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Highly efficient enrichment of phosphopeptides by a magnetic lanthanide metal-organic framework

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ABSTRACT

Highly efficient enrichment of phosphopeptides from complex biological samples is crucial prior to mass spectrometry analysis due to the low abundance and ion suppression effects. In this study, a facile route was designed for preparation of a magnetic erbium(Er)-based metal-organic framework (denoted as Fe_3O_4@PDA@Er(btc)), which was synthesized with 1,3,5-benzenetricarboxylic acid(H_3btc) as ligand and grafted on the polydopamine (PDA) – coated Fe_3O_4. The as-prepared material exhibited ultra-high sensitivity (detection limit of 20 amol/μL) and selectivity at a low mass ratio of β-Casein/BSA (1:500). Moreover, it was also investigated for enrichment of phosphopeptides from human serum, which provided a promising technique for highly efficient enrichment of low-abundance phosphorylated peptides in the practical application.

1. Introduction

Protein phosphorylation, one of the most important post-translational modifications (PTMs), plays a key role in biological processes including cell cycle, [1] cell function, [2] signal transduction [3] and other interactions. Mass spectrometry (MS) is the most common tool for phosphoproteome research in terms of high-throughput experimental platform development [4] and the contribution of MS to phosphoproteomics was reviewed in 2011 [5] Identification of phosphopeptides remains quite difficult because of the low abundance and low ionization efficiency. Therefore, enrichment of phosphopeptides from complex bio-samples is quite crucial for MS analysis [5,6].

Various strategies were applied for phosphopeptides enrichment such as immobilized metal affinity chromatography (IMAC), [7,8] metal oxide affinity chromatography (MOAC), [9,10] immunoprecipitation, [11] chemical modifications [12] and ion exchange chromatography [13]. Among all these strategies, IMAC and MOAC are the most widely used for efficient enrichment of phosphopeptides nowadays. IMAC relies on the attraction of metal cations (Ti^{4+}, Al^{3+}, Fe^{3+}) and negatively charged phosphate groups, [14] while MOAC relies on the affinity of oxygen in the phosphate groups and the surface of metal oxide, including TiO_2, [15,16] ZrO_2, [17,18] etc. TiO_2-metal oxide has been demonstrated to be potential in phosphopeptide enrichment thanks to its high salt tolerance. Ti^{4+} and Zr^{3+} have been reported to be a strong binding between metal ions and phosphopeptides. However, strong binding also brought some dismerits, like co-enriching some large-molecule proteins. Limited surface area and poor hydrophilicity from IMAC and MOAC also restrain the performance of capturing phosphopeptides. As IMAC or MOAC-based methods still suffer from shortcomings such as troublesome preparation techniques, sample loss due to the centrifugation, nonspecific adsorption, [19] it is in urgent need to develop innovative strategies and novel materials for phosphoproteome research.

Recently, great efforts have been devoted to the synthesis of metal-organic frameworks (MOFs), which are consisted of metal ions linked together by organic ligands [20]. MOFs, a new class of porous materials, have been employed in gas adsorption, separation, [21] catalysis, [22] and so on, owing to its adjustable pore structure, high surface area and in-pore functionality and outer-surface modification [23]. To date, some magnetic MOF matreials have been applied in phosphopeptide enrichment but with tedious and time-consuming synthesis (including several cycles in self-assembly) [24,25] Furthermore, the element chosen for MOFs to enrich phosphopeptides was limited by Fe-MOF [24,25] and Zr-MOF, [26,27] while Er-MOF was almost not applied in phosphoproteomics research. Who once applied Er-MOF in phosphopeptide enrichment, but gained relatively poor enrichment effect [28]. Herein, a facile route was proposed for preparation of a magnetic metal-organic framework by modifying erbium-MOF on the polydopamine (PDA)-coated magnetic microspheres (denoted as Fe_3O_4@PDA@Er(btc)) showing strong magnetic responsiveness, excellent hydrophilicity, and high surface area and anticipating a

