Journal of Chromatography A, 1468 (2016) 49–54

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Magnetization of 3-dimentional homochiral metal-organic frameworks for efficient and highly selective capture of phosphopeptides

Xiaoyue Qi[a], Cuilan Chang[b], Xinyuan Xu[a], Yiding Zhang[a], Yu Bai[a], Huwei Liu[a,*]

[a] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
[b] Dow Chemical Company (China) Investment Co., Ltd, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 26 July 2016
Received in revised form
20 September 2016
Accepted 21 September 2016
Available online 21 September 2016

Keywords:
Affinity probes
Phosphopeptides
Enrichment
Chiral MOFs

ABSTRACT

Enrichment of phosphopeptides based on various affinity probes prior to mass spectrometry detection is usually required due to the low abundance and low ionization efficiency of phosphopeptides. In this work, a 3-dimentional homochiral metal-organic frameworks (MOFs) was modified with magnetic nanoparticles using a facile method and then utilized for phosphopeptides capture with high efficiency and specificity. Based on magnetic solid phase extraction, a rapid and efficient method was developed and the whole enrichment procedure could be easily finished within 10 min. Efficient and highly selective capture of phosphopeptides from tryptic digests and human serum was achieved. This affinity probe showed satisfactory reproducibility of the particle synthesis and could be recycled for at least seven times. With all the advantages mentioned above, this strategy is of great potential for routine application in phosphoproteomes.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Reversible phosphorylation of proteins is a crucially significant mechanism in cell signal regulation, physiological and pathological processes [1]. The research in phosphoproteomics provides a comprehensive understanding of underlying mechanism, as well as involved diseases, including cancer [2,3], Alzheimer's [4], Parkinson's [5], cardiovascular diseases [6] and schizophrenia [7]. It is estimated that, phosphorylation occurs predominantly in serine (≈90%), followed by threonine (≈10%) and tyrosine (≈0.05%) [8]. Mass spectrometry (MS) based phosphoproteomics is an important technique used in the qualitative and quantitative analysis of phosphorylated proteins in biological samples. Due to the low abundance and low ionization efficiency of phosphopeptides, effective analytical techniques for enriching the phosphopeptide constituents from phosphoprotein digests are prerequisite to facilitate the mass spectrometric characterization and analysis of phosphorylation events. Prior to MS analysis, immobilized metal affinity chromatography (IMAC) has been one of the available methods for phosphopeptides enrichment. However, the selectivity of most metal ions is limited with contamination from nonspecific binding of non-phosphopeptides, especially when applied in highly complex samples, e.g., whole-cell lysates or extracts [9].

To address this issue, some kinds of novel affinity probes have been developed for phosphopeptides enrichment, such as metal oxide nanoparticles [10–12], functional graphene-based materials [13] and metal-organic frameworks (MOFs) [14,15]. Metal oxide (e.g., TiO_2) affinity chromatography (MOAC) has been studied for years, which conventionally requires centrifugation at high speed during the enrichment process. The problems of time-consuming and labour-intensive in MOAC could be solved by magnetic substrates derived from magnetic solid phase extraction (MSPE), since Fe_3O_4 nanoparticles present unique properties of superparamagnetism, high magnetic susceptibility, high coercivity and low Curie temperature [9]. Benefit from their easy manipulation by an external magnetic field, novel Fe_3O_4-modified functional materials have been applied for rapid capture of low-abundance peptides. Considerable efforts are made to combine Fe_3O_4 core with the phosphate-affinity properties of different coating materials, such as Ce^{4+}-chelated magnetic microspheres [16], Fe_3O_4@TiO_2 [17], Fe_3O_4@ TiO_2-ZrO_2 [18], polyethyleneimine-modified magnetic nanoparticles [19] and many others. Despite these successful cases, the design and facial preparation functional anchors for phos-

* Corresponding author.
E-mail address: hwliu@pku.edu.cn (H. Liu).

http://dx.doi.org/10.1016/j.chroma.2016.09.046
0021-9673/© 2016 Elsevier B.V. All rights reserved.

phopeptides enrichment are still appealing with high specificity and efficiency.

