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Designed Synthesis of Titania Nanoparticles Coated Hierarchially Ordered Macro/Mesoporous Silica for Selective Enrichment of Phosphopeptides

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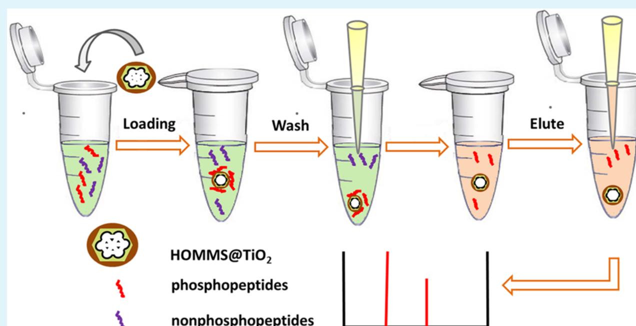
Supporting Information

ABSTRACT: Metal oxide affinity chromatography (MOAC) is a powerful technique in phosphoproteome research. However, the achievement of highly specific enrichment and sensitive detection of phosphopeptide by MOAC remains a big challenge since the lack of high specificity and large binding capacity of conventional MOAC materials. In this work, a new MOAC material, TiO_2-coated hierarchically ordered macro/mesoporous silica (denoted as HOMMS@TiO_2) composites, was prepared via a facile process. The HOMMS@TiO_2 composites were demonstrated to have low limit of detection (8 fmol) and great specificity with a very rapid enrichment speed (within 1 min). These experimental results have demonstrated that the HOMMS@TiO_2 exhibit great potential in phosphoproteome research.



KEYWORDS: *HOMMS@TiO_2, selective enrichment, phosphoproteomics, mass spectrometry*

INTRODUCTION

Protein phosphorylation is one of the most important post-translational modifications (PTMs). Phosphorylation of protein is involved in many biological processes including protein stability, cell division, DNA damage repair, enzyme activity, and signal transduction.[1,2] Thus, to identify the phosphorylation sites and quantify their dynamic changes is crucial. Mass spectrometry (MS) is the most important technique for phosphoproteomics reserach. However, to identify the phosphoproteins remain a challenge because phosphopeptides often coexist with plenty of nonphosphopeptides, which lead to the suppression of the signal of phosphopeptides. Therefore, isolation and extraction of phosphopeptides from complicated samples is necessary.[3−5]

To date, a variety of phosphoproteomics strategies for phosphopeptide enrichment have been developed, such as using antibodies,[6] chemical-modification strategies,[7] immobilized metal affinity chromatography (IMAC),[8,9] and metal oxide affinity chromatography (MOAC).[10−12] Because of the bridging bidentate binding between the surface of the metal oxide and the phosphate group,[13−15] MOAC methods have been demonstrated to be more potential in phosphoproteome research, such as TiO_2,[16,17] ZrO_2,[18,19] and Al_2O_3.[20,21] Due to high selectivity and high salt tolerance,[22,23] TiO_2-based MOAC materials[24−26] have been demonstrated to be the most potential materials for phosphopeptide enrichment.

Ordered mesoporous materials, due to the striking properties of meso-channels and quantum effects,[27] have attracted intensive attention since their discovery.[28,29] The unique property promotes their wide applications in many areas. Although mesoporous materials have a large surface area, diffusion is resistance because of long transportation distance.[30,31] While macroporous materials can provide efficient mass transport, their surface area is relatively lower. Hierarchically ordered macro/mesoporous materials have both large surface area and efficient mass transportation[32] and are desired to have potential performance in practical applications.

Herein, TiO_2-coated hierarchially ordered macro/mesoporous silica (denotes as HOMMS@TiO_2) composites were synthesized and used as the novel MOAC material for phosphoproteomcs study. Compared with the previous MOAC materials,[33,34] the as-made HOMMS@TiO_2 composites had uniform shapes and fast mass transport. The HOMMS@TiO_2 composites with above unique merits are anticipated to have potential performance in phosphoproteome analysis.

