Fusing Atmospheric Pressure ECD (AP-ECD) with nanospray to track Post-Translational Modifications with a "standard" mass spectrometer

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Overview

We have recently added the capability of performing ECD to our nanospray CID Q-ToF. A modified AB Sciex source was interfaced with a QStar XL Q-ToF so that ions from a nanospray emitter could be exposed to photoelectrons generated from acetone to induce electron capture dissociation. With the photoionization lamp off, and no photoelectrons present, ionized peptides were admitted into the MS as in traditional nanospray - allowing conventional high sensitivity LC-MS. When the photoionization lamp was on, the resulting photoelectrons caused ECD in source. C- and z-type fragment ions were admitted into the mass spectrometer, allowing high sensitivity LC-AP-ECD- MS. AP-ECD is also able to produce fragment ions with labile PTMs retained, allowing for both sequencing and localization of the PTMs, equivalent to modern ECD or ETD available on specialized instruments. The new AP-ECD source can be incorporated in-line on any atmospheric pressure ionization instrument; in fact, switching to this source has enabled our Q-ToF MS to identify labile modifications on peptides at the fmol level with no need for ion trapping.

Introduction

The comprehensive analysis of proteins requires sophisticated instrumentation, and mass spectrometers are currently the preferred tools for these analyses. In contrast to traditional CID, electron capture dissociation and its related technique, electron transfer dissociation, offers direct identification and localization of labile PTMs, but generally requires specialized mass spectrometers. In CID, energy from collisions can be randomized throughout the molecule causing the weakest bonds to break first leading to only partial sequence coverage. Importantly, labile PTMs, such as glycosylations, are lost prior to backbone fragmentation so that they cannot be localized. In comparison, ECD and ETD happens on a much faster time scale so that the fragmentation energy isn't randomized causing bond cleavages to be more evenly distributed along the backbone. Importantly, fragment ions retain their labile modifications allowing for localization.

The potential for photo generated electrons to be used for peptide dissociation was first reported by Laprévote et al. using an unmodified PhotoSpray™ atmospheric pressure photoionization source. Subsequent work in our lab with a modified PhotoSpray™ lamp gave promising results of infused peptides at the mid fmol level. This most recent, purpose built, iteration can be interfaced with chromatography, making it a useful addition to API mass spectrometers for tracking PTMs at the low fmol levels.

Methods

- Peptides were dissolved in water acidified with 0.1% formic acid
- Substance P, trypsin digest of BSA and glycosylated MUC5AC 3 were examined.
- 1 uL of sample solution was injected via a Famos LC onto a lab made C18 analytical column
- Upon ignition of the photoionization lamp, photoelectrons produced from acetone are captured by the analyte cations, and ECD fragments are formed in source.
- Mass Spectrometry was performed on an Applied Biosystems/ MDS Sciex QStar XL.

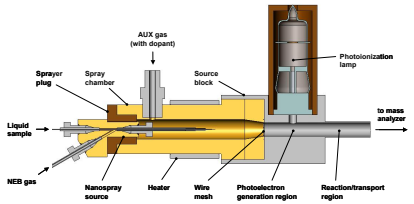


Figure 1: AP-ECD apparatus integrated into existing LC

Electrons are produced from interactions between photons generated from a modified photoionization lamp and acetone by the reaction:

$$h\nu_{(energy = 10.0\ eV)} + acetone_{(IE = 9.69\ eV)} \longrightarrow acetone^{+} + e^{-}$$

Ions are sprayed from the emitter into the spray chamber where they mix with acetone dopant and are carried past the photon source. Photons of sufficient energy ionize the acetone dopant releasing electrons that can interact with the analytes causing dissociation.

Results

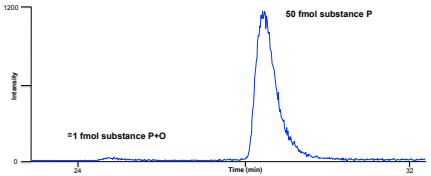


Figure 2a: 50 fmol on column substance P
Extracted Ion Chromatogram of C5 from a 50 fmol injection of substance P. Note the peak to the left attributed to a substance P+O M(O) side product estimated at 2% by peak area

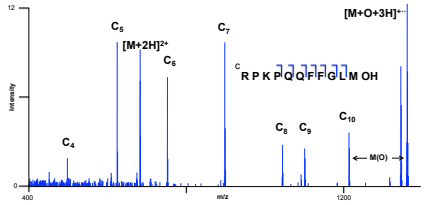


Figure 2b: Mass Spectra of substance [P+O]⁺
Mass spectra of ≈1 fmol [P+O]⁺ with strong signal to noise response and good backbone cleavage.

