

Intact Protein Sequencing Using ETD and PTR in a Dual-Pressure Linear Ion Trap

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Overview

Purpose: To evaluate the performance of proton transfer reaction (PTR) in a linear ion trap for charge reduction following electron transfer dissociation (ETD) and to investigate the utility of ETD with PTR for intact protein sequencing.

Methods: Experiments were performed using LTQ XL ETD ion trap and LTQ Velos dual-pressure linear ion trap mass spectrometers, both equipped with ETD and PTR, under LTQ 2.6 instrument control software with developer's kit. Benzoic acid anions, which are generated in the chemical ionization source at the rear of the instrument, were used as PTR reagent.

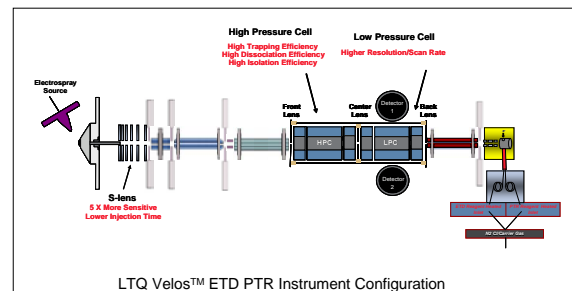
Results: When intact protein ETD analysis is performed on a unit-resolution instrument, the resulting spectra are information rich, yet contain multiply charged product ions which can not be resolved adequately. PTR following ETD reduces charge carried by product ions so that they are resolved at unit resolution. Using different PTR time, ETD-PTR of intact proteins generates very informative and well-resolved spectra. The improved sensitivity, resolution, and faster scan rate of the LTQ Velos mass spectrometer resulted in extensive sequence coverage of intact proteins of up to 30kDa. With product ions of +5, +6 and even +7 charge resolved in full, zoom scan mode, the LTQ Velos instrument identified many c/z⁺ product ions as big as 10 kDa in the complex spectrum of intact myoglobin and carbonic anhydrase in a single 10 minute experiment. Number of identified ions, thus the sequence coverage was significantly improved in LTQ Velos compared to LTQ XL.

Introduction

Electron transfer dissociation (ETD) is an beneficial tool for intact protein analysis because it is relatively insensitive to the size, amino acid composition and post-translational modifications of proteins, therefore randomly cleaves protein / peptide backbone bonds. ETD of intact proteins performs with high efficiency, generating very informative, yet extremely complex spectra which contain highly charged product ions that are difficult, or even impossible to resolve at unit resolution. Proton transfer reaction (PTR) following ETD was developed to reduce spectral complexity. PTR removes protons from the multiply charged product ions, generating a simplified spectrum that contains product ions of resolved charge states at unit resolution. PTR has recently been implemented, under software control, in the LTQ XL ion trap and the new LTQ Velos dual-pressure linear ion trap. Shown below is the instrument configuration of LTQ Velos ion trap with ETD and PTR. The new technologies in LTQ Velos instrument not only improve the sensitivity by 5-10 times, but also improve trapping, isolation and dissociation efficiencies. The dual-pressure trap provides two times faster overall scan speed compared to LTQ XL instrument. Here, the performance of ETD PTR for intact protein analysis is compared between the LTQ XL and LTQ Velos instruments.

Methods

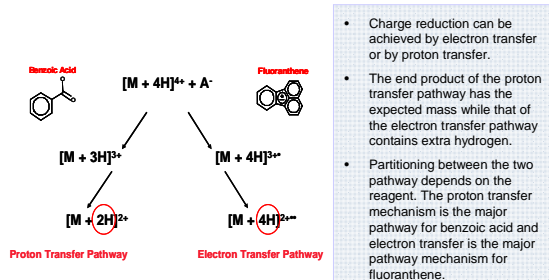
Standard peptides were purchased from Anaspec. Intact proteins were purchased from Sigma. Desalted intact protein was diluted in acetonitrile / water / formic acid (50:50:0.1) to a final concentration of 1 to 5 pmol/μL. The sample was directly infused using static nanospray with a 4 micron tip (Picotip™, New Objective). ETD with PTR was performed using Thermo Scientific LTQ XL ETD and LTQ Velos ETD mass spectrometers with PTR capability under LTQ 2.6 instrument control software with developer's kit. Benzoic acid anions which are generated in the chemical ionization source at the rear of the instrument were used as PTR reagent. The anion target was 2e5. Activation time was 5 - 10 msec for ETD or for 25 -50 msec for PTR.



