Overview

Purpose: To investigate potential approaches to intact protein analysis using Electron Transfer Dissociation (ETD) or Linear Ion Trap Mass Spectrometry.

Methods: A fraction of the sample was isolated by reversed-phase chromatography and then analyzed by ETD LC/MS/MS. The peptide mass fingerprints (PMF) were searched against the SwissProt database. The combined sequence coverage from ETD and CID is 55.5%.

Results: The intact MUPs were digested with trypsin. The ETD and CID spectra were acquired in a LTQ linear ion trap mass spectrometer. The MS/MS spectra were results of up to 3000 transients. The MS/MS spectra were analyzed using SEQUEST to identify the peptides. The identified peptides were then searched against the SwissProt database.

Conclusions: The combination of ETD and CID methods provides increased sequence coverage compared to the use of either method alone. The ETD method is particularly useful for the analysis of large peptides and moderate-size intact proteins.

Analysis of Mouse Urinary Proteins: A Combined Electron Transfer / Collision-Induced Dissociation Strategy with Linear Ion Trap Mass Spectrometry

Zhig Hao1; Jennifer Zhang1; Sarah R Hart1; Duncan HL Robertson2; Robert J Beynon1; Simon J Gaskell1 and Andreas FR Huhmer1

1University of Manchester, Manchester, United Kingdom; 2University of Liverpool, Liverpool, United Kingdom; 3Thermo Electron Corporation, San Jose, CA

Electron Transfer Dissociation (ETD) on the LTQ linear ion trap mass spectrometer is a very effective tool for the analysis of large peptides and moderate-size intact proteins. ETD shows ECD-like polypeptide fragmentation patterns, and is implemented on the linear ion trap mass spectrometer. ETD on the LTQ linear ion trap generated spectra and ion series which were comparable to those from FT-ICR instruments. A novel fragmentation method, electron transfer dissociation (ETD) shows ECD-like polypeptide fragmentation patterns, and is implemented on the linear ion trap mass spectrometer.

**Figure 1**

Electron Transfer Dissociation (ETD) of Intact MUPs. A: Full MS spectrum of intact MUPs. The inset shows the sequence of MUPs. B: Comparision of ETD and CID spectra. C: Full MS spectrum of ETD of intact MUPs containing tryptic changes in the sequence of MUPs. D: CID spectra of intact MUPs containing tryptic changes in the sequence of MUPs. The Combined sequence coverage from ETD and CID is 55.5%.

**Figure 2**

Sequential coverage of ETD and CID on intact MUPs. Fragments generated by 10% CID on intact MUPs. Fragments generated by 10% ETD on intact MUPs. The Combined coverage from ETD and CID is 60.3% vs. 60.1% for ETD and CID, respectively. The Combined coverage from ETD and CID is 60.3%. The Combined coverage from ETD and CID is 60.3% vs. 60.1% for ETD and CID, respectively.

**Figure 3**

The Combined coverage from ETD and CID is 60.3% vs. 60.1% for ETD and CID, respectively.

**Figure 4**

Identification of MUPs performed using "bottom-up" ETD. A: Accurate mass and sequence of the intact MUPs. The peptides identified by 10% ETD which contain modifications are in black. Those with modifications not seen in CID are in gray. ETD spectra of the identified peptides which contain modifications are in red. Fragments generated by CID in the MS/MS spectrum are in red. Those with mutations are in black. Other modifications are in gray. The Combined sequence coverage from ETD and CID is 55.5%.