Development and Evaluation of Atmospheric-Pressure Electron Capture Dissociation (AP-ECD) for the LC/MS Analysis of Protein Digests

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Novel Aspect

update on the development and performance of a novel AP-ECD source

Introduction

Atmospheric-pressure electron capture dissociation (AP-ECD) is an emerging technique for peptide analysis, suitable in principle for use with any electrospray mass spectrometer. We have recently developed a novel AP-ECD ion source utilizing nanospray for peptide ionization and photoionization of a dopant for electron generation. This source provides extensive production of c- and z-type ECD fragments from multiply-charged peptides, with detection limits in the low fmol range for samples delivered by infusion. To obtain this performance, however, manual procedures have to date been required for data collection and processing, and consequently our AP-ECD source has yet to be successfully utilized in on-line LC/MS applications. We aim to develop a fully automated AP-ECD method using our source, and then to evaluate its performance in the LC/MS analysis of protein digests.

Methods

Model proteins (e.g. BSA) will be enzymatically digested, separated by on-line LC (Ultimate LC, LC Packings), then delivered to the AP-ECD source and a Q-ToF instrument (QStar XL, AB Sciex) for mass analysis. The complete method will be automated to the greatest extent possible, to better demonstrate how it might be used in real applications. Also, as part of the performance evaluation, the data files generated by the method will be input into standard search engines (e.g. Mascot) to assess the compatibility of AP-ECD with conventional data processing tools.

Preliminary Data

To obtain high quality AP-ECD spectra of peptides with our source, it is important to eliminate background signals from nanosprayed solvent clusters/impurities, precursor "nozzle-skimmer" CID products, and photoionization by-products, since there is no precursor selection stage in-source and all the ions exiting the source contribute to the spectra generated. We have recently shown (paper submitted to JASMS) that the nanospray background continuum and CID products can be easily removed by subtracting background spectra (acquired with the photoionization lamp off) from the raw AP-ECD spectra (acquired with the lamp on). An additional subtraction step is required to remove the background of photoionization by-products, and there are a couple of ways to do this. Through these

procedures, clean, low-background spectra of ECD products formed at atmospheric pressure are attainable. To date, however, the data acquisition and processing procedures required to achieve this performance have been manual, and thus impractical for on-line LC applications. Automation of the method will require a means of switching the photoionization power supply in-synch with the data acquisition method, so that ECD and background spectra can be collected alternately, continuously throughout the LC run. Implementation of this scheme is a work in progress. Once completed, the performance of the automated method will be evaluated in the LC/MS analysis of various protein digests.