Atmospheric Pressure Electron Capture Dissociation (AP-ECD): Further Development and Evaluation for Localization of Labile PTMs on Sulfopeptides and Glycopeptides

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

[1] The Biomedical Research Centre, [2] The Department of Chemistry, 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 modifications to the instrument.

• We have previously shown the effectiveness of the AP-ECD source for tryptic protein digests and localization of phospho modifications on peptides at low fmol levels.

• Here, we demonstrate the use of AP-ECD in the LC/MS analysis of model sulfated and glycosylated peptides and explore the interface energetics on retention of these labile modifications.

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. The AP-ECD source is suitable for any electrospray instrument.

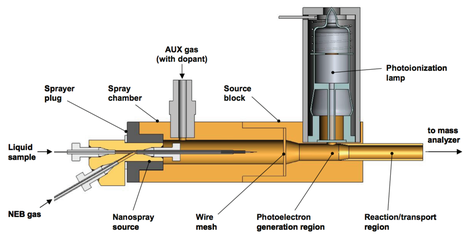


Figure 1: *AP-ECD source*

• Multiply-charged peptide ions are created within the heated spray chamber by the enclosed nanospray source. These ions are transported through the spray chamber by a flow of gas to the photoelectron generation region.

• Electrons are produced from interactions between photons, generated from a photoionization lamp, and acetone dopant (D) added to the auxiliary (AUX) gas: $D + h\nu \longrightarrow D^+ + e^-$

• Photogenerated electrons interact with peptide ions to cause in-source ECD preserving labile modifications. The ions are then introduced to the mass spectrometer.

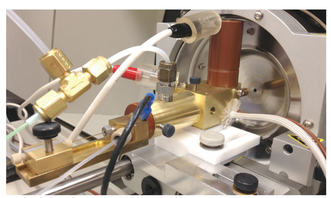


Figure 2: *AP-ECD interfaced with a QStar XL Q-ToF from AB Sciex*

Experimental

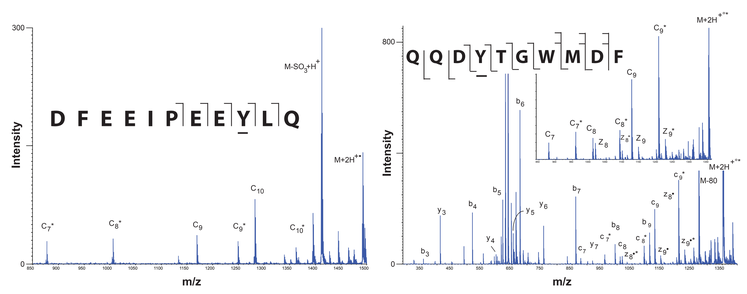
• AP-ECD source: uses nanospray emitters (New Objective) for peptide ionization and a PID lamp (Heraeus Noblelight) for generation of electrons via photoionization of the acetone dopant (0.2 ul/min); the auxiliary gas is high-purity nitrogen (~7 l/min). The source temperature was varied from 100 to 150 °C. The nanospray voltage was 2.8 kV and the voltage of the ion source block was 1.2 kV. The declustering potential was varied from 40 to 100 volts.

• Mass Spectrometer: unmodified QStar XL Q-ToF from AB Sciex; scan TOF MS (1 sec accumulation/scan)

• Chromatography: LC packings Ultimate with Famos autosampler, column 10 cm x 75 um RP C18; flow = 200 nl/min; A standard gradient of: mobile phase: A= 0.1% formic acid in water, B = 0.1% FA in acetonitrile/ water.

• Samples: Solutions containing 100 fmol to 1 pmol of peptide were injected on column.

Results - Sequencing and Localization



A: *AP-ECD spectra of sulfated hirudin*

B: *AP-ECD spectra of sulfated caerulein*

C: AP-ECD spectra of glyco EPO

D: *AP-ECD spectra of glyco MUC5AC*

Figure 3 (a-d): AP-ECD spectra of two sulfo and two glyco peptides. The peptide sequences were determined from the extensive fragmentation. AP-ECD could also localize the modifications. Of special note was the ability to determine the location of the sulfo group on Caerulein in contrast to previously reported efforts.

Results - Parameters affecting retention of modification

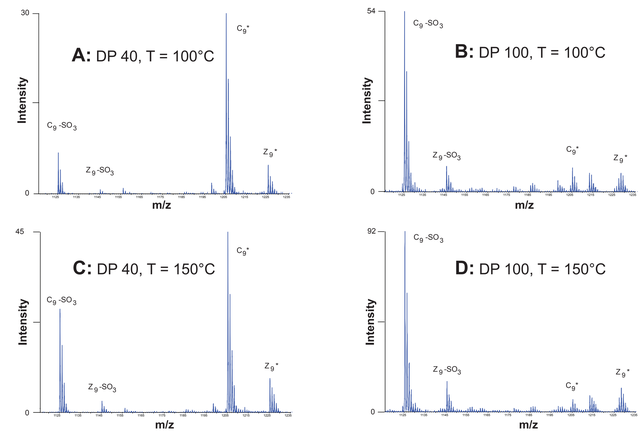


Figure 4 (a-d) illustrates the effect of DP and temperature on representative c and z ions of sulfated caerulein where desulfated ions are more prevalent at higher DP and higher temperature.

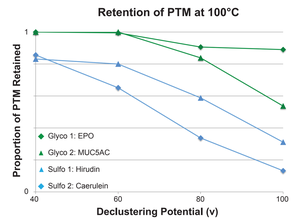


Figure 5a: DP *effect on PTM retention at 100°C*

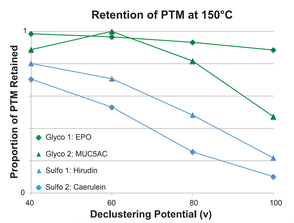


Figure 5b: *DP effect on PTM retention at 150°C*

The effect of varying declustering potential and temperature for two sulfo and two glyco peptides is shown in figure 5. At high DP and temperature, the loss of modifications was greater than at low DP and temperatures. The loss of sulfo modifications was greater than the loss of glyco modifications.

Conclusions

• Localization of sulfation and glycosylations and sequence determination was achieved by AP-ECD.

• The effect of DP and source temperature was determined. Retention of PTMs was greatest at low DP and temperatures.

• AP-ECD could be useful tool for the targeted analysis of modified peptides and proteins

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