Protein N- and C-terminal Sequencing Using Electron Transfer Dissociation Mass Spectrometry

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Abstract

Purpose: To apply electron transfer dissociation (ETD) mass spectrometry to top-down protein sequencing. To optimize such application for protein N- and C- terminal sequencing.

Methods: ETD was applied to top-down protein sequencing both in an LTQ Orbitrap XL ETD hybrid mass spectrometer and in an LTQ XL linear ion trap mass spectrometer equipped with ETD and proton transfer reaction (PTR) functionality. ETD with accurate mass and high resolution was employed in the Orbitrap instrument to study optimized reaction conditions for protein N- and C-terminal sequencing. In the unit-resolution linear ion trap, PTR was used to reduce the product ion charge state after ETD. The resulting spectra contain product ions of resolvable charge states at unit resolution.

Results: ETD is advantageous for sequencing intact proteins, because it randomly cleaves protein backbone bonds, generating information rich spectrum. Different ETD reaction times allow access to different parts of the protein, with shorter reaction times favoring the overall sequence coverage and longer reaction times increasing the terminal sequence coverage. When Orbitrap detection is available, high-resolution, accurate-mass ETD spectra of intact proteins are readily interpreted. Without high resolving power, PTR following ETD is needed to reduce product ion charge state, thus simplifying the spectrum for data analysis.

Introduction

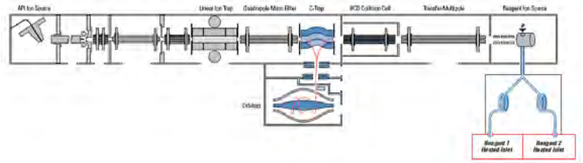
Mass spectrometry has drawn more and more attention as an alternative technology to traditional protein N-, as well as C-terminal, sequencing. Electron transfer dissociation (ETD) mass spectrometry is particularly advantageous for sequencing applications because ETD is relatively insensible to the size, the amino acid composition, and post-translational modifications of proteins, therefore randomly cleaves peptide backbone bonds. ETD of intact proteins is highly efficient, generating very informative, yet extremely complex spectra that contain highly charged product ions that are difficult, or even impossible to resolve at unit resolution. ETD technology was recently implemented in a hybrid linear ion trap - Orbitrap mass spectrometer whose high resolution and mass accuracy facilitate analysis of intact proteins using ETD. For unit resolution instruments, proton transfer reaction (PTR) following ETD was developed to reduce spectral complexity. PTR removes protons from multiply charged product ions, generating simplified spectra that contain product ions at charge states resolvable at unit resolution. In this study, the utility of an LTQ Orbitrap XL ETD and an LTQ XL ETD with PTR for intact protein sequencing was investigated.

Materials & Methods

Standard proteins were purchased from Sigma. Desalted intact protein was diluted in acetonitrile / water / formic acid (50:50:0.1) to a final concentration of 2 µM to 5 µM. The sample was directly infused using static nanospray with a 4 micron tip (Picotip™, New Objective). ETD was performed using a Thermo Scientific LTQ Orbitrap XL ETD or LTQ XL ETD with PTR. In the LTQ Orbitrap XL ETD™, as shown below, fluoranthene radical anions were generated in a reagent (CI) ion source. The anions were passed through a transfer multipole, HCD collision cell, c-trap, and quadrupole mass filter to the linear ion trap. The quadrupole operates as a low mass filter, ensuring purity of the reagent anions. Individual charge states of the protein molecular ions were selected for isolation and ETD in the linear ion trap. The resulting fragment ions were transferred to the Orbitrap for detection. The anion target was adjusted from 3e5 to 5e5 and the activation time was 2 - 100 msec. The resolving power of the Orbitrap was selected at 60,000 (FWHM). Data was collected for 5 min (200-300 micro scans).

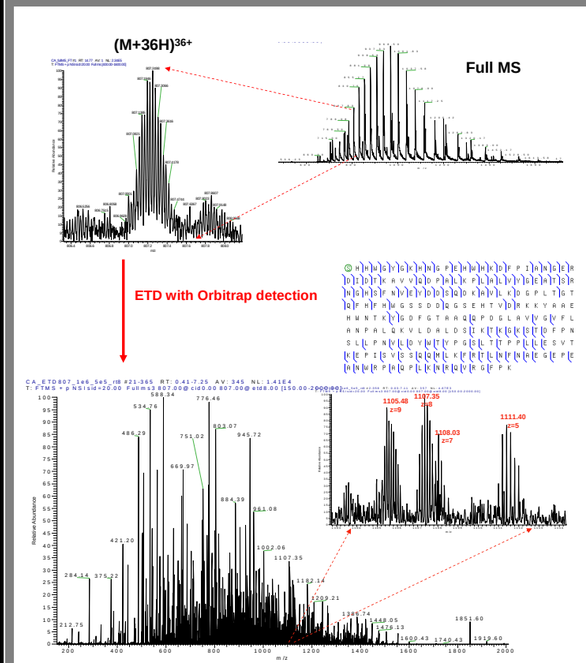
PTR was performed using LTQ XL™ with ETD and PTR capability under LTQ 2.6 instrument control software with developer's kit. Benzoic acid anions generated in the chemical ionization source at the rear of the instrument were used as a PTR reagent. The anion target was adjusted from 1e5 to 2e5 for both ETD and PTR. PTR reaction time was from 10 -100 msec.

