

# 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 linear ion trap mass spectrometers for charge reduction following electron transfer dissociation (ETD), to investigate the utility of ETD with PTR for intact protein sequencing, and to compare the results obtained by two different ion trap mass spectrometers.

**Methods:** Common intact proteins of varying sizes were analyzed on LTQ XL ETD linear ion trap and LTQ Velos dual-pressure linear ion trap mass spectrometers equipped with ETD and PTR.

**Results:** ETD-PTR analysis of intact proteins up to 30 kDa generated very informative and well-resolved spectra. Using full zoom scan mode, the LTQ Velos instrument fully resolved product ions of +5, +6 and even +7 charge. In a single 10-minute experiment, it identified many c/z<sup>+</sup> product ions as large as 10 kDa in the complex spectra from intact proteins. The number of identified ions, and thus the sequence coverage, was higher in the LTQ Velos instrument. This was attributed to its greater sensitivity, resolution, and scan rate.

## Introduction

Electron transfer dissociation (ETD) is a favored tool for intact protein analysis because it is relatively insensitive to the size, amino acid composition and post-translational modifications of proteins. It randomly cleaves protein / peptide backbone bonds, generating very informative, but extremely complex spectra. These spectra can be difficult or even impossible to resolve at unit mass resolution due to the high charge states of the product ions. Therefore, charge reduction of product ions is necessary to generate a simplified spectrum that contains product ions of resolved charge states at unit resolution.

As illustrated in Figure 1, charge reduction, and hence reduction of spectral complexity, can be achieved through either extended electron transfer or through proton transfer reaction. Both processes are carried out using the same equipment. Partitioning between the two pathways depends on the reagent used. When the reagent is fluoranthene, electron transfer is the dominant pathway. When the reagent is benzoic acid, proton transfer is the major pathway.

Charge-reduced product ions from extended ETD reaction times often contain extra hydrogens that cause a mass shift as shown in both Figure 1 and Figure 2. These ions are usually not assigned during database searches (the colored peaks in Figure 2 are ions identified by the database search). Extended ETD reaction also generate internal fragments as noise peaks which complicate the data analysis. ETD-PTR, on the other hand, produces charge-reduced product ions with expected masses for database searches. ETD-PTR spectra are also less noisy. Sequence coverage from ETD-PTR experiments is better than that from extended ETD experiments (figure 2).

FIGURE 1. Charge reduction can be achieved through electron transfer or proton transfer.

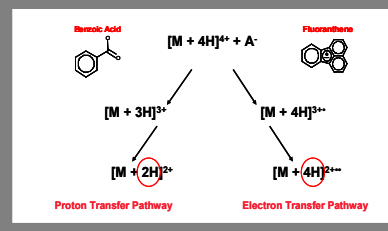
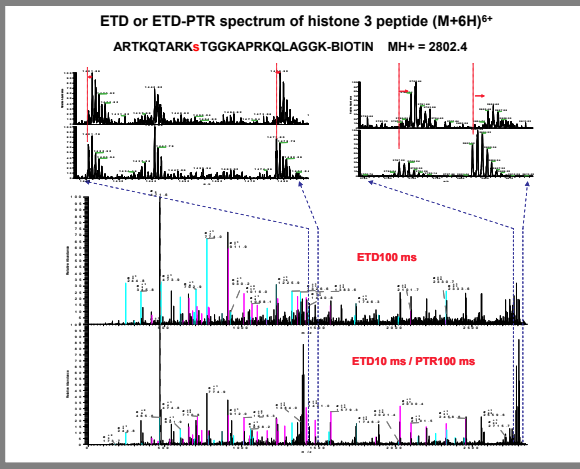


FIGURE 2. Extended ETD can produce mass shifts (red arrows) that reduce the number of product ions identified (colored peaks) by database searching. ETD-PTR does not produce mass shifts and results in more product ions identified.



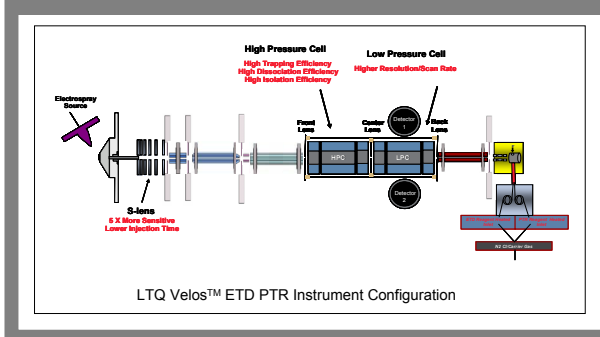
## Methods

Standard peptides were purchased from Anaspec. Intact proteins were purchased from Sigma. Desalted intact proteins were 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).

