

A Simple and Efficient Concept for ETD and PTR in a Spherical Ion Trap

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Introduction

ETD (electron transfer dissociation) is the preferred method for the analysis of post-translational modifications (PTM) in proteins. It surpasses internal heating and generates rapid N-Cα bond cleavage along the amino acid backbone, leaving labile bonds like those to PTMs intact. For ETD, an excess of radical anions generated in a negative chemical ionization (nCI) source is added to multiply charged peptide cations in the ion trap. Fluoranthene is a commonly used ETD reactant. The addition of ETD to a spherical trap is very simple. Shown here is the setup as well as its extremely high efficiency.

Methods

- **HCTultra PTM Discovery** ion trap, incl. ETD and PTR (proton transfer reaction).
- Fluoranthene and a derivative is used for both modes. Anions are generated by an external nCI source.
- Scan speed for enhanced resolution (0.35 u peak width) 8100 u/sec.
- Scan range 50 – 3000 u
- Mass accuracy ± 0.15 u

Results

The spherical trap has a number of inherent features for fast and effective ETD/PTR:

1. The simultaneous storage of cations and anions:
 - the same transfer line as for the analytical ions can be used (fig. 1). This provides for a simple, space-effective setup.

- the ETD process starts as soon as the anions enter the trap, leading to fast and direct fragmentation (tab.1).
2. Cations and anions are mixed and compressed in a small globular volume resulting in an excellent ion-ion-cross section, highly efficient (fig. 2) and most sensitive ETD (fig. 3)
 3. The high m/z range of the spherical trap of 3000u with isotope mass resolution (fig. 4) is essential for a good sequence coverage in both ETD and PTR of proteins.

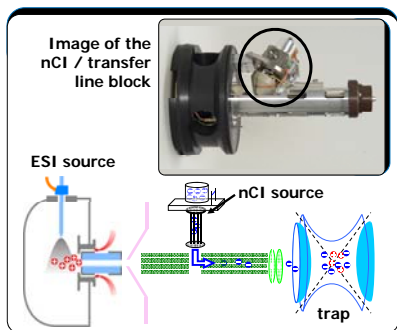


Fig. 1: Setup of the nCI source for ETD in the spherical HCTultra ion trap. The nCI source is built into the transfer line.

Time (ms)	HCTultra
Cation injection	20
Precursor isolation	60
Anion injection	1-5
Ion-ion reaction (ETD)	70
Prescan	-
Enhanced res. scan (m/z 100–2000)	235
Total	~390 msec

Table 1: Total data acquisition time for efficient ETD-MS/MS. No prescan is needed.

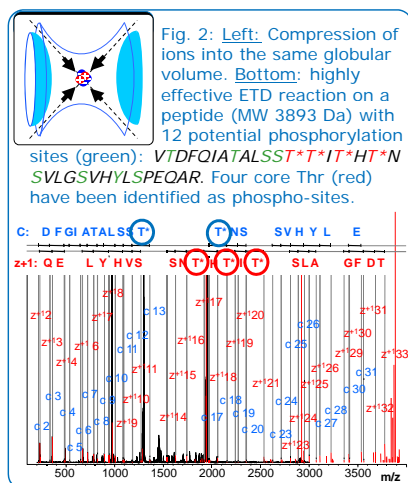


Fig. 3: DB search result of a nanoLC run with ETD-MS/MS of a 5 fmol Enolase tryptic digest (top). Sequence coverage is 32% with excellent data quality (bottom).

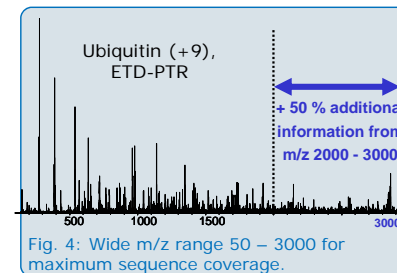


Fig. 4: Wide m/z range 50 – 3000 for maximum sequence coverage.

PTR is added for the fragmentation of larger peptides or small proteins in the top-down approach. Following the ETD reaction, PTR anions reduce the charge states of the highly charged fragments into “ion trap readable” numbers. While the ETD reactant is a strong electron donor (like fluoranthene), the PTR reactant must have a high proton affinity for efficient proton stripping: proposed were e.g., benzoate or PDCH. Handling several reactant reservoirs, plus different nCI sources, becomes complicated and prevents from effective and automated MS/MS. The HCTultra uses a single nCI and a single reactant. Fluoranthene is used for ETD. For PTR, its derivative, a basic anion, generated by modifying nCI parameters is used. A fast and automated switch-over between ETD and PTR is possible within a few ms.

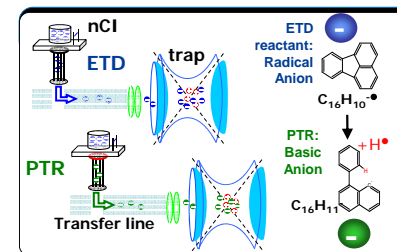


Fig. 5: Setup of ETD & PTR in the spherical trap.

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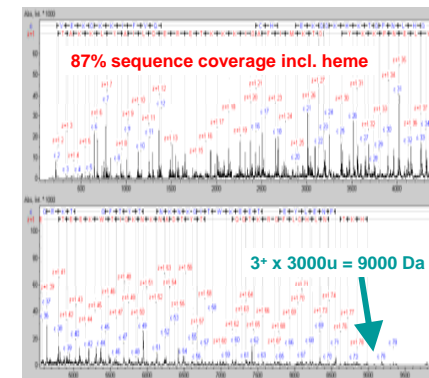


Fig. 6: ETD & PTR of intact Cytochrome c (MW 12360 Da) after deconvolution. The covalently bound heme stays intact with the protein (true ETD dissociation). The maximum reachable fragment mass of ~ 9000 Da is due to the m/z range of 3000u and the obtainable resolution of at least up to 3+ ions.

Conclusions

The advantages of the spherical ion trap for ETD & PTR could be shown:

1. Simultaneous storage of peptide cations and ETD/PTR anions
2. High m/z range of 3000 u with sufficient resolution for at least up to 3+ ions
3. Easy and fast operation of ETD / PTR just by changing electrical potentials in the nCI source
4. Combination of resolution for 3+/(4+) charge states and of high scan speed of 8100 u/sec
5. High mass accuracy (< 0.15 Da) for excellent database search scores.

Ion Trap
ETD / PTR