Talanta 135 (2015) 81–86

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Preparation of on-plate immobilized metal ion affinity chromatography platform via dopamine chemistry for highly selective isolation of phosphopeptides with matrix assisted laser desorption/ionization mass spectrometry analysis

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ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form
22 December 2014
Accepted 25 December 2014
Available online 2 January 2015

Keywords:
On-plate IMAC platform
Dopamine chemistry
Selective enrichment and isolation of phosphopeptides
Mass spectrometry analysis

ABSTRACT

In this study, a novel on-plate IMAC technique was developed for highly selective enrichment and isolation of phosphopeptides with high-throughput MALDI-TOF-MS analysis. At first, a MALDI plate was coated with polydopamine (PDA), and then Ti^{4+} was immobilized on the PDA-coated plate. The obtained IMAC plate was successfully applied to the highly selective enrichment and isolation of phosphopeptides in protein digests and human serum. Because of no loss of samples, the on-plate IMAC platform exhibits excellent selectivity and sensitivity in the selective enrichment and isolation of phosphopeptides, which provides a potential technique for high selectivity in the detection of low-abundance phosphopeptides in biological samples.

1. Introduction

Protein phosphorylation is one of the most essential and universal post translational modifications (PTMs) of proteins, which is involved in almost all aspects of cell life, such as cell growth, division, migration and differentiation [1–3]. With the intention of studying these bioprocesses, much effort has been devoted to develop the methods and techniques of systematic identifying and characterizing phosphoproteins. Mass spectrometry (MS) strategies, the workhorse for detection and characterization of phosphoproteins, have emerged as a powerful technique due to its high speed and high throughput features, such as matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis [4–6]. Unfortunately, the large amount of non-phosphopeptides contained in various biological samples suppresses the detection of phosphopeptides, which calls for the urgent demand of selective isolation of phosphopeptides from biological samples before MALDI-TOF-MS analysis.

Plenty of traditional off-target methods, such as immobilized metal ion affinity chromatography (IMAC), metal oxide affinity chromatography (MOAC) and functionalized nanoparticles, have been developed for selective enrichment and isolation of phosphopeptides before MALDI-TOF-MS analysis [7–11]. Zou et al. prepared Fe_3O_4@SiO_2@$(HA/CS)_{10}$-Ti^{4+} nano-materials for selective enrichment of phosphopeptides [12,13]. In our lab, Yan et al. developed polydopamine-coated grapheme with Ti^{4+} or TiO_2 immobilized as a platform for phosphoproteome analysis, which leads to excellent selectivity and sensitivity in the isolation of phosphopeptides for MALDI-MS analysis. However, the above methods mentioned will result in a series of problems, such as inevitable loss of samples, waste of materials and potential contaminants. In Tsai's work, a tip was packed with Ni-NTA silica resin and immobilized multimetal on it to enrich phosphopeptides, which could avoid the above problems mentioned. However, it was hard for it to realize high throughput analysis, and it also needed to elute during the process of phosphopeptides enrichment like other off-target methods [14]. To avoid these problems, on-target isolation prior to MALDI-TOF-MS analysis has been developed and attracted much attention recently, which involves in on-plate MOAC platform for the selective isolation of phosphopeptides. Lu et al. synthesized alumina hollow spheres for on-plate isolation of phosphopeptides [15]. Tan et al. functionalized a MALDI target plate with magnetic nanoparticles for phosphopeptides isolation [16]. Zeng et al. developed on-plate selective isolation of peptides/proteins for direct MALDI MS analysis [17]. Nonetheless, on-plate MOAC platform for selective isolation will

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http://dx.doi.org/10.1016/j.talanta.2014.12.041
0039-9140/

lead to destruction of the ion source of mass spectrometry, owing to the access of those nanoparticles impacted by laser [18,19]. Therefore, it is crucial to develop novel on-plate isolation techniques for highly selective enrichment of phosphopeptides.

Many researches have proved that dopamine (DOPA) can self-polymerize on various substrates (such as metal) in mild condition [20–23]. Moreover, the metal ions such as Ti^{4+} can be directly immobilized on the surface of polydopamine (PDA) in mild conditions through the catechol groups in PDA coating [24,25] Based on these, in this study, we developed a novel on-plate IMAC platform, Ti^{4+}-immobilized MALDI plate by PDA layer, for isolation of bound phosphopeptides in direct MALDI-TOF MS analysis. At first, the MALDI plate was coated with PDA. And then Ti^{4+} was immobilized on the PDA layer. Our method avoided the problems of off-target methods and the MOAC platform. And it also realizes the high throughput analysis of phosphopeptides since the pretreatment is finished on the MALDI plate directly. Moreover, the prepared on-plate IMAC platform was applied to the highly selective enrichment and isolation of phosphopeptides without elution both in protein digests and human serum, which greatly shortened the enrichment time. Owing to no loss of samples, the Ti^{4+}-immobilized PDA-coated MALDI target plate (Ti^{4+}-PDA-plate) exhibits excellent selectivity and sensitivity in the isolation of phosphopeptides for MALDI-MS analysis.

