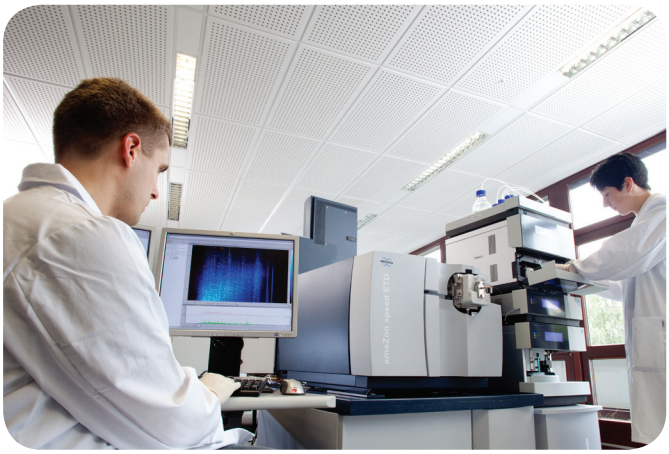

Technical Note # TN-42

Phosphoproteome mapping with the amaZon speed ETD

Introduction

Phosphorylation of proteins – one of the essential regulatory mechanisms in eukaryotic cells – is driven by the action of kinases. These kinases influence the information flow through signaling processes and consequently regulate cellular functions such as cell cycle, growth control and differentiation. Altered regulation of these processes is observed in a variety of diseases, including many cancers. The ability to detect, characterize and ultimately quantify the phosphoproteins altered in these pathologies is an important step toward a better understanding of cellular regulation.

Against a high background of abundant proteins, the low concentration level and ionization efficiencies of phosphorylated proteins and peptides make them difficult to analyze directly using mass spectrometry. MS instruments that are capable of covering a wide dynamic range and also offer alternative ionization techniques such as CID and ETD are required.

The amaZon speed ETD instrument is the latest and most powerful member of the performance-proven Bruker ion trap series. Compared to its predecessor, this flexible instrument offers a dramatically improved spectral duty cycle, covers a wider dynamic range and is the most sensitive ETD system on the market. The neutral-loss triggered ETD capabilities of the amaZon speed ETD (Fig. 1) enable full characterization of any phosphorylation site by combining ETD and CID information.

The zero-adjustment CaptiveSpray™ ion source provides robust, sensitive and easy-to-use ionization. Coupled with the state-of-the-art data evaluation capabilities of Bruker's ProteinScape™ software package, the amaZon speed ETD is a powerful tool for addressing the most challenging proteomic analyses. This study demonstrates the performance of this solution for a human cell line phosphoproteome mapping experiment.

Experimental

Human Raji B cell lysate was subjected to gel-assisted digestion [Mol. Cell. Proteomics 2008, 7, 1983-1997]. Subsequent phosphopeptide purification was performed as previously described [J. Proteome Res. 2008, 7, 4058-4069]. Eluted peptide samples were vacuum-dried, reconstituted in 0.1% (v/v) TFA (40 µL), and desalted and concentrated using ZipTips™ (Millipore, Bedford, CA). LC-MS/MS alternating CID/ETD acquisition was performed on a dual ion funnel amaZon speed ETD ion trap instrument (Bruker Daltonik, Bremen, Germany) coupled to an Ultimate 3000 nano-LC system (Dionex, California, USA) and equipped with a CaptiveSpray™ ion source (Bruker-Michrom, Auburn, CA).

Acquisitions were performed using a scan speed of 8,100 u/s in MS and 52,000 u/s in MS/MS. MS/MS acquisitions were performed in alternating CID/ETD mode with 5 precursors and active exclusion. Singly charged ions were permanently excluded. Precursor ion isolation for MS/MS was carried out using SMART isolation mode.

Three technical replicates were performed to assay the system's robustness and capability of delivering reliable and reproducible results.

Protein searches were performed using Mascot™ against a dedicated database. Carbamidomethylation was considered as a fixed modification whereas methionine oxidation and serine/threonine phosphorylation were considered as variable modifications. All results were stored and further analyzed using Bruker's ProteinScape 2.1 software package.

Results

The speed of acquisition and the high quality of the obtained CID and ETD spectra – combined with the use of dedicated bioinformatics software – enabled efficient mapping of phosphorylation sites for the majority of the peptides studied (Fig.2).

Using the amaZon speed ETD system, analysis of enriched phosphopeptides from a human cell line led to the reproducible identification of an average of 320 proteins in three LC runs. Of these proteins, 313 were phosphoproteins (Fig.3). In total, 506 phosphorylated peptides were found in all three replicates.

