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Selective enrichment of phosphopeptides by titania nanoparticles coated magnetic carbon nanotubes

Yinghua Yan [a,b], Zhifang Zheng [a,b], Chunhui Deng [a,b,*], Xiangmin Zhang [a,b], Pengyuan Yang [a,b]

[a] Department of Chemistry, Fudan University, Shanghai 200433, China
[b] Institutes of Biomedical Science, Fudan University, Shanghai 200433, China

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ABSTRACT

Selective enrichment of phosphoproteins or phosphopeptides from complex mixtures is essential for mass spectrometry (MS)-based phosphoproteomics. In this work, for the first time, titania nanoparticles coated magnetic carbon nanotubes (denoted as MagCNTs@TiO_2 composites) were synthesized through a facile but effective solvothermal reaction for selective enrichment of phosphopeptides. The MagCNTs@TiO_2 material demonstrated low limit of detection (20 fmol), along with an exceptional great specificity to capture phosphopeptides from a tryptic digest of the mixture of a nonphosphorylated protein BSA and a phosphorylated protein β-casein with molar ratios of BSA/β-casein up to 200:1. In addition, the high magnetic susceptibility allowed convenient separation of the target peptides by magnetic separation. Experimental results demonstrated that the MagCNTs@TiO_2 composites showed excellent potential for the selective enrichment of phosphopeptides for MS analysis.

1. Introduction

Magnetic microspheres have attracted wide interest because of their wide range of current and potential applications in the biomedical field [1–6]. They allow mechanical sorting, trafficking, and other forms of micro-manipulation to be easily performed in biological systems simply via the application of an external magnetic field [7,8]. Along this line, the application of magnetic microspheres to proteomics research has also attracted much attention [9,10]. As one of the most important and ubiquitous post-translational modifications (PTMs), protein phosphorylation is a key regulator of almost all aspects of cellular processes in both prokaryotes and eukaryotes [11,12]. Mass spectrometry (MS) has become a powerful technique for determining the phosphorylation profiles of proteins in phosphoproteome research due to its high sensitivity, high-throughput, and simplicity in identification of phosphorylation sites and quantification of changes in phosphorylation states [13,14]. However, the identification and characterization of phosphoproteins remains a challenge due to their low abundance and low ionization efficiency [15]. The selective enrichment of phosphoproteins or phosphopeptides from complex mixtures is therefore essential for MS-based phosphoproteomics. To date, various affinity materials and techniques have been introduced to specific capture phosphopeptides. Immobilized metal ion affinity chromatography (IMAC) is the most commonly used method to enrich phosphopeptides, which relies on the affinity of the phosphate groups to metal ions immobilized on a matrix. In recent years, metal oxide nanoparticles such as TiO_2, HfO_2, and ZrO_2 have been demonstrated to be more potential in phosphopeptides analysis than conventional IMAC because such oxides rely on specific and reversible chemisorption of phosphate groups on their amphoteric surface and have less non-specific binding [16–18]. In particular, recent advances in TiO_2 synthesis have led to the development of the current state of the art phosphate-adsorbing materials, which have a higher enrichment capacity and better selectivity than solid oxides [19,20].

Carbon nanotubes (CNTs) were first discovered in 1991 by Iijima [21], from then on, intensive studies have been carried out to explore their applications in various fields [22,23]. To date, many hybrid nanomaterials based on CNTs have been reported, which combine the fantastic physical–chemical properties of both carbon nanotubes and functional nanoparticles or molecules [24–26], such as magnetic nanoparticle–CNTs and gold nanoparticle–CNTs. These reports demonstrated that CNTs were useful support material because of their ability to prevent the macroscopic aggregation of nanoparticles. Therefore, it can be expected that, metal oxides nanoparticles that show affinity to phosphopeptide and can coated on CNTs to form CNTs-based composites may have potential performance on enrichment of phosphopeptide. However, when the phosphopeptide-bound materials are harvested using centrifugation, high-molecular-weight non-phosphopeptides are sedimented at high

* Corresponding author at: Department of Chemistry, Fudan University, Shanghai 200433, China. Fax: +86 21 65641740.
E-mail address: chdeng@fudan.edu.cn (C. Deng).

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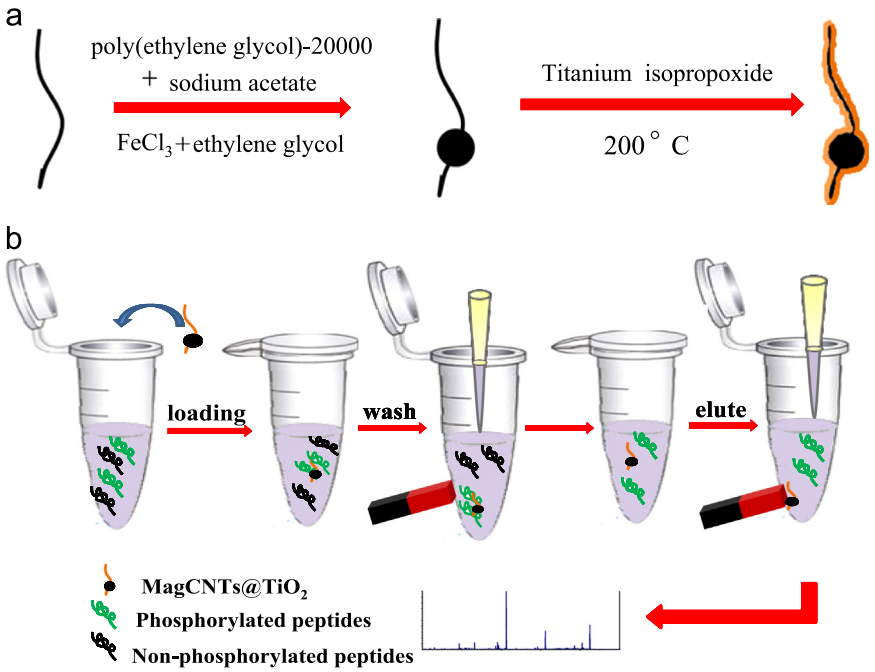

