

Large Peptide Sequencing Using Electron Transfer Dissociation

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Overview

Purpose: To apply electron transfer dissociation (ETD) to large peptide and intact protein sequencing. To enhance ETD performance on peptide of low charge density using automated supplemental activation (SA).

Methods: ETD of standard large peptide and protein digests was performed using LTQ XL ETD with automated supplemental activation enabled under instrument control software. ETD of intact ubiquitin was performed using the LTQ Orbitrap XL ETD™.

Results: When carrying sufficient charge, large peptide can be readily fragmented using ETD. Direct, unambiguous sequencing of 8559 Da ubiquitin with close to 100% sequence coverage was achieved using ETD with Orbitrap detection. However, ETD fragmentation efficiency decreases with the increase of precursor mass to charge ratio. Using supplemental activation which enhances ETD performance on peptides with low charge density, large peptides were successfully identified from partially digested protein.

Introduction

Conventional methodology of protein mass spectrometry using a bottom-up approach has limitations with respect to characterization of protein isoforms. In the bottom-up approach, proteins are enzymatically digested into a collection of peptides of relatively short size making the post-analysis assembly of protein isoform information challenging. The analysis of large peptides or intact proteins would reduce or even overcome this problem, thus, provide more complete sequence coverage, including site-specific modifications or mutations.

Electron transfer dissociation (ETD), compared to collisional activation, is relatively insensitive to the size of peptides, therefore offers a great opportunity for large peptide sequencing. From multiply charged precursors of large peptides, ETD generates information rich spectra containing full series of c and z types of product ions. With the increase of precursor ion charge state, fragment ions are often obtained carrying charge of +5 or even higher which are not well resolved in unit resolution instruments. Data interpretation of such complex, information rich, yet not well resolved ETD spectra would be difficult. High resolution and accurate mass would greatly facilitate analysis of complex ETD spectra. ETD was recently implemented with a hybrid linear ion trap – LTQ Orbitrap XL ETD mass spectrometer. An orbitrap mass analyzer with ETD capabilities was applied here for direct, unambiguous sequencing of large peptide or small intact proteins.

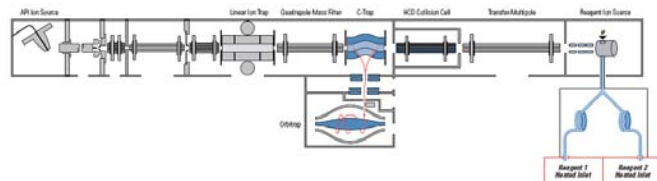
It has been observed that ETD fragmentation efficiency decreases with the increase of precursor m/z, regardless of precursor charge or peptide molecular mass. A supplemental activation approach can enhance ETD performance by converting the non-dissociative electron transfer product into c and z type of ions (1). Automated implementation of supplemental activation on precursor ions of charge +2 through +6 is now available under instrument control software. In this study, the automated supplemental activation was evaluated using standard peptides and applied to large peptide identification from protein digest.

Methods

Standard peptide was purchased from Anaspec. Ubiquitin was purchased from Sigma-Aldrich. Human IgG protein were from CalBiochem. The human IgG protein was reduced, alkylated and partially digested by LysC to produce relatively large peptides.

For static infusion, peptides or the intact protein was dissolved in acetonitrile/water/formic acid (50:50:0.1) at a concentration of 2 μM to 10 μM. The spray voltage was 1.8-2.0 kV for flow rate of 3 μL/min.

ETD of standard peptide and protein digest was performed using a Thermo Scientific LTQ XL ETD. ETD of intact ubiquitin was performed using the LTQ Orbitrap XL ETD in which a chemical ionization (CI) source and a flatapole and a quadrupole are coupled to the orbitrap analyzer via the c-trap. Fluoranthene radical anions commute to the LTQ via an added flatapole, the HCD collision cell, the c-trap and a quadrupole operating as a low pass filter, as shown below. Individual charge states of the protein molecular ions were selected for isolation and ETD in the linear ion trap and fragment ions were subsequently detected in orbitrap. The anion target was adjusted from 3 to 5 e5 and the activation time was 20 - 100 msec. The resolving power of the orbitrap was selected at 60,000 (FWHM).

