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

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Introduction

- ECD and ETD are normally performed using late-model ion trapping instruments, in which peptide or protein ions are trapped, isolated, then reacted with electrons or anions to produce characteristic fragment ions.
- We have developed a novel in-source atmospheric pressure (AP)-ECD method as an alternative to conventional *in vacuo* ECD or ETD, capable of providing ECD functionality to all types of electrospray mass spectrometers, without modification of the instrument.
- Here, we demonstrate the use of AP-ECD in the LC/MS analysis of a protein digest.

AP-ECD Method

- The AP-ECD source is comprised of a sprayer, a spray chamber, and a source block with a photoionization detector (PID) lamp, suitable for use with any electrospray instrument.

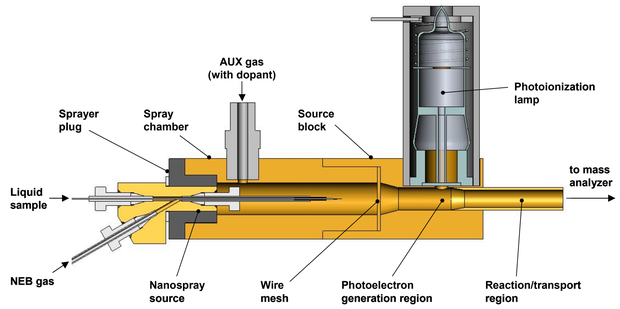


Figure 1. AP-ECD source.

- In operation, multiply-charged peptide ions are created within the spray chamber by the enclosed nanospray source. These ions are transported through the spray chamber by a flow of gas to a downstream source block whose central channel may be irradiated by the photoionization lamp; the source block is heated to "activate" the nanosprayed ions prior to and during ECD.
- When the lamp is switched off, peptide ions pass through unaffected, and the source operates as a normal nanospray source. When the lamp is switched on, photoelectrons are generated by photoionization of the dopant (e.g. acetone) added to the auxiliary (AUX) gas.
- Photoelectrons are then captured by the peptide ions in the downstream atmospheric pressure reaction/transport zone, resulting in ECD, and the fragment ions produced are delivered through the vacuum interface of the instrument for subsequent mass analysis.

Data Processing

- Raw AP-ECD spectra contain a background of solvent and impurity ions, formed by both electrospray and photoionization, because there is no precursor isolation in-source. Also, depending upon the conditions, there can be substantial levels of interface ("nozzle-skimmer") CID products of residual (post-EC) sample precursors.
- The ECD products can be isolated by a two-step post-acquisition data processing procedure:

  1) Background subtraction [involves selecting the chromatographic peak and adjacent area(s) to be subtracted, then implementing the regular background-subtract function; removes the background of ESI- and APPI-generated solvent and impurity ions]

  2) CID-product subtraction (requires acquisition of spectra without electron production – i.e. with the lamp off – which may then be subtracted from the raw AP-ECD spectra)

Experimental

- AP-ECD source: uses nanospray emitters (New Objective) for peptide ionization and a PID lamp (Heraeus Noblelight) for generation of electrons via photoionization of the acetone dopant (0.2 μl/min); the auxiliary gas is high-purity nitrogen (~9 slpm); source T ~120ºC. See **Figure 1**.
- Mass Spectrometer: unmodified QStar XL Q-ToF from AB SCIEX; scan = TOF MS (200-1800 Da, 2 sec accumulation/scan); interface Declustering Potential (DP) = 50/90 V (50/50 time split).
- Chromatography: LC Packings Ultimate with Famos autosampler; column = 15 cm x 75 μm ID RP C18; flow = 300 nL/min; mobile phase: A = 0.1% formic acid in water, B = 0.1% FA in 80/20 acetonitrile/water; gradient = 10 - 50%B over 90 minutes; 1 μl full-loop injection.
- Sample: 0.1 μM bovine serum albumin (BSA) tryptic digest; **100 fmol** on-column.
- Data acquisition: separate runs performed with lamp on and lamp off, to enable post-acquisition removal of CID products (see Data Processing).

Results

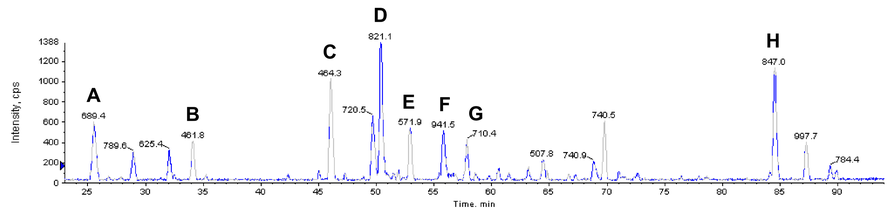


Figure 2. AP-ECD base peak chromatogram of 100 fmol BSA tryptic digest. Peak labels indicate spectra below.

