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Polydopamine-coated eppendorf tubes for Ti^{4+} immobilization for selective enrichment of phosphopeptides

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ABSTRACT

Mass spectrometric technique has emerged as a preferred technique in the analysis of protein phosphorylation. Owing to the low stoichiometry of phosphopeptides and the signal suppression effect by non-phosphopeptides, there is a demand for efficient enrichment of phosphopeptides. The selective enrichment of phosphopeptides in modified eppendorf tubes prior to mass spectrometry analysis, which can minimize sample loss as well as nonspecific interferences effectively, has become a hot topic in current proteomics field. In our work, an easy-to-use phosphopeptide-selective eppendorf tube was initially prepared, with its inner surface being modified with a Ti^{4+}-immobilized polydopamine (PDA) layer. The unique Ti^{4+}-immobilized PDA-modified eppendorf tubes (EP tube@PDA-Ti^{4+}) are investigated for its application in selective enrichment of phosphopeptides from complex biological samples. Due to the high Ti^{4+} loading amount on the surface of PDA, the EP tube@PDA-Ti^{4+} exhibits remarkable phosphopeptide enrichment ability in protein digests and human serum, which presents a powerful evidence for its high selectivity in detecting the low-abundance phosphopeptides from complex biological samples.

1. Introduction

Protein phosphorylation is a common post-translational modification (PTM) in mammalian systems, which can broaden biological functionality of proteins. Protein phosphorylation affects around 30% of a proteome and plays an important role in major regulatory machinery for many complex biological processes, such as intracellular signaling transduction and cellular growth and division [1–3]. However, owing to the complexity of the phosphoproteome, the low stoichiometry of phosphopeptides, the signal suppression effect by non-phosphorylated peptides in protein digests, and the dynamic nature of signaling networks during mass spectrometry analysis, phosphopeptides may be difficult to be detected by mass spectrometry [4]. Therefore, more and more techniques have been developed to selectively enrich phosphopeptides from highly complex mixtures in order to increase detection sensitivity for efficient phosphopeptides identifications, such as immobilized metal ion affinity chromatography (IMAC), metal oxide affinity chromatography (MOAC), strong cation exchange chromatography, mesoporous nanostructure materials, chemical-modification strategies and immunoprecipitation [5–9].

Among these techniques, IMAC and MOAC are the most effective and convenient and have been widely applied in the selective enrichment of phosphopeptides. For example, Lu et al. reported synthesis of Fe_3O_4@mesoporous TiO_2 microspheres for selective enrichment of phosphopeptides for phosphoproteomics analysis [10] and modified graphene with TiO_2 for specific capture of phosphopeptides [11]. Yan et al. reported functionalized carbon nanotubes with titania nanoparticles for selective enrichment of phosphopeptides for mass spectrometry analysis [12]. Xu et al. reported synthesis of magnetic microspheres with immobilized metal ions for selective enrichment and analysis of phosphopeptides [13].

Recently, it has been proved that dopamine (DOPA), a biomolecule with catechol and amine functional groups found in high concentration in the adhesive protein Mefp-5 (Mytilus edulis foot protein 5) secreted from mussels, has several advantages, such as excellent environmental stability, good biocompatibility and splendid hydrophilicity. DOPA can generate into a thin adherent polydopamine (PDA) films on a variety of substrates via the oxidative self-polymerization of the PODA in a basic solution [14]. In addition, the catechol groups in PDA coating are capable of strong metal ions coordination [15,16], which can facilitate to in situ deposit metal ions on the surfaces of PDA at mild conditions. More recently, in our group, PDA has successfully been developed as a novel chelating ligand for metal ions to achieve enrichment of phosphopeptides [17,18].

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In order to reduce sample loss due to adsorption on container walls and lead to high-throughput analyses, IMAC and MOAC have been developed to on-plate selective enrichment and pipette-tips selective enrichment in the recent years. For example, Lu et al. modified the MALDI target plate with alumina hollow spheres proposed for pre-treatment of phosphopeptides in biological samples [19]. Hsieh et al. developed a titanium dioxide nanoparticle pipette-tip for selective enrichment of phosphorylated peptides [20]. However, to our knowledge, there is no eppendorf tubes (EP tubes) strategy using IMAC for the selective enrichment of phosphopeptides. EP tubes, which are used to store up liquid, are commonly requisites in laboratory. It can eliminate carry-over and minimize the sorbent consumption to develop IMAC to EP tubes selective enrichment since there is no need for magnetic separation or high-speed centrifugation. Therefore, developing EP tube enrichment technique for phosphopeptides is very interesting and important.

In this study, an easy-to-use phosphopeptide-selective EP tube based on dopamine chemistry was initially prepared, with its inner surface being modified by a Ti^{4+}-immobilized PDA layer. The performance of the as-prepared EP tube@PDA-Ti^{4+} was investigated for its application in selective enrichment of phosphopeptides from complex biological samples. Due to the high Ti^{4+} loading amount on the surface of polydopamine, the EP tube@PDA-Ti^{4+} exhibits remarkable ability of enrichment for phosphopeptides in the presence of numerous nonphosphopeptides in protein digests and human serum. The excellent hydrophilic property, selectivity, sensitivity, detection limit, reusability and stability were also proven.