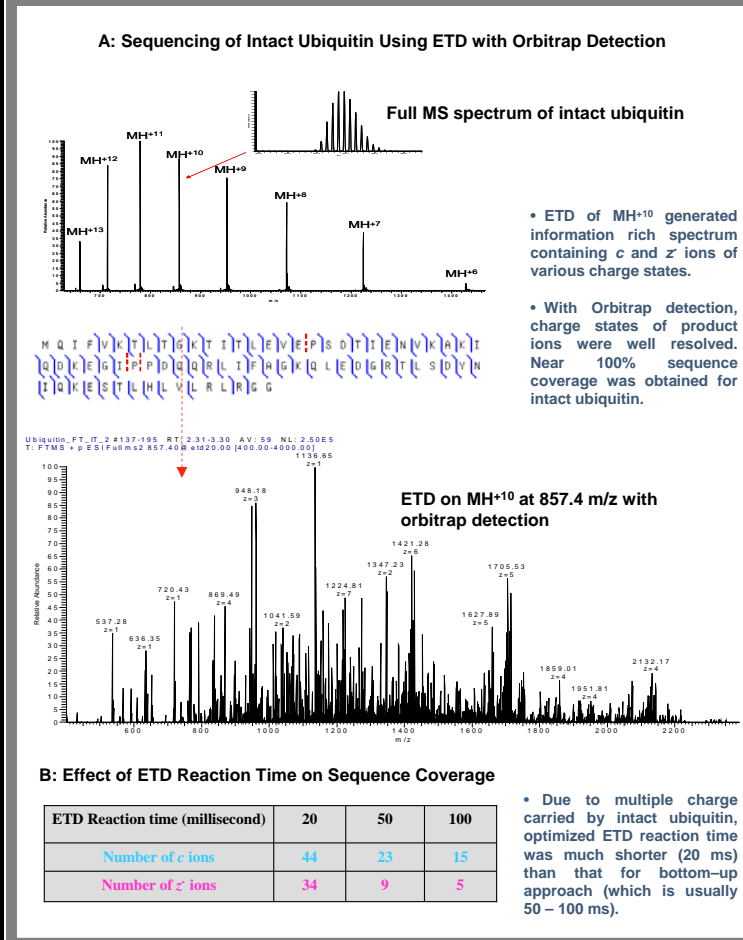


A Thermo Scientific Surveyor™ HPLC equipped with a Micro AS and nanospray source was interfaced with the LTQ XL ETD for online peptide separations using a C18 column. Spectra were acquired automatically using a Data Dependent™ ETD MS/MS instrument method (1 full MS plus 2 ETD spectra on each of the 3 most intense peaks with and without SA enabled).

ProSightPC™ was used for ETD spectra analyzed with high resolution mode on the orbitrap analyzer. ZCore database search algorithm within Proteome Discoverer software was used to identify peptides from ETD spectra of protein digests.

Results

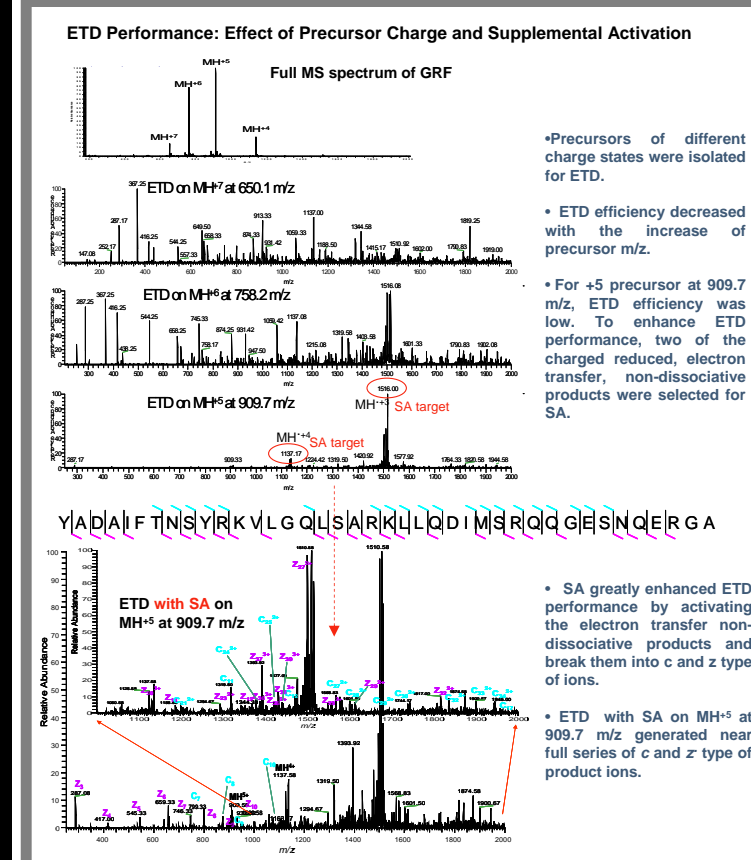
FIGURE 1. ETD of intact ubiquitin with orbitrap detection. A. MH⁺¹⁰ at 857.4 m/z from the Full MS spectrum were isolated and fragmented using ETD. B. Effect of ETD reaction time on sequence coverage