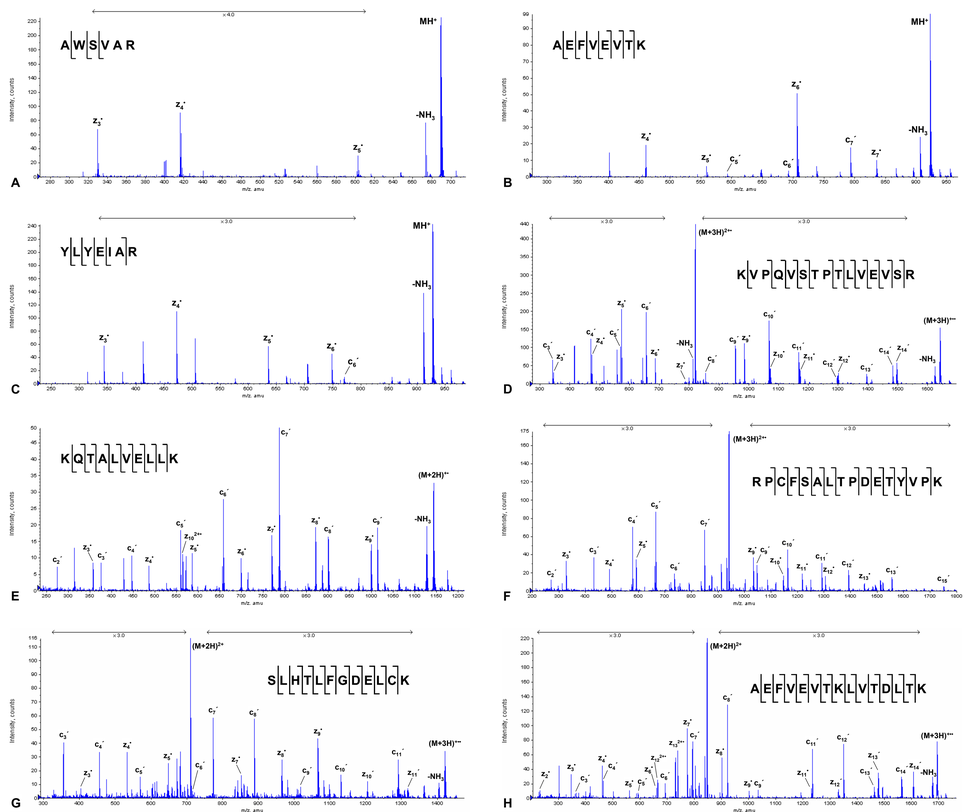


Figure 3. AP-ECD spectra of BSA tryptic peptides. Peptides A-C: 2+ precursors only; peptides D-H: 2/3/(4)+ precursors.

Results (cont'd)

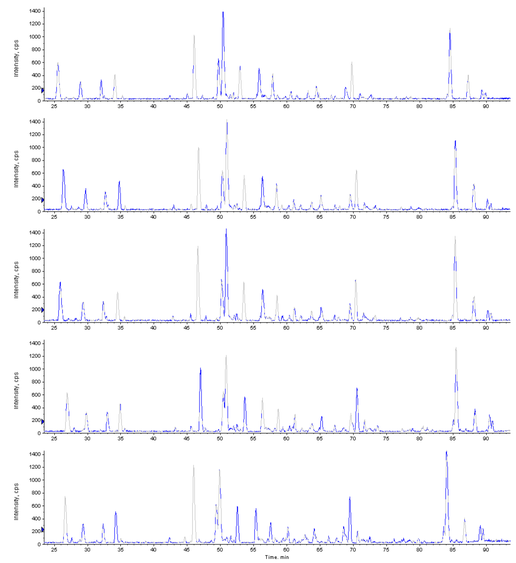
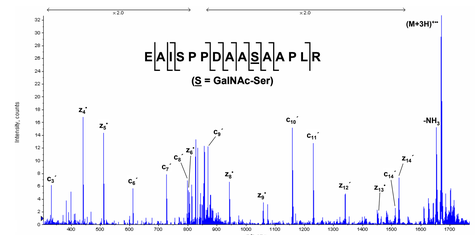


Figure 4. AP-ECD base peak chromatograms from 5 consecutive 100 fmol injections of BSA tryptic digest, demonstrating overnight stability for the method (12+ hours continuous operation).



BONUS. AP-ECD spectrum of glycosylated Erythropoietin (EPO) (117-131) (100 fmol on-column), demonstrating that labile modifications may be retained during fragmentation.

Conclusions

- AP-ECD is suitable for the LC/MS analysis of tryptic protein digests, as well as peptides with labile modifications.
- Method sensitivity provides low-fmol on-column detection limits.
- Performance (sequence coverage) is comparable to "activated ion" ECD/ETD.
- Sample prep and chromatography are required to isolate peptides prior to introduction to the source (because of parallel fragmentation).
- AP-ECD could be a useful tool for the targeted analysis of peptides and proteins.

Acknowledgments

- Major funding for this work was from an "Invention - Tools, Techniques and Devices" Catalyst Grant from the Canadian Institutes for Health Research (CIHR).
- Additional funding was from a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada, and also UBC.