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Mesoporous Fe_2O_3 microspheres: Rapid and effective enrichment of phosphopeptides for MALDI-TOF MS analysis

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Abstract

Mesoporous Fe_2O_3 microspheres have been successfully synthesized by the polymerization (urea and formaldehyde)-induced ferric hydroxide colloid aggregation. The urea-formaldehyde resin was removed by calcination in air. The obtained mesoporous Fe_2O_3 materials have spherical morphology with uniform particle size of ~3.0 μm and porous surface with large inter-particle pores of ~48.0 nm. The surface area is as large as ~33.3 m^2/g and the pore volume is 0.31 cm^3/g. The mesoporous Fe_2O_3 microspheres were used for the enrichment of phosphopeptides for the first time, in which high sensitivity, selectivity and capacity of specifically enriched phosphopeptides were achieved under a mild condition in a relative short time. After enriched from tryptic digest products of β-casein by the novel mesoporous Fe_2O_3 microspheres, phosphopeptides can be selectively detected with high intensity in MALDI-TOF mass spectrometry. Elimination of "shadow effect" was observed by using mesoporous Fe_2O_3 microspheres, and the detectable limitation is 5×10^{-10} M. This material is also effective for enrichment of phosphopeptides from the complex tryptic digests of commercial phosphoprotein casein, with much more phosphorylated sites (26 in 27 of total) and higher signal/noise ratio in the MALDI-TOF mass spectrometry, compared to commercial Fe_2O_3 nanoparticles. It shows a great potential application in the field of rapid and effective isolation of phosphopeptides.

Keywords: Ferric oxide; Mesoporous materials; Mass spectrometry; Phosphopeptide; Specific enrichment

1. Introduction

Protein phosphorylation is the best known post-translational modification and one-third of the proteins expressed in mammalian cells are phosphorylated at serine, threonine and tyrosine residues [1,2]. The reversible phosphorylation of proteins plays an important role in regulating cellular functions including signal transduction, metabolism and apotosis. In order to better understand the molecular basis of these processes, it is important to identify these phosphorylation sites of proteins. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), which is a robust and sensitive analytical method, has been successfully utilized to map the phosphorylation sites [3,4]. Prior to the positive MALDI mass analysis, the selective enrichment of phosphopeptides from complex mixtures is necessary to enhance the detection, because the signals are often suppressed by that of the co-existing abundant non-phosphorylated peptides [5].

Immobilized metal-ion affinity chromatography (IMAC) chelating Fe(III) ions have been substantially developed as a reliable method to purify and concentrate the phosphopeptides in the last few years [6–11]. In this method, immobilized (chelated) Fe (III) ions show high affinity for the oxygen atoms in the side chains of serine, threonine and tyrosine under acidic conditions, and high-pH elution disrupts this interaction after the separation of other nonphosphopeptides. The Fe^{3+}-IMAC beads binding target peptides can also be directly analyzed by MALDI-MS to simplify the sample preparation with the elimination of phosphopeptides elution from the metal ion column [12,13]. Recently, some new materials [14–25], including the nanoparticles incorporating Fe^{3+} [21–24], have been successfully employed to effectively isolate phosphopeptide from proteolytic mixtures. However, the present approach for the se-

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lective enrichment requires a long loading time (>60 min) or a high acidity of loading solution such as 0.1% trifluoroacetic acid (TFA) solution (pH ∼2) to avoid the nonspecific binding of nonphosphopeptides containing multiple acidic residues. As a result, novel carriers for rapid and effective extraction of phosphopeptides are still attractive.

Ferric oxide (Fe_2O_3) materials are also good candidates for the enrichment of phosphopeptides, because it has been proved to specifically react with phosphate groups under an acidic condition [26,27]. Meanwhile it requires high surface area, large pore size and porosity to achieve fast and efficient enrichment. As a result, mesoporous Fe_2O_3 materials are speculated to be a promising substrate. However, it is significantly difficult to synthesize mesoporous ferric oxides, because it is hard to control the hydrolysis and condensation of ferric precursors. Until now, there are only a few reports of mesoporous Fe_2O_3 [28–32], and the morphology has not been discussed yet. As a substrate, the morphology is a very important factor affecting the sorption and separation of phosphopeptides. And for the micro-scaled spheres, the large particle will make it rapid and easy to the trap and elution treatment. It is therefore critical to synthesize mesoporous Fe_2O_3 with specific morphology.

