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ZrO_2 doped magnetic mesoporous polyimide for the efficient enrichment of phosphopeptides

Xi-Ming Wang, Zhi-Yong Guo, Yue Zhang, Ming-Li Chen*, Jian-Hua Wang*

Research Center for Analytical Sciences, Department of Chemistry, College of Sciences, Northeastern University, Box 332, Shenyang 110819, China

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ABSTRACT

Fe_xO_y and ZrO_2 nanoparticles co-doped layered porous polyimide, polyimide-Fe_xO_y-ZrO_2 is prepared with a one-step strategy, shortly termed as PI-Fe_xO_y-ZrO_2. The layered and porous structure of the polymer offers a supported platform for metallic oxide anchoring, exhibiting a mesopore size of 3.93 nm and providing a surface area of 198.47 $m^2\ g^{-1}$. The metallic oxides were uniformly and highly dispersed in the PI-Fe_xO_y-ZrO_2 nanocomposite with percentages of 15.81 and 20.53 wt% for Fe and Zr, respectively. The magnetic Fe_xO_y provides driving force for rapid separation. The high doping of ZrO_2 facilitates effective enrichment of phosphopeptides, even at a very low mass ratio of 1:1000 for tryptic digest of phosphopeptides/non-phosphopeptides, e.g., β-casein/BSA in this particular case. In addition, the PI-Fe_xO_y-ZrO_2 nanocomposite exhibits better adsorption performance to phosphopeptides with respect to commercial titanium dioxide nanoparticles. The effectiveness of low-abundant phosphopeptides isolation and enrichment from human serum is further identified and demonstrated by means of MALDI-TOF MS and LC-ESI-MS/MS.

1. Introduction

Post-translational modifications of proteins are among the most crucial tools for signaling in cellular networks [1–4]. Protein phosphorylation has become a major regulatory machinery in regulating many complicated biological processes, e.g., cell cycling, cell growth, cell differentiation [1] and several diseases like cancers [5], Alzheimer's disease [6] and diabetes [7]. Currently, mass spectrometry-based techniques are preferential for the characterization of protein phosphorylation due to its wide dynamic range, ultra-high sensitivity and high analytical speed. However, low dynamic stoichiometry and severe signal interference from their high abundance native counterparts make mass spectrometric analysis of phosphopeptides still a great challenge in the absence of sample pretreatment. In this respect, an effective capture and enrichment for phosphopeptides from complicated biological sample matrixes in advance of MS analysis is highly desired.

Numerous strategies have been applied to the separation of phosphorylated proteins and peptides from the nonphosphorylated counterparts. These include immobilized metal ion affinity chromatography (IMAC) [8], molecular imprinting [9,10], chemical modification [11], strong cation exchange (SCX) [12,13], strong anion exchange (SAX) [14], amine-based affinity [15] and metal oxide affinity chromatography (MOAC) [16]. Among these approaches, immobilized metal affinity chromatography (IMAC) has been most widely used. In practice, IMAC requires the immobilization of metal ions, especially Fe^{3+}, Zr^{4+} and Ti^{4+} [17–22]. Recently, metal oxide affinity chromatography (MOAC) [23–25], relying on the interactions among the target analytes and the metal oxide, have gained more attention because of their excellent properties for phosphopeptides enrichment. A few metal oxides, e.g, TiO_2 [26], ZrO_2 [27], SnO_2 [28], Fe_2O_3 [29] and Ta_2O_5 [30], have been demonstrated to be effective adsorbents for the enrichment of phosphopeptides as they seem to have higher selectivity to phosphopeptides due to their reduced nonspecific binding.

Microporous or mesoporous organic polymers (MOPs) with high surface areas, microporosity and mechanical stability are much attractive in various application fields, e.g., gas storage and separation [31,32], catalysis [33], drug delivery [34] and pollutant removal [35]. The synthetic flexibility of MOPs make them superior to inorganic microporous materials such as zeolites and activated carbons. Therefore, various protocols have been reported for the synthesis of MOPs, including metal catalyzed coupling reactions [36], click reactions [37] and cyclotrimerization [38]. It is noted that aromatic polyimides (PIs) are among the most useful classes of high-performance polymers with excellent thermal and thermo-oxidative stability, solvent resistance, favorable electrical insulating properties as well as superior mechanical characteristics. However, they are rarely employed as absorbents for proteome enrichment, especially for peptide isolation.

