Implementation of Electron-transfer dissociation (ETD) and Electron-transfer/higher-energy collision dissociation (EThcD) on a modified Orbitrap hybrid MS

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Abstract

Purpose: Electron-transfer dissociation (ETD) and Electron-transfer/higher-energy collision dissociation (EThcD) were implemented on the Thermo Scientific™ Orbitrap™ Excedion Pro™ hybrid mass spectrometer enabling fast and efficient fragmentation for a variety of applications.

Methods: HeLa tryptic digest was separated via liquid chromatography and analyzed using DDA-ET(hc)D as reference to investigate fragmentation performance. Reaction time scaling as well as supplemental activation energy were varied systematically to optimize fragmentation quality and speed.

Results: Optimized ETD parameters, i.e., charge-dependent reaction times and normalized supplemental activation energies, are presented. High ion densities during reaction allow short ETD reaction times to be used even for low charge state precursors. MS2 scan rates of more than 15 Hz are demonstrated for EThcD. Improved fragmentation quality is shown compared to pure HCD fragmentation based on higher achievable cross correlation scores.

Introduction

Electron-transfer dissociation (ETD) is a well-established fragmentation technique complementary to collisional fragmentation approaches.[1] Fragmentation is induced by electron transfer from radical anions to multiply charged precursor cations in ion-ion reactions. Dissociation of the resulting unstable radical cations causes formation of primarily *c*- and *z-type* ions contrary to the *b*- and *y-type* ions resulting from collisional activation of peptides or proteins. Consequentially, ETD can offer additional sequence information that is especially useful for top- and middle-down experiments, de novo sequencing, as well as identification and localization of labile post-translational modifications.[2] In this study, a novel approach enabling fast and efficient ETD including optional supplemental activation for electron-transfer/higher-energy collision dissociation (EThcD) using the Orbitrap Excedion Pro mass spectrometer is presented.

Materials and methods

Sample preparation

Thermo Scientific™ Pierce™ HeLa Digest standard 20 µg/vial was reconstituted by adding 200 µL of 0.1 % Formic acid. The vial was subsequently sonicated for 5 min, followed by multiple sample aspiration and release cycles with a pipette to dissolve it completely.

LC/MS method

HeLa tryptic digest samples were separated on a 25 cm x 75 µm Thermo Scientific™ Aurora Ultimate™ column using a Thermo Scientific™ Vanquish™ Neo UHPLC system in direct injection configuration. Flow rate was kept at 200 nl/min during a 19.5 min gradient. The column with integrated emitter was inserted into a Thermo Scientific™ EASY-Spray™ source. The Orbitrap Excedion Pro MS was operated in a data-dependent acquisition (DDA) mode at a resolving power of 60,000 for MS1 and of 15,000 for MS2, respectively. Isolation window was set to $\Delta m/z$ = 1.2, cycle time was set to 1.5 sec. Precursor mass range was set to m/z = 375-1500, MS2 scan range was set to automatic. Settings for ETD fragmentation were systematically varied to determine optimal conditions.

Data analysis

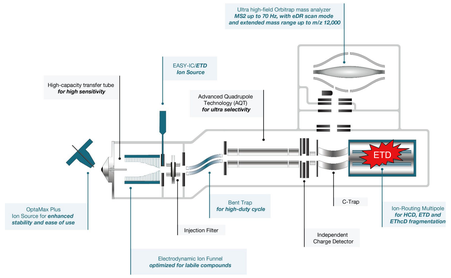
Post-processing of the acquired raw data was performed using Thermo Scientific™ Proteome Discover™ 3.1.1.102 software as well as custom data analysis scripts.

Instrumental setup

Hardware and software implementation

A schematic of Orbitrap Excedion Pro MS with ETD and EThcD capabilities is shown in Figure 1. Alternative fragmentation is enabled inside the ion routing multipole (IRM) by upgrading its functionality to allow dual polarity ion trapping. The latter is achieved by new electronics that allow radio-frequent (RF) voltages to be applied to the entrance and exit lens.

Figure 1. Schematic of the Orbitrap Excedion Pro mass spectrometer including ETD and EThcD functionality.



ETD is then realized by simultaneous storage of reagent anions and precursor cations in the IRM. The detailed sequence of an ET(hc)D MS2 scan is shown schematically in Figure 2.

Figure 2. Sequence of sub-events during an EThcD scan: Precursor injection → ETD Preparation → Reagent injection → Reaction → [Supplemental activation] → Orbitrap injection, parallelized to transient acquisition.

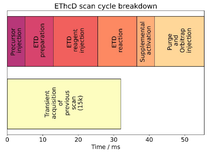
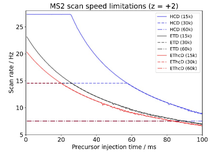


Figure 3. Exemplary scan rates for ETD/EThcD/HCD using resolutions of 15k/30k/60k as a function of precursor injection time for a precursor charge state of z = +2.



After the precursor ions were injected into the IRM front, they are transferred into the back of the cell while the polarity of the instrument's front-end ion optics is changed during the ETD preparation step. In the following sub-event reagent is injected into the IRM. To this end, fluoranthene radical anions are formed by the EASY-IC/ETD source and transferred to IRM using optimized soft settings. Reagent and precursor are stored separate from each other utilizing the axial DC gradient of the IRM.

As soon as the reaction is triggered, the gradient is switched off to allow reagent and precursor to mix and form fragment ions. At the same time, RF voltage is applied to the end caps of the IRM preventing ion losses of either ion polarity. Once the reaction time is completed, created ions can be activated collisional by an optional supplemental activation step. To realize this, ions can be transferred back to the C-Trap and then accelerated into the IRM using a defined energy. This is especially useful to overcome non-dissociative ETD events, or form b/y ions in addition to c/z-fragments created by ETD. Finally, all ions are injected into the Orbitrap for mass analysis. During transient acquisition new ions can be processed.

