Application Note 00698

Rapid-Fire Electron Capture Dissociation for Post-Translational Modification Studies

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Introduction

Since the initial discovery of electron capture dissociation (ECD) in 1998 by Zubarev et al. [1], ECD has made a tremendous impact in driving mass spectrometry to the forefront of structural analysis of biological molecules. ECD, which is currently solely an FTMS technique, has become an important method in distinguishing isomeric or enantiomeric structures and analyzing the protein conformers in vacuo. It has also proven to be invaluable for top-down proteomics, specifically for the analysis of post-translational modifications (PTM) of proteins and peptides.

Although the mechanism of ECD is not yet thoroughly understood, it is thought to involve a recombination of an electron with protons to create very active hydrogen radical sites. The electrons captured by the peptide ion recombine with the protons in the ion. The energy released from the recombination causes the peptide ions to fragment as illustrated in Figure 1. The fragmentation occurs primarily at the N-Ca bond, yielding an extensive series of c- and z-type fragments.

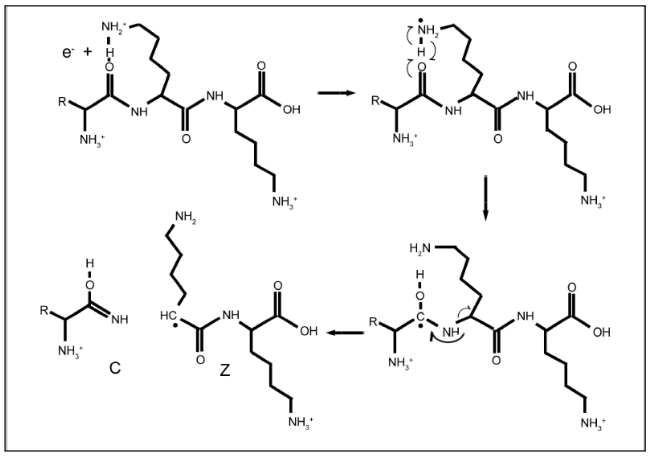

Figure 1 Mechanism for formation of c- and z-type ions in the ECD process.

The advantage of applying ECD fragmentation to the PTM studies is the non-selectivity of the process. The side-chains remain intact and the labile PTMs remain attached to the backbone during this non-ergodic (semi-predictable) process.

There are two main reasons why FTICR is an optimal instrument for ECD studies. First, the FTICR has a unique capability to obtain a very minimal difference between the energy of the multiply protonated ions and emitted electrons trapped in the ICR cell. This maximizes the efficiency of the electron capture. Also, the ECD efficiency is highest for electron energies <1 eV. Continuous efforts are made to create these conditions in other types of mass spectrometers, but currently ECD is primarily an FTICR technique. Second, although the fragmentation proceeds at a very high rate, several milliseconds are required to ensure the electron capture by most precursor ions. The required trapping time exceeds the storage capability of most other types of mass spectrometers.

In the current application note, first the data acquisition and analysis of ECD fragmentation of melittin will be discussed to illustrate the efficiency and the suitability of the Rapid-Fire ECD module. Then we demonstrate the application of ECD to study phosphorylation of a peptide; the ECD spectra of phosphorylated angiotensin II are discussed.

Methods

A 0.5 µM solution of honey bee venom melittin ($C_{131}H_{229}N_{39}O_{31}$; M.W. of 2846.46; Sigma M4171) was prepared in 50:50:2 water: methanol: acetic acid. The sample was infused at a flow rate of 2 µL/min through the electrospray ionization source. This corresponds to approximately 30 femtomoles of melittin per spectrum. The analysis was performed with Varian's Rapid-Fire ECD Module combined with the 901-MS (QFT™) 7.0 T instrument.

The Varian 901-MS with the Rapid-Fire ECD Module is well suited for ECD studies because low energy electrons can be trapped in the analyzer cell simultaneously with multiply-protonated ions created in the electrospray source. The fragmentation efficiency has been optimized using a high current dispenser cathode mounted close to the analyzer cell. A single electron beam of 30-50 ms duration is typically sufficient for extensive fragmentation. In addition, IRMPD fragmentation can be performed simultaneously with ECD to yield complimentary b- and y-type fragments.