Results

FIGURE 1. Electron transfer and proton transfer for charge reduction

Charge reduction using electron transfer or proton transfer



ETD or ETD-PTR spectrum of histone 3 peptide (M+6H)⁶⁺



- For analysis on a unit resolution instrument charge reduction of product ion is desired when precursor ion of high charge state are produced, as is the case in intact protein analysis.
- Both extended ETD reaction and PTR can be used to reduce the product ion charge state.
- Charge reduced product ions from extended ETD reaction times often contain extra hydrogen which cause a mass shift (insert). These ions are usually not assigned during database searches (the colored peaks are ions identified by the database search). Extended ETD reaction times also generate internal fragments as noise peaks which complicate the data analysis.
- PTR, on the other hand, produces charge reduced product ions with expected masses for database searches. ETD-PTR spectra also contain less noise. Thus sequence coverage from ETD-PTR experiments is better than that from extended ETD experiments (more colored peaks).

FIGURE 2. Sequencing of intact ubiquitin using ETD-PTR.

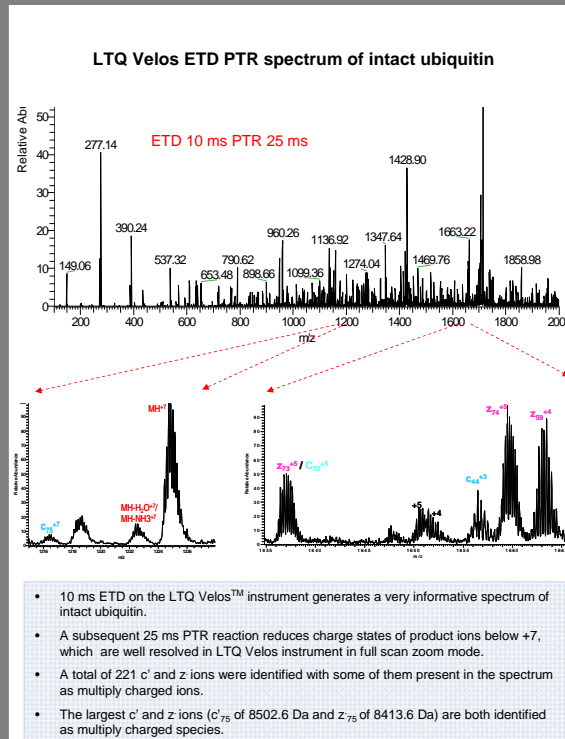


FIGURE 3. Top-down sequencing of myoglobin (top) or carbonic anhydrase (bottom) using LTQ Velos ETD with PTR. Results were from 10 min data averaging.

