Effect of Phosphorylation on Ionization Efficiency during Mass-spectrometry Experiments

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The field of proteomics is currently advancing at high speed, combining stable isotope labeling, peptide enrichment techniques, high accuracy measurement instruments and sophisticated bioinformatics analysis. The recent developments have allowed not only for precise identification of the peptides, proteins and post-translational modifications in a complex mixture, but also for quantification of the identified proteins and the observed modifications [1].

The workflow of a mass spectrometry-based analysis includes several main steps. The protein sample is subjected to protease digestion, which results in a mixture of peptides of defined lengths. To reduce the complexity of the sample, HPLC is used to separate the resulting peptides. As a mass spectrometer uses electromagnetic fields to distinguish the different peptides and to measure mass-to-charge ratios, ionization of the peptides is required prior to their introduction into the mass spectrometer. The measured mass spectra are then analyzed with advanced bioinformatics analysis.

In this study, we focus on identifying differences in the efficiency of the ionization step between phosphorylated and unmodified peptides. Different factors, which might have an effect on electrospray ionization, are investigated. The findings of such an analysis would have an important implication for future phosphoproteomics studies, as they would give a general idea on the difficulty that could be expected in detecting phosphorylation sites with specific properties. Furthermore, a recently introduced method for computing absolute stoichiometry of phosphorylation in large-scale data sets relies on the accurate measurement of both phosphorylated and unmodified peptides for correct quantification [2]. Thus it is of great interest and importance to assess the effect that phosphorylation might have on ionization efficiency and therefore on the precision of the quantification.

During peptide electrospray ionization [3] the peptides, eluting from the chromatography column, are subjected to a high electrical potential with respect to the entrance into the mass analyser. The liquid effluent containing the peptides is dispersed, resulting in positively charged droplets. The solvent evaporates and as a result of the high electrical fields and multiple fission steps very small droplets, each containing on average a single analyte ion, are generated. During that process the peptides can exhibit different protonation states, with the majority of the tryptic peptides being doubly protonated.

The ionization efficiency can be measured by comparing the ion chromatograms of the two forms of a peptide of interest – the phosphorylated and the unmodified one – with the calculated site stocheometries. Furthermore, we study how those differences vary with respect to protonation state of the peptides, ion size, amino acid content of the peptide, total number of modifications in the peptide as well as the their exact positions.

References:

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