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Zirconia layer coated mesoporous silica microspheres used for highly specific phosphopeptide enrichment

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ABSTRACT

Zirconia layer coated mesoporous silica microspheres with mesostructured cellular foams (MCFs) were prepared by NH_3/water vapor-induced internal hydrolysis method. Zirconia layer coated MCF microspheres were characterized by SEM, XRD, N_2 sorption, UV, and chromatographic analysis, and explored for enrichment of phosphopeptides. ZrO_2/MCF microspheres in solid-phase extraction (SPE) mode demonstrated much higher selectivity and higher efficiency towards phosphopeptide enrichment than bulk ZrO_2 particles. In particular, the selectivities of ZrO_2/MCF microspheres towards multi-phosphopeptides are even higher than that of the widely used commercial TiO_2 microparticles. The ZrO_2/MCF microspheres were also applied to enrich endogenous phosphopeptides from human serum, and twelve endogenous phosphorylated peptides could be specifically enriched.

1. Introduction

Protein phosphorylation is recognized as one of the most important post-translational modifications (PTMs), which involves in a large number of biological processes, such as cellular growth migration, differentiation, intercellular communication and metabolisms. Characterization of the phosphorylation sites of the proteins is crucial for understanding the role of protein phosphorylation in these biological processes [1–3]. However, the low abundance and sub-stoichiometric modification-site occupancy of PTMs can result in lessened ion signals of the phosphorylated peptides in mass spectrometry (MS) analysis, which will be suppressed by the large excess of non-phosphorylated peptides in protein digests. Therefore, a specific enrichment step targeting phosphopeptides from peptide mixture is prerequisite before MS analysis [4]. Considerable efforts, including immunopurification through phosphoprotein antibody [5,6], strong cation chromatography [7,8], strong anion chromatography [9], and affinity chromatography, have been expended to develop methods for the isolation of phosphopeptides aiming at the phosphate functional groups. Commonly used affinity based method is immobilized metal ion affinity chromatography (IMAC) [10–12] using Fe(III), Ga(III), or other metals. However, nonspecific binding of non-phosphorylated acidic peptides and the complexity of factors affecting phosphopeptide binding and release often result in low specificity for target phosphopeptides. Recently, phosphopeptide-enrichment strategies, based on metal oxide affinity chromatography (MOAC), have been widely used in large-scale phosphoproteome due to the specific and reversible affinity of phosphate groups to the amphoteric surface of metal oxides [13–18]. Microparticles of titanium dioxide (TiO_2) [13–15], zirconium oxide (ZrO_2) [16], aluminum hydroxide ($Al(OH)_3$) [17] and other metal oxides [18] have been explored in phosphopeptide isolation and exhibited higher specificity for trapping phosphate groups than IMAC. However, the relatively low surface areas of these microparticles limit their applications. Owning to their potential higher surface areas than particles of microscale, titania-coated magnetic iron oxide (Fe_3O_4@TiO_2) [19], and ZrO_2 nanoparticles [20], have been attempted by centrifugation and demonstrated higher capacities to trap phosphopeptides.

Mesoporous materials possess of unique textural properties, such as high surface area, flow-through ordered structure and narrow pore size distribution. Such large surface areas, together with the many active surface sites, can be translated into even higher loading capacity for binding phosphate groups. Mesoporous MCM-41 silica nanoparticles immobilized with titanium ion have been used to selectively capture phosphopeptides from complex peptide and protein mixtures with high sensitivity based on MALDI-TOF-MS detection, and four phosphorylated peptides from serum samples can be observed after enrichment with Ti(IV)-MCM-41 [21]. Very recently, ZrO_2 nanoparticles with mesoporous structure have demonstrated higher selectivity than commercial

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ZrO_2 microparticles for phosphopeptide enrichment [22]. However, the co-sedimentation of peptides in centrifugation procedure and large elution volumes embarrassed the nanoparticle efficiency in phosphopeptide enrichment. Therefore, metal oxide microparticles with high surface area which can be used in columns or tips are desired for phosphopeptide analysis. However, most of synthesized mesoporous ZrO_2 is thermal instability and will collapse by calcination at high temperatures, and it is challenging to control the morphology of the mesoporous ZrO_2 to obtain micro particles. Therefore, supporting zirconia on mesoporous silica microspheres is an alternative way to improve its dispersion and hence the surface area.

Due to the interfacial electronic and structural interactions between the metal oxide and silica support, the structural features and physical-chemistry properties of metal oxide layer in highly dispersed state on the surface of silica supports differ greatly to that of the bulk crystalline metal oxides [23–25]. Furthermore, these metal oxides dispersed mesoporous oxide materials can preserve the unique textural properties. Therefore, these metal oxides dispersed oxide materials have been widely applied as catalysis, separation, adsorption and host materials [26–29]. TiO_2 modified macroporous silica foams demonstrated higher efficient and sensitive phosphopeptide enrichment than TiO_2 nanoparticles. Based on the strong affinity of 4-coordinated Ti^{IV} species for the phosphate group, the highly dispersed Ti^{IV} species on the silica surface showed preferential enrichment of multi-phosphorylated peptides with very low detection limit and high selectivity [30]. Micrometer-sized three-dimensional siliceous mesostructured cellular foam (MCF) [31,32] spheres with large pore size have been synthesized and used as packing materials in high-performance liquid chromatography [33,34]. However, the ordered structure could be destroyed by the formation of bulk crystalline metal oxide, which puzzled high loadings of metal oxide on the surface of mesoporous silica [29]. Ogura and coworkers have recently resolved this disturbance and coated high loading of ZrO_2 on the surface of mesoporous SBA-15 silica without pore blocking by NH_3/water vapor-induced internal hydrolysis method [35].

