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Facile synthesis of titania nanoparticles coated carbon nanotubes for selective enrichment of phosphopeptides for mass spectrometry analysis

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ABSTRACT

In this work, titania nanoparticles coated carbon nanotubes (denoted as CNTs/TiO_2 composites) were synthesized through a facile but effective solvothermal reaction using titanium isopropoxide as the titania source, isopropyl alcohol as the solvent and as the basic catalyst in the presence of hydrophilic carbon nanotubes. Characterizations using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) indicate that the CNTs/TiO_2 composites consist of CNT core and a rough outer layer formed by titania nanoparticles (5–10 nm). Measurements using wide angle X-ray diffraction (WAXRD), zeta potential and N_2 sorption reveal that the titania shell is formed by anatase titania nanoparticles, and the composites have a high specific surface area of about 104 m^2/g. By using their high surface area and affinity to phosphopeptides, the CNTs/TiO_2 composites were applied to selectively enrich phosphopeptides for mass spectrometry analysis. The high selectivity and capacity of the CNTs/TiO_2 composites have been demonstrated by effective enrichment of phosphopeptides from digests of phosphoprotein, protein mixtures of β-casein and bovine serum albumin, human serum and rat brain samples. These results foresee a promising application of the novel CNTs/TiO_2 composites in the selective enrichment of phosphopeptides.

1. Introduction

Protein phosphorylation is one of the most important and ubiquitous post-translational modifications (PTMs). It is a key regulator of almost all aspects of cellular processes in both prokaryotes and eukaryotes [1,2]. Mass spectrometry (MS) is a powerful technique for determining the phosphorylation profiles of proteins in phosphoproteome research because of its high sensitivity, high-throughput, and simplicity in identification of phosphorylation sites and quantification of changes in phosphorylation states [3–5]. However, phosphopeptides can be suppressed by non-phosphopeptides in MS detection due to their low abundance and low ionization efficiency [6]. To solve these problems, various affinity materials and affinity enrichment techniques have recently been introduced for the enrichment of phosphopeptides, such as immobilized metal ion affinity chromatography (IMAC), metal oxides affinity chromatography (MOAC) and metal oxide mesoporous materials etc. [7–14]. Generally, the basic principle for these selective enrichment techniques can be viewed as a Lewis acid–Lewis base interaction. The phosphate groups of phosphopeptides act as bidentate ligands (Lewis bases) which can effectively bind metal atoms (Lewis acids) upon contacting metal oxides or immobilized metal ions [15]. Following this principle, increasing efforts have been devoted to designing high performance nano-sorbents that possess high surface area, integrated functionalities, and novel nanostructures with an aim to achieve a highly selective, efficient, convenient and fast enrichment of phorsphopeptides from complex samples as well as an improved phosphopeptides recovery [14].

Since carbon nanotubes (CNTs) were first discovered in 1991 by Iijima [16], intensive studies of their properties have been carried out to explore their applications in various fields [17–21]. A great variety of CNTs-based hybrid nanomaterials have been reported, including gold nanoparticle-CNTs, magnetic nanoparticle-CNTs, and functional probe molecules-modified CNTs, which combine the fantastic physic-chemical properties of both carbon nanotubes and functional nanoparticles or molecules [22–26]. Moreover, carbon nanotubes (CNTs) were demonstrated as a unique support for nanomaterials. For instance, Chen et al. reported Pt nanoparticles-CNTs composites obtained by the microwave assisted polyol synthesis method, and successfully used the composites for methanol electrooxidation [27]. Peng et al. synthesized ceria supported on carbon nanotubes (CeO_2/CNTs) via a controlled sol–gel coating method. CeO_2 nanoparticles were found to uniformly deposit on the surface of CNTs without self-aggregation, which endowed the composites with high surface area for efficient removal of arsenate from water [28]. All these previous reports clearly indicate that CNTs are a useful support material for both in situ growth and post

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loading of functional nanoparticles due to their ability to prevent the macroscopic aggregation of nanoparticles, especially precious metal nanoparticles and active metal oxides. Therefore, it can be expected that, metal oxides nanoparticles that show affinity to phosphopeptide and can be coated on CNTs to form a CNTs-based nanocomposite have a promising application in phosphopeptide analysis.

Herein, we rationally designed and synthesized titania nanoparticles coated carbon nanotubes (CNTs/TiO_2 composites) through a facile solvothermal reaction for selective enrichment phosphopeptides for mass spectrometry analysis. The obtained CNTs/TiO_2 composite has an affinity metal oxide shell constructed by titania nanoparticles which can selectively enrich phosphopeptides. SEM, TEM and zeta potential observations reveal that the obtained CNTs/TiO_2 composites maintained the tubular structure but with rough surface morphology, and the walls of CNTs were fully coated by titania nanoparticles of 5–10 nm. WAXRD and N_2 sorption characterizations indicate that the titania nanoparticles have an anatase crystalline phase, and the surface area of the CNTs/TiO_2 composites is 104 m^2/g as a result of the close packing of nanoparticles on CNTs. Enrichment study shows that the CNTs/TiO_2 composites possess high performances in selective enrichment of phosphopeptides from complex peptide mixtures, its detection is 2×10^{-10} M, which suggests that the CNTs/TiO_2 composites are promising nanomaterials for specific enrichment of phosphopeptides.