Analyses were performed using Thermo Scientific LTQ XL and LTQ Velos ion trap mass spectrometers equipped with ETD and PTR. LTQ 2.6 software with developer's kit provided instrument control. Benzoic acid anions generated in the chemical ionization source at the rear of the instrument were used as the PTR reagent. The anion target was 2e5. Activation time was 5 - 10 ms for ETD and 25 - 50 ms for PTR.

The LTQ Velos™ ion trap features several innovations (Figure 3) not present in the LTQ XL™ instrument. These include S-lens ion optics and a dual-pressure ion trap. The S-lens optics improve ion transmission. The dual-pressure ion trap improves ion trapping, isolation, dissociation, and scanning efficiencies. Together, these innovations improve sensitivity by 5 - 10 times and also improve mass resolution and scan rate compared to the LTQ XL instrument.

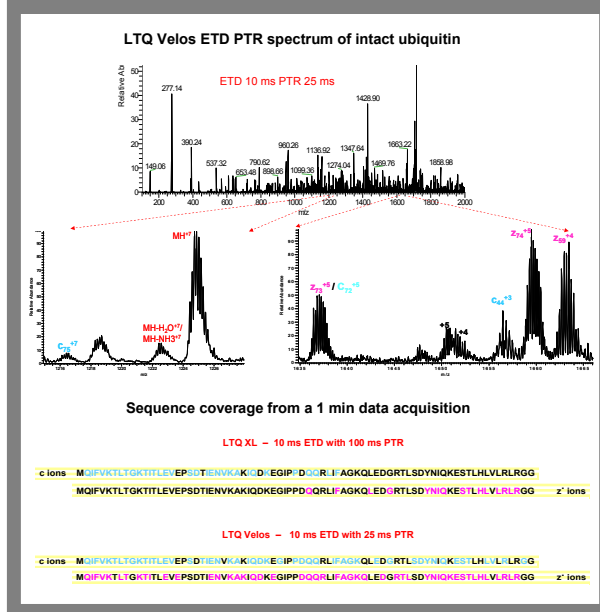
FIGURE 3. LTQ Velos with ETD.



## Results

Intact ubiquitin was analyzed using both the LTQ XL and LTQ Velos instruments. PTR reaction times were adjusted individually on each instrument (LTQ XL: 100 ms, LTQ Velos: 25 ms) to reduce the charge states of the product ions enough that they could be resolved under full scan zoom mode (LTQ XL: +1 to +3, LTQ Velos: +1 to +7). On both instruments, ETD-PTR with spectral averaging of 1 minute resulted in extensive sequence coverage. The LTQ Velos instrument operated at 10 ms ETD and 25 ms PTR (Figure 4) produced significantly better coverage; a total of 221 c<sup>+</sup> and z<sup>+</sup> ions were identified. Some of them were present in the spectra as multiply charged ions. The largest c<sup>+</sup> and z<sup>+</sup> ions (c<sub>25</sub> of 8502.6 Da and z<sub>25</sub> of 8413.6 Da) were both identified as multiply charged species. The superior performance of the LTQ Velos was attributed to its increased sensitivity, mass resolution, and scan rate.

FIGURE 4. Sequencing of intact ubiquitin using ETD-PTR.



Intact myoglobin and carbonic anhydrase were also analyzed using both instruments. As shown in Figure 5, using the LTQ XL ion trap with ETD and PTR, extensive sequence coverage is obtained for ubiquitin and myoglobin proteins while less ions are identified in a larger protein such as carbonic anhydrase due to the limited resolving power.

Averaging 10 minutes of ETD-PTR spectra, the LTQ Velos produced even better sequence coverage (Figure 5 and Figure 6). A total of 155 and 187 c/z<sup>+</sup> ions were identified from intact myoglobin and carbonic anhydrase, respectively. Under this experimental condition, the largest c/z<sup>+</sup> product ions identified were c<sub>96</sub> of 10610.7 Da and z<sub>94</sub> of 10267.6 Da for myoglobin, and c<sub>83</sub> of 9326.6 Da and z<sub>87</sub> of 9936.2 Da for carbonic anhydrase.