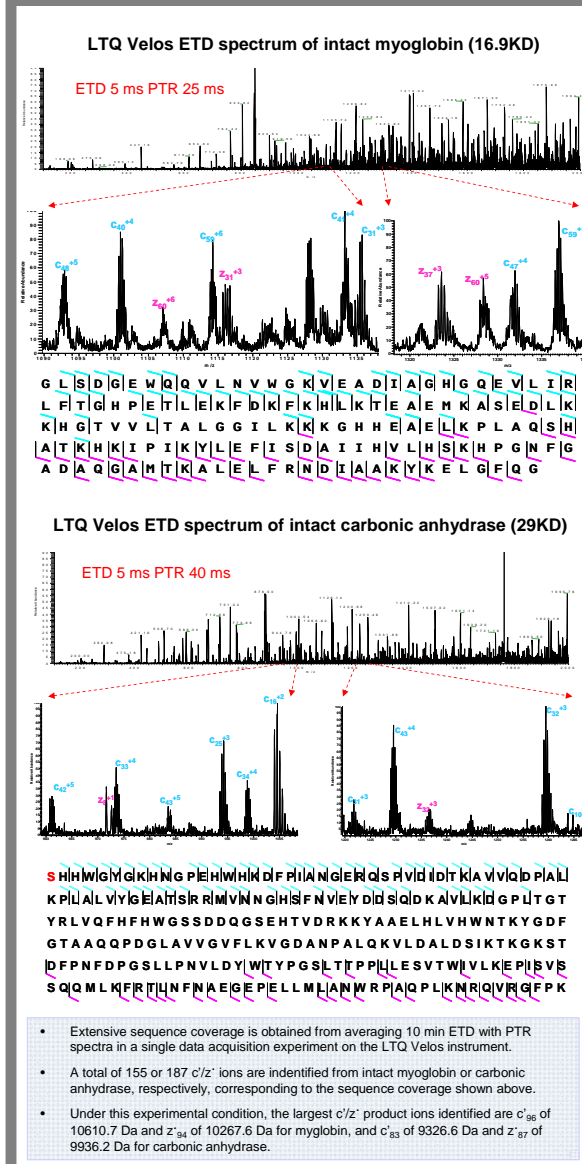
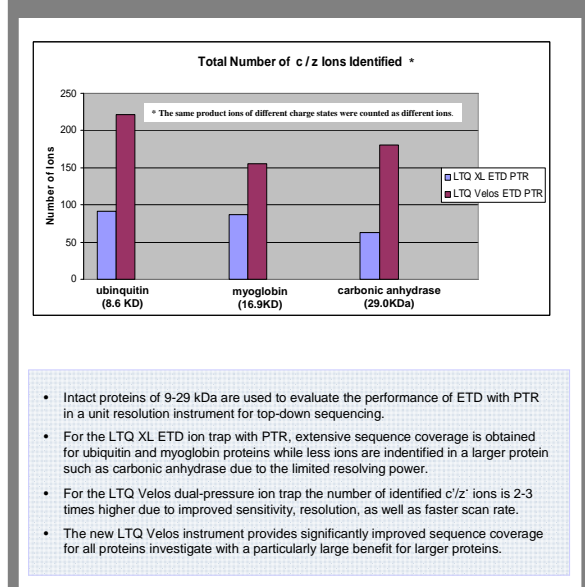


FIGURE 4. Improved identification from top-down sequencing using ETD with PTR in a dual-pressure linear ion trap.



Conclusions

- PTR following ETD reduces charge carried by product ions, thus the complexity of the ETD spectrum.
- Charge reduction of product ions can also be achieved by extending the ETD reaction time. However extended ETD reactions produce c⁺/z⁺ ions containing one or more extra hydrogen, especially for ions at higher m/z. Spectra from extended ETD reactions also look noisy due to secondary fragmentations.
- By subtracting protons, PTR following ETD generates charge reduced c/z⁺ ions that do not contain extra hydrogen. These ions show the expected masses and are more easily interpreted using standard data analysis software.
- Improved sensitivity, resolution, as well as faster scan rates in the LTQ Velos dual-pressure linear trap result in extensive sequence coverage of intact proteins of up to 30kDa in a single 10 minute experiment.
- With product ions of +5, +6 and even +7 charge resolved in full zoom scan mode, LTQ Velos instrument identifies many c/z⁺ product ions as big as 10 kDa in the complex spectrum of intact myoglobin and carbonic anhydrase.
- The number of identified ions, and thus the sequence coverage for the protein is significantly improved in the LTQ Velos dual-pressure ion trap compared to the LTQ XL ion trap.

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