Application Note: 30081

Electron Capture Dissociation on the Finnigan LTQ FT–Preserving Post-translational Modifications during Peptide Fragmentation

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Key Words

- Finnigan™ LTQ FT™
- ECD
- FTICR-MS
- Post-translational Modification (PTM)
- Proteomics

Introduction

Electron capture dissociation (ECD) has recently evolved as an alternate activation method, especially for peptide and protein sequencing with Fourier-transform ion cyclotron resonance-mass spectrometry (FTICR-MS). With ECD, multiply charged cations are irradiated with low energy electrons produced by an emitter cathode behind the ICR cell. Electron capture produces a radical cation $[M+nH]^{(n-1)+\bullet}$ which can dissociate by a rapid, facile fragmentation of the N-C^{α} bond of the peptide chain, producing mainly c- and $z^{\bullet}$-type fragment ions.[1]

In contrast to ECD, the most commonly used activation methods such as collision induced dissociation (CID) and infrared multiphoton dissociation (IRMPD) induce dissociation by vibrational excitation of the precursor. Since these activation methods induce "ergodic" processes, i.e. they add internal energy to the precursor slower than the rate of energy randomization, usually cleavage of the weakest bonds within the precursor is observed. Within peptides, the backbone amide bond has the lowest energy barrier to dissociation and predominantly b- and y-type fragment ions are formed. However, substituents added in co- and post-translational modifications often have lower energy barriers than those of backbone cleavage. This can result in more complex tandem mass spectra and potentially the loss of the information on the attachment site of these substituents.

Due to the non-ergodic nature of ECD, co- and post-translational modifications are preserved. ECD allows site specific analysis of phosphorylation,[2,3] O- and N-linked glycosylation,[4-7] and sulfation.[8] ECD holds much promise as a supplementary dissociation technique to CID for unambiguous protein identification, *de novo* sequencing[9] and detailed protein characterization.[10]

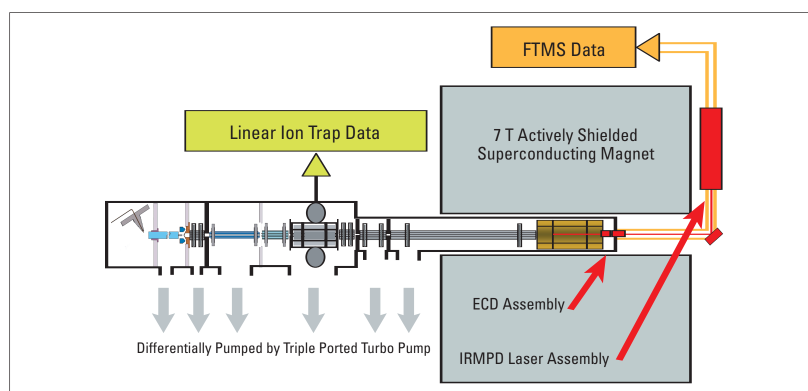
ECD shows higher degrees of fragmentation, allowing the distinction of leucine and isoleucine residues[11-13] and even between D- and L-amino acids.[14] ECD can also provide additional information of the amino acid composition by careful examination of side chain losses.[15-17]

However, the overall efficiency of ECD is typically lower than that obtained with CAD. Long ion accumulation, activation, and detection times in the ICR cell together with the need for the addition of multiple spectra makes the use of ECD for on-line separation of complex peptide or protein mixtures quite challenging.

The Finnigan LTQ FT hybrid linear ion trap/FTICR mass spectrometer provides a means to perform ECD analysis on complex mixtures of peptides and proteins on an LC timescale. The use of ion accumulation and isolation in the linear ion trap prior to transfer to the ICR cell for ECD and product ion detection allows improved cycle times (< 1.5 seconds), resulting in the acquisition of multiple ECD scans for each peak eluted. High cleavage efficiency is achieved by the use of a cathode with large electron emitting surface[18].

The cathode is positioned at the back of the ICR cell, slightly off-axis to allow the simultaneous use of ECD and IRMPD (see below).

