English abstract

The DNA holds the genetic information of all proteins in the cell, and expressed proteins undergo post-translational modifications (PTMs) that alter their function, cellular localization or turnover. Phosphorylation is a reversible PTM that switches protein function and activity, making it a key regulator of many cellular processes (cell cycle, DNA reparation, cell motility...). Disruption of these mechanisms by unrestrained phosphorylation can cause severe damage to the cell, like uncontrolled division or migration to other parts of the organism, leading to tumor generation and metastasis in cancer. In mammalians, phosphorylation mainly occurs in serine, threonine and tyrosine residues, but recent studies have demonstrated that histidine phosphorylation is also involved in protein regulatory networks. Due to the low abundance of this PTM and its dynamic behavior, detection and quantification of proteins that would aid in inhibiting aberrant signaling pathways is challenging.

Mass spectrometry-based phosphoproteomics aims to identify, localize and quantify phosphorylation sites in proteins, but MS instruments are not sensitive enough to identify phosphopeptides from crude samples as the abundance and ionization efficiency of non-phosphopeptides are much higher. Therefore, phosphoproteomics relies on the efficient isolation of phosphopeptides through enrichment strategies like immunoprecipitation with antibodies, immobilized metal-ion affinity chromatography (IMAC) and metal oxide affinity chromatography (MOAC). However, limitations and biases in these methods can hamper quantitation and identification of phosphopeptides and other acid-labile modifications like phosphohistidine. For this reason, new and improved strategies are in constant development for better and more efficient analysis of the phosphoproteome.

The goal of this research was to design and optimize novel methods for phosphopeptide enrichment purposes that will eventually contribute in MS-based phosphoproteomics research. We improved phosphopeptide enrichment methods by revisiting Zr(IV)-IMAC and benchmarking it against Ti(IV)-IMAC and TiO_2. We demonstrated that Zr-IMAC is a viable technique for large-scale phosphoproteomic studies for efficient, selective, and automated phosphopeptide enrichment (Paper I). Furthermore, we investigated molecularly imprinted polymers (MIPs) as a new technology for selective capture of histidine-phosphorylated (pHis) peptides, and we provided proof of concept for future protocols that will benefit labile phosphorylation protein studies (Paper II). We also attempted to optimize Zr-IMAC for pHis-peptide enrichment by varying solvent pH, and compared quantitative phosphoproteomics methods in an industrial setting. These studies provide novel strategies for phosphopeptide enrichment in large-scale MS-based phosphoproteomics analysis with potential applications in biological and biomedical research.