For the angiotensin II experiment 1 µM solution of phosphorylated angiotensin II was prepared in the previously mentioned solvent. The above infusion flow rates and instrumentation were applied.

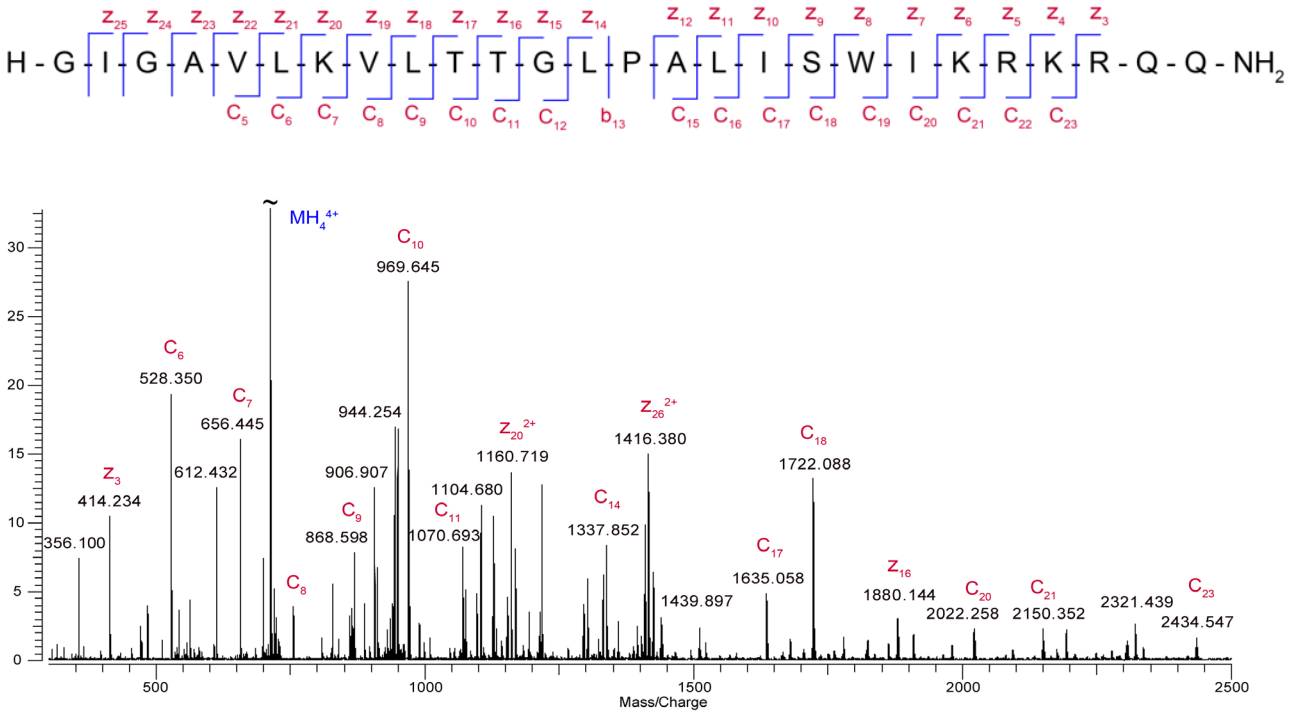

Figure 2 Quadrupole isolation and ECD fragmentation of the +4 charge state of melittin (*m/z* 712) using a 50 ms electron beam pulse.

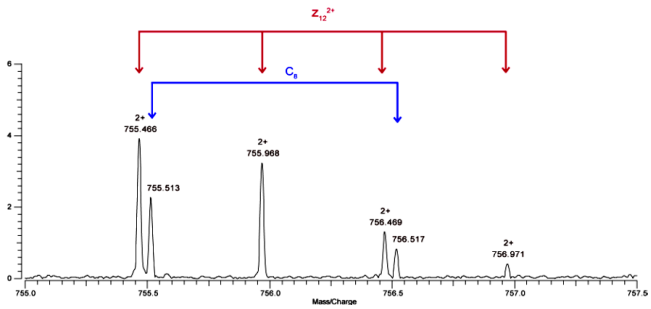

Figure 3 A close-up from the spectrum shown in Figure 2.