Herein, a novel hierarchical and porous metal oxide affinity

* Corresponding authors.
E-mail addresses: chenml@mail.neu.edu.cn (M.-L. Chen), jianhuajrz@mail.neu.edu.cn (J.-H. Wang).

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chromatography (MOAC) material was designed by chelating acetylacetonate and polyamide acid (PAA). During the imidization process, PAA is dehydrated and condensed into polyimide (PI). At the same time, acetylacetonate is converted to metal oxide. The obtained nanocomposite was magnetic, possessing a fine porous structure, large surface area and moderate pore volume, with uniformly and highly dispersed metallic oxide. These properties bring an excellent capacity for the enrichment of phosphopeptides with respect to the non-phosphopeptides. In practice, the composite was further employed for the enrichment of endogenous phosphopeptides from human serum.

2. Experimental section

2.1. Materials

Iron (III) nitrate nonahydrate ($Fe(NO_3)_3{\cdot}9H_2O$), zirconium chloride ($ZrCl_4$), 3,3′,4,4′-benzophenonetetracarboxylic dianhydride (BTDA) were purchased from Aladdin Chem. Co. Ltd (Shanghai, China). 4,4′-diaminobiphenyl (BZD), ammonium hydroxide ($NH_3{\cdot}H_2O$, 25%), acetylacetone (ACAC) and N,N-Dimethylformamide (DMF) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). β-Casein, bovine serum albumin (BSA), dithiothreitol (DTT), iodoacetamide (IAA), ammonium bicarbonate (ABC, 99.5%), 1–1-(tosylamido) − 2-phenyl-ethyl chloromethyl ketone (TPCK)-treated trypsin, acetonitrile (ACN, chromatographic grade), formic acid (FA) and trifluoroacetic acid (TFA) were acquired from Sigma (St. Louis, USA). These reagents were at least of analytical-reagent grade and used as received without any further treatment. Human whole blood from a healthy volunteer was provided by the Hospital of Northeastern University.

2.2. Preparation of polyamic acid (PAA)

In a typical synthesis procedure, 1.78 g of benzidine (BZD) as the diamine monomer was dissolved in 50 mL DMF, then 3.11 g of 3,3′,4,4′-benzophenonetetracarboxylic dianhydride (BTDA) as the dianhydride monomer was added into the mixture. The pre-polymerization reaction was allowed to carry out for 10 h by stirring continuously at ambient temperature, and a brownish viscous PAA solution was obtained.

2.3. Preparation of PI-Fe_xO_y-ZrO_2

0.38 g of $ZrCl_4$ and 1.00 g of ($Fe(NO_3)_3{\cdot}9H_2O$) were first added into 1 mL and 0.75 mL ACAC, respectively. The two solutions were then mixed into a 10 mL centrifuge tube containing 2 mL of DMF to get a brown solution for further use.

The above Fe-Zr-ACAC solution was drop-wisely added into 10 mL PAA solution under magnetic stirring for 10 h. Afterwards, the hybrid was transferred into a Teflon-inner autoclave to further polymerization at 180 °C for 10 h. The mixture was allowed to cooling down naturally to room temperature, and the precipitate in the autoclave was filtrated through a 0.22 μm membrane followed by washing with DMF and ethanol alternatively for three times. Finally, the brown color PI-Fe_xO_y-ZrO_2 powder was collected by vacuum drying for overnight and calcination at 280 °C for 5 h.

