Journal of Chromatography A, 1316 (2013) 62–68

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Ultrathin-yttrium phosphate-shelled polyacrylate-ferriferrous oxide magnetic microspheres for rapid and selective enrichment of phosphopeptides

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ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form 3 October 2013
Accepted 3 October 2013
Available online 5 October 2013

Keywords:
Ultrathin shell
Phosphopeptide
Magnetic microsphere
Yttrium phosphate
MALDI-TOF MS

ABSTRACT

Rapid and selective enrichment of phosphopeptides from complex biological samples is essential and challenging in phosphorylated proteomics. We present the direct growth of the ultrathin YPO_4 shell on the surface of polyacrylate capped secondary Fe_3O_4 microspheres (PA-Fe_3O_4@YPO_4) for the rapid and selective trapping phosphopeptides from complex samples. The prepared PA-Fe_3O_4@YPO_4 could be rapidly harvested in the presence of an applied magnetic field and easily re-dispersed in solutions after removing the external magnet. The ultrathin YPO_4 shell on super-hydrophilic PA-Fe_3O_4 has the advantages of fast adsorption/desorption dynamics and low non-specific adsorption, thus trapping of phosphopeptides from the tryptic digests mixture of β-casein/BSA with molar ratio of 1/300 is achieved in 20 s adsorption/desorption time. Two phosphopeptides can still be detected with a signal to noise ratio (S/N) over 3 when the amount of β-casein was as low as 8 fmol.

1. Introduction

Phosphorylation of proteins is one of the most important post-translational modifications of proteins. It plays an important role in a large number of complex biological processes, such as signal transduction, metabolism, and cell differentiation, proliferation and apoptosis [1–3]. Many human diseases (e.g. cancer) are related to the deregulation of protein phosphorylation [4–8]. Recently, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been demonstrated to be a powerful tool for the analysis of protein phosphorylation. However, due to their low dynamic stoichiometry and the signal suppression of non-phosphopeptides, the identification of phosphopeptides is still challenging. Therefore, it is essential to enrich phosphopeptides from complex samples before MALDI-TOF MS analysis.

Many techniques have been reported for phosphopeptides enrichment [9]. Immobilized metal affinity chromatography (IMAC) that relies on the interactions between metal–ligand complexes and phosphate groups is a most widely used strategy [10]. Various metal ions (e.g. Ti^{4+} [11], Zr^{4+} [12], and Fe^{3+} [13]) have been used in the IMAC for the selective enrichment and detection of phosphopeptides from digested phosphoproteins. Adsorbents coated with metal oxides such as TiO_2 [14], ZrO_2 [15], ZnO [16], Al_2O_3 [17], Ga_2O_3 [18], SnO_2 [19], La_2O_3 [20], and Fe_2O_3 [21] (known as the metal oxide affinity chromatography (MOAC) method) have also been demonstrated to be effective for the enrichment of phosphopeptides [22]. Recently, rare earth metal orthophosphates ($REPO_4$) such as $YbPO_4$, $GdPO_4$, and YPO_4 were used as the adsorbents for effective and high-selective trapping and labeling of phosphopeptides [23], while the high-speed centrifugation was required to harvest the phosphorylated-peptide-bound materials and thus resulted in time-consuming as well as the introduction of high-molecular-weight non-phosphorylated peptides.

The magnetic composites, with intrinsic magnetic features, are charming for the convenient and fast bioseparation [24–26], targeted drug delivery [27,28] and medical diagnosis [29,30]. Recently, the application of magnetic composites in proteomics research has also been high-lighted [31–34]. The secondary Fe_3O_4 particles composed of small Fe_3O_4 nanocrystals (the domain size less than 30 nm) have been considered as the idea cores of the magnetic composites due to the advantages of increasing the magnetization while remaining the superparamagnetic characteristics [35]. Such advantages are very beneficial for their reduplicative use in separation science due to the rapid magnetic separation and easy redispersibility of the magnetic composites into solutions.