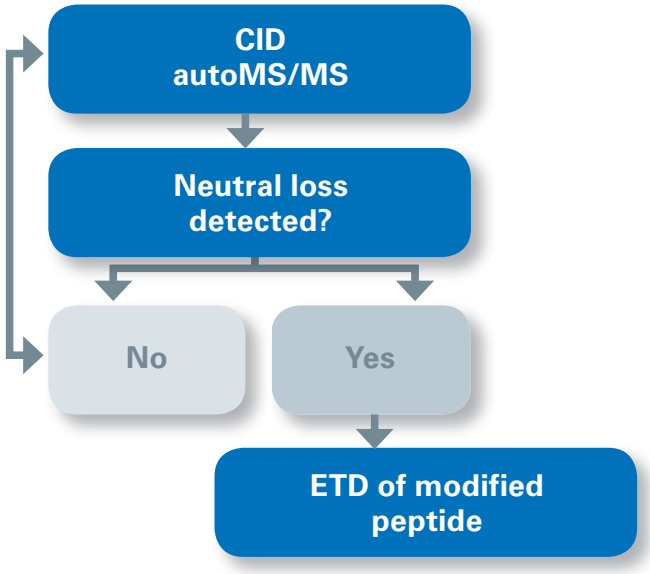

Figure 1: The advanced fragmentation modes of the amaZon speed ETD – such as CID neutral-loss triggered ETD – enable the extraction of maximum information from the sample by exploiting the complementary aspects of each fragmentation technique.

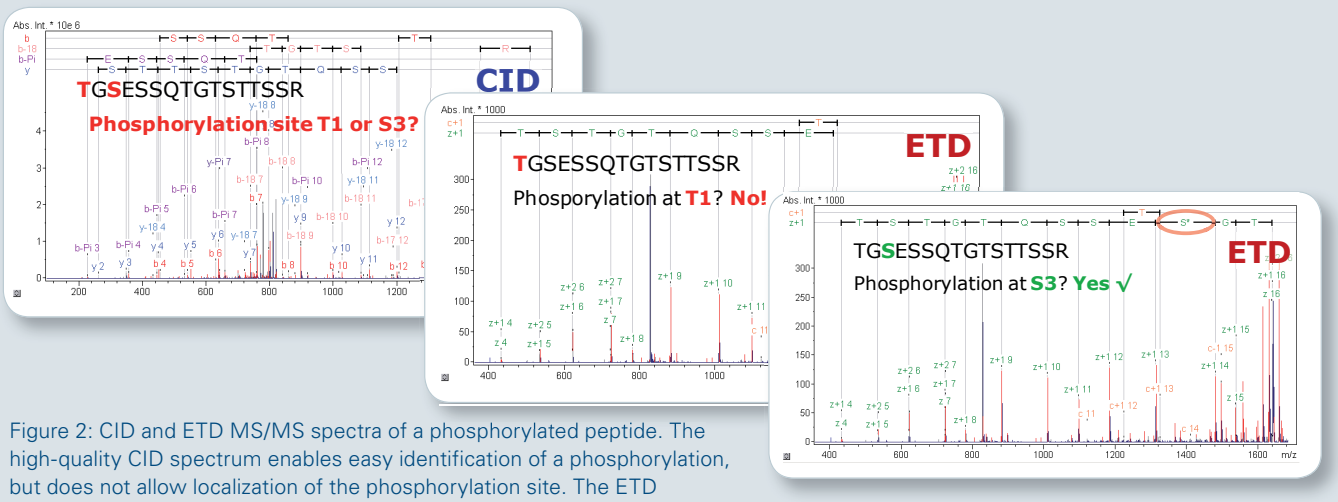

Figure 2: CID and ETD MS/MS spectra of a phosphorylated peptide. The high-quality CID spectrum enables easy identification of a phosphorylation, but does not allow localization of the phosphorylation site. The ETD fragmentation however, allows the labile phosphate group that is usually cleaved in CID to remain in position. This makes it possible to automate phosphorylation site assignment by scoring the different localization hypotheses. In this case, the phosphorylated amino acid residue is serine 3, and not threonine 1.

Conclusion

Analysis of automated neutral loss triggered CID/ETD spectra allows unambiguous assignment of phosphorylation sites. At the same time, the duty cycle is enhanced; maximizing the protein identification rate and sequence coverage.

Data processing and evaluation with the ProteinScape software package offers easy access to powerful bioinformatics tools which convert data into useful biological information.

Combined with the Bruker ProteinScape software package, the amaZon speed ETD represents a robust and powerful tool for phosphoproteome mapping and characterization.

	Number of identified proteins	Phosphoproteins	Total number of identified peptides	Non-phosphorylated peptides
First run	341	333	724	35
Second run	327	320	687	27
Third run	294	288	593	26

Figure 3: Summary of the results obtained in the three replicate measurements (4 µL injected each time). Protein identification was performed using the Mascot search engine against human entries in the Swiss-Prot database.

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