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Recent advances in phosphopeptide enrichment: Strategies and techniques

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ABSTRACT

Phosphoproteomics has become one of the most active research areas in proteomics studies. Phosphopeptide enrichment is a critical and indispensable step in phosphoproteomics. To date, a variety of strategies and techniques have been developed for the selective enrichment of phosphopeptides. With the progress of science and technology, novel methods are being continually developed to enhance the specificity and selectivity of the enrichment strategies. In this review, we summarize and discuss recent advances of strategies for phosphopeptide enrichment and highlight novel techniques developed in this research field. In addition, strategies for specific phosphopeptide enrichment including multi-phosphopeptides and endogenous phosphopeptides are also summarized and discussed.

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Abbreviations: AGE, allyl glycidyl ether; BSA, bovine serum albumin; CS, chitosan; DVB, divinylbenzene; EDMA, ethylene dimethacrylate; GO, graphene oxide; HA, hyaluronate; HSA, human serum albumin; IDA, iminodiacetic acid; IMAC, immobilized metal affinity chromatography; MCNC, magnetic colloid nanocrystal cluster; MHMSS, magnetic hollow mesoporous silica sphere; MOAC, metal oxide affinity chromatography; MS, mass spectrometry; multi-P, multi-phosphopeptides; MWNT, multiwalled carbon nanotube; NTA, nitrilotriacetic acid; PA, polyacrylate; PAA, poly(acrylic acid); PDMS, polydimethylsiloxane; PEG, poly(ethylene glycol); PEGMP, poly(ethylene glycol methacrylate phosphate); PGMA, poly(glycidyl methacrylate); PHEMA, poly(2-hydroxyethyl methacrylate); PMAA, poly(methylacrylic acid); PVPA, poly(vinylphosphonic acid); SAX, strong anion exchange chromatography; SCX, strong cation exchange chromatography; SI-ATRP, surface-initiated atom transfer radical polymerization; TOF, time of flight; UPLC, ultra performance liquid chromatography.

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1. Introduction

Protein phosphorylation is a reversible posttranslational modification mediated by kinases and phosphatases. The addition and removal of phosphate groups will alter the conformation and surface charge distribution of proteins, leading to a change in enzymatic activity and protein–protein interactions. Therefore, protein phosphorylation is closely related to a variety of cellular processes including metabolism, growth, proliferation, differentiation, and apoptosis [1].

The most common phosphorylation sites of proteins in eukaryotic cells are usually located on serine, threonine, and tyrosine residues, with an approximate ratio of 1800:200:1. It is estimated that approximately 700,000 potential phosphorylation sites exist in a eukaryotic cell [2]; however, the phosphorylation of protein is dynamic and only a small portion of sites are simultaneously phosphorylated at a specific moment. Therefore, the content of phosphoproteins is relatively low (only 1–2% of the entire amount of protein is estimated to be phosphorylated at a specific moment), and phosphopeptide abundances after proteolytic digestion are much lower [3]. The regulation of phosphorylation is a very sophisticated cascade reaction process, and disruption of these regulatory pathways is likely to result in various kinds of diseases, including cancers [4]. Hence, the analysis of protein phosphorylation is a challenging but significant task.

Currently, mass spectrometry (MS)-based techniques have become the foremost choice for phosphoproteomics analysis because of their high sensitivity and accuracy [5,6]. However, the detection and identification of phosphopeptides by MS are still challenging. In the case of MS techniques, data-dependent acquisition methods are often used for untargeted phosphoproteomics analysis. Due to the limitation of the analyzer sampling frequency, only the peptides of high concentration can be identified. Unfortunately, the content of phosphopeptides is often low, and they are less likely to be detected in the presence of abundant non-phosphopeptides. In addition, the ionization efficiencies of phosphopeptides are considered to be lower than that of non-phosphorylated peptides due to the additional phosphate group(s), which further makes phosphopeptide identification difficult [7]. In short, because of their low abundance and lower ionization efficiency compared with non-phosphopeptides, the isolation and enrichment of phosphopeptides from complex biological samples prior to MS analysis are crucial to the study of phosphoproteome.

Methods for phosphopeptide enrichment have been extensively studied. Furthermore, novel approaches and techniques are being continually developed. Therefore, comprehensive review and discussion of the recent advances in phosphopeptide enrichment are urgently needed. In this review, we summarize strategies for phosphopeptide enrichment and highlight novel techniques developed in this research field. Details of other related issues, such as separation methods for the pre-fractionation of phosphopeptide and MS technology for the identification of phosphorylation sites, are not discussed here. The principles and new developments of each method for phosphopeptide enrichment are summarized. Various kinds of techniques applied in the traditional enrichment strategies are then highlighted. Finally, specific enrichment strategies for multi-phosphopeptides and endogenous phosphopeptides are discussed.

2. Strategies for phosphopeptide enrichment

After decades of development, a variety of strategies have been established for phosphopeptide enrichment. The most commonly used strategies are summarized in Fig. 1, including immobilized metal affinity chromatography (IMAC) [8], metal oxide affinity chromatography (MOAC) [9], inorganic salt affinity chromatography [10], coprecipitation [11], ion exchange chromatography [12], chemical derivatization [13], and immunoprecipitation [14]. Among them, IMAC, MOAC, inorganic salt affinity chromatography, and coprecipitation are of the inorganic ion affinity chromatography type, all of which are based on the affinity interaction between metal ions and the phosphate group of phosphopeptides. IMAC and MOAC are the most extensively used methods due to their high selectivity and sensitivity. Ion exchange chromatography takes advantage of the charge difference between phosphopeptides and non-phosphopeptides for the separation, which is frequently used to prefractionate phosphopeptides and in combination with other enrichment methods, such as IMAC and MOAC. The principles and new developments of these phosphopeptide enrichment methods are described in detail in the following sections.

2.1. Immobilized metal affinity chromatography

In the IMAC strategy, metal ions are immobilized on supporting substrates through chelators, and then used for phosphopeptide enrichment based on the affinity of the phosphate group to metal ions. IMAC was initially used to separate phosphoproteins [15]. With the development of the "bottom-up" strategy and biological MS, it is now primarily used for phosphopeptide enrichment. The chelators that were initially used in IMAC for phosphopeptide enrichment

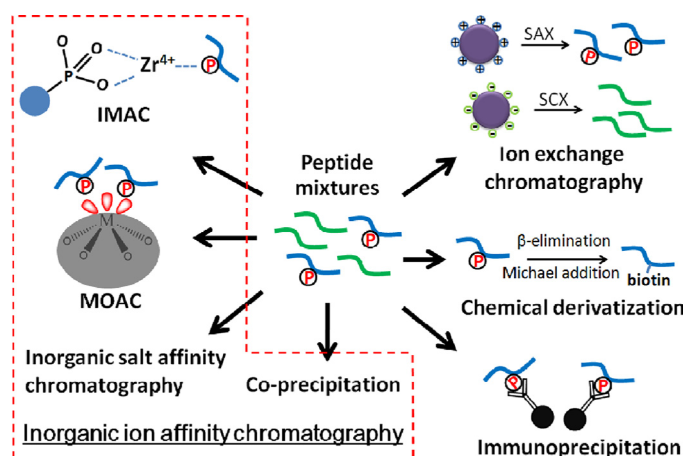

Fig. 1. The commonly used strategies for phosphopeptide enrichment, including IMAC, MOAC, inorganic salt affinity chromatography, coprecipitation, ion exchange chromatography, chemical derivatization, and immunoprecipitation.

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