Overall, the LTQ Velos dual-pressure ion trap identified 2 - 3 times as many product ions as the LTQ XL ion trap for each of the intact proteins. It also provided significantly better sequence coverage, particularly for larger proteins. This was attributed to its greater sensitivity, mass resolution, and scan rate.

FIGURE 5. Improved identification from top-down sequencing using ETD with PTR in a dual-pressure linear ion trap.

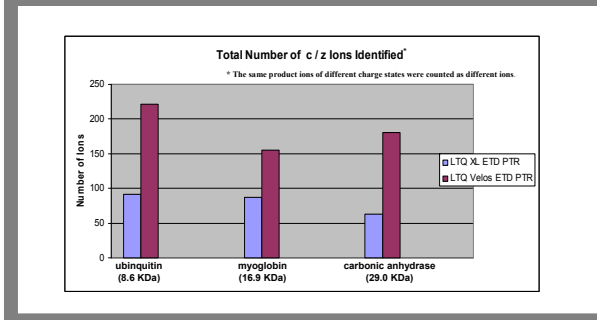
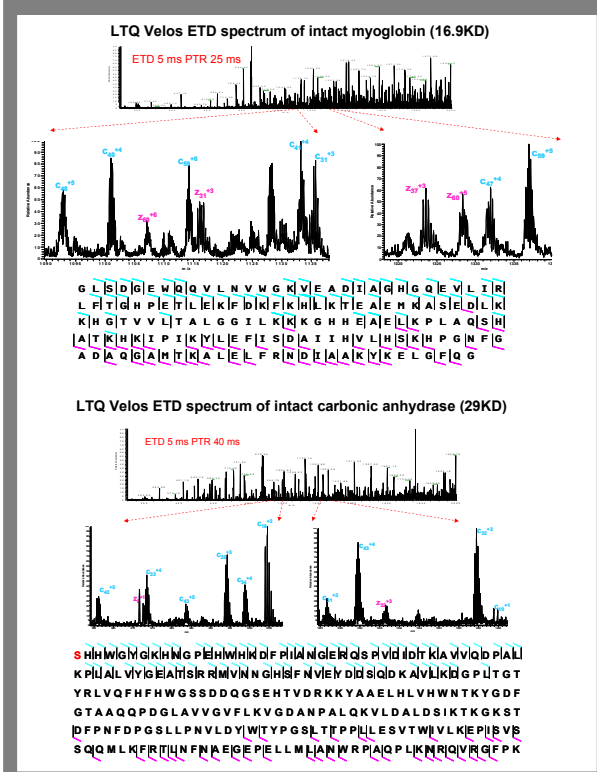


FIGURE 6. Top-down sequencing of myoglobin (top) and carbonic anhydrase (bottom) using LTQ Velos ETD with PTR. Results are from 10 min data averaging.



## Conclusions

- Electron transfer dissociation (ETD) is very useful for intact protein analysis, but it produces complex spectra that can be difficult or even impossible to resolve at unit mass resolution due to the high charge states of the product ions.
- Electron transfer or proton transfer can be used to reduce the charge levels on product ions, making them more amenable to analysis. Proton transfer avoids mass shifts and spectral noise common in electron transfer.
- ETD combined with charge reduction by proton transfer reaction (PTR) allowed both the LTQ XL and LTQ Velos ion trap mass spectrometers to analyze intact proteins of 9 - 29 kDa.
- The LTQ XL ion trap produced good sequence coverage for intact ubiquitin and myoglobin proteins but identified somewhat fewer ions in the larger carbonic anhydrase protein due to the instrument's more limited resolving power.
- The LTQ Velos dual-pressure ion trap identified 2 - 3 times as many c/z<sup>+</sup> product ions, and hence produced significantly greater sequence coverage, for all proteins analyzed. The difference was greater for larger proteins.
- In a single 10-minute experiment, the LTQ Velos ion trap produced extensive sequence coverage for intact proteins up to 30 kDa.
- In full zoom scan mode, the LTQ Velos instrument was able to resolve product ions of +5, +6 and even +7 charge. It identified c/z<sup>+</sup> product ions as large as 10 kDa in the complex spectra of intact myoglobin and carbonic anhydrase.

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