Although the magnetic composites have found application for enrichment of phosphopeptides, the marriage of Fe_3O_4 particles and YPO_4 is seldom reported [36]. Herein, we present the direct

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http://dx.doi.org/10.1016/j.chroma.2013.10.010

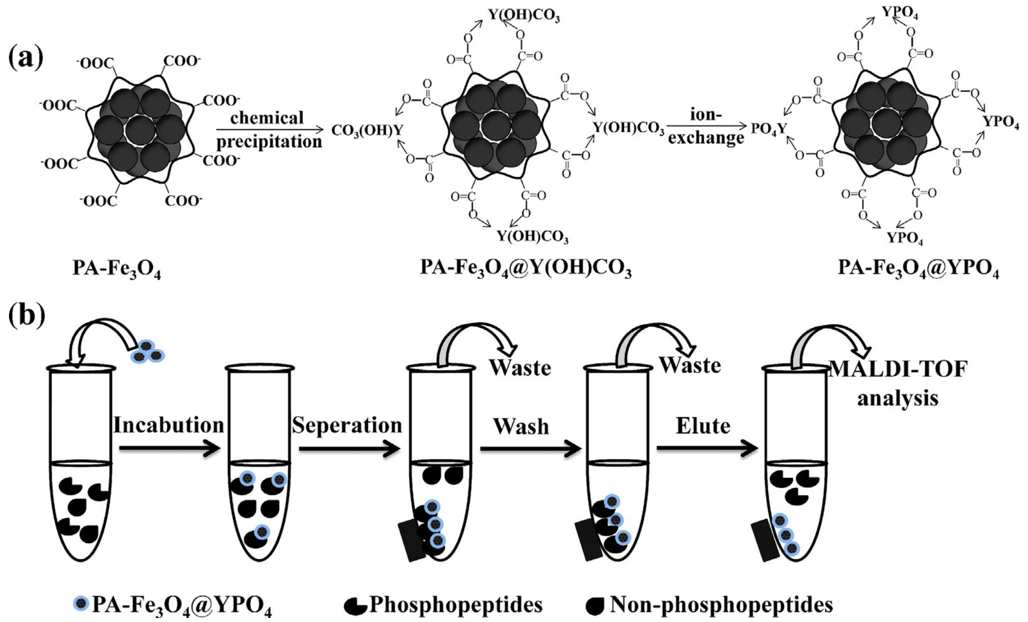

Fig. 1. Schematic illustration of (a) the fabrication of the PA-Fe_3O_4@YPO_4 microspheres and (b) the enrichment of phosphorylated peptides using PA-Fe_3O_4@YPO_4 microspheres.

growth of the ultrathin YPO_4 shell on polyacrylate capped secondary Fe_3O_4 microspheres (PA-Fe_3O_4@YPO_4) for the rapid and selective trapping phosphopeptides from complex samples (Fig. 1). The ultrathin YPO_4 shell was grown in situ onto the surface of secondary PA-Fe_3O_4 microspheres, directed via the surface abundant COO^- groups that were highly affinitive to the rare-earth metal ions. Except for the merits of rapidly collection in the presence of an applied magnetic field and easily re-disperse in solutions after removing the external magnet, the prepared PA-Fe_3O_4@YPO_4 microspheres displayed the charming characteristics of fast adsorption/desorption dynamics and the low co-adsorption of massive interference than the Fe_3O_4@hYPO_4 [36] due to the ultrathin YPO_4 shell on the hydrophilic PA-Fe_3O_4. To the best of our knowledge, no research considering the thickness of shells on the adsorption/desorption and non-specific adsorption of core–shell composites for enriching phosphopeptides has been reported yet.