2.4. Characterizations

SEM images are implemented on a SU8010 Scanning electron microscope (Hitachi, Japan). TEM images and Energy-dispersive X-ray spectra (EDS) are recorded on a Tecnai G2 F20 Transmission electron microscope (FEI, USA). X-ray photoelectron spectroscopy (XPS) scanning curves of native PI and the PI-Fe_xO_y-ZrO_2 composite are obtained on an ESCALAB 250 surface analysis system (Thermo Electron, England) with an Al Kα 280 eV excitation source. FT-IR spectra from 4000 to 500 cm^{-1} are performed on a Nicolet 6700 spectrophotometer (Thermo Electron, USA) with a resolution of 2.0 cm^{-1}. X-ray diffraction (XRD) patterns are carried out on an X′Pert Pro MPD X-ray diffractometer (PANalytical BV, Holland) with Cu Kα radiation at λ 1.54 Å. Magnetic characterization is performed with a vibrating sample magnetometer (VSM) on a Model 6000 physical property measurement system (Quantum, USA) at 300 K. Thermogravimetric analyses (TGA) are carried out with a TGA 290 C analyzer (Netzsch, Germany) with a heating rate of 10 °C min^{-1} under nitrogen protection. Nitrogen sorption/desorption experiment is carried out on a Micromeritcs Tristar 3000 analyzer (USA). Zeta potentials are recorded on a Nano-ZS90 laser nanoparticle analyzer (Malvern, England).

2.5. Preparation of tryptic digests of proteins and treatment of serum

A trypsin solution of 10 mg mL^{-1} was first obtained by dissolving 10 mg of trypsin into 1 mL ABC buffer (50 mM, pH 8.2). A mixture contains 5 mg β-casein and 1 mL ABC buffer was boiled for 5 min and cooled down naturally, followed by digestion at 37 °C for 16 h with the addition of 20 μL trypsin solution. As a model of non-phosphopeptides, 20 mg of BSA was dissolved into 5 mL of ABC buffer. For reducing the disulfide bonds in BSA, 200 μL of DTT (1 mmol L^{-1}) was added and the solution was maintained at 60 °C for 1 h. Then, 74 mg of IAA was introduced to alkylate the protein in dark for about 40 min. Subsequently, the solution was incubated with 80 μL of trypsin at 37 °C for 16 h. Finally, the two tryptic digests were stored at − 20 °C for future use.

Human whole blood without anticoagulation was placed in a blood-collecting vessel for 1 h, followed by centrifugation at 2000 rpm for 10 min to collect human serum. 25 μL of human serum was added into 250 μL of loading buffer (49% H_2O, 50% ACN, 1% TFA, v/v/v) with gentle blending for 30 min at 25 °C. The mixture was vortexed for 3 min with a rotation speed at 1000 rpm, and then the supernatant was collected and stored at − 20 °C for further use.

2.6. Phosphopeptides enrichment with PI-Fe_xO_y-ZrO_2

2 mg of the PI-Fe_xO_y-ZrO_2 composite was pre-washed by 50% ACN and kept in a centrifuge tube after equilibrated with 1 mL loading buffer. Meanwhile, 20 μL of β-casein digest was transferred into the centrifuge tube and mixed with the PI-Fe_xO_y-ZrO_2 composite. The mixture was gently incubated at room temperature for 30 min. After removal of the supernatant, the precipitate was washed twice with 1 mL of loading buffer to remove the non-specifically bound peptides for twice. The captured phosphopeptides were then eluted twice by $NH_3{\cdot}H_2O$ (100 μL, 5 wt%). After magnetic separation, the supernatant containing phosphopeptides was collected in a centrifuge tube.

For phosphopeptides enrichment from human serum, 2 mg of the PI-Fe_xO_y-ZrO_2 composite was used for the treatment of 250 μL diluted serum in 750 μL loading buffer. The mixture was incubated for 30 min to facilitate adsorption of phosphopeptides. After removal of the supernatant and cleaning twice with 1 mL of loading buffer to eliminate the non-specifically adsorbed species, 100 μL of $NH_3{\cdot}H_2O$ (5 wt%) was added and the mixture was incubated for 15 min to recover the captured phosphopeptides. This operation was repeated for twice and the eluent was combined for further analysis by MS.

2.7. MALDI-TOF MS analysis and database processing

1 μL of peptide solution was first deposited on a MALDI plate, and then 1 μL of DHB matrix aqueous solution was deposited. MALDI-TOF MS experiments were performed using an ultrafleXtreme time-of-flight mass spectrometer (Bruker, Bremen, Germany) in positive-ion reflector mode with a 337 nm nitrogen laser. The spectra were acquired with an available accelerating voltage of 20 kV, and the laser intensity was kept constant. The mass spectra covering the mass range from m/z 700–3500 were recorded and analyzed by flex Analysis software (version 3.3).