Data analysis was performed using Thermo Scientific ProSightPC 1.0 software for deconvoluted high resolution ETD spectra with a fragment tolerance of 10 ppm, or manually for ETD-PTR spectra. All the search results were manually evaluated.



Results

FIGURE 1. Electron transfer dissociation of intact carbonic anhydrase (MW= 29KDa) with Orbitrap detection



- **Intact carbonic anhydrase ions carrying 36 charges were isolated for ETD.**
- **ETD generated an informative, complex spectrum.**
- **Charge states of the product ions were resolved using Orbitrap detection.**
- **Extensive sequence coverage was obtained from the deconvoluted accurate-mass spectrum.**

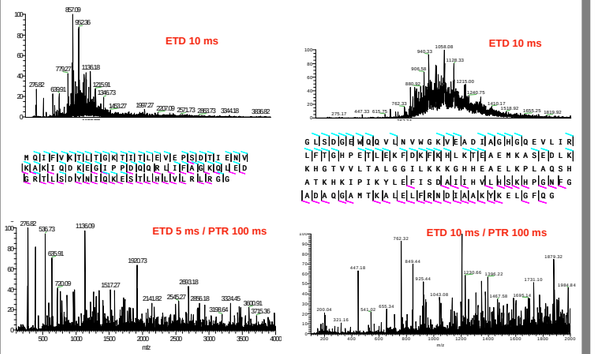
FIGURE 2. Effect of ETD reaction time on sequence coverage

Effect of ETD reaction time on total sequence and terminal sequence coverage

Reaction time (ms)	Total number of c ions (out of 239)	Total number of z ions (out of 239)	Number of c ions for N-terminal 30 amino acid (out of 27)	Number of z ions for C-terminal 30 amino acid (out of 26)
2	65	51	21	11
4	84	61	25	16
8	78	68	25	22
20	66	64	25	23
50	41	44	26	25
100	38	35	25	22

- **Optimized ETD reaction time for an intact protein is much shorter than for a peptide.**
- **Shorter reaction time generated larger product ions and better overall sequence coverage.**
- **Extended ETD reaction produced mostly shorter, terminal sequence ions, which is an ideal result for protein N- and C- terminal sequencing.**
- **Extended ETD reaction also generated *c/z* type ions containing extra hydrogen (c+1 and z+1 ions) from multiple electron transfer (data not shown). Such product ions can be missed by data analysis software due to the unexpected mass shift.**
- **For carbonic anhydrase in this study, 4 - 8 millisecond of ETD reaction produced the best overall sequence coverage while 50 millisecond of ETD resulted in the best N- and C-terminal sequence coverage. Under this condition, 26 (out of 27) c ions for N-terminal 30 amino acids (with three Pro), and 25 (out of 26) z ions for C-terminal 30 amino acids (with four Pro) were identified.**

FIGURE 3. Without high resolution, PTR is needed to simplify the ETD spectra by reducing the charge state of the product ions



- **When high resolving power is not available, PTR following ETD is needed to reduce spectral complexity.**
- **ETD without PTR generates information-rich spectra which are difficult to resolve at unit resolution (top panel). These spectra are hard, or even impossible to interpret.**
- **PTR removes protons from multiply charged product ions, generating a simplified spectrum containing product ions with charge states that allow them to be resolved at unit resolution (bottom panel). ETD-PTR spectra of intact protein can thus be interpreted and sequence information can be obtained (middle panels).**

Conclusions

- **LTQ Orbitrap XL ETD or LTQ XL ETD with PTR are promising tools for protein N- and C- terminal sequencing.**
- **ETD is particularly advantageous because it randomly cleaves protein backbone bonds, generating information-rich spectra.**
- **With Orbitrap detection, the complex, well-resolved, accurate-mass ETD spectra of intact proteins are easily interpreted.**
- **In the LTQ XL, PTR is available to reduce the ETD spectrum complexity so that terminal sequence information can be obtained from intact proteins.**