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Iron affinity gel and gallium immobilized metal affinity chromatographic technique for phosphopeptide enrichment: a comparative study

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ABSTRACT

Protein phosphorylation is central to most cellular processes. Despite the importance and widespread occurrence of this modification, phosphoproteome analysis of complex biological samples still remains a challenge. The present study has been undertaken to compare iron affinity immobilized metal ion affinity chromatography (Phos-Select™ IMAC) and Gallium immobilized metal ion affinity chromatography (Ga-IMAC) to enrich and characterize phosphopeptides from the whole-cell lysate of Jurkat cells. Our results indicated that enrichment was dependent on the isoelectric point (pI) and molecular mass of the proteins and it was found to be better in case of PHOS-Select IMAC (51.2%) as compared to Ga-IMAC (28.8%). This study compared and analysed phosphopeptide profile of Jurkat cells by two different IMAC techniques and provides advancement in phosphopeptide enrichment methods.

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Introduction

Reversible protein phosphorylation, principally on serine, threonine or tyrosine residues, is one of the most important and well-studied post-translational modifications. It has been estimated that one-third of mammalian proteins may be phosphorylated [1,2]. Many disease pathologies, such as Alzheimer's disease, are characterized by changes in phosphorylation levels of various proteins [3,4]. Despite the importance and widespread occurrence of protein phosphorylation in cellular system, identification of phosphopeptides and the site of protein phosphorylation still remains a big challenge. Phosphopeptides have been isolated earlier from complex protein mixtures by mass spectrometry (MS) method [5,6] and two-dimensional (2D) phosphopeptide mapping method, which was commonly used for analysing protein phosphorylation [7]. In recent times, most phosphoproteomic studies are conducted by immobilized metal ion affinity chromatography (IMAC) followed by MS. IMAC was a widely used affinity medium for phosphopeptide enrichment [8,9] and was introduced by Porath in 1975 [10,11]. IMAC, usually Fe^{3+}-IMAC, and metal oxide affinity chromatography, mainly titanium dioxide (TiO_2) chromatography, are the most frequently used enrichment techniques for S/T-phosphorylated peptides [12]. The principle of IMAC is based on the affinity of transition metal ions to histidine and cystine in aqueous solution [13]. The ions commonly used are of borderline or soft metals, such as Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}. Protein retention in IMAC depends on the number and type of pendant groups that can interact with the metal [14]. Interaction between ions and protein is dependent on variables such as pH, temperature, solvent, nature of immobilized metal and chelate, ligand density and protein size. Proteins are usually eluted by a decreasing pH gradient or by an increasing gradient of a competitive agent, such as imidazole, in a buffer [14]. Elucidation of phosphopeptides from complex biological samples requires isolation and sequencing of phosphopeptides derived from protein digests. Identification of specific phosphorylated residues and actual site(s) of phosphorylation in proteins still remains a labour-intensive, time-consuming challenge and is a very complicated process [15,16]. Moreover, phosphoproteins are post-translationally modified proteins and are present in low abundance, which tend to coexist with their unphosphorylated isoform within the cell. Hence, enrichment protocols are required for identification of particular peptides. The enrichment strategies are generally based on chemical modifications, affinity chromatography or immunoprecipitation by phospho-specific antibodies [17] and most frequently affinity chromatography is used using immobilized antibodies to phosphorylated amino acids [18,19]. Currently, IMAC is the most widely used technique for the enrichment of phosphopeptides [20], and is based on the

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