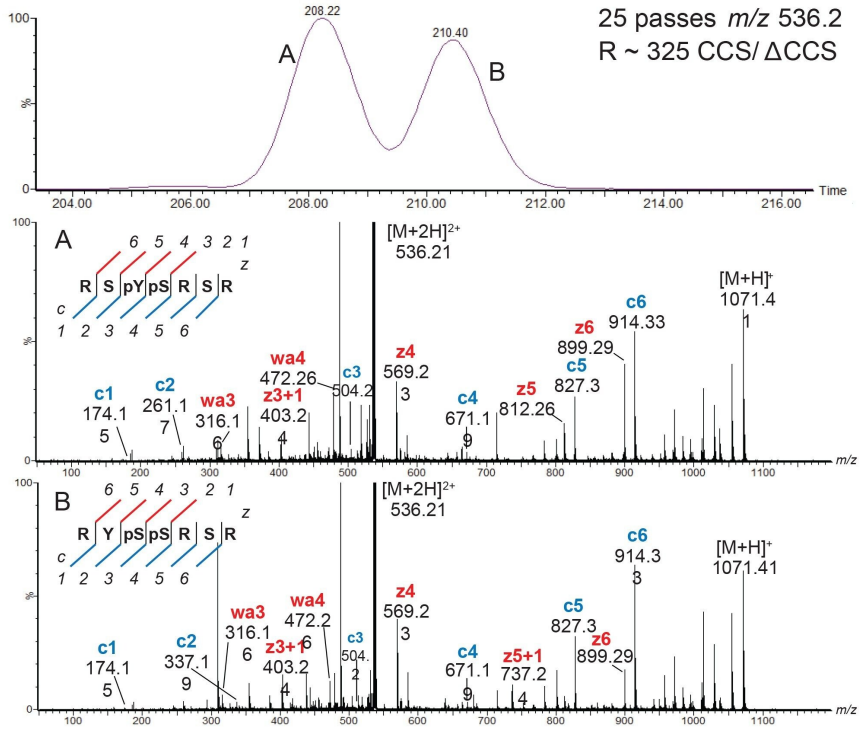

Figure 2. Separation of the 2+ species of the phosphopeptide mixture by 25 passes of cyclic ion mobility. Top: Two major distributions (labelled A and B) were observed in the peptide mixture after 25 passes of mobility (R~325 CCS/ΔCCS), suggesting one for each isomer. Assigning ECD product ions at each arrival time shows species A to be peptide RSpYpS RSR and B to be RYpSpSRSR.

Enhancing Sequence Coverage With Mobility Separation of ECD Product Ions

Top-down protein product ion spectra are highly complex and congested due to the wide range of product ions and charge states generated during the dissociation process. Also, in ECD there is the high abundance precursor

ion that can overlap in *m/z* with key fragments. To demonstrate the ability of ion mobility to disentangle these ions, pre-IMS ECD was performed on the 10+ charge state of bovine ubiquitin.

Figure 3 shows the top-down ECD ion mobility mass spectrum of the 10+ charge state of bovine ubiquitin. The product ions are separated according to their mass, shape and charge in the drift vs *m/z* space. This increase in peak capacity makes the product ions easier to assign due to a reduced overlap in the *m/z* dimension. A primary driver of the mobility separation is charge, with the singly-charged product ions separated out into a distinct series (Figure 3). The rest of the product ions inhabit a narrower drift vs *m/z* region due to the square root relationship to charge, with these ions being multiply-charged, larger and more conformationally diverse.

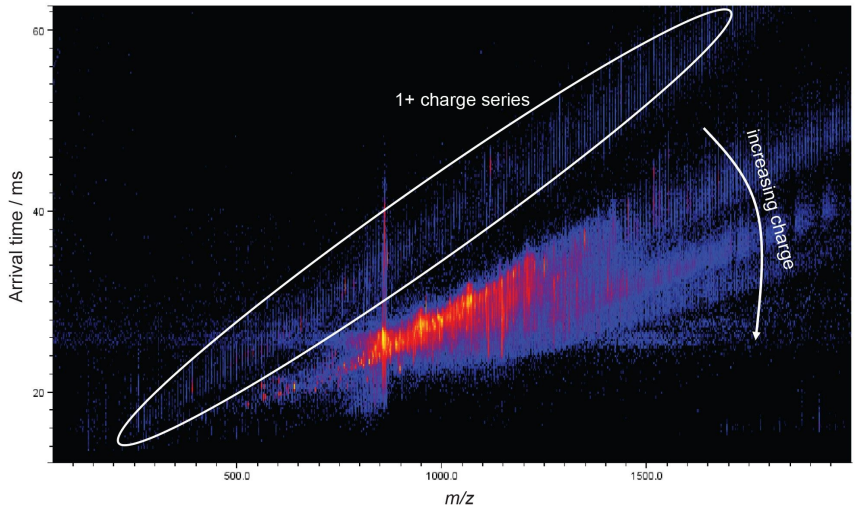

Figure 3. ECD product ions are separated by cyclic ion mobility. The different product ions have different masses, shapes, and charges and so separate accordingly. The singly charged ions (1+) are separated into a distinct series.

