Atmospheric Pressure Electron Capture Dissociation (AP-ECD): Localization of Labile Post-Translational Modifications on Sulfopeptides

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Overview

We are evaluating our in-source atmospheric pressure (AP)-ECD method as an alternative to conventional in vacuo ECD or ETD, capable of providing ECD capability to all types of electrospray mass spectrometers, without modification of the instrument.

Here, we demonstrate AP-ECD localizing modifications of sulfopeptides and survey previous AP-ECD results of glyco and phospho peptides.

Introduction

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 sulfations, are lost prior to backbone fragmentation so that they cannot be localized. In comparison, ECD and ETD happen 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.

AP-ECD Source

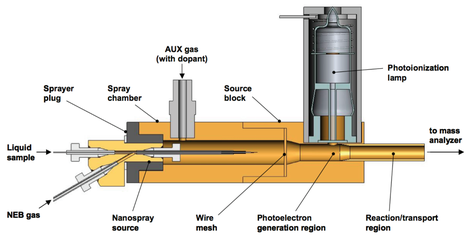

Figure 1: *AP-ECD source*

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

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

Photogenerated electrons interact with ions to cause in-source electron capture dissociation preserving labile modifications.

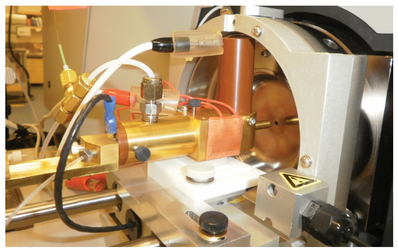

Figure 2: *AP-ECD interfaced with an AB Sciex source*

Results

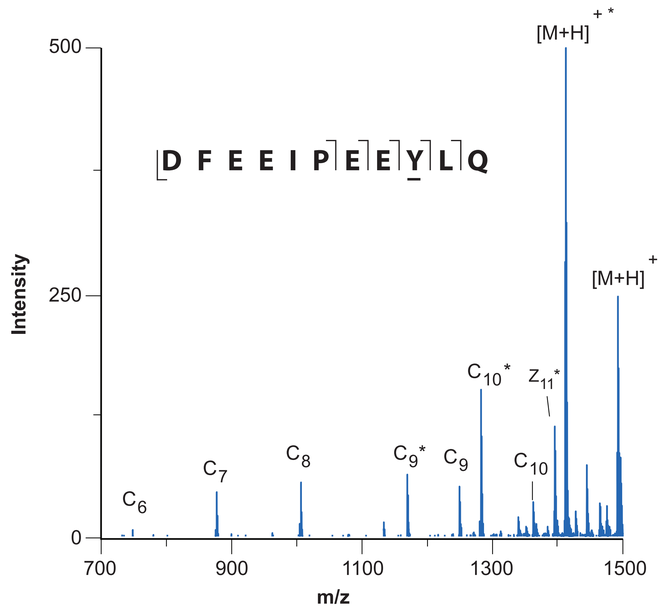

Figure 3: *AP-ECD spectra of sulfated hirudin*

The AP-ECD spectra of sulfated hirudin above shows retention of sulfate on many c-ions allowing localization of the labile modification. Loss of SO_3 was found in some c-ions, denoted C *.

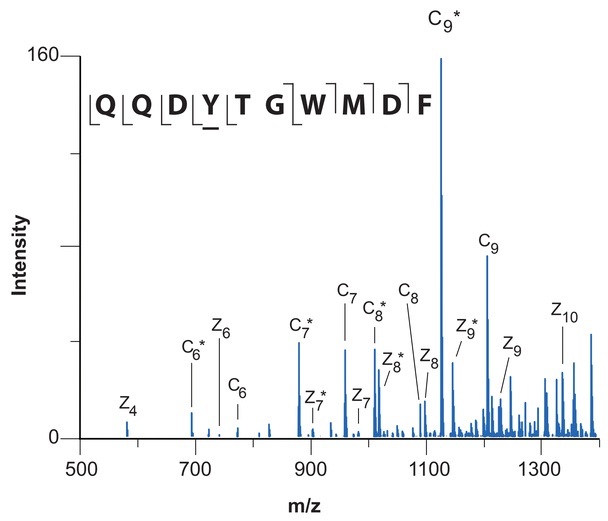

Figure 4: *AP-ECD spectra of sulfated caerulein*

The AP-ECD spectra of sulfated caerulein shows retention of sulfate on many c and z-ions allowing localization of the labile modification. Loss of SO_3 was found in some ions, denoted C *. Appearance of sulfated ions from caerulein is in contrast to previous FT-ICR ETD data (Medzihradszky et al., J. Am. Mass Spectrom, 2007, 1617-1624).

Other Results

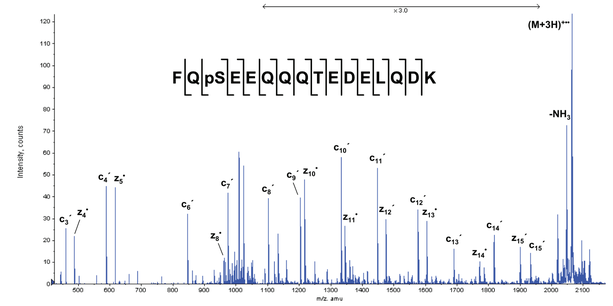

Figure 5: Localization of a Phosphopeptide

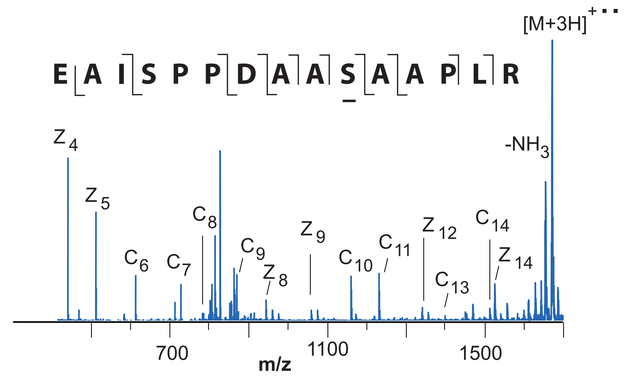

Figure 6: Localization of a Glycopeptide

In previous work AP-ECD has successfully localized modifications on both phosopho and glyco peptides.

Conclusions

AP-ECD has successfully localized sulfo modifications on two peptides. Combined with previous work where AP-ECD localized modifications on glyco and phospho peptides AP-ECD is proving to be a useful tool for targeted analysis of modified peptides and proteins.

Acknowledgements

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 of Canada, and also UBC.

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