

Combining novel fragmentation and front-end enrichment techniques for highly increased sensitivity and selectivity of phosphopeptide detection

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The characterization of serine and threonine phosphorylation is usually challenging. The commonly applied collision-induced dissociation (CID) results in neutral loss of the labile phosphate group with often insufficient further fragmentation of the peptide chain itself, and subsequently very limited sequence information. We will present a novel concept of MS/MS in the ion trap by combining CID and electron transfer dissociation (ETD), which is particularly suitable for phosphorylation identification due to its non-ergodic nature: the prompt fragmentation along the amid backbone following the electron capture leaves the amino acid-phosphate bond intact..

Since phosphopeptides are often present at very low concentrations and more difficult to ionize, they are prone to suppression by other peptides in nanoESI, in particular in highly complex mixtures. Therefore, a front-end enrichment is highly recommended. Here, various methods are presented and discussed, ranging from functional surfaces on magnetic beads to TiO₂ columns.

Digests of standard proteins spiked with sub-stoichiometric phosphopeptide amounts and phosphopeptide enriched digests from Arabidopsis were analyzed using nanoLC-MS/MS.

The comparison of ETD and CID spectra shows the benefits of ETD, where dephosphorylation of the parent ion was not observed. CID in contrast shows generally the phosphate loss as the most abundant signal, indicating the phosphorylation presence. The combination of both fragmentation methods within one acquisition cycle provided improved fragmentation data and enabled the unambiguous determination of sequence and phosphorylation site