In this study, zirconia layer coated MCF silica microspheres were synthesized and characterized thoroughly. The zirconia layer coated mesoporous MCF silica ZrO_2/MCF microspheres were explored in solid-phase extraction (SPE) mode to enrich phosphopeptides from standard tryptic protein digests, and further used for enrichment of the endogenous phosphopeptides from human serum.

2. Experimental

2.1. Chemicals and reagents

The following reagents were used: toluene, NH_3/H_2O (25%), pyridine, and acetone (analytical grade) from Tianjin Kermel (Tianjin, China); methanol from Shandong Yuwang Chemicals (Dezhou, China); dimethyloctadecylchlorosilane from Alfa-Aesar (Ward Hill, MA, USA); trifluoroacetic acid (TFA) from Dima Technology Inc. (Richmond Hill, USA); α-casein, ammonium bicarbonate, and lactic acid (LA) from Sigma–Aldrich (St. Louis, MO); trypsin (sequencing grade) from Promega (Madison, WI); formic acid (FA) from Acros (Geel, Belgium); acetonitrile (CH_3CN) from Merck (Darmstadt, Germany); urea from Shenyang Lianbang Chemicals (Shenyang, China); 3-hydroxypropionic acid (HPA) from TCI Chemicals (Japan); NH_3/H_2O (10%) from Fluka (Switzerland); TiO_2 from GL Sciences (Japan); GELoader tips from Eppendorf (Hamburg, Germany); and C18-AQ from Sunchrom (Friedrichsdorf, Germany). All the water used in experiments was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Synthesis and characterization of ZrO_2(12, 24)/MCF

2.2.1. Synthesis of ZrO_2(12, 24)/MCF

Mesoporous MCF silica particles were synthesized according to published procedure [36] and our previous work [34]. Synthesis of ZrO_2/MCF was according to published NH_3/water vapor-induced internal hydrolysis method [35]. Briefly, 12% (m/m) ZrO_2 equivalent of precursor of $ZrO(NO_3)_2{\cdot}2H_2O$ was wet-impregnated into the pores of MCF silica under stirring at 60 °C until dryness, and then further heated at 100 °C for 6 h. By repeating the above procedures, 24% (m/m) zirconia equivalent of precursor of $ZrO(NO_3)_2{\cdot}2H_2O$ impregnated MCF can be obtained. The zirconia precursor loaded MCF silica (1.0 g) was put in an open glass and kept inside the autoclave which contained a 14% ammonium hydroxide (20 mL) without contact with the ammonium hydroxide. The sample was heated at 100 °C for 12 h and calcined under air at 500 °C for 5 h. Then ZrO_2(12, 24)/MCF materials were obtained, where 12 and 24 represents the % (m/m) of ZrO_2 loaded into the MCF spheres.

2.2.2. Characterization of ZrO_2(12, 24)/MCF

Scanning electron microscopy (SEM) images were taken on a HB-600 electron microscope with an accelerating voltage of 25 kV. Powder X-ray diffraction (XRD) was performed on a Rigaku D/max 3400 diffractometer (Cu Kα radiation, λ = 0.1542 nm) at 20 kV and 50 mA. N_2 adsorption–desorption experiments were undertaken isothermally at 77 K on an automatic ASAP 2000 Micromeritics apparatus. Prior to measurement, the materials were out-gassed at 573 K for 10 h. BET surface areas were calculated from adsorption data in the relative pressure range from 0.01 to 0.2. The total pore volume was evaluated at a relative pressure about 0.99. Pore size distributions were analyzed with the supplied BJH software package from the adsorption branches of the isotherms. Ultraviolet–visible diffuse reflectance spectrum was recorded on a JASCO V-550 UV–visible spectrophotometer. The Van Deemter chromatographic experiments were performed on the HPLC system (Agilent 1100 Series, USA) consisted of a quaternary pump, an auto-sampler, a degasser, an automatic thermostatic column compartment, and a diode array UV detector.

2.3. Phosphopeptide enrichment

2.3.1. Trypsin digestion of α-casein

1 mg α-casein was dissolved in 1 mL of ammonium bicarbonate (50 mM, pH 8.0) and digest in trypsin for 18 h at 37 °C with a 1:40 (w/w) enzyme-to-protein ratio.

2.3.2. Phosphopeptide enrichment from standard phosphoprotein digests

250 μg of ZrO_2(12, 24)/MCF and MCF beads, 1 mg of TiO_2, or 500 μg of ZrO_2 beads were packed in GELoader tips, respectively. The ZrO_2(12, 24)/MCF, MCF, and ZrO_2 tips were equilibrated with 20 μL of 100 mg/mL 3-hydroxypropionic acid (HPA) in 0.1% TFA/80% CH_3CN and then 1 μL of α-casein digested solution was loaded on the ZrO_2/MCF, MCF and TiO_2 tips. After successive washing with 20 μL of 100 mg/mL HPA in 0.1% TFA/80% CH_3CN and 20 μL of 0.1% TFA/80% CH_3CN, the bound phosphopeptides were eluted with 20 μL of 2% ammonium hydroxide. For TiO_2 tips, 100 mg/mL HPA was replaced to 300 mg/mL lactic acid. The eluted solution was acidified with 10% FA and desalted with C_{18} microcolumns before ESI-Q-TOF-MS analysis.

2.3.3. Endogenous phosphopeptide enrichment from human serum

1 mg of ZrO_2(12)/MCF was slurried in 100 μL of 0.1% TFA/80% CH_3CN, packed onto SPE microcolumn, and then equilibrated with 90 μL of 100 mg/mL HPA in 0.1% TFA/80% CH_3CN. An aliquot