2. Experimental

2.1. Instrumentation

Transmission electron microscopic (TEM) characterization was performed on a JEOL 100 CXII transmission electron microscope (JEOL, Tokyo, Japan) operating at a 200 kV accelerating voltage. The morphology and microstructure of the PA-Fe_3O_4@YPO_4 were characterized by high resolution transmission electron microscopy (HRTEM) and energy-dispersive X-ray (EDX) on a Tecnai G2 F20 field emission transmission electron microscope (FEI, Hillsboro, American & Holland) operating at a 200 kV accelerating voltage. The X-ray diffraction (XRD) spectra were recorded with a Rigaku D/max-2500 X-ray diffractometer (Rigaku, Tokyo, Japan) using Cu K_α radiation ($\lambda = 1.5418$ Å). The Fourier transform infrared (FTIR) spectra (4000–400 cm^{-1}) in KBr were recorded with a Nicolet MAGNA-560 FTIR spectrometer (Nicolet, Madison, USA). The thermogravimetric analysis (TGA) was performed on a PTC-10A thermal gravimetric analyzer (Rigaku, Japan) from room temperature to 700 °C at a ramp rate of 10 °C min^{-1}. The magnetic properties were studied using a LDJ 9600-1 vibrating sample magnetometer (LDJ Electronics Inc., Troy, USA) at room temperature by cycling the field from −6 to 6 kOe.

2.2. Chemicals and reagents

All reagents were at least of analytical grade and used without further purification. $FeCl_3{\cdot}6H_2O$, CH_3COONa, and $CO(NH_2)_2$ were brought from Guangfu fine Chemical Research Institute (Tianjin, China). Ethylene glycol (EG), trifluoroacetic acid (TFA) and 2,5-dihydroxybenzoic acid (DHB) were from Aladdin (Shanghai, China). β-Casein and trypsin from bovine milk were purchased from Sigma–Aldrich (Shanghai, China). Anhydrous ethanol and acetonitrile (ACN) were from Kangkede Fine Chemical Research Institute (Tianjin, China). $NH_4H_2PO_4$ was from Tianjin Chemical Reagent No. 2 Plant (Tianjin, China). $Y(NO_3)_3{\cdot}6H_2O$ and $NH_3{\cdot}H_2O$ were from Alfa Aesar (Tianjin, China) and Tianjin Yingda Rare Chemical Reagents Factory (Tianjin, China), respectively. Sodium acrylate and ammonium bicarbonate were from Heowns (Tianjin, China).

2.3. Synthesis of PA-Fe_3O_4

PA-Fe_3O_4 was synthesized via a solvothermal reaction [37]. Briefly, $FeCl_3{\cdot}6H_2O$ (0.540 g) was dissolved in EG (20 mL) under magnetic stirring to form a clear yellow solution. Then CH_3COONa (1.2 g), and sodium acrylate (0.3 g) were added into the above solution under vigorous stirring. The obtained homogeneous yellow solution was transferred into a 30 mL Teflon-lined stainless-steel autoclave and heated at 200 °C for 10 h. The autoclave was then cooled to room temperature. The obtained PA-Fe_3O_4 was washed several times with water and ethanol with the help of magnet, and then dried in vacuum overnight.

2.4. Synthesis of PA-Fe_3O_4@YPO_4 core–shell microspheres

The fabrication of PA-Fe_3O_4@YPO_4 (Fig. 1) involved two steps [23,26,38–40]. Firstly, the precursor PA-Fe_3O_4@$Y(OH)CO_3$ were prepared by a chemical precipitation method. Briefly, $Y(NO_3)_3{\cdot}6H_2O$ (0.072 g), and $CO(NH_2)_2$ (0.375 g) were dissolved into deionized water (50 mL) at room temperature to form a clear solution. The as-prepared PA-Fe_3O_4 (0.020 g) were added and well dispersed into the above solution with the assistance of ultrasonication for 20 min. The resultant mixture was subsequently transferred to a round-bottomed flask and heated to 90 °C for 2 h with vigorous