

Enhancing Linear Ion Trap Mass Spectrometry ETD Performance Using Supplemental Activation

Zhiqi Hao, Jae C Schwartz, John E P Syka and Andreas FR Hühmer
Thermo Fisher Scientific, San Jose, CA, USA

Overview

Purpose: To evaluate enhanced ETD performance in a linear ion trap using supplemental activation (SA)

Methods: SA was performed in an LTQ XL ETD instrument under instrument control software

Results: ETD fragmentation efficiency decreases with an increase in precursor mass to charge ratio. Supplemental Activation (SA) enhances ETD efficiency by activating charge reduced, non-dissociated, intact product ions to produce c/z type product ions. SA is needed for most 2+ precursors and precursors of high m/z.

Introduction

Electron transfer dissociation (ETD) is relatively indifferent to peptide amino acid composition and post-translational modifications compared with CID. However, ETD is relatively inefficient for doubly charged precursors or precursors having high m/z. To overcome this problem, a collisional supplemental activation method (SA) has been implemented in the LTQ XL™ and LTQ Orbitrap XL™ with ETD systems. In this study, the effect of supplemental activation on ETD performance in the LTQ XL was assessed. The utility of ETD with SA for enhanced peptide identification and PTM analysis was investigated.

Results

FIGURE 1. Peptides identified with high confidence using ETD and CID. Peptides were plotted based on their precursor charge and m/z.

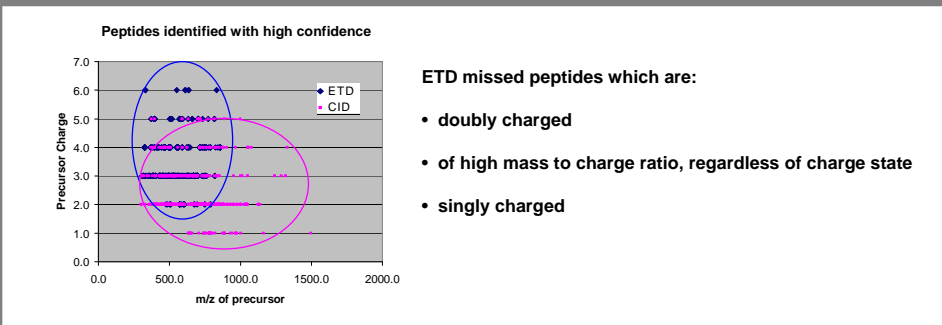
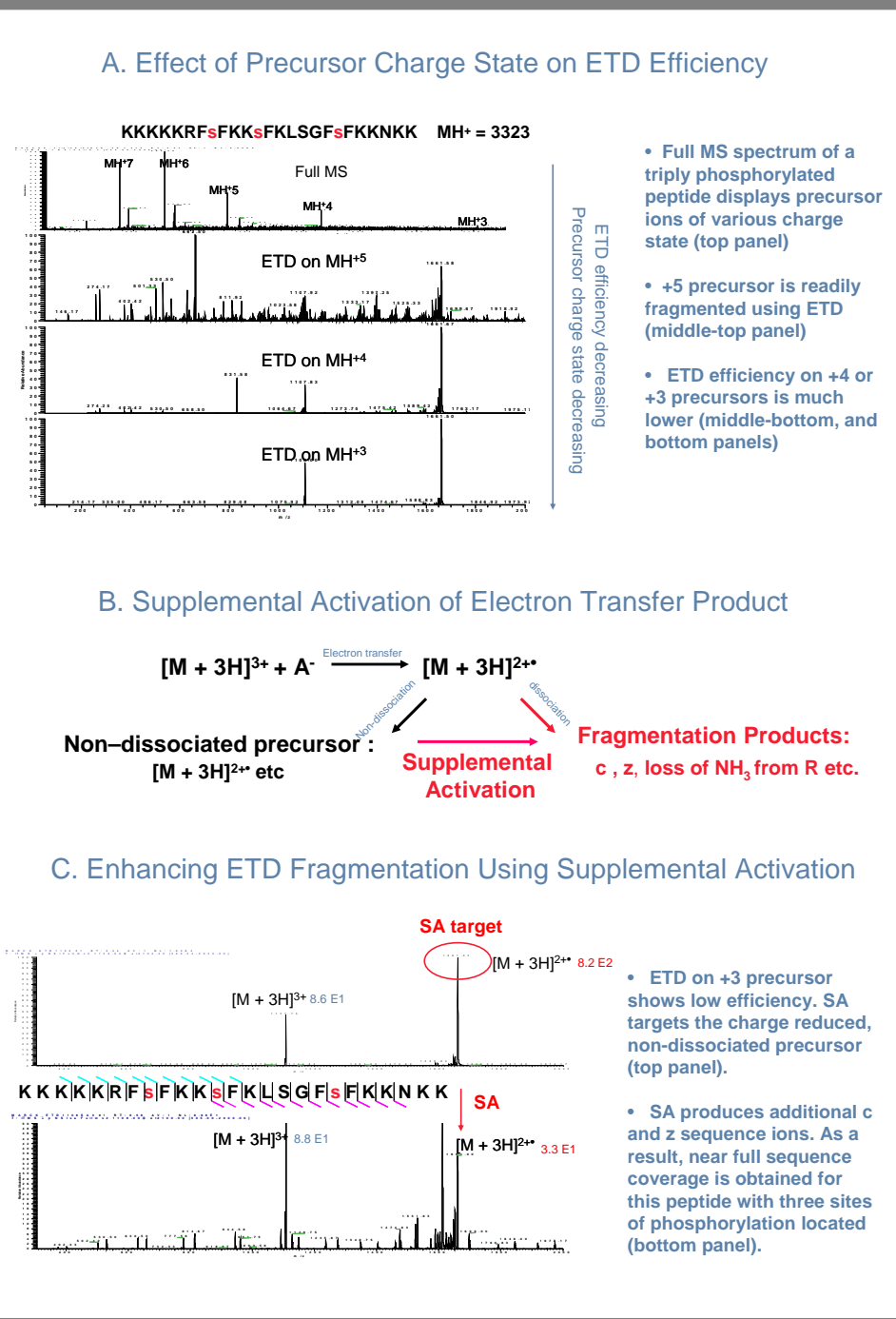