By exploiting the ion mobility dimension, clearer product ion spectra are obtained (Figure 4) allowing more confident assignment and less demanding deconvolution. Indeed, Figure 5 shows the results of the top-down sequencing of ubiquitin. In the case where ion mobility is utilized, sequence coverage is boosted from 89% to 97%. Furthermore, the number of product ions detected is doubled, which increases the redundancy in product ion assignments and therefore the confidence in the sequencing results.

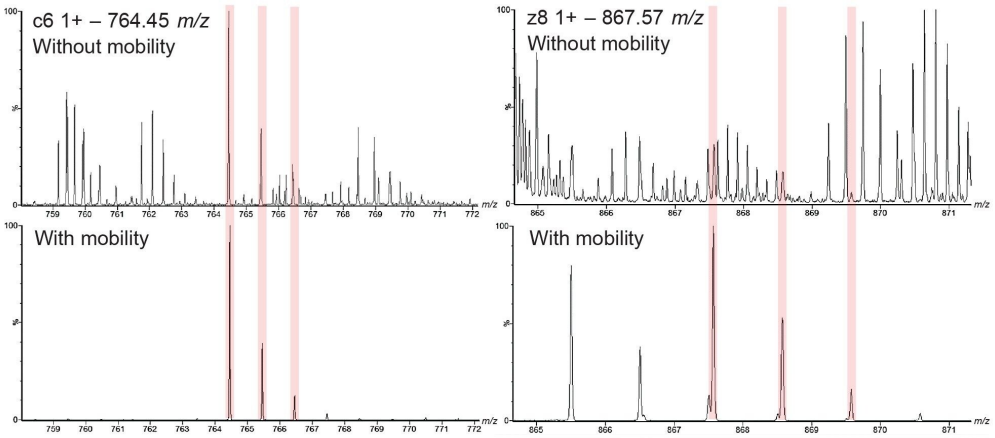

Figure 4. Examples of ion mobility separation of product ions in top-down ECD spectra. The c6 (top left) and z8 (top right) ions exist in congested regions of the spectrum. Separation using ion mobility simplifies the mass spectra (bottom) to make assignment easier.

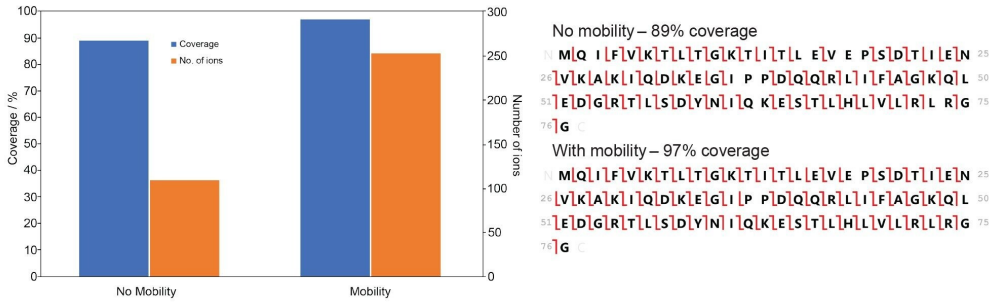

Figure 5. Left - Graph showing the increase in information obtained from topdown sequencing of bovine ubiquitin. When utilizing the ion mobility dimension the number of ions detected increases by over 100% from 110 to 253. This leads to an improvement in sequence coverage from 89% without mobility to 97% with mobility and an increase in redundancy, boosting confidence in results. Right – Coverage maps from Prosight Lite (5).

Conclusion

In this application brief we have demonstrated the power of ECD when implemented on the SELECT SERIES Cyclic IMS Mass Spectrometer. The ability to place the ECD pre- or post-IMS provides the ultimately flexibility for protein and peptide characterization experiments. The high resolving power mobility separation enables the separation of isomeric phosphopeptides and when coupled to ECD the peptides can be fully sequenced and identified. Furthermore, when implementing the ECD cell pre-IMS, the mobility separation can be utilized to increase the number of ions detected in top-down characterization experiments. Together with the high resolving power time-of-flight mass analyzer, these capabilities offer an attractive platform for protein researchers in the fundamental academic field and the pharmaceutical industry.

References

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