Discussion

Figure 2 shows the MS/MS spectrum of the +4 charge state of melittin. The quadruply-charged parent ion, occurring with an *m/z* value of 712 Da, was isolated using the quadrupole mass filter and fragmented using the Rapid-Fire ECD Module with a 50 ms electron beam pulse. A single scan was acquired to obtain the spectrum shown in Figure 2. The complexity of this MS/MS spectrum is intensified due to the overlapping charge states of the ECD fragments. An example of the overlapping c_8 and z_{12}^{2+} fragments is shown in Figure 3, a close-up of the spectrum shown in Figure 2.

The acquired data is analyzed using Varian PeakHunter™ software. Figure 4 shows a screenshot of this software. PeakHunter is a tool to interrogate and accurately analyze complex fragmentation (MS/MS) and LC/MS type of data. It automatically determines a default noise threshold, picks peaks, determines isotopic clusters and determines the charge states for each isotopic envelope. Figure 4 demonstrates how the monoisotopic masses have been calculated and the peptide envelopes are visualized in order to compare the acquired data on the top of the screen to the theoretical data shown in the bottom of the screen. The monoisotopic peaks listed as neutral and singly-charged masses on the left, can be conveniently copied as text or XML format to another software package.

The *de novo* sequencer in PeakHunter is able to accurately assign even very complex overlapping spectra as shown in Figures 2 and 3. The list of obtained fragment assignments is shown in Table 1.

The average mass error for the identified fragments was 0.98 ppm for this experiment.

Ion	*m/z*	Ion	*m/z*	Ion	*m/z*
Z3	414.2339	C6	528.3504	A7	612.4317
Z19 [+2]	563.3478	C7	656.4454	Mel [+3]	949.2587
Z6	827.5328	C24 [+3]	863.8863	Y19 [+2]	1104.1786
Z23 [+3]	868.2101	C9	868.5979	Y20 [+2]	1168.2261
Z26	943.9189	C25 [+3]	906.5725	?	1359.3551
Z17 [+2]	989.5893	C10	969.6455		
Z19 [+2]	1096.1695	C11	1070.6932		
Z20 [+2]	1160.2170	C21 [+2]	1075.1772		
Z23 [+2]	1302.3154	C23 [+2]	1217.2752		
Z24 [+2]	1330.8264	C14	1337.8515		
Z [+2]	1415.8786	C15 [+2]	1408.8886		
Z11	1439.8963	C17	1635.0561		
Z12	1510.9334				
Z14	1721.0702				
Z16	1879.1394				
Z17	1980.1865				

Table 1 List of assignments for the ECD spectrum of melittin shown in Figure 2.