RESULTS AND DISCUSSION

The designed synthesis strategy of HOMMS@TiO_2 composites was shown in Scheme 1(a). At first, we synthesized HOMMS according to the previous method.[40] HOMMS@TiO_2 composites were synthesized via a hydrothermal method; the hydrothermal method can also be used for the coating of other metal oxides (such as SnO_2, ZrO_2) on macro/mesoporous

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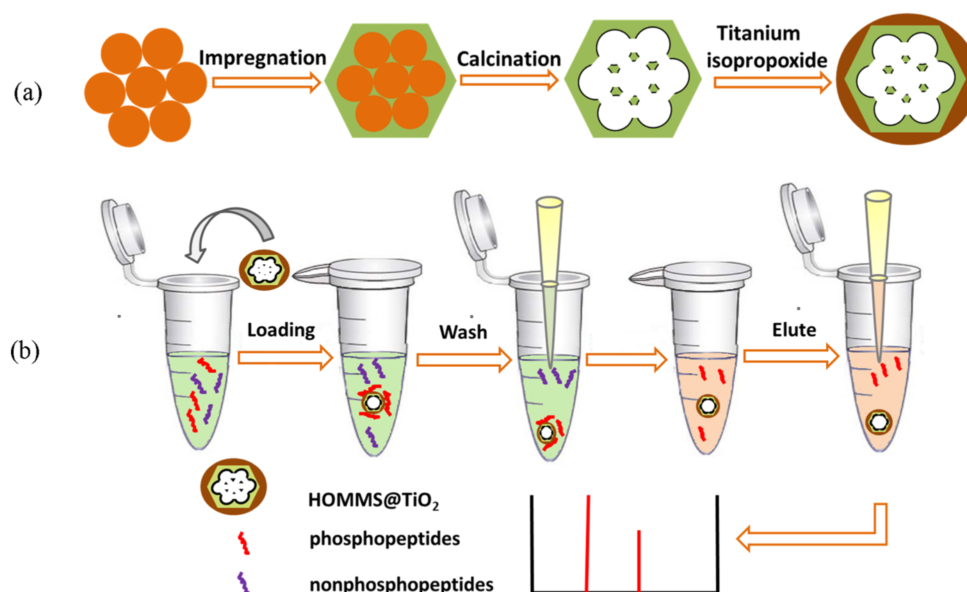
Scheme 1. (a) Schematic Illustration of the Synthetic Procedure for Preparation of HOMMS@TiO_2 and (b) Workflow of Phosphopeptides Enrichment from a Biological Sample Using HOMMS@TiO_2



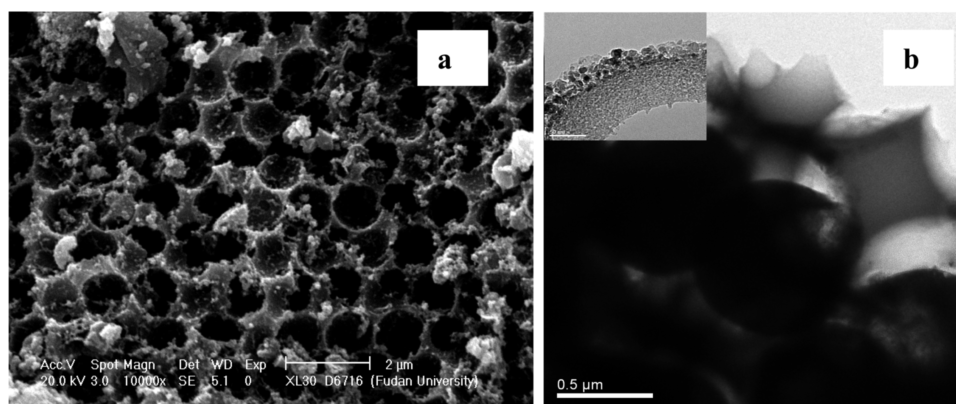


Figure 1. Images of SEM (a) and TEM (b) of HOMMS@TiO_2.