Figure 1 shows a single scan ECD MS/MS spectrum of the doubly charged precursor of substance P at *m/z* 674.37. The spectrum exhibits intense ECD fragment



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ion peaks. Bearing in mind that the cyclic structure of proline does not allow formation of c- and z-type fragments, all possible N-C$^{\alpha}$ bonds are cleaved, allowing even *de novo* sequencing of peptides with unknown amino acid sequences.

The CID MS/MS spectrum of substance P is shown in Figure 2. The doubly charged peptide precursor ions were subjected to CID in the linear ion trap and the fragment ions were transferred into the ICR cell and detected. The spectrum looks somewhat more complex compared to the ECD spectrum. The fragment ion peaks of this spectrum are sufficient to identify substance P in a database search, but *de novo* sequencing would be a challenge.

As mentioned above, labile post-translational modifications are preserved in ECD. This is due to the fact that the amide carbonyl has a higher H$^{\bullet}$ affinity than the amino acid side chains and, therefore, the backbone N-C$^{\alpha}$ bonds are cleaved preferentially.

Figure 3 shows the ECD MS/MS spectrum of an O-glycosylated peptide carrying one N-acetylglycosamine (GlcNAc) moiety attached to one of its serines.

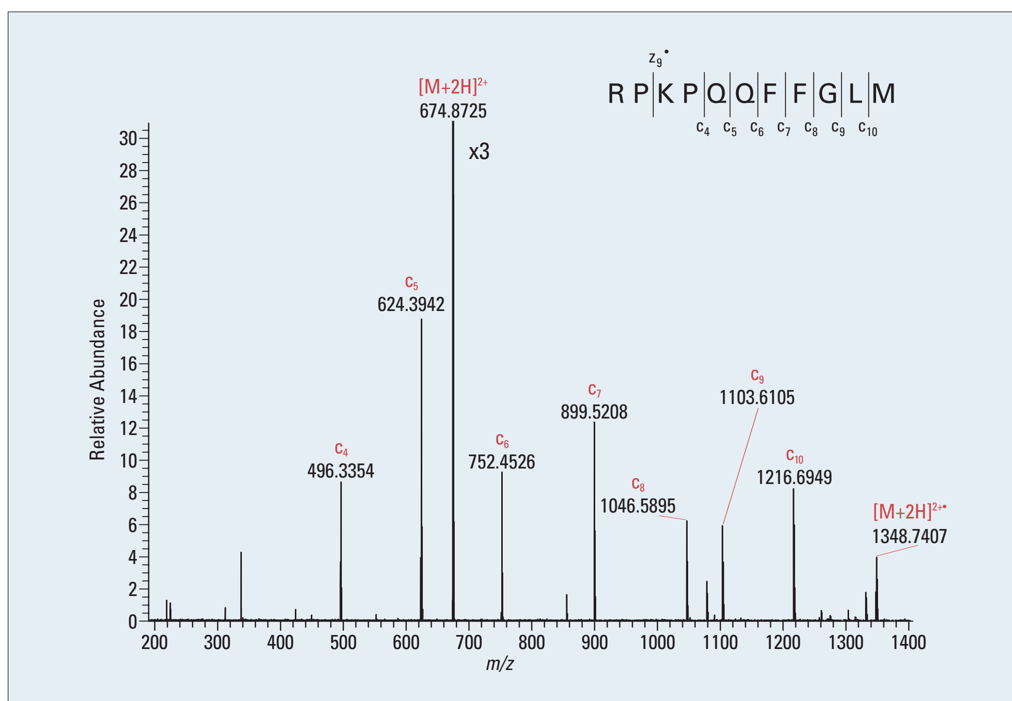

Figure 1: ECD spectrum of substance P, single scan at 100,000 resolution (FWHM)

