

ECD and CID of Peptide Ions in a Hybrid Linear Ion Trap-Permanent Magnet FT ICR Mass Spectrometer

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OVERVIEW

● First demonstration of the Electron Capture Dissociation (ECD) in an FT ICR instrument with a permanent magnet (PM)

● Implementation of the Collision Induced Dissociation (CID) in the Linear Ion Trap (LIT) of the PM FT ICR instrument

INTRODUCTION

The design and the performance of the LIT-PM FT ICR instrument equipped with an atmospheric ionization source were reported elsewhere^{1,2} (Please also see the ASMS 2007 poster MPD 058). Currently, several modifications to the reported design are being implemented in order to further improve the instrument's performance and extend its analytical capabilities. The capability of performing tandem mass spectrometry (MS/MS) experiments is of vital importance for analytical applications such as proteomics. In this work, we demonstrate the feasibility of implementing MS/MS in the PM FT ICR instrument. Two MS/MS methods, CID in the LIT and ECD in the ICR cell were employed to fragment the peptide ions generated by ESI.

INSTRUMENTATION

MODIFICATIONS FOR CID

The linear-ion-trap region of the hybrid LIT-PM FT ICR mass spectrometer^{1,2} was modified to perform CID as detailed in the following schematics (Figure 1).

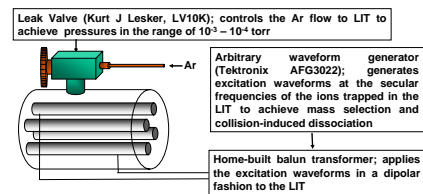


Figure 1. Modifications in the LIT region for CID

ASSEMBLY FOR ECD

The dispenser cathode assembly used in the ECD experiments is illustrated below (Figure 2).

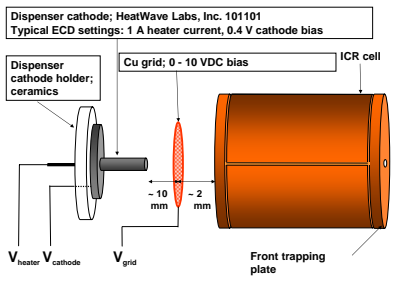


Figure 2. The dispenser cathode assembly mounted after the ICR cell.

PRELIMINARY RESULTS

CID IN THE LINEAR ION TRAP

Performing CID for the ions trapped in the LIT includes several steps such as the isolation of the parent ions of interest followed by the application of the RF waveforms at the ions' resonance frequencies to cause their collision-induced fragmentation with the buffer gas. The mass selection of the fibrinopeptide A 2+ ions from a mixture of six peptides in the LIT is demonstrated in Figure 3. This was achieved using a SWIFT waveform calculated in MathCAD using an algorithm described by Doroshenko et. al.³ that contained all the resonance frequencies for the mass range shown except for a notch (center 86 kHz and width of 8 kHz) at the resonance frequency of fibrinopeptide A 2+ ions.

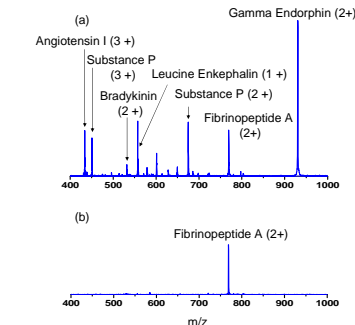


Figure 3. Demonstrating the mass selection of fibrinopeptide 2+ ions from a six-peptide mixture. (a) LIT-PM FT ICR mass spectrum for a six peptide mixture (b) The same peptide mixture after the mass selection waveform was applied to select the fibrinopeptide A 2+ ions.

Figure 4 is an MS/MS spectrum obtained for bradykinin 2+ ions showing several fragment ions resulting from the CID with Ar gas introduced into the LIT.

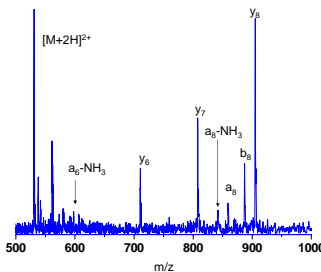


Figure 4. An LIT-PM FT ICR CID Spectrum for bradykinin 2+ ions

ELECTRON CAPTURE DISSOCIATION

ECD is a fragmentation technique that typically produces c and z type fragments for peptide and protein ions^{4,5,6}. In that sense it is orthogonal to CID which predominantly produces b, a and y fragments. The main difficulties for implementing ECD in the PM FT ICR instrument are the greatly increased pressure in the ICR cell region due to the cathode outgassing (with the "hot" dispenser cathode used) and the relatively high inhomogeneity of the magnetic field of the permanent magnet that could affect the introduction of the low-energy electron beam into the ICR cell. Figure 5 shows an ECD spectrum obtained in the LIT-PM FT ICR instrument for substance P. The lifetime of the signal was ~ 5 ms due to the increased pressure and limited the resolution in the spectrum. The inset shows the ECD efficiency for substance P vs cathode DC voltage bias. ECD efficiency was determined as the (total fragment ion intensity)/ (parent ion intensity) after ECD. The optimal cathode voltage bias was found to be 0.4 V at a heater current of 1 A and a trapping plate voltage of 1.3 V.

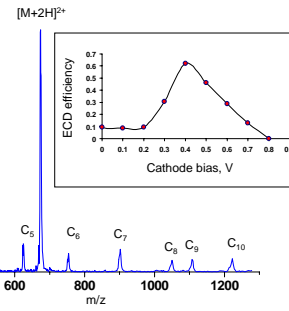


Figure 5. An ECD spectrum for substance P. Estimated ICR cell pressure is $10^7 - 10^6$ torr. Irradiation time is 4 s. Inset: ECD efficiency vs cathode voltage bias. Estimated ICR cell pressure is $10^7 - 10^6$ torr.

CONCLUSIONS AND FUTURE DIRECTIONS

For the first time, ECD and CID ion fragmentation methods were successfully incorporated into the LIT-PM FT ICR mass spectrometer demonstrating the MS/MS capability of the instrument.

The performance of the current ECD setup will improve after achieving lower pressure conditions. In order to implement this, efforts are underway to increase the pumping efficiency in the ICR cell region and to employ "cold cathodes" for ECD experiments.

Also, an increase in the detection sensitivity will improve the signal-to-noise ratio of the MS/MS signals. For this purpose, the preamplification system will be replaced with a more sensitive one in the future.

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