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Designed synthesis of Graphene @titania @mesoporous silica hybrid material as size-exclusive metal oxide affinity chromatography platform for selective enrichment of endogenous phosphopeptides

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ABSTRACT

In this work, a novel size-exclusive metal oxide affinity chromatography (SE-MOAC) platform was built for phosphoproteome research. The operation for preparing graphene @titania @mesoporous silica nanohybrids (denoted as G@TiO_2@$mSiO_2$) was facile and easy to conduct by grafting titania nanoparticles on polydopamine (PD)-covered graphene, following a layer of mesoporous silica was coated on the outermost layer. The G@TiO_2@$mSiO_2$ nanohybrids exhibited high sensitivity with a low detection limit of 5 amol/μL (a total amount of 1 fmol) and high selectivity for phosphopeptides at a mass ratio of phosphopeptides to non-phosphopeptides (1:1000). The size-exclusive capability of the nanohybrids were also demonstrated by enriching the phosphopeptides from the mixture of Bovine Serum Albumin (BSA), α-casein, and β-casein digests with a high mass ratio (β-casein digests: α-casein: BSA, 1:500:500), which was attributed to the large surface area and ordered mesoporous channels. In addition, the G@TiO_2@$mSiO_2$ nanohybrids were employed to capture the endogenous phosphopeptides from human serum successfully.

1. Introduction

Due to the high potential for discovering biomarkers associated with human diseases, [1,2] peptidome, which refers to the peptides expressed in biological organs, tissues, cells, or biofluid, has been given close attention by scientists. Over the years, due to the rapid development of bio-mass spectrometry (MS), MS-based proteomics has been a forceful analytical technique to finish the qualitative and quantitative analysis of endogenous peptides [3–5]. Nevertheless, the analysis of endogenous phosphopeptides still suffers from serious challenges, these were supposed to be blaming the low abundance, low ionization efficiency of endogenous phosphopeptides, the interference of the high-abundant proteins and salt in bio-samples [6]. To overcome these complicated problems, enriching endogenous phosphopeptides from bio-samples has been confirmed to be quite a simple, quick, and efficient way.

Traditional protocols for enriching endogenous peptides from complex bio-samples are centrifugal ultrafiltration [7–10] and solid-phase extraction [11–13]. But the low efficiency and weakness in serum analysis are considerable deficiencies. So far, multitudinous strategies have been developed for selective enriching phosphopeptides through different affinity mechanisms, including ion-exchange chromatography [14], immobilized metal affinity chromatography (IMAC) [15–20], and metal oxide affinity chromatography (MOAC) [21–23]. Generally speaking, MOAC is considered more reliable than other methods, such as TiO_2, ZrO_2, Al_2O_3, Ga_2O_3, SnO_2, ZnO, Ta_2O_5, Nb_2O_5, CeO_2, etc [21,23–38]. It takes effect by forming the bridge bidentate binding between the metal oxides and the phosphate groups. Among all of the metal oxides which were mentioned above, TiO_2-based MOAC materials possessed the excellent performance including high recovery and high salt tolerance, exhibiting a better result. However, traditional MOAC materials possessed poor ability of protein tolerance. The results were easily to be interfered by the existence of high-abundant proteins. Considering this, most of the bio-samples have to be digested before treated by MOAC methods. The pretreatment step greatly increased complexity of the sample and brought much work in following data analysis.

In consideration of the remarkable properties of mesoporous materials, including extremely large specific surface area, uniformed mesoporous tunnels, narrow pore size distribution, and continually adjustable pore size, various mesopore-dependent

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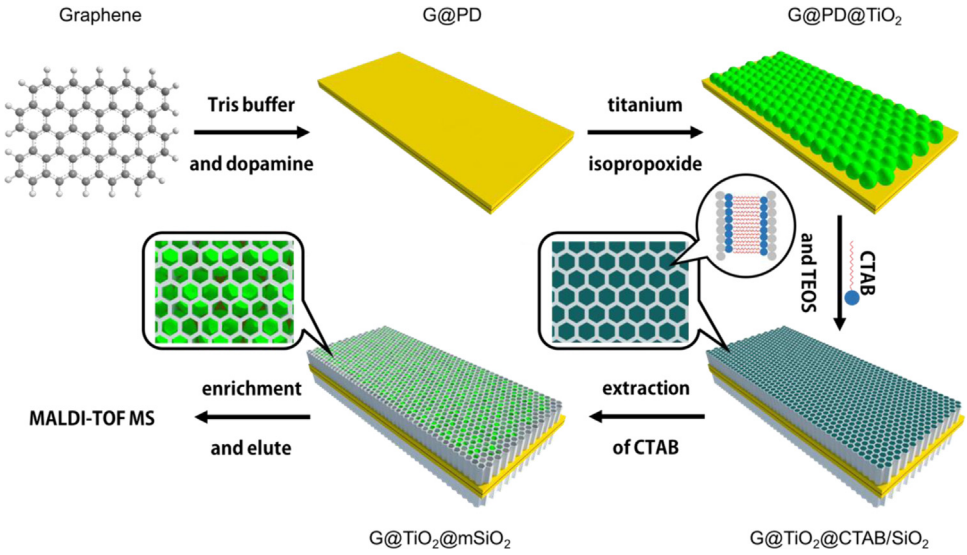