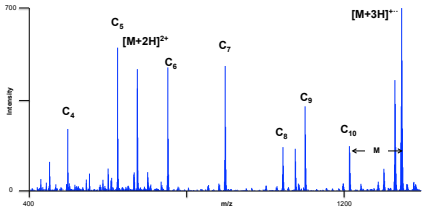


Figure 2c: Mass Spectra of Substance P
Mass spectra of ≈50 fmol with strong signal to noise signal and good backbone cleavage.

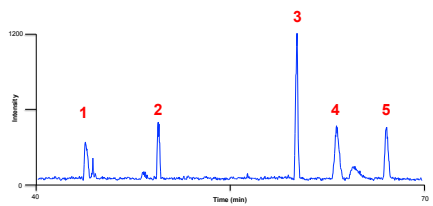
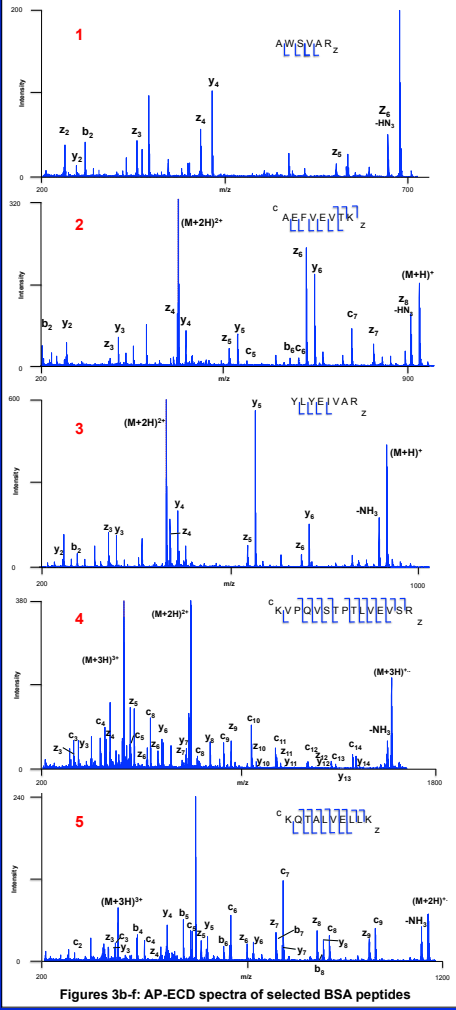


Figure 3a: Chromatogram of peptides from BSA digest



Figures 3b-f: AP-ECD spectra of selected BSA peptides

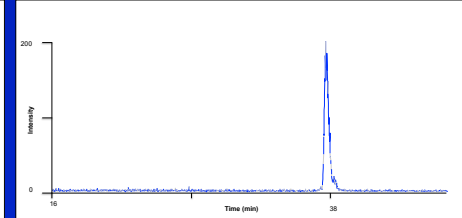


Figure 4a: 250 fmol on column Glycosylated MUC5AC 3

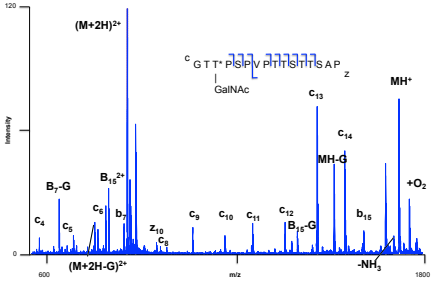


Figure 4b: AP-ECD mass spectra with retention of the GalNAc modification

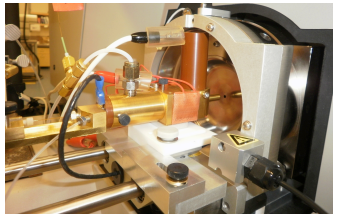


Photo 1: AP-ECD apparatus interfaced with AB Sciex Source

Conclusions

- AP-ECD has been interfaced with nanoLC-MS
- AP-ECD was found to be sensitive to the low fmol levels
- AP-ECD is suitable for tryptic digests
- Labile PTMs are retained in AP-ECD

Acknowledgements

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