Electron Transfer Dissociation

Improving characterization of post-translational modifications

Tandem mass spectrometry is often used for the identification of proteins. Collision-induced dissociation (CID), the form of fragmentation typically used between stages of MS, works well for protein identification, but is less effective for the identification of post-translational modifications (PTMs). The new Agilent 6340 Ion Trap LC/MS can perform both CID and a different form of fragmentation: electron transfer dissociation (ETD). Ions produced through ETD complement those produced by CID, and together they present a fuller picture of both protein identities and the precise locations of chemical modifications.

Over the past few decades, MS-based protein identification strategies have been widely employed. Recent developments in separations and mass spectrometry instrumentation have enabled techniques such as shotgun proteomics whereby entire proteomes are enzymatically digested, chromatographically separated, and analyzed via tandem MS to obtain sequence information (Figure 1). Ion trap mass spectrometers have been an important instrument in proteomics analysis, and in particular the identification of post-translational modifications (PTMs).

Typically, tandem MS in an ion trap mass analyzer is achieved by a fragmentation process known as collision-induced dissociation (CID). During this process, the amide bonds are cleaved, producing a spectrum containing peptide fragment ions that differ in mass by a single amino acid. This permits the amino acid sequence to be read (de novo sequencing) or the measured peptide masses can be searched against a protein database.

While this technique has proven very effective for proteomics analysis, a major limitation has been the characterization of protein PTMs by ion trap MS. CID fragmentation of peptides containing important post-translational modifications such as phosphorylation or glycosylation commonly causes loss of the phosphate or sugar moiety, respectively.
Recently, an alternative fragmentation technique known as electron transfer dissociation (ETD) was developed. ETD offers several advantages for the characterization of protein PTMs. It is a softer fragmentation process that preserves labile chemical modifications such as phosphorylations or glycosylations.

ETD generates mostly c and z ions that complement the b and y ions commonly produced by CID. When ETD fragmentation is combined with CID fragmentation on the new Agilent 6340 Ion Trap LC/MS, the precise location of chemical modifications such as phosphorylations can be deduced in a single analytical run with high confidence.

Figure 2 shows MS/MS spectra of a phosphopeptide eluted during a data dependent LC/MS/MS experiment on a 6340 Trap equipped with the Agilent HPLC-Chip Cube MS interface (Figure 3). The top trace shows the MS/MS spectrum obtained from traditional collision-induced dissociation. The
spectrum is dominated by a peak corresponding to the loss of the phosphate moiety. It provides minimal sequence information with only a few b and y ions. This precludes the identification of the peptide sequence. By contrast, the ETD spectrum shown in the bottom trace displays a rich fragmentation pattern corresponding to cleavages across the peptide backbone. This enables easier sequence determination including the precise identification of the amino acid residue containing the post-translational modification (t).

ETD-type information was previously only achievable via electron capture dissociation (ECD) using a Fourier transform mass spectrometer (FT-MS). ECD analysis on an FT-MS instrument is a relatively slow process, and is not compatible with typical shotgun proteomics experiments done on a chromatographic timescale. ETD on the 6340 Trap is a fast process that is readily compatible with fast, high-resolution chromatography such as that achieved with HPLC-Chips.

Beyond incompatibility with a chromatographic timescale, there are numerous other disadvantages associated with the operation of an FT-MS instrument: ultra-high vacuum, high-field magnets, dedicated operator, and an approximately $1 million dollar pricetag. By contrast, the Agilent 6340 Ion Trap LC/MS with ETD permits characterization of PTMs at a price-point, and with the ease of use, that makes the technique vastly more accessible.

Figure 3. Agilent 6340 Ion Trap LC/MS equipped with an Agilent HPLC-Chip Cube MS interface.
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