silica. SEM and TEM were used to characterize the morphology of the prepared HOMMS@TiO_2. As seen from SEM in Figure 1a, the as-made materials had hierarchically ordered macro/mesoporous structure, and numerous tiny TiO_2 nanoparticles were found to deposit on HOMMS. Macropores with about 1 μm diameter was clearly observed from TEM (Figure 1b). The inlet of TEM also displayed that numerous tiny TiO_2 were successfully coated on HOMMS. Wide-angle X-ray diffraction patterns of the HOMMS@TiO_2 composites (Figure S1) displayed typical diffraction peaks of anatase TiO_2 (JCPDS card no. 21-1272). The broad diffraction peaks implied that the titania particle was very small. The BET surface area of HOMMS@TiO_2 was 137 m^2/g, pore volume was 0.15 cm^3/g, and pore size was 3.1 nm characterized by N_2 adsorption−desorption isotherms (Figure S2). The BET surface area of HOMMS was 255 m^2/g, pore volume was 0.46 cm^3/g, and pore size was 4.6 nm (Figure S3). The surface area and pore size of HOMMS@TiO_2 was smaller than HOMMS. This can be explained by that large amounts of TiO_2 were coated on HOMMS.

The procedure to enrich phosphopeptides from complex peptides using HOMMS@TiO_2 was displayed in Scheme 1b. In brief, HOMMS@TiO_2 composites were incubated with the tryptic digest of proteins for 1 min, and then HOMMS@TiO_2 were separated. The captured phosphopeptides on the composites were detected using MALDI-TOF MS. The capacity of HOMMS@TiO_2 for specific capturing phosphopeptides was first tested using standard phosphopeptides. Direct analysis of 1×10^{-6} M β-casein by MALDI-TOF MS was shown in Figure 2a. We found the spectrum was dominated by nonphosphorylated peptides, and no phosphopeptides peaks were observed. In comparison, after enrichment by HOMMS@TiO_2, the peaks for phosphopeptides dominated the spectrum, and there were almost no nonphosphorylated peptides peaks (Figure 2b). Furthermore, two phosphopeptides derived from α-casein (1 and 2) were also detected. A dephosphorylated fragment of phosphopeptide through loss of H_3PO_4 was marked with a circumflex. It is worth mentioning that the time needed for phosphopeptide loading and elution were both within 1 min, and the whole enrichment process could be completed within 10 min. The enrichment time by this material was much shorter than previous reports.[35,36] The high enrichment efficiency and the time-saving merit might be due to the macro/mesoporous structure.

We use complex peptides with the molar ratio of BSA to β-casein up to 1000:1 to further evaluate the specificity of

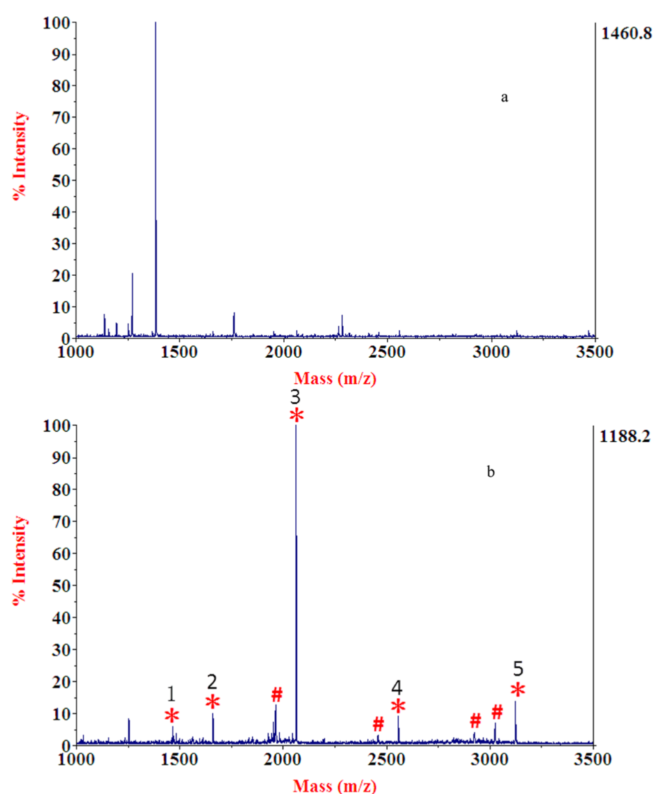


Figure 2. MALDI mass spectrum of peptides derived from β-casein (a) before enrichment and (b) enriched by HOMMS@TiO_2, where the * indicates the phosphopeptides, and # indicates the metastable losses of phosphoric acid.