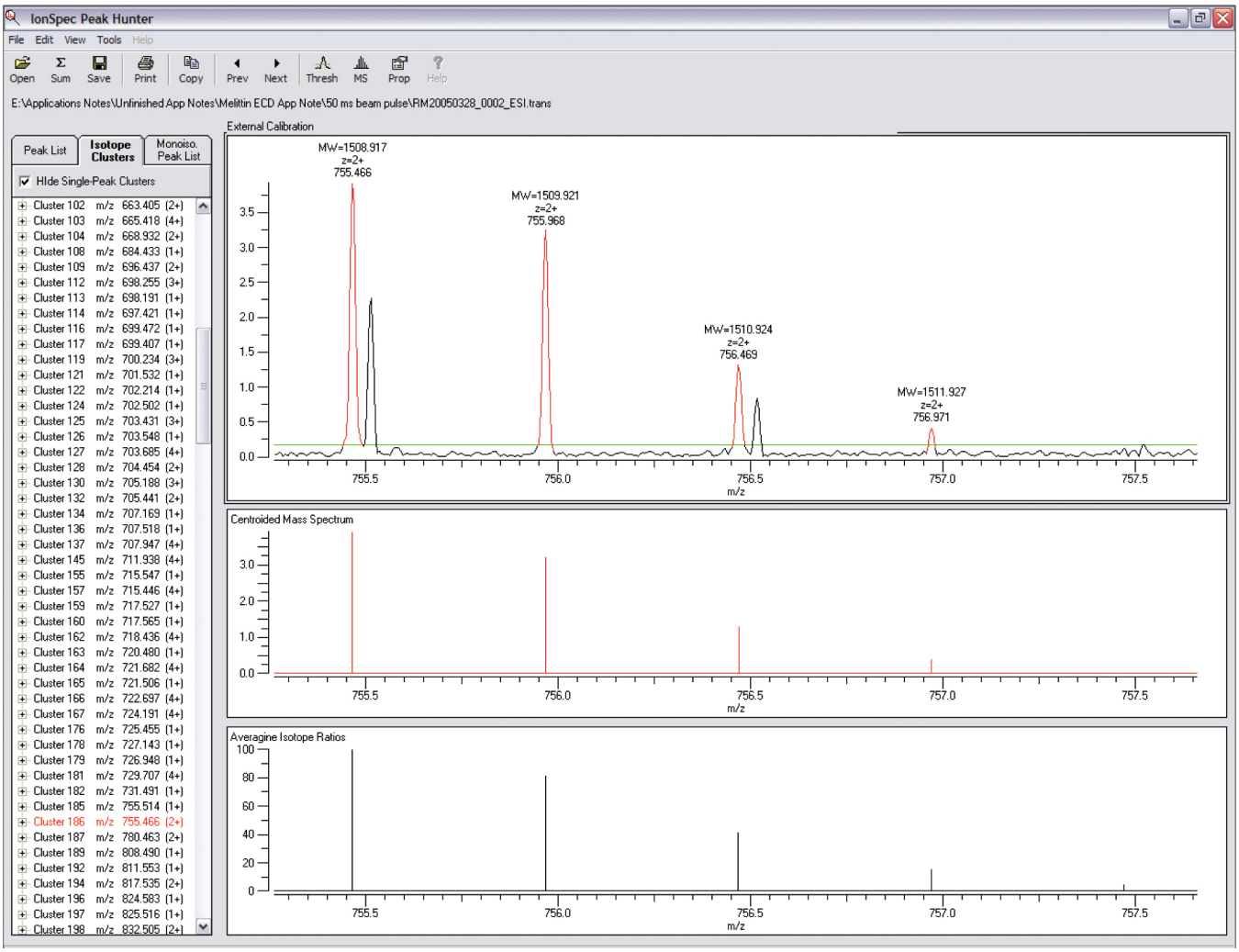

Figure 4 PeakHunter™ software interrogates the complex MS/MS spectrum of melittin to accurately identify isotope clusters.

Post-translational modifications such as phosphorylation, glycosylation, and sulfation are usually lost in CID and IRMPD experiments. The rational is that these groups are easily cleaved off because they form weak covalent bonds with the amino acid side chains. This is unfortunate because phosphorylation is an ubiquitous regulatory mechanism. It can result in the activation or termination of many important cellular events, including cell signaling growth, and differentiation. Cancer, inflammatory diseases, metabolic disorders, and neurological diseases are among those in which protein phosphorylation plays an important role.

One of the remarkable features of ECD is that post-translational modifications are retained in fragments. Figure 5 demonstrates this for phosphorylated Angiotensin II, Asp-Arg-Val-pTyr-Ile-His-Pro-Phe. The doubly-charged peptide at *m/z* 563.758 was selected by the RF/DC quadrupole and injected into the analyzer cell. The electron beam from the dispenser cathode was fired for 50 ms and 20 scans were accumulated. The peak at *m/z* 1126.509 results from capture of an electron by the doubly-charged ion. It is called a charge-reduction peak. Related to this is a fragment 81 Da lower at *m/z* 1045.464 which results from loss of H_2PO_3. The sequence at the top of Figure 4 shows that the arginine (R) residue second from the N-terminus holds the positive charge and accounts for an abundant series of c-type fragments. The site of phosphorylation is revealed by the large gap of 243.03 Da between c_3 and c_4: *m/z* 631.260 – *m/z* 388.230 = 243.03.