2. Experimental

2.1. Chemicals and reagents

β-casein, bovine serum albumin, L-1-tosylamido-2-phenylethyl-chloromethyl ketone (TPCK) treated trypsin (from bovine pancreas), ammonium bicarbonate (NH_4HCO_3), trifluoroacetic acid (TFA), 3-(trihydroxysilyl)propyl methylphosphate, 2,5-dihydroxybenzoic acid (DHB) and Tris were purchased from Sigma Chemical (St. Louis, MO). Dopamine hydrochloride was purchased from Alfa Aesar Johnson Malthey Company. $Ti(SO_4)_2$ was purchased from Sinopharm Chemical Regents Co. Ltd (Shanghai, China). Acetonitrile was purchased from Shanghai Lingfeng Chemical Reagents Co. Ltd (Shanghai, China). Eppendorf tubes were purchased from Axygen Inc. All aqueous solutions were prepared using Milli-Q water purified by Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Modification of the inner surface of eppendorf tubes

The synthetic protocol for preparation of EP tube@PDA-Ti^{4+} is presented in Fig. 1a. Firstly, Tris (12.1 mg) was dissolved in 10 mL distilled water by sonication to form 10 mM Tris buffer. 200 μL solution of PDA (2 mg/mL, dissolved in 10 mM Tris buffer) was added into eppendorf tube (500 μL). The tube was exposed to light and capped and left at room temperature for 24 h to produce a PDA coating on the inner surfaces of eppendorf tube. The obtained PDA-coated eppendorf tubes were washed with Milli-Q water (400 μL) three times by sonication for 1 min and dried in vacuum at 50 °C over night (0.085 mbar).

Secondly, 200 μL solution of $Ti(SO_4)_2$ (100 mM, aqueous solution) was added into the eppendorf tubes modified by PDA, and left at room temperature for 2 h to immobilize Ti^{4+} cation. The resultant eppendorf tubes were washed with Milli-Q water (400 μL) three times by sonication for 1 min and dried in vacuum at 50 °C over night (0.085 mbar).

2.3. Characterization

A Philips XL30 electron microscope (The Netherlands) was employed to record scanning electronic microscope (SEM) images of materials operating at 20 kV. A LabRam-1B Raman spectrometer was employed to record the Raman spectra with a laser at an excitation wavelength of 632.8 nm at room temperature.

2.4. Sample preparation

The protein (bovine serum albumin or bovine β-casein) was dissolved in 25 mM NH_4HCO_3 buffer (pH 8.3) containing proteomic-grade trypsin (2%, w/w) at 37 °C with overnight shaking. The digested products were diluted to various concentrations and stored below 0 °C. Human serum was diluted to ten times with 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) aqueous solution (v/v).

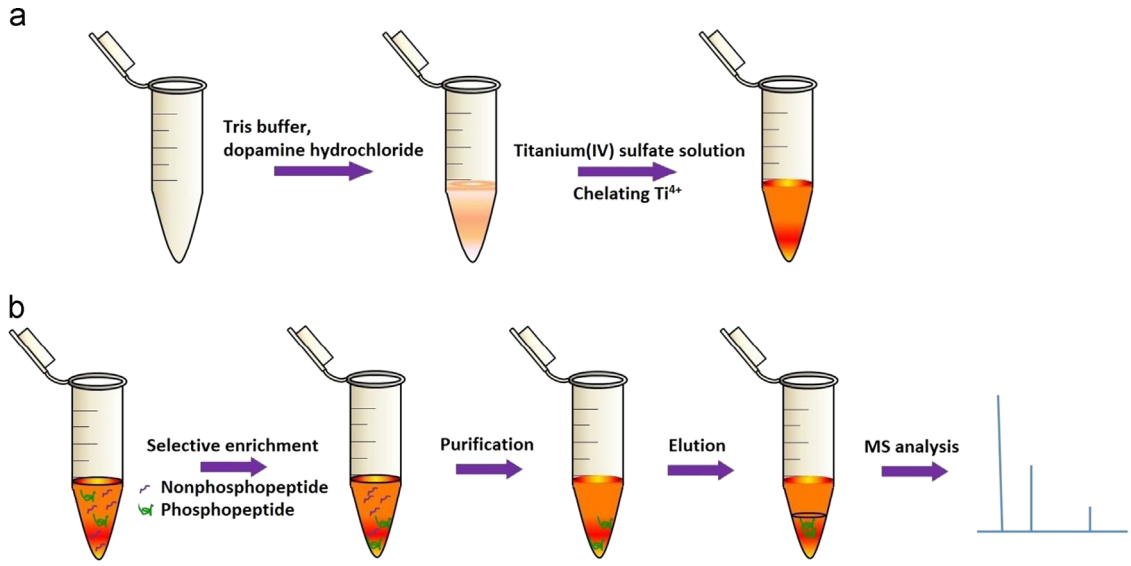

Fig. 1. (a) The synthetic procedure for Ti^{4+}-immobilized PDA-modified eppendorf tubes. (b) The procedure of phosphopeptides enrichment by utilizing Ti^{4+}-immobilized PDA-modified eppendorf tubes as adsorbent.