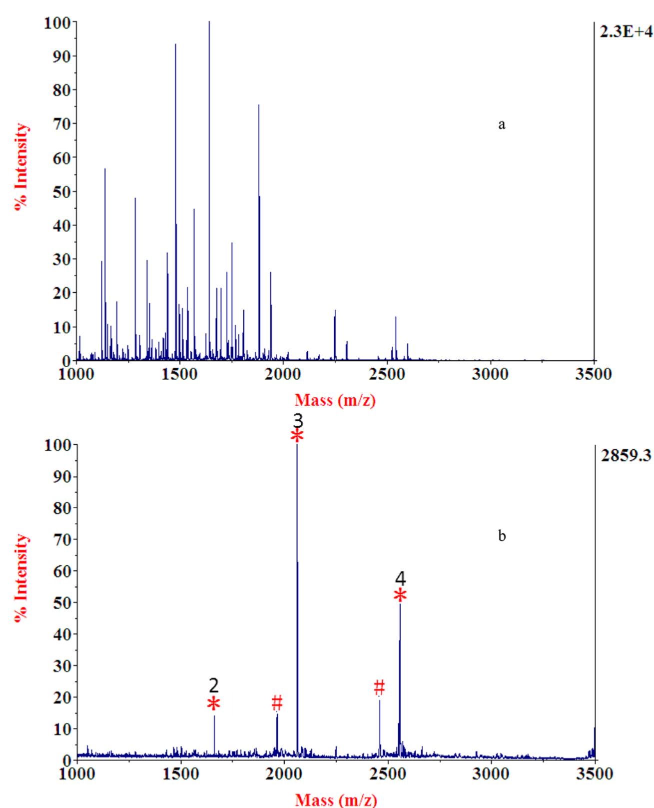


Figure 3. MALDI mass spectrum of peptides derived from a peptide mixture of β-casein and BSA at a molar ratio of 1:1000: (a) without enrichment and (b) enriched by HOMMS@TiO_2, where the * indicates the phosphopeptides, and # indicates the metastable losses of phosphoric acid.

HOMMS@TiO_2 toward phosphopeptides. As shown in Figure 3a, without enrichment, nonphosphopeptide peaks were of high intensity, and no phosphopeptide peaks were observed. However, after incubation with HOMMS@TiO_2, the peak of phosphopeptides dominated the spectrum, and there were no nonphosphopeptides peaks (Figure 3b). The result indicated that HOMMS@TiO_2 could specific capture phosphopeptides.

To study the capacity of HOMMS@TiO_2, 200 μL of the standard phosphopeptide (pSADGQHAGGLVK) with different concentrations was enriched using the fixed amount of HOMMS@TiO_2, the supernate was analyzed by MALDI-TOF MS. The enrichment capacity of HOMMS@TiO_2 was about 1.0 mg g^{-1} (Figure 4a). The detection limit using this approach was 80 fmol, shown in Figure 4b. Even when the total amount of β-casein was only 8 fmol, the targeted phosphopeptide could also be detected (Figure 4c).

In order to understand the influence of pore-structure and surface area on high enrichment capacity and detection sensitivity, comparison of our material with the previous two materials[16,37] were performed (Table S1). Compared with the previous TiO_2-based methods,[17,24−26,37] all three materials with mesopores have large surface area and low detection limit for the analysis of phosphopeptides. This shows that surface area of the MOAC materials plays an important role in the enrichment of low-concentration phosphopeptides. In the three materials, the two materials with macroporous pore, Fe_3O_4@ mesoporou/macroporous TiO_2 and HOMMS@TiO_2, have better selectivity and shorter enrichment time. This shows that the macropore of the MOAC materials can provide efficient mass transport. In addition, our material has large enrichment capacity. In summary, in the MOAC materials, the macropore can provide efficient mass transport and improve enrichment selectivity; and the mesopore can provide high surface area and improve the detection limit.

