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

Davin Carter [1], Jason Rogalski [1], Damon Robb [2], Michael Blades [2], Juergen Kast [1,2]
[1] UBC Biomedical Research Centre, [2] UBC Chemistry

Novel aspect
Localization of PTMs of sulfopeptides and glycopeptides by AP-ECD

Introduction
AP-ECD mass spectrometry is an emerging technique for analysis of labile Post-Translational Modifications. Labile PTMs are difficult to localize by traditional CID but are important for diagnosis and treatment of critical illness. In contrast to CID, ECD offers direct identification and localization of PTMs but requires specialized mass spectrometers whereas AP-ECD can be adapted to any API instrument. We have previously shown that AP-ECD is applicable at the fmol level, is effective on chromatographic time scales for mixtures and can localize modifications on phospho and glyco peptides. We have expanded our analysis to localize modifications of sulfo and additional glyco peptides and are optimizing interface energetics for our AP-ECD source.

Methods
Electrons generated from a photoionization lamp are used for Electron Capture Dissociation in a custom designed source that is interfaced with a QStar XL™. Using a Famos autosampler, samples are injected onto a lab-made C18 column and eluted using a standard peptide gradient. After the analytical column but prior to the mass spectrometer, peptides are exposed to photogenerated electrons. The electrons are produced from interactions between photons produced the photoionization lamp and a dopant, acetone. Ions are admitted into the QToF mass spectrometer where high sensitivity measurements of c and z ions take place. Simple data processing was done using Analyst.

Preliminary data
We are testing whether sulfo modifications on peptides can be localized by AP-ECD. Using caerulein, QQD(sulfo)YTGWMDF, we compared our AP-ECD results to those previously published for FT-ICR ECD. In the previous report of FT-ICR ECD analysis of Caerulein, the molecular ion and all applicable c and z ions displayed characteristic losses of 80 amu, loss of SO_3. In contrast, AP-ECD spectra show that the sulfo group is retained on many c and z ions (c_5-c_9 & z_7-z_9) and on the molecular ion, allowing for localization. Another sulfo peptide was successfully analyzed and the modification localized in hirudin, DFEEIPEE(sulfo)YLQ. The sulfo modification was retained on fragment ions (c_9 and c_{10}) allowing for localization and demonstrating AP-ECD usefulness where FT-ICR ECD had failed.

We are also examining AP-ECD for glycopeptides. In previous work, we successfully used AP-ECD in the analysis of EPO (EAISPPDAA(glyco)SAAPLR) and produced near complete sequence coverage localizing the glyco modification in both c and z ions. Another glycosylated peptide, MUC5AC3 (GT(glyco)TPSPVPTTSTTSAP), was analyzed and found to retain the glycosylation on various c ion fragments but due to the structure of the peptide the modification could not be localized. A lack of basic residues and multiple prolines made ECD/ETD difficult on MUC5AC3. We will be expanding to more glycosylated peptides to demonstrate the effectiveness of AP-ECD for O-linked monosaccharides.

We aim to more fully characterize AP-ECD parameters for optimal ion transmission and to limit in-source CID of labile modifications. We have investigated the effects of varying declustering potential from 40 to 150 volts and source temperature from ambient to 150°C. Generally, larger declustering potentials improve ion transmission but cause increased CID fragmentation leading to loss of labile modifications. Similarly, increased source temperature improves ion signal but causes preferential loss of modifications.

Session: Peptides: Ion Activation/Dissociation Strategies