Measured MS2 scan rates for ETD/EThcD/HCD are plotted in Figure 3 as a function of the precursor ion injection time for a doubly charged precursor ion.

Calibration of reaction times

ETD reaction times t_{rct} were calculated automatically based on the precursor charge state z and a reaction constant k, according to the following equation:[3]

$$t_{rct} = \frac{2 \cdot f}{k_{MRFA} \cdot z^2}$$

A scaling factor f can be used to control the reaction extent of the pseudo-first reaction that is using an excess of reagent anions. By default (f=1) the precursor depletion is tuned to reach 1/e² ≈ 13.5 %.

The reaction constant k of the singly-protonated peptide MRFA is determined using a calibration procedure measuring the ion depletion as a function of reagent injection time and reaction time, as shown in Figure 4 and 5.

Figure 4. Logarithmic plot of the precursor depletion as function reaction time and reagent injection time.

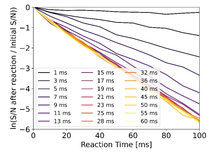
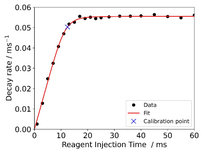


Figure 5. Resulting decay rates as function of reagent injection time with the determined calibration constant k_{MRFA}.



A charge-dependent calibration of reaction times can improve fragmentation performance substantially over fixed reaction times due to the pronounced charge dependency of ET(hc)D.

Results

Optimization of ET(hc)D conditions

A tryptic HeLa digest standard was chosen as a challenging reference sample due to its high complexity and low charge state distribution to test the performance of the ET(hc)D implementation. A series of ET(hc)D-DDA experiments using different settings was conducted to determine optimal fragmentation settings for best MS spectral quality while maintaining good scan rates.

The determined Sequest XCorr scores and the number of high confidence PSMs as function of the supplemental activation (SA) energy and reaction time (RT) scaling factor f are shown in form of heatmaps in Figure 6 and 7, respectively.

Figure 6. Average Sequest XCorr score for high confidence assignments as function of supplemental activation energy and reaction time scaling factor.

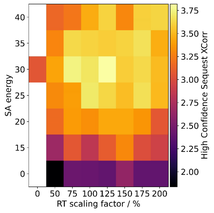
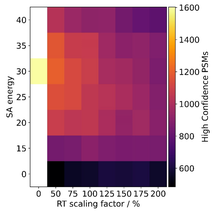


Figure 7. Number of high confidence PSMs as function of supplemental activation energy and reaction time scaling factor.



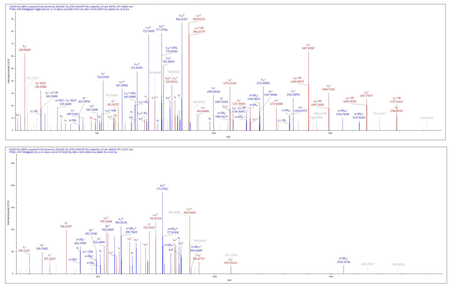
The two figures contain the average results for duplicates measured with ETD (SA energy = 0), HCD (RT scaling factor = 0) and EThcD (SA energy and RT scaling factor > 0) fragmentation.

With respect to the Sequest cross correlation score XCorr (Fig. 6), best results are obtained for EThcD using SA energies of 30 (normalized collision energy, NCE) and reaction time scaling of close to 100%, only minor differences are observed between 75% and 125%.

Regarding the number of PSMs (Fig. 7), reducing the RT scaling factor can help to increase the analysis depth by increasing the achievable scan speed. SA energies of 30 (NCE) deliver the highest number of PSMs.

Exemplary EThcD and ETD spectra employing determined optimal conditions, i.e. using a supplemental activation energy of 30 (NCE) as well as a reaction time scaling of 100%, for the peptide TSSAQVEGGVHSLHSYEK are shown in the following Figure 8.

Figure 8. MS2 spectra of TSSAQVEGGVHSLHSYEK using EThcD with z = +3 (top) and ETD with z = +4 (bottom).



Performance indicators for the used fragmentation techniques are compared in Table 1.

EThcD exhibits lower numbers of PSMs than HCD due the lower MS2 scan rate but provide better scores indicating improved confidence in assignment. ETD without supplemental activation is falling behind the other approaches due the high abundance of low charge state precursors.

Table 1. Comparison of different fragmentation techniques available on the Orbitrap Excedion Pro mass spectrometer based on various metrics for LC-MS DDA runs of a tryptic HeLa digest.

	PSMs	XCorr	# MS2	ID rate	MS2 rate* / Hz
HCD	1602.8	3.06	16267	0.099	21.7
EThcD	1086.4	3.68	12471	0.087	14.2
EThcD-50%	1202.1	3.42	12969	0.093	15.2
ETD	571.875	2.42	12856	0.044	14.9

* Median scan rate between retention time of 5 min and 20 min filtered for MS2.

Conclusions

- Electron-transfer dissociation (ETD) and Electron-transfer/higher-energy collision dissociation (EThcD) were implemented on the Orbitrap Excedion Pro MS
- Tryptic digest of HeLa was investigated as complex reference standard by LC-MS / DDA-ET(hc)D to determine a set of good fragmentation settings for peptide samples
- EThcD MS2 scan rates of up to 15 Hz were observed with Sequest XCorr scores indicating improved confidence in PSM assignment over HCD and highlighting the potential of ET(hc)D for applications like de novo sequencing, as well as identification and localization of labile post-translational modifications

References

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