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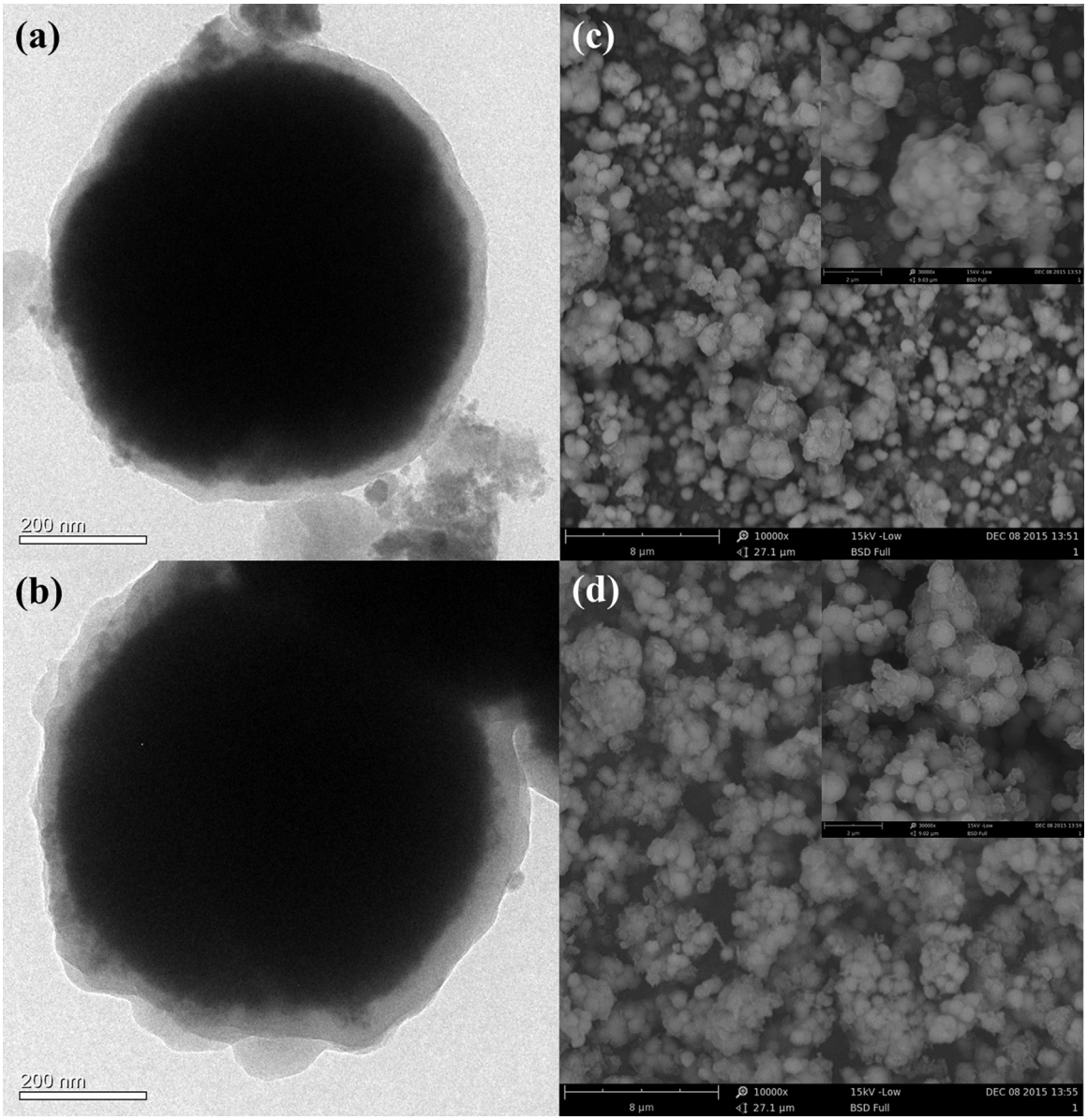

Fig. 1. TEM images of (a) Fe_3O_4@PDA and (b) Fe_3O_4@PDA@Er(btc); SEM images of (c) Fe_3O_4@PDA and (d) Fe_3O_4@PDA@Er(btc).

great performance in phosphopeptides enrichment with ultra-high sensitivity and selectivity.

2. Experimental

2.1. Synthesis of Fe_3O_4@PDA@Er(btc)

The synthetic route for Fe_3O_4@PDA@Er(btc) is presented in Scheme S1 in SI. At first, the Fe_3O_4 magnetic microspheres were synthesized via a widespread solvo-thermal reaction [29]. Then, the magnetic core was coated with a PDA layer through the spontaneous polymerization of dopamine in alkaline solution. A PDA layer could enhance the biological amphipathy and Er^{3+} could be much more easily deposited on the surface with the help the hydroxyl of PDA. The obtained Fe_3O_4@PDA (50 mg) was dispersed in 40 mL ethanol of MOF precursors, containing 80 mg $Er(NO_3)_3 \cdot 5H_2O$ and 84 mg H_3btc at 70 °C for 2 h. The product was collected by magnetic separation and washed with ethanol and then kept in the vacuum oven.

2.2. Characterization and measurements

Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Energy dispersive X-ray (EDX), Magnetization measurement, Fourier transform infrared spectra (FT-IR), Raman spectra (Raman), powder X-ray diffraction patterns (XRD) and Zeta potential measurements were measured and detailed information were illustrated in Supporting Information.

2.3. Sample preparation

BSA and β-Casein were respectively dissolved in NH_4HCO_3 buffer (25 mM, pH 8.3) and incubated with trypsin (trypsin: β-Casein is 1:50, w/w) at 37 °C for 16 h. Human serum was diluted 10 fold with loading buffer.

2.4. Enrichment of phosphopeptides from β-Casein digests

The workflow of phosphopeptides is illustrated in Scheme S2 in SI. 200 μg of Fe_3O_4@PDA@Er(btc) was added into 200 μL peptide