2. Experimental

2.1. Chemicals

Bovine β-casein, bovine serum albumin (BSA), L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) treated trypsin (from bovine pancreas), 2, 5-dihydroxybenzoic acid (DHB) and ammonium bicarbonate (NH_4HCO_3) were purchased from Sigma Chemical (St. Louis, MO). Acetonitrile was purchased from Merck (Darmstadt, Germany). All aqueous solutions were prepared using Milli-Q water by Milli-Q system (Millipore, Bedford, MA). Carbon nanotubes (multi-walled) with diameter of 20–40 nm were purchased from Nanoport Company (Shenzhen, China). Titanium(IV) isopropoxide (95%) and diethylamine (99%) were purchased from Alfa Aesar.

2.2. Synthesis of CNTs/TiO_2 composites

The pristine CNTs were firstly activated by treating in a concentrated nitric acid (65 wt%) at 100 °C under magnetically stirring for 12 h. The black dispersion was then diluted with water, and the CNTs were collected and washed with deionized water by filtration until the pH value of the filtrate reaches neutral. The acid-treated CNTs were dried in vacuum at 60 °C overnight for further use. The CNTs/TiO_2 composites were synthesized according to previous report with some modification [29]. Briefly, the activated CNT powder (30 mg) was dispersed in isopropyl alcohol (IPA, 50 mL) under sonication for 0.5 h. Then, 0.02 mL of diethylamine was added into the obtained stable dispersion followed by stirring for 5 min. Afterwards, 1.5 mL of titanium isopropoxide was added. After further stirring for 5 min, the solution was transferred into a Teflon-lined stainless-steel autoclave (100 mL capacity) for heating at 200 °C for 24 h in an oven. After reaction, the autoclave was taken out and left to cool down to room temperature. The product was collected by centrifugation and washed thoroughly with ethanol, and finally dried at 60 °C for 8 h. The dried sample was annealed at 400 °C in N_2 for 2 h with a heating rate of 1 °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 JEOL 2011 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). Nitrogen sorption isotherms were measured at 77 K with a Micromeritcs Tristar 3000 analyzer (USA). Before measurements, the samples were degassed in a vacuum at 200 °C for at least 6 h. The Brunauer-Emmett-Teller (BET) method was utilized to calculate the specific surface areas (S_{BET}) using adsorption data in a relative pressure range from 0.05 to 0.35.

2.4. Sample preparation

Bovine serum albumin was reduced with dithiothreitol [DTT] and carboxamidomethylated with iodoacetamide. Bovine serum albumin and bovine 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 with 50% acetonitrile and 0.1% trifluoroacetic acid [TFA] aqueous solution (v/v).

2.5. Preparation of the lysate of rat brain

Rats were sacrificed and the brains were quickly removed and placed in ice-cold homogenization buffer consisting of 7 M urea, 2 M thiourea and a mixture of protease inhibitor (1 mM phenylmethanesulfonylfluoride) and phosphatase inhibitors (0.2 mM Na_3VO_4, 1 mM NaF). After mincing with scissors and washing to remove blood, the brains were homogenized in a Pottter-Elvejhem homogenizer with a Teflon piston, using 5 mL of the homogenization buffer per 1 g of tissue. The suspension was homogenized for approximately 2 min, vortexed at 0 °C for 30 min, and centrifuged at 22,000*g* for 1.5 h. The supernatant contained the total brain proteins. Appropriate volumes of protein sample were precipitated as above, lyophilized to dryness, and redissolved in reducing solution (6 M guanidine hydrochloride, 100 mM NH_4HCO_3, pH 8.3) with the protein concentration adjusted to 2 mg/mL. Then, 200 mg of this protein sample (100 mL volume) were mixed with 10 mL of 0.5 M DTT. The mixture was incubated at 37 °C for 1 h, and then 20 mL of 0.5 M 2-iodoacetamide were added and incubated for an additional 30 min at 37 °C in the dark. The protein mixtures were exchanged into 50 mM NH_4HCO_3 buffer, pH 8.5, and incubated with trypsin (40:1) at 37 °C overnight.

2.6. Enrichment of phosphopeptides from tryptic digestion of standard proteins

In a typical process, a suspension of CNTs/TiO_2 composites (10 μL, 2 mg/mL) was added to 200 μL of a peptide mixture originating from tryptic digestion. The mixture was then vibrated at 25 °C for 30 min. The phosphopeptide-loaded CNTs/TiO_2 composites were collected by centrifugation and washed with 200 μL of a 50% acetonitrile and 0.1% TFA water solution three times. Subsequently, an aqueous solution of NH_4OH (5 μL, 0.4 M) was added to elute the captured phosphopeptides and the eluate was analyzed by MALDI–TOF MS.