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Short communication

Core-shell structured magnetic metal-organic framework composites for highly selective detection of *N*-glycopeptides based on boronic acid affinity chromatography

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ABSTRACT

Boronic acid affinity chromatography (BAAC) is one of the most significant methods in glycoproteomics research due to its low bias towards glycopeptides and easy enrichment process. In this work, core-shell structured magnetic metal-organic framework (MOF) composites with abundant boronic acid groups were designed and synthesized for selective glycopeptide enrichment based on BAAC. The as-prepared core-shell structured magnetic MOF composites (denoted as Fe_3O_4@PVP/PEI@MOF (B)) inherited strong magnetic responsiveness from the Fe_3O_4 core as well as ultrahigh surface area and abundant boronic acid sites from the MOF shell. The affinity between boronic acid and *cis*-diols groups endowed the composites with improved sensitivity (0.5 fmol/μL) and selectivity (1:100) towards glycopeptides, achieving remarkable results in glycopeptides detection from standard glycoprotein digests as well as complex bio-samples. As a result, a total of 209 *N*-glycosylation peptides from 89 different glycoproteins were identified from human serum digests, indicating its broad prospect in glycoproteome study.

1. Introduction

As one of the most significant post-translational modifications (PTMs), protein glycosylation plays a key role in lots of biological processes [1], including molecular recognition [2], signal transduction [3], cell–cell interaction [4], and so on. Abnormal glycosylation is closely linked to cancer progression, and it has been reported that glycoproteins and glycopeptides could act as biomarkers of some kinds of diseases [5,6]. Therefore, the identification of glycosylation sites and glycopeptides has great meaning in medical diagnosis. Mass spectrometry (MS) is one of the most powerful tools to identify protein glycosylation due to its high throughput, high resolution and fast speed [7,8]. To our disappointment, because of the low abundance of glycopeptides in biological samples and poor ionization efficiency of glycopeptides in MS analysis, it is a great challenge to identify glycopeptides directly [9]. Therefore, highly selective enrichment of glycopeptides from the complicated bio-samples is crucial prior to MS analysis.

Up to now, many methods have been developed for glycopeptide enrichment based on various strategies, such as hydrazine chemistry [10], hydrophilic interaction chromatography (HILIC) [11], lectin affinity chromatography [12], boronic acid chemistry [13,14] and so on. The hydrazine chemistry needs commercially available hydrazide resins, which are expensive and hard to be separated. The hydrophilic interaction chromatography lacks of sufficient selectivity because some hydrophilic non-glycopeptides may be enriched simultaneously [15]. Among them, boronic acid and its analogous derivatives modified materials are demonstrated to be efficient in glycopeptides enrichment, due to their low bias towards glycopeptides and convenient enrichment process [16]. However, most of these materials have limited amount of boronic acid sites on the materials, resulting in unsatisfactory enrichment efficiency of glycopeptides. Thus, designing a material with high content of boronic acid is quite necessary.

In recent years, metal-organic frameworks (MOFs), a special kind of porous materials, has gained great attention owing to its ultrahigh surface area, tunable pore properties and easy procedure of functionalization. This fascinating material which is consisted of metal ions and organic ligands has gained extensive use in separa-

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tion [17], adsorbents [18,19], drug delivery [20,21], gas adsorption [22,23], and so on. For the past few years, the application of MOFs in proteomics has emerged, including enzyme immobilization [24], endogenous peptide enrichment [25], phosphopeptide enrichment [26–28], glycopeptide enrichment [29,30], etc. Based on this, we proposed a new idea of creating a novel material, combining the boronic acid chemistry and MOF, to explore its potential application in glycoproteome analysis.

Therefore, a novel boronic acid functionalized magnetic metal-organic framework (Fe_3O_4@PVP/PEI@MOF (B)) was designed and synthesized in a facile route by modifying MOF with boronic acid on polyvinyl pyrrolidone (PVP) and polyethylenimine (PEI) coated magnetic microspheres. Boronic acid functionalized MOFs were easily obtained by employing 1, 4-phenylenebisboronic acid (denoted as PBA) and terephthalic acid (TPA) as organic ligands. The PBA can provide a large amount of boronic acid sites in MOFs with the help of TPA's participation. In comparison to former reported MOFs with boronic acid [13], the as-prepared Fe_3O_4@PVP/PEI@MOF (B) contained much more boronic acid groups, which is the key to improve the glycopeptide enrichment efficiency. The introduction of the polymer made it much easier to compose a core-shell structure. What's more, this material owned strong magnetic responsiveness which was gained by an easy-to-prepare and time-saving synthesis route. The obtained novel MOFs demonstrated outstanding performance in selective enrichment of glycopeptide from both digests of standard glycoproteins as well as complex bio-samples, with great selectivity and sensitivity, showing its great value in glycoproteome study.