Herein, a facile process of polymerization-induced ferric hydroxide colloid aggregation is demonstrated for the synthesis of mesoporous Fe_2O_3. The obtained mesoporous Fe_2O_3 materials show microspherical morphology with particle size of ca. 3 μm and large pore size of ca. 48 nm. The mesoporous Fe_2O_3 spheres can be used as a substrate for the enrichment of phosphopeptides. Our results show that nonphosphopeptides from tryptic digest can be effectively removed and phosphopeptides can be selectively detected in MALDI-TOF mass spectra. The treatment condition is mild and the loading time is relatively short. Meanwhile the detection limit is significantly low. In addition, the large pore sizes of mesoporous Fe_2O_3 may favor the elimination of the shadow effect during the direct MS analysis of phosphopeptides trapped on Fe_2O_3 beads.

2. Experimental

2.1. Chemicals

Ferric chloride (AR 98.0%), sodium hydroxide (AR 96.0%), urea (AR 99.0%), formaldehyde solution (AR 37.0–40.0%) and commercial ferric oxide nanoparticles were purchased from Shanghai Medical Corp. 2,5-Dihydroxybenzoic acid (DHB), acetic acid (AA), trifluoroacetic acid (TFA) and acetonitrile (ACN) were purchased from Merck (Germany). Trypsin, β-casein and casein (from milk) were purchased from Sigma (MO, USA). The water used was obtained from a Milli-Q water purification system (Millipore, MA, USA). Other reagents were purchased from Shanghai Chemical Reagent Company (China).

2.2. Synthesis

Mesoporous Fe_2O_3 materials were prepared via the polymerization-induced colloid aggregation method by using ferric hydroxide as a colloid precursor, urea and formaldehyde as the monomer agents, according to the method in our previous work [33]. For a typical synthesis, 20 mL of 6 M $FeCl_3$ solution and 20 mL of 12 M NaOH solution were heated to 60 °C separately. The hot NaOH solution was added into $FeCl_3$ solution slowly under violent stirring, after that stirring for additional 10 min at 60 °C, then ferric hydroxide colloid was formed. After the colloid was cooled down to 13 °C, 3.6 g of urea and 3.6 mL of formaldehyde solution (36 wt%) were added subsequently. The mixture was stirred for 30 s and then kept static for 2 h. The precipitate was filtered, washed with water and ethanol, and then dried. As-synthesized urea-formaldehyde resin/ferric hydroxide composites were calcined at 550 °C in air for 3 h to yield mesoporous Fe_2O_3 microspheres.

2.3. Characterization

X-ray diffraction (XRD) patterns were recorded on a Bruker D4 X-ray diffractometer with Ni-filtered Cu$K\alpha$ radiation (40 kV, 40 mA). Scanning electron microscopy (SEM) images were obtained on a Philips XL 30 microscope operated at 20 kV. Transmission electron microscopy (TEM) images were obtained with a JEOL 2011 microscope operated at 200 kV. For TEM measurements, the samples were dispersed in ethanol, and then dipped and dried on Cu grids. Nitrogen adsorption–desorption isotherms were measured at 77 K by using a Micromeritics ASAP Tristar 3000 system. The samples were degassed at 180 °C for 8 h on a vacuum line. The Brumauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas. The pore size distributions were derived from the adsorption branches of the isotherms based on the Barrett–Joyner–Halenda (BJH) model.

2.4. Proteolysis and enrichment of phosphopeptides

β-Casein and casein were respectively dissolved in 25 mM NH_4HCO_3 solution, and trypsin at a weight ratio of 1:50 with the proteins was added into the protein solutions. The digestion proceeded at 37 °C for 18 h. To perform an enrichment, Fe_2O_3 microsphere suspension (10 μL, 5 mg mL^{-1} in pure water) and tryptic casein digest were mixed in 200 μL of loading solution containing 0.1% acetic acid and 30% acetonitrile (pH ∼4), and then incubated for 5 min at room temperature. After centrifugation, the deposited particles with trapped phosphopeptides were resuspended (or eluted) by 20 μL of 1.0 M ammonia water (pH ∼10). 0.5 μL of resuspension (elution) was applied onto the MALDI plate for MS analysis with 2,5-dihydroxybenzoic acid (0.35 μL, 10 mg mL^{-1} in water solution containing 0.1% TFA and 50% ACN) as the assisted matrix.

2.5. MALDI-TOF mass analysis

MS analysis was performed on a 4700 proteomic analyzer (Applied Biosystems, USA). A laser (337 nm, 200 Hz) was used as a desorption/ionization source. The instrument was used in reflector-positive mode with an acceleration voltage of 15 kV.