Evaluation of a nanospray Atmospheric Pressure - Electron Capture Dissociation (AP-ECD) ionization source for the analysis of Post-Translational Modifications.

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Novel Aspect

Hardware and method development allowing for high sensitivity ECD of post-translationally modified peptides on traditional API instruments.

Introduction

Understanding the causes and effects of many biological functions demands a mechanistic understanding of Post-Translational Modifications (PTMs) of peptides and proteins, with tandem mass spectrometry being a powerful tool in these investigations. 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 a previously described apparatus, photo-induced electrons were generated at atmospheric pressure to create an in-source ECD interface that could be adapted to any API mass spectrometer. A new, second generation fragmentation/ionization source allows for integration with chromatography, making it a useful addition to API mass spectrometers for tracking PTMs in proteomic investigations.

Methods

A modified AB Sciex PhotoSpray™ source was interfaced with a QStar XL™ Q-ToF so that ions from a nanospray emitter could be selectively exposed to photoelectrons 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 and fragment ions were admitted into the mass spectrometer, allowing high sensitivity LC-AP-ECD-MS/MS. The performance of this source for the analysis of modified peptides was studied using synthetic phosphorylated, O- and N- glycoslated, sulfated and acylated peptides.

Preliminary Data

The new second generation AP-ECD source can produce nanospray sensitivity (low fmol) for peptides, and be interfaced to dissociate peptides from a chromatographic eluent stream. AP-ECD is also able to produce fragment ions with labile PTMs retained, allowing for both sensitive sequencing and localization of the PTMs, equivalent to modern ECD or ETD

available on specialized instruments. The new source, however, can be incorporated in-line on any API instrument. In fact, switching to this source has enabled our Q-ToF MS to localize labile modifications on peptides at the fmol level with no need for ion trapping. For example, the O-GalNAc modification on the glycosylated peptide, HLLVSNVGGDGEEIER, is not normally directly localizable on our instrument, as CID preferentially dissociates the labile modification producing either a neutral loss of 101.5Th from the doubly charged precursor, or marker ions at 204.1Th, 186.1Th and 168.1Th. Only with the AP-ECD source are we able to directly detect and localize the modifications. A c-ion series (c_4-c_{12}) is produced in source with no evidence of cleavage of the glycosidic bond. This source has produced sensitive ECD-MS/MS spectra for singly and multiply phosphorylated peptides, in addition to peptides modified with O-linked sugars. Current studies are benchmarking the effect of dopant flows (and available photoelectrons), declustering potentials, charge state and peptide size on the c-ion yield from labile post-translationally modified peptides relative to the previous generation sources, focusing on those that are difficult to study by CID - phosphorylation, O- and N-linked sugars, sulfation and acylation.