Scheme 1. Schematic illustration of (a) synthetic procedure for the fabrication of the MagCNTs@TiO_2 composites and (b) the selective process for the enrichment of phosphorylated peptides using MagCNTs@TiO_2 composites and magnetic separation..

rotation speeds. We therefore formulated a rational design to combine magnetic nanomaterials with porous TiO_2, with the aim of achieving the simple and efficient separation and enrichment of the phosphopeptides from complex samples.

Herein, we synthesized titania nanoparticles coated magnetic carbon nanotubes (MagCNTs@TiO_2 composites) through a facile solvothermal reaction, and applied them for highly selective enrichment of phosphopeptides. The synthesized procedure for the MagCNTs@TiO_2 composites is illustrated in Scheme 1a. To demonstrate the highly selective enrichment ability for phosphopeptides, MagCNTs@TiO_2 composites were applied to enrich the phophopeptides in different biological samples for mass spectrometry analysis (Scheme 1b).

2. Experimental

2.1. Materials and chemicals

Trifluoroacetic acid (TFA), β-casein, ammonium bicarbonate (NH_4HCO_3), bovine serum albumin, trypsin from bovine pancreas. 3-(trihydroxysilyl)propyl methylphosphate and 2,5-dihydroxybenzoic acid (DHB, Sigma). Acetonitrile was purchased from Merck (Darmstadt, Germany). Human serum was supplied by Zhongshan Hospital. All aqueous solutions were prepared using Milli-Q water by Milli-Q system (Millipore, Bedford, MA).

2.2. Synthesis of MagCNTs@TiO_2 composites

The magnetic CNTs were prepared via a modified hydrothermal method reported by Jia et al. [27]. Briefly, multiwalled CNTs (400 mg, diameter 20–40 nm, Shenzhen Nanotech Port Co., Ltd.) were dispersed into 50 mL concentrated nitric acid at 60 °C with magnetic stirring for 7 h. Then the pretreated CNTs (150 mg) and $FeCl_3 \cdot 6H_2O$ (810 mg) were dispersed into 40 mL ethylene glycol solution with trisodium citrate (0.15 g), sodium acetate (3.6 g) and poly(ethylene glycol)-20000 (1.0 g) by ultrasonication and stirring for 3 h. The mixture was sealed in the autoclave to be heated at 200 °C for 10 h. Finally the gained MagCNTs were washed and collected.

The MagCNTs@TiO_2 composites were synthesized according to previous report with some modification [28]. Briefly, the MagCNT powder (30 mg) was dispersed in isopropyl alcohol (50 mL) under sonication for 0.5 h. Then, 0.02 mL of diethylamine was added and stir for 5 min. Afterwards, 1.5 mL of titanium isopropoxide was added. The solution was transferred into a autoclave for heating at 200 °C for 24 h. The product was collected and washed thoroughly, and finally dried at 60 °C for 8 h. The dried sample was annealed at 400 °C in air for 2 h with a heating rate of 1.0 °C/min.

2.3. Characterization and measurements

Scanning electron microscopy (SEM) images were obtained on a Philips XL30 electron microscope (Netherlands) operating at 20 kV. Transmission electron microscopy (TEM) images were taken with a JEOL2011 microscope (Japan) operating at 200 kV. Wide-angle X-ray diffraction (WAXRD) patterns were recorded on a Bruker D4 X-ray diffractometer (Germany) with Ni-filtered Cu KR radiation (40 kV, 40 mA).

2.4. Sample preparation

Bovine serum albumin was reduced with dithiothreitol [DTT] and carboxamidomethylated with iodoacetamide. Then bovine serum albumin and β-casein were dissolved in 25 mM NH_4HCO_3 buffer at pH 8.3 and treated with trypsin (2% w/w) for 16 h at 37 °C, respectively. Human serum was diluted 10 times with 50% acetonitrile and 0.1% trifluoroacetic acid [TFA] aqueous solution (v/v).

2.5. Enrichment of phosphopeptides from tryptic digestion of standard proteins

MagCNTs@TiO_2 composites (200 μg) were added to 200 μL of a peptide mixture originating from tryptic digestion. The mixture was vibrated at room temperature for 30 min. After washed with 200 μL of