Evaluation of a Nanospray Atmospheric Pressure-Electron Capture Dissociation (AP-ECD) Ionization Source for the Analysis of Post-Translational Modifications

Damon Robb,[1] Jason Rogalski,[2] Davin Carter,[2] Michael Blades,[1] and Juergen Kast[2];

The Department of Chemistry[1] and The Biomedical Research Centre,[2] The University of British Columbia, Vancouver, Canada

Introduction

• Electron capture dissociation (ECD) and electron transfer dissociation (ETD) have emerged as powerful tools for the analysis of post-translational modifications.

• 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 model phosphorylated and glycosylated peptides.

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.

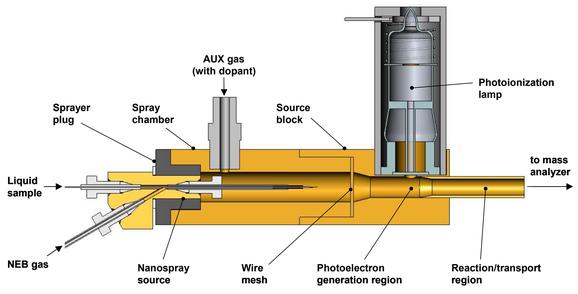
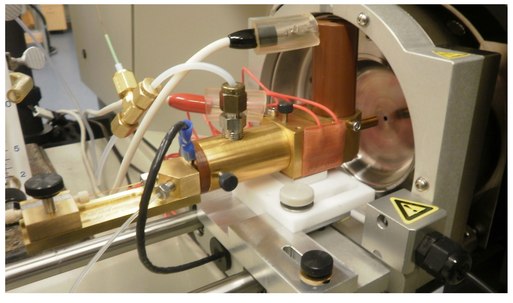


Figure 1. AP-ECD source.

• 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.



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 (2 sec accumulation/scan); interface Declustering Potential (DP) = 60/100 V (50/50 time split) for phosphopeptides, and 50/90 V for glycopeptides.

• 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 for phosphopeptides and 10 - 40%B over 40 minutes for glycopeptides; 1 μl full-loop injection.

• Samples: 0.1 μM solutions of phosphorylated and glycosylated peptides (from AnaSpec and ProteaBiosciences, respectively); **100 fmol** on-column for each peptide.

• Data acquisition/processing: separate runs performed with lamp on and lamp off, to enable post-acquisition removal of CID products.

Results - Phosphopeptides

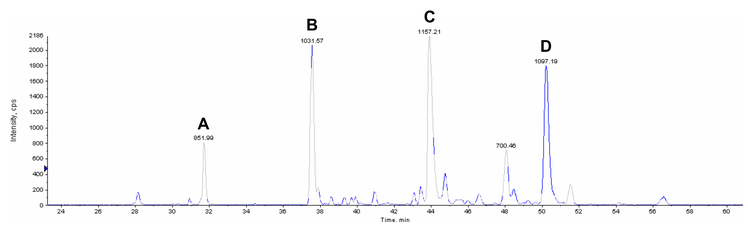


Figure 2. AP-ECD base peak chromatogram of a phosphopeptide mixture (100 fmol each). Peak labels indicate spectra below.

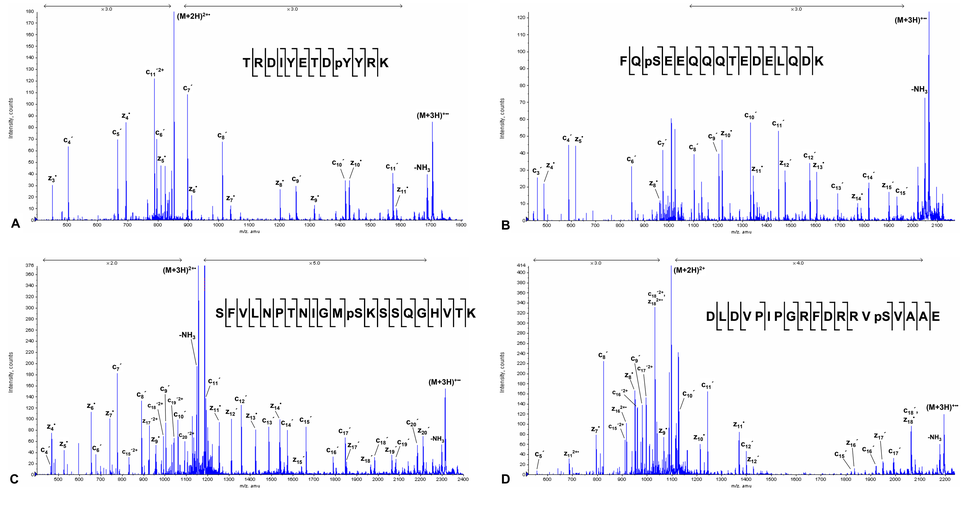


Figure 3. AP-ECD spectra of phosphorylated peptides (100 fmol). Phosphorylated peptides dissociate to produce high-coverage fragment ion sequence ladders, while retaining the modification in its native state. Charge-directing residues (especially arginine) likely affect the distribution of fragment ions produced (evident in **D**). In sum, high S/N for phosphorylation-containing fragment ions is achievable from low-fmol sample quantities.

Results - Glycopeptides

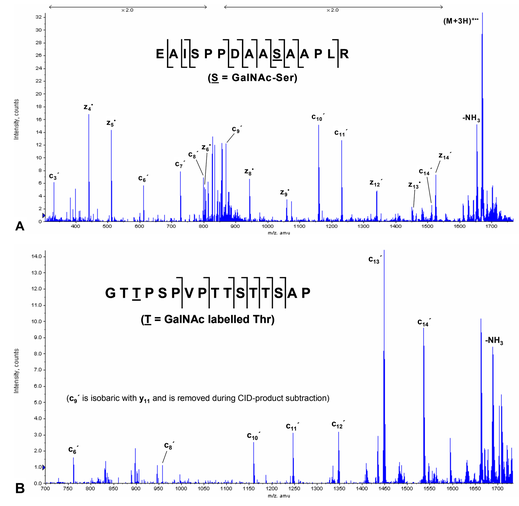


Figure 4. AP-ECD spectra of model glycopeptides (100 fmol).

A. Glycosylated Erythropoietin (EPO) (117-131), demonstrating near-complete sequence coverage with no loss of the CID-labile GalNAc modification.

B. Glycosylated MUC5AC 3, a difficult sample for ECD/ETD because there are no basic residues, multiple prolines, and a y-ion isobaric with a c-ion; regardless, the spectrum again demonstrates that the labile GalNAc modification is retained during AP-ECD.

Conclusions

• AP-ECD is suitable for the LC/MS analysis of phosphorylated and glycosylated peptides.

• 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 modified 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.