Conclusions

- Large peptide or intact protein carrying sufficient charge can be readily fragmented using ETD, producing information rich spectrum.
- Direct, unambiguous sequencing of 8559 Da ubiquitin with close to 100% sequence coverage was achieved using ETD and Orbitrap detection.

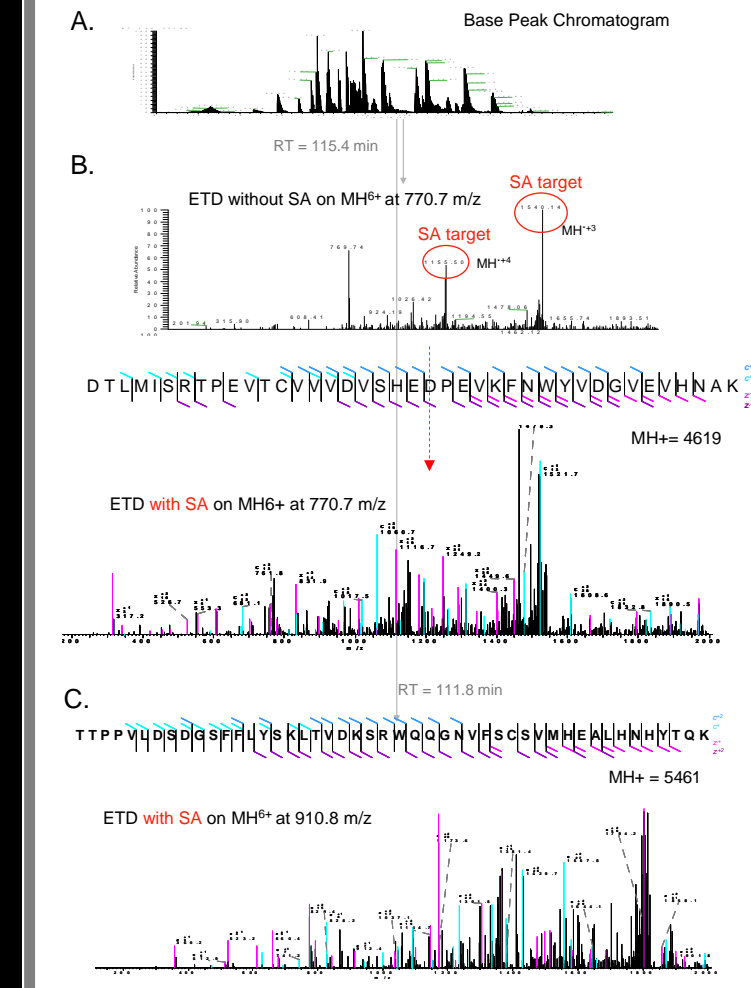
FIGURE 2. Effect of precursor charge state on ETD efficiency and enhancing ETD performance using supplemental activation. From full MS spectrum of standard peptide GRF, precursors of different charge state were isolated for ETD. ETD efficiency decreased with the increase of precursor m/z. For larger m/z at 909.7 when ETD efficiency was low, supplemental activation was enabled to enhance ETD performance.



Conclusions (continued)

- Optimized ETD reaction time for intact ubiquitin at 857.4 m/z was much shorter than that for small to medium sized peptides.
- ETD efficiency decreases with the increase of precursor mass to charge ratio. Automated supplemental activation enhances ETD performance by selectively activating electron transfer, non-dissociative products.
- Using ETD with supplemental activation, peptides of more than 5000 Da which are of relatively low charge density were successfully identified from the partially digested protein.

FIGURE 3. Identification of large peptides from Human IgG digest using ETD with supplemental activation. A. Base peak chromatogram of Human IgG partial LysC digest. B. ETD spectra with and without SA for a peptide (MH⁺ = 4619) eluted at 115.4 min. C. ETD with SA spectrum for a peptide (MH⁺ = 5461) eluted at 111.8 min.



References

Good MD, Wirtala M, McAlister GC and Coon JJ. *Mol Cell Proteomics*. 2007 Nov;6(11):1942-51.

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