2. Experimental

2.1. Materials

1, 4-phenylen ediboronic acid, ferric nitrate nonahydrate were from Adamas-beta, polyethylenimine (PEI, Mw25000), terephthalic acid (TPA), dimethylformamide (DMF, 99%), and acetone (99.5%), ammonium bicarbonate (NH_4HCO_3), horseradish peroxidase (HRP), bovine serum albumin (BSA), trypsin from bovine pancreas, 2, 5-dihydroxy-benzoic acid (DHB), dithiothreitol (DTT) and indoaceamide (IAA) were purchased from Sigma-Aldrich. ACN (HPLC-grade), trifluoroacetic acid (TFA) and formic acid (FA) were purchased from Merck (Darmstadt, Germany). PNGase F was purchased from Genetimes Technology. Human serum was donated by Zhongshan Hospital (Shanghai, China). All aqueous solutions were prepared using Milli-Q water by Milli-Q system (Millipore, Bedford, MA). Other chemicals were all of analytical grade.

2.2. Apparatus

Transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive X-ray (EDX), fourier transform infrared spectra (FT-IR), raman spectra (Raman), powder X-ray diffraction patterns (XRD) and magnetization measurements were measured and detailed information was shown in Supporting Information.

2.3. Synthesis of Fe_3O_4@PVP/PEI@MOF (B)

Fe_3O_4@PVP/PEI@MOF (B) was prepared in a facile route (Fig. 1a). First, magnetic Fe_3O_4 was synthesized by a traditional solvothermal reaction. Then, the magnetic microspheres were modified with the polymer of polyvinyl pyrrolidone and polyethylenimine according to a previous report [31]. The obtained PVP/PEI coated magnetic particles (denoted as Fe_3O_4 @ PVP/PEI) were dispersed in the mixed solvent of ACN and DMF, with the dissolved ferric nitrate nonahydrate, 1,4-phenylenebisboronic acid (PBA) and terephthalic acid (TPA) as MOFs precursors. Then the mixed solution was heated at 120 °C for 2 h. During the process of reaction, the metal salt and organic ligands were added again every 30 min for three times. In the end, Fe_3O_4@PVP/PEI@MOF (B) was washed with DMF, ethanol and deionized water three times respectively and dried in vacuum oven at 50 °C.

2.4. Enrichment of glycopeptides from biological samples

Briefly, 200 μg of Fe_3O_4@PVP/PEI@MOF (B) was washed with loading buffer for several times and then added into 100 μL peptide mixture. The solution was incubated at 37 °C for 45 min 50 mM NH_4HCO_3 was selected as the loading buffer here to form an alkaline environment. The material was then separated with a magnet and washed with the loading buffer. The captured glycopeptides were eluted by the solution of 50%ACN/49%H_2O/1%TFA at 37 °C for 30 min. The obtained eluent was deposited on MALDI plate and analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

For human serum enrichment, 1 mg of Fe_3O_4@PVP/PEI@MOF (B) was added into 100 μL human serum digests. The enrichment and elution process was the same with that of enrichment of standard glycoprotein tryptic digests. The eluent was lyophilized and incubated with 1 μL PNGase F at 37 °C for 16 h. After lyophilization, it was redissolved and analyzed by nano high performance liquid chromatography – mass spectrometry (Nano-HPLC–MS/MS). The human Uniprot-SwissProt database (release 2015_03_11, with 20199 entries) was used to identify glycosylation peptides.

2.5. MALDI-TOF MS and nano-LC–MS/MS analysis

All MALDI-TOF-MS experiments were carried out in a reflector positive mode by an AB Sciex 5800 MALDI-TOF mass spectrometer (AB Sciex, USA) at 200 Hz, 20 kV, with Nd-YAG laser at 355 nm.

For Nano-LC–MS/MS analysis, firstly, solvent A of water with 0.1% formic acid and solvent B of ACN with 0.1% formic acid was prepared. The lyophilized peptide sample was redissolved in 10 μL solvent A and then separated by Nano-LC for on-line electrospray tandem mass spectrometry analysis. The EASY-nLC 1000 system (Thermo Fisher Scientific, Waltham, MA) connected to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an online nano-electrospray ion source was used to perform the experiment. The peptide sample was loaded on the analytical column (Acclaim PepMap C18, 75 μm × 25 cm) with a linear gradient. The column was re-equilibrated at preliminary stage for 15 min with a flow rate of 300 nL/min. electrospray voltage of 2.0 kV versus the inlet of the mass spectrometer was used. The Orbitrap Fusion mass spectrometer was performed in the data-dependent mode to switch automatically between MS and MS/MS acquisition. Survey full-scan MS spectra (m/z 350–1500) were acquired in Orbitrap with a mass resolution of 120 000 at m/z 200. The AGC target was set to 1 000 with a maximum injection time of 50 ms. MS/MS acquisition was operated in Orbitrap in 3 s cycle time. The intensity threshold was 50 000, with the maximum injection time of 100 ms. The AGC target was set to 200 000 with isolation window was 2 m/z. Ions with charge states of 2+, 3+, and 4+ were orderly fragmented by HCD(higher energy collisional dissociation) with NCE (normalized collision energy) of 35% with fixed first mass at 110. A microscan was recorded using dynamic exclusion of 30 s.

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