Scheme 1. Schematic diagram of the synthetic route for preparation of G@TiO_2@$mSiO_2$.

methods have been developed and exhibited outstanding size-exclusive ability and selectivity for the enrichment of target peptides. By taking advantage of the existence of mesopores, target peptides were allowed to enter into channels and exclude the large-size proteins off the materials. These methods have done a marvelous job in selectively enriching phosphopeptides form sophisticated samples, all the results of which arose from treating with tanglesome mixture of peptides and proteins [15,38–43]. In our previous work, size-exclusive magnetic graphene/mesoporous silica composites were synthesized by immobilizing titanium(IV) on the pore walls for selective enrichment of endogenous phosphorylated peptides [15]. Although this method exhibited excellent results, the materials have its own shortcomings. The titanium(IV) was not only immobilized on the pore walls but also on the external surface of the mesoporous silica layer. So, some impurity, such as phosphoproteins, may be absorbed on the external surface to influence the results. On the other hand, metal ions may desorb from the materials as they were immobilized through coordination function. Therefore, we anticipated to design and synthesize the novel nanocomposites combining the merits of high sensitivity, selectivity and size-exclusion, which were provided by TiO_2-based MOAC materials and mesoporous materials, to selectively enrich endogenous phosphopeptides for peptidome analysis.

Herein, the novel size-exclusive MOAC materials of graphene @titania @mesoporous silica (denoted as G@TiO_2@$mSiO_2$) were designed and synthesized by grafting titania nanoparticles on graphene, which had been covered with polydopamine as the coupling linker in advance, and then mesoporous silica were coated on both sides of the titania grafted graphene sheet. Surprisingly, the novel materials showed great enrichment performance by capturing phosphopeptides from digestion of a standard phosphoprotein β-casein and also from mixture of β-casein digests and interference proteins and non-phosphopeptides. In addition, G@TiO_2@$mSiO_2$ nanohybrids were also employed to enrich the endogenous phosphopeptides from human serum successfully.

2. Results and discussion

2.1. Synthesis and characterization of G@TiO_2@$mSiO_2$ nanohybrids

The synthetic route for the preparation of G@TiO_2@$mSiO_2$ nanohybrids is illustrated in Scheme 1. In brief, dopamine autopolymerized on the surface of acidulated graphene at room temperature (G@PD), then titania nanoparticles were grafted onto G@PD through a hydrothermal reaction (G@TiO_2), and finally a layer of mesoporous silica was directly coated on both sides of G@TiO_2 via a sol–gel process to obtain G@TiO_2@$mSiO_2$ nanohybrids.

Scanning Electron Microscopy (SEM) image in Fig. S1, supporting information (SI), showed that the titania microparticles were dispersed on the surface of G@PD. Transmission Electron Microscopy (TEM) image indicated that mesoporous silica was resoundingly coated on the outermost layer of nanohybrids, which had uniform pore channels (Fig. 1). G@TiO_2@$mSiO_2$ nanohybrids were further affirmed by N_2 adsorption experiment. The typical IV type curve of mesoporous structure can be observed in Fig. 2, which could be ascribed to the existence of outmost $mSiO_2$. The sudden raise of P/P_0 from 0.25 to 0.75 suggested the uniform pore-size distribution of G@TiO_2@$mSiO_2$ nanohybrids. The Brunauer–Emmett–Teller surface area was calculated as 321 m^2/g. From the inset of Fig. 2, main pore-size is estimated to be 3 nm, which means that the G@TiO_2@$mSiO_2$ nanohybrids were workable to allow target peptides to enter into channels and exclude the large-size proteins by taking advantage of the mesopores at the same time. As seen from wide-angle X-ray Diffraction patterns, the strong diffraction peak of (002) plane was at around 26° (Fig. S2 in SI), implying that the graphene layers remained unchanged during the whole synthesis process The series of diffraction peaks of (101), (004), (200), (105), and (204) are all the typical diffraction peaks of anatase TiO_2. The Energy-Dispersive X-ray analysis (EDX) (Fig. S3 in SI) of the G@TiO_2@$mSiO_2$ nanohybrids revealed the existence of C, N, Ti, O, and Si elements. All above characteristics suggested that the PD has been successfully coated on the surface of acidulated graphene, the titania nanoparticles were successfully grafted on the outer layer of G@PD and the mesoporous silica layer was also coated on the outmost layer of G@TiO_2 successfully.

2.2. Selective enrichment of phosphopeptides by using G@TiO_2@$mSiO_2$ nanohybrids

The operation sequence of enriching phosphopeptides from the mixture is exhibited in Scheme 2. Namely, the mixture of protein tryptic digestion and G@TiO_2@$mSiO_2$ nanohybrids were vibrated for 30 min and washed with 50% acetonitrile and 0.1% TFA aqueous