This agrees exactly with the expected mass for phosphorylated tyrosine. The phosphate group adds HPO_3 (79.9663 Da) to the tyrosine side chain. The other gaps match up well with the expected amino acids. Notice that c_6 is missing in the sequence, which is consistent with the proline at position 7.

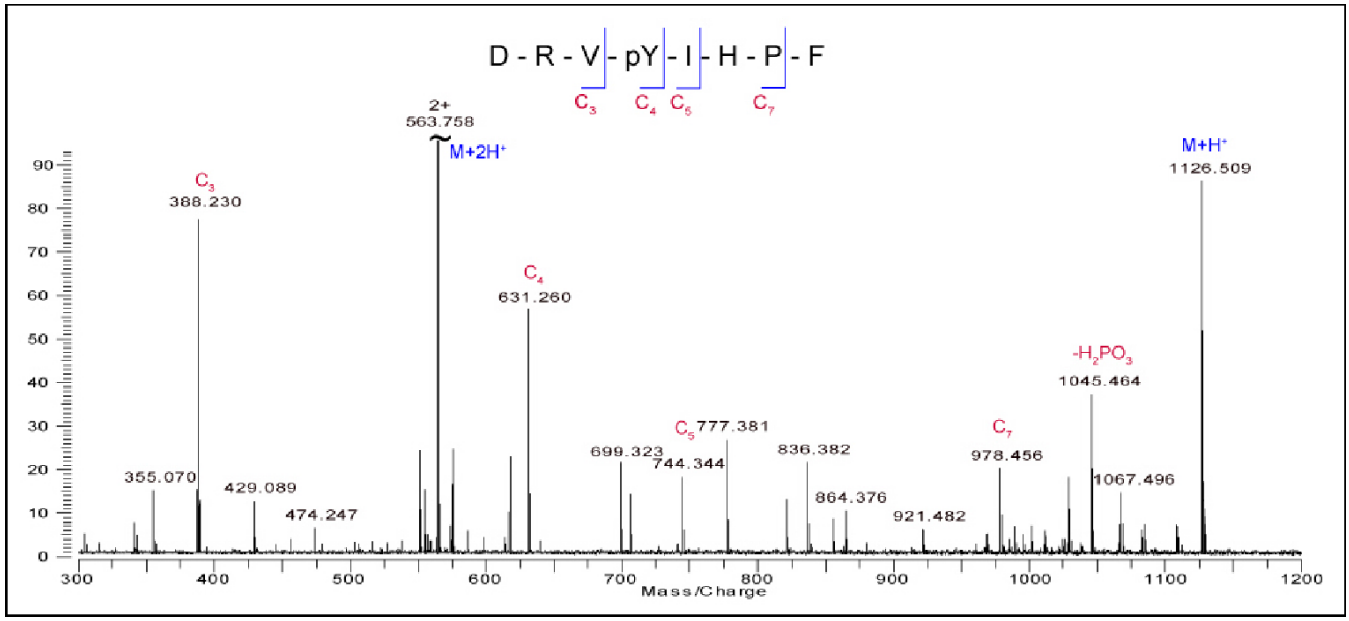

Figure 5 ECD mass spectrum of the doubly-charged peptide at *m/z* 563.758 of phosphorylated angiotensin II.

Conclusions

Although electron capture dissociation is a relatively new technique, it has already become an important tool in fragmentation studies performed with FTMS instruments. The advantage of this rapid process is that it cleaves the proteins and peptides non-selectively leaving the side chains intact. This makes ECD an invaluable tool for structural studies including the determination of isomeric or enantiomeric structures of peptides and analyzing the post-translational modification states of proteins.

References

[1] Zubarev, R. A. *Current Opinion in Biotechnology* 2004, 15, 12-16.

These data represent typical results.
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