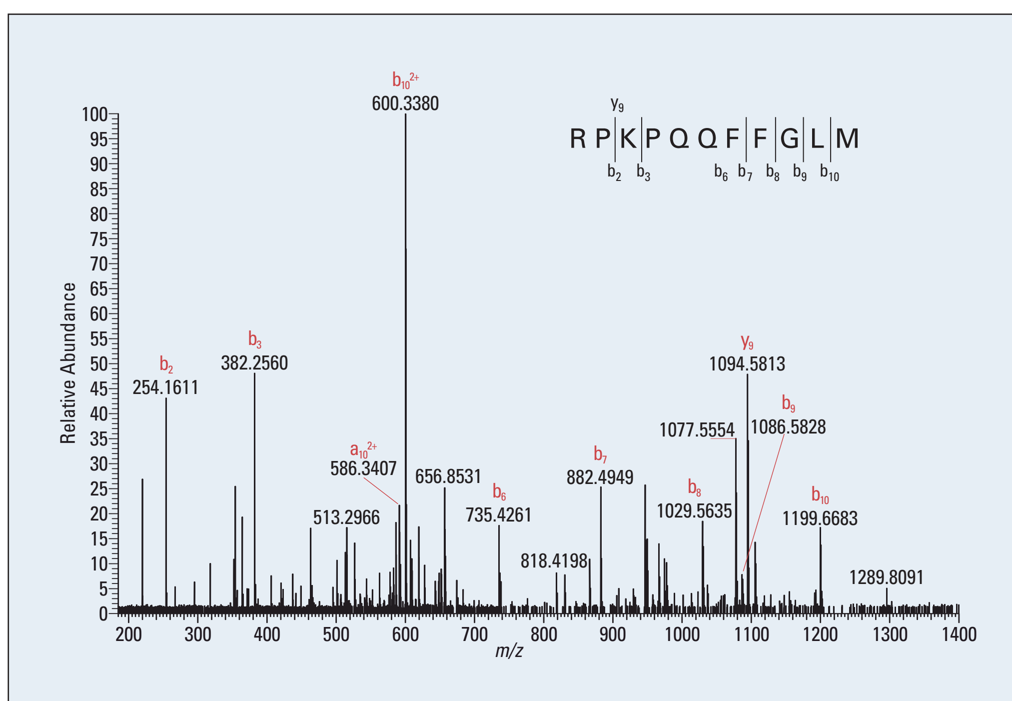

Figure 2: CID spectrum of substance P, single scan at 100,000 resolution (FWHM)

Interestingly, ECD of this glycosylated peptide gives rise to z- and $z^{\bullet}$-type fragment ion peaks in addition to one c-type fragment ion peak. The fragment ion peaks z_4 to z_7 are one Dalton heavier than expected and can be explained by direct $H^{\bullet}$ capture or H rearrangement and is a previously observed minor pathway in ECD.[19]

However, the mass difference between the z_6 and z_7-fragment ion peaks corresponds to the mass of a serine carrying a GlcNAc moiety, whereas the mass difference between the z_4 and z_5-fragment ion peaks corresponds to an unmodified serine residue. Although no peak for $z_{11}^{\cdot}$- fragment ions can be seen in the spectrum, the mass difference between the $z_{10}^{\cdot}$- fragment ion peak and the mass of the intact peptide corresponds to unmodified serine and proline.

The CID spectrum of the doubly charged precursor of this glycosylated peptide is shown in Figure 4.