Encouraged by the emerging advantages of HOMMS@TiO_2, the novel material was further applied to analyze phosphopeptides of a real complex sample, human serum. As shown in Figure 5b, after enrichment with HOMMS@TiO_2, four phosphopeptides could be clearly detected; the detailed phosphopeptides information was listed in Table S2. In contrast, before enrichment, nonphosphopeptides dominated the spectrum (Figure 5a). The results were as good as previous reports.[38,39] The results clearly indicated that our approach was effective and specific in capturing phosphopeptides from real biological samples.

CONCLUSION

In conclusion, a new facile route was proposed for the synthesis of HOMMS@TiO_2. HOMMS@TiO_2 was specific and effective toward phosphopeptides. What is more, HOMMS@TiO_2 could have selective enrichment of phosphopeptides from human serum. This work was anticipated to have potential performance in phosphoproteome research.

EXPERIMENTAL SECTION

Chemicals. β-casein (Sigma); trifluoroaceticacid (TFA); trypsin; acetonitrile; ammonium bicarbonate; 3-(trihydroxysilyl)propyl methylphosphate and 2,5-dihydroxybenzoic acid (DHB); dopamine hydrochloride; P123 (Mav = 5800 g/mol); polyvinylpyrrolidone (PVP, Aldrich); 2,2-azobisisobutyronitrile (AIBN); styrene (St); tetraethyl orthosilicate (TEOS). Milli-Q water by Milli-Q system

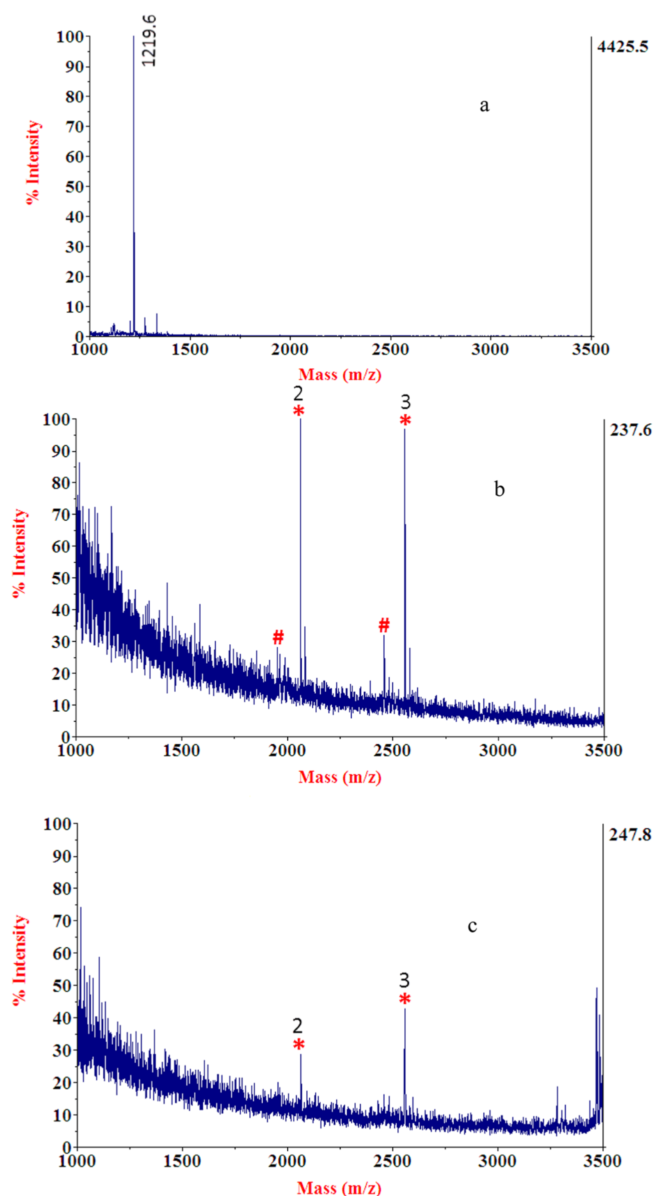


Figure 4. MALDI mass spectrum of phosphopeptide pSADGQHAGGLVK (0.4 μg/μL, 200 μL), after enrichment (a). MALDI mass spectra of phosphopeptides enriched by HOMMS@TiO_2, with the tryptic digests of β-casein amount as (b) 80 fmol and (c) 8 fmol. The * indicates the phosphopeptides, and # indicates the metastable losses of phosphoric acid.