FIGURE 2. A. Effect of precursor charge state on ETD efficiency. B. Supplemental activation (SA) of electron transfer product. C. Enhancing ETD fragmentation using SA.



Methods

Standard peptides were purchased from Anaspec. Protein was reduced, alkylated and enzymatically digested to produce peptides. Samples were analyzed in either infusion or LC/MS/MS mode. Supplemental activation was implemented in the LTQ XL with ETD under instrument control software. After ETD, the non-dissociated precursor ions were automatically activated without an additional isolation step. Thus, fragment ions generated during the initial ETD remained in the final spectrum. SA was performed with low collision energy 10 (%) under a low activation q of 0.15. Spectra obtained under these conditions contain almost exclusively c/z type ions.

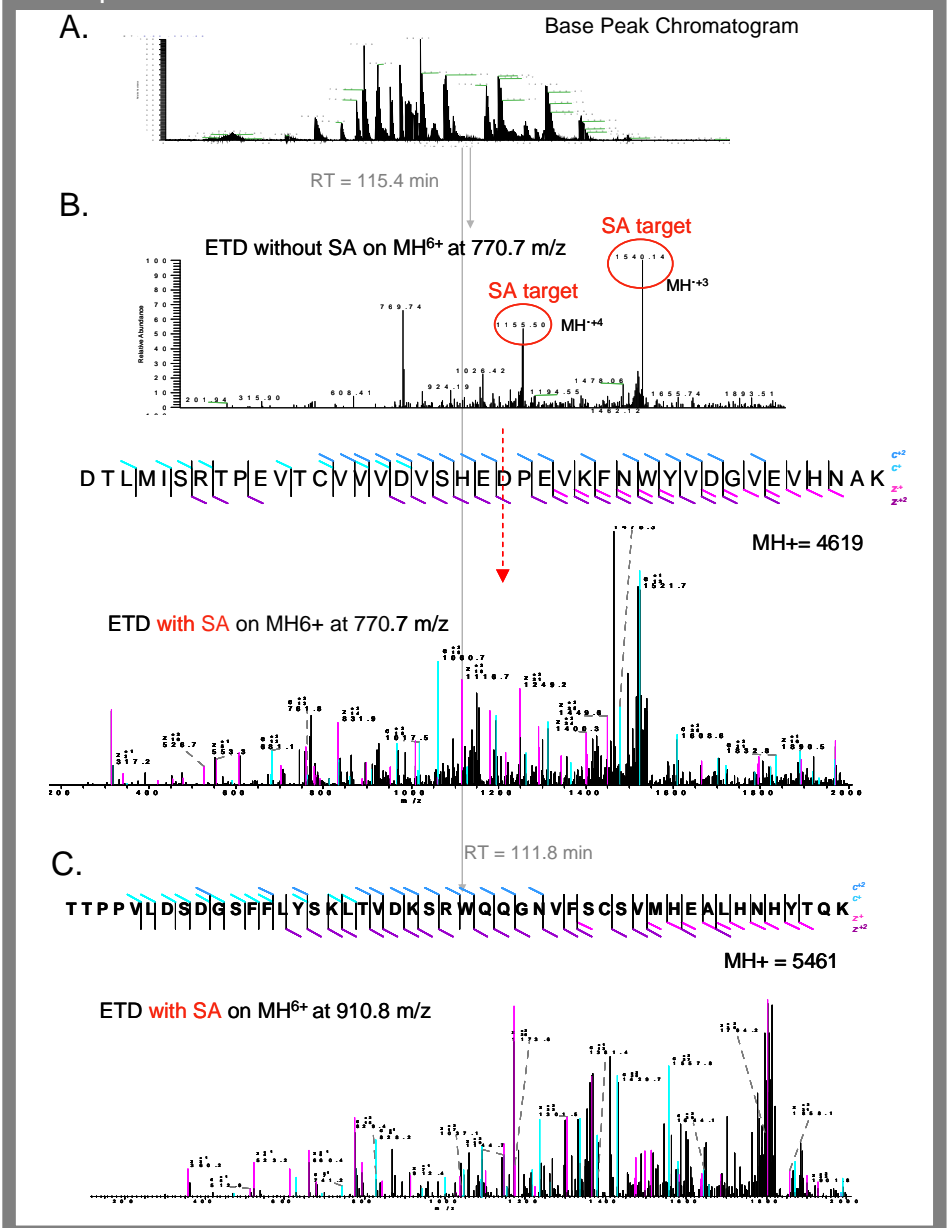
A Thermo Scientific Surveyor™ HPLC equipped with a Micro AS and nanospray source was interfaced with the LTQ XL ETD for online peptide separations using a C18 column. Spectra were acquired automatically using a Data Dependent™ ETD MS/MS instrument method (1 full MS plus 2 ETD spectra on each of the 3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

Data analysis was performed using the ZCore algorithm in Proteome Discoverer 1.0 software.

Results

Great enhancements to sequence coverage are possible, particularly for peptides with larger m/z. Even peptides of great length and high charge benefit from supplemental activation in terms of sequence coverage obtained. In figure 3 we see 2 typical examples of improved data following SA. Peptides of mass 4618 and 5460 (MH^+ of 4619 and 5461) showed greatly improved sequence coverage for ETD with SA compared with ETD alone. These peptides were of 40 and 47 residues in length respectively.

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. Sequence coverage information in figure 3B and 3C is for ETD with SA spectra.



Conclusions

- Supplemental Activation (SA) enhances ETD efficiency by activating charge reduced, non-dissociated, intact product ions to produce c/z type product ions.
- SA has been implemented in the standard instrument control software for the LTQ XL ETD.
- SA is compatible with labile PTMs, such as phosphorylation and glycosylation (data not shown).
- SA is necessary for most 2+ precursors and precursors of higher charge state, usually above 900 m/z.

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