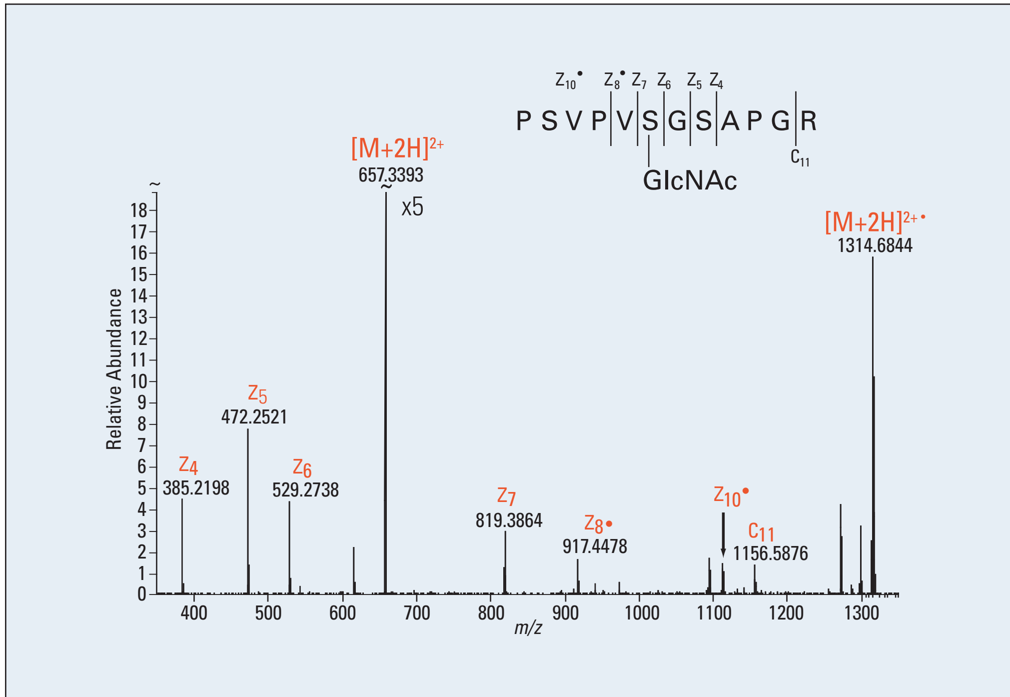

Figure 3: ECD spectrum of the O-glycosylated peptide

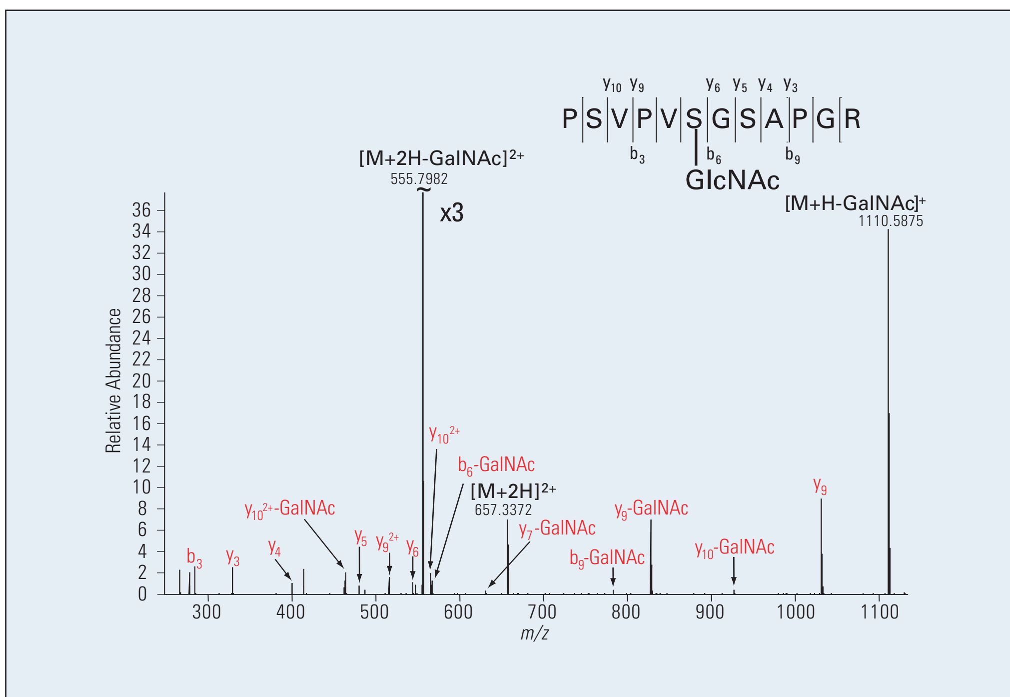

Figure 4: CID spectrum of the O-glycosylated peptide

It can be clearly seen in the spectrum that the major fragmentation pathway under CID conditions is the loss of the neutral sugar moiety. The spectrum also reveals fragment ions with the sugar moiety still attached and, in addition, fragments with the loss of the neutral sugar. The abundant peaks for y_9- fragment ions can be explained by the presence of proline in the peptide sequence which is known to be prone to facile fragmentation under CID conditions.

Conclusion

ECD is an effective alternate dissociation method, especially for the analysis of post translational modifications on an LC time scale. With the LTQ FT, additional confidence in the results is provided by accurate mass assignment of the fragment ions.

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