(Millipore, Bedford, MA) was used through the whole experimental process.

Preparation of HOMMS@TiO_2. At first, HOMMS were prepared according to the previous report,[40] and then HOMMS@TiO_2 composites were synthesized by the hydrothermal methods.[41]

Characterization and Measurements. Scanning electron microscopy (SEM) images were characterized at 20 kV on a Philips XL30 electron microscope (Netherlands). Transmission electron microscopy (TEM) images were characterized at 200 kV on a JEOL2011 microscope (Japan). The Brunauer−Emmett−Teller (BET) method was utilized to calculate the specific surface areas.

Sample Preparation. Bovine serum albumin (BSA) was denatured at 95 °C for 10 min and then reduced with DTT and IAA, and then BSA and β-casein were digested using the solution digest method. Human serum was diluted with water.

Enrichment of Phosphopeptides from Tryptic Digestion of Standard Proteins. 200 μg of HOMMS@TiO_2 was added into 200 μL of a peptide mixture and vibrated at 25 °C for 1 min. Subsequently, HOMMS@TiO_2 was eluted with NH_4OH (5 μL, 0.4 M) for 1 min and analyzed by MALDI-TOF MS.

MALDI-TOF MS Analysis. 0.5 μL of the eluate was deposited on the plate, and then 0.5 μL of DHB aqueous solution was deposited and analyzed by MALDI−TOF MS.

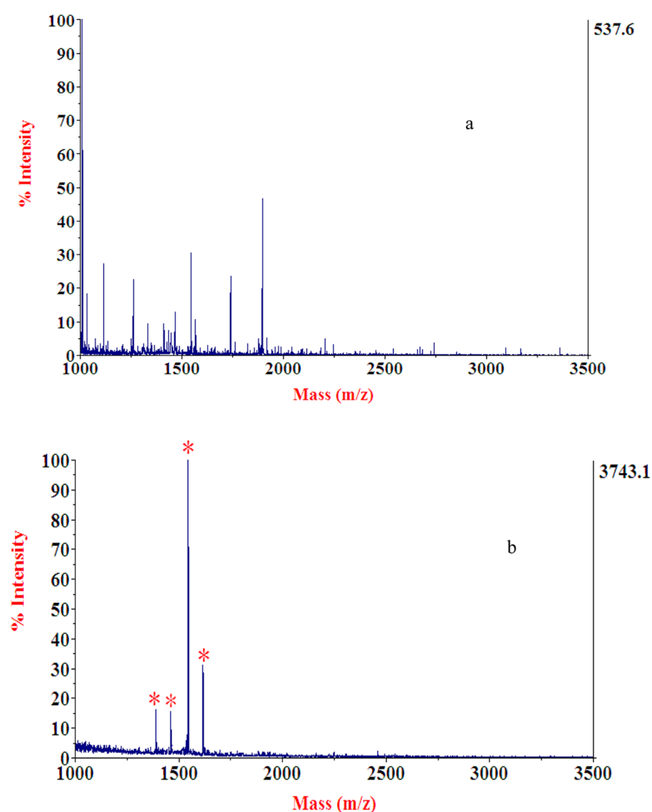


Figure 5. MALDI mass spectrum of peptides derived from human serum (a) without enrichment and (b) enriched by HOMMS@TiO_2, where the * indicates the phosphopeptides, and # indicates the metastable losses of phosphoric acid.

■ ASSOCIATED CONTENT

Supporting Information

Figures S1−S3 and Tables S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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