2. Experimental

2.1. Chemicals and reagents

Dopamine hydrochloride was purchased from Alfa Aesar Johnson Malthey Company (Beijing, China). $Ti(SO_4)_2$ and NH_4OH were purchased from Sinopharm. Chemical Regents Co. Ltd. (Shanghai, China). The stainless-steel plate (384 Opti-TOF 123 mm × 81 mm SS) was purchased from AB Sciex (Massachusetts, USA). The standard peptide was purchased from China Peptides Co., Ltd. (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris), trifluoroacetic acid (TFA), β-casein, bovine serum albumin (BSA), trypsin (from bovine pancreas, TPCK treated), ammonium bicarbonate (NH_4HCO_3), and 2, 5-dihydroxybenzoic acid (DHB) were purchased from Sigma Chemical (St. Louis, MO). Acetonitrile was purchased from Shanghai Lingfeng Chemical Reagents Co. Ltd. (Shanghai, China). Distilled water was purified by a Milli-Q system (Milford, MA, USA). All other chemicals and reagents are of the highest grade and commercially available.

2.2. Preparation of IMAC plate

The synthetic strategy of IMAC plate is shown in Fig. 1. First, 400 mg of dopamine hydrochloride was dissolved in 200 mL of Tris buffer (10 mM, pH 8.5). The stainless-steel plate was washed with distilled water and ethanol several times and dried at room temperature. Then the clean target plate was immersed in the prepared solution of dopamine at room temperature for 24 h to form a PDA coating on the surface of the plate. The obtained PDA-coated plate was washed with distilled water for several times and immersed again in the aqueous solution of $Ti(SO_4)_2$ (100 mM) at room temperature for 2 h to immobilize Ti^{4+}. The product plate was washed with distilled water for several times and dried at room temperature.

2.3. Characterization

A Phillips XL30 electron microscope (Netherlands) was used to record scanning electronic microscope (SEM) images of materials which operated at 20 kV. An energy dispersive X-ray spectroscopy (EDX) was employed to identify the composition of materials.

2.4. Sample preparation

The protein (bovine serum albumin or bovine β-casein) was dissolved in NH_4HCO_3 buffer (25 mM, pH 8.3) and treated with proteomic-grade trypsin (2%, w/w) at 37 °C for 16 h. The digested products were stored below 0 °C. Human serum was centrifuged and the supernatant was stored below 0 °C.

2.5. On-plate selective enrichment and isolation of phosphopeptides

As shown in Fig. 1, the enrichment of phosphopeptides was performed by the modified MALDI plate. The IMAC plate was washed with 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) water solution three times. The digests of β-casein, the mixture digests of β-casein and BSA and human serum were diluted to various concentrations with 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) aqueous solution (v/v). Then 1 μL diluted digests were pipetted onto the modified plate and incubated for 30 min at room temperature and washed with 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) aqueous solution (v/v) several times to remove nonspecific adsorption. Finally, 1 μL of DHB aqueous solution (20 mg/mL, 50% acetonitrile and 1% H_3PO_4) was added at the spots as a MALDI matrix for further analysis by MALDI-TOF-MS.

2.6. MALDI-TOF MS analysis.

MALDI-TOF MS experiments were performed by a Proteomic Analyzer (mode 5800, AB Sciex, Framingham, MA, USA) with the Nd: YAG laser at 355 nm, a repetition rate of 200 Hz and an acceleration voltage of 20 kV in the positive ion reflection mode within a scan range of 1000–3500 *m*/*z*.

3. Results and discussion

Herein, a novel IMAC plate was prepared (Fig. 1). The synthetic IMAC plate was applied in the selective enrichment and isolation of phosphopeptides, which could minimize the loss of samples, the consumption of materials and reduce potential contaminants. Moreover, there was no need of elution, which resulted in the fast and high-throughput MALDI MS analysis after the phosphopeptides enrichment and isolation. The Ti^{4+} based IMAC plate was characterized by scanning electronmicroscopy (SEM) and energy dispersive X-ray spectroscopy (EDX).

3.1. Preparation and characterization of IMAC plate

Dopamine hydrochloride can self-polymerize oxidatively on the surface of various substrates in mild condition. The MALDI plate is silver metallic luster before modified with PDA. After the self-polymerization of DOPA, the color of MALDI plate changes to light brown, which indicates the successful polymerization of DOPA. The morphology of the PDA-modified plate was evaluated by SEM characterization. As shown in Fig. 2a, after immersing in DOPA solution for 10 h, DOPA monomer has a tendency to form free PDA (Fig. 2a) and we can also see that the MALDI plate exhibits the characteristic surface of metal defects. As time goes by, the free PDA particles grow up into PDA films [26]. In Fig. 2b, after modified with PDA for 24 h, the surface of MALDI target plate is firmly coated with a robust PDA layer. The thickness of PDA layer is determined by the concentration of DOPA solution and polymerization time. After modification of PDA, plenty of Ti^{4+} in the addition of titanium (IV) sulfate precursor was immobilized on the