MOFs are regarded as a competitive porous material with high surface area, tunable structures and availability of in-pore and outer surface modification and functionalization [20]. MOFs were successfully applied as an affinity material for the selective enrichment of phosphorylated peptides in 2013 [21]. Zhao et al. designed a hydrophilic core-shell-shell structured magnetic MOF as a novel immobilized metal ion affinity platform for phosphoproteome research [14]. However, the specificity of this magnetic MOFs was unsatisfactory. In the last decade, chiral MOFs have been reported to be used as stationary phases for chromatographic separation [22], luminescent sensors [23], enantioselective catalyst [24], and carriers for drug delivery [25] due to the host-guest interactions. To discover more potential applications of chiral MOFs, a 3D homochiral MOFs was designed to trap phosphopeptides as an interesting attempt.

Herein, the aim of this work was to design and synthesize a 3D homochiral MOFs modified with magnetic nanoparticles through a facile reaction for phosphopeptides capture in complex biological samples with high efficiency and selectivity.

2. Materials and methods

2.1. Chemicals and materials

L-Lactic acid and 1,4-benzenedicarboxylic acid (H_2bdc) were obtained from Sigma-Aldrich (St. Louis, MO, USA). $Zn(NO_3)_2{\cdot}6H_2O$, *N,N*-dimethylformamide (DMF), ammonium hydroxide ($NH_3{\cdot}H_2O$) and acetic acid (HAC) were supplied by Beijing Chemical Company (Beijing, China). Fe_3O_4 nanoparticles were purchased from Beijing Nano Chen Technology Development Co. Ltd. HPLC grade acetonitrile was purchased from Dikma Technology (Richmond, VA, USA). β-casein, α-casein, trypsin, 2,5-dihydroxybenzoic acid (2,5-DHB) were obtained from Sigma-Aldrich. Trifluoroacetic acid (TFA) was bought from J&K Technologies Inc. Ammonium bicarbonate (NH_4HCO_3) were purchased from Fluka. Nonfat milk was produced by Yili Industrial Group Co., Ltd. (Inner Mongolia, China). Human serum was obtained from Peking University Hospital (Beijing, China). Purified water was bought from Hangzhou Wahaha Group. (Hangzhou, Zhejiang, China). All reagents and solvents employed were used as supplied without further purification.

2.2. Instrumentation

The instrument parameters of matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-ToF-MS) (Bruker, German) were set as follows: ion source 1, 19.00 kV; ion source 2, 15.85 kV; lens, 9.80 kV; reflector, 20.06 kV; laser frequency, 30 Hz; laser energy, 50%. Composition of matrix: 20 mg/mL 2, 5-DHB in 50% (v/v) ACN, and 5% (v/v) phosphoric acid.

2.3. Synthesis and characterization of ZnBLD particles and Fe_3O_4@ZnBLD composites

100 μg Fe_3O_4 nanoparticles were added into the mixture of $Zn(NO_3)_2{\cdot}6H_2O$ (10 mmol), 1,4-benzenedicarboxylic acid (5 mmol), and L-lactic acid (5 mmol) in DMF solution followed by sonication for 30 min, and then heated at 140 °C for 2 h with stirring at high speed of 1500 r/min. Brown crystals were then collected, and washed with DMF and ethanol for six times. After careful washing, the materials were dried in the oven overnight to yield final products. In order to control the quality of Fe_3O_4@ZnBLD particles, the reaction temperature, reaction time and stirring rate were strictly set and remained the same from batch to batch. The synthesis method of ZnBLD particles was the same as described above without the adjunction of Fe_3O_4 nanoparticles.

The as-synthesized material was characterized by scanning electron microscopy (SEM, Hitachi S4800, 5 kV), X-ray diffraction (XRD, Rigaku Dmax-2400, Cu Kα radiation, 40 kV, 100 mA) and transmission electron microscopy (TEM, FEI Tecnai T20, 200 kV). The magnetization curve of Fe_3O_4@ZnBLD particles was got at room temperature.

2.4. Preparation of standard protein digests

β-Casein or α-casein was dissolved in 50 mM NH_4HCO_3 solution, and then a certain amount of trypsin was added to the solution at a 1:40 (w/w) enzyme-to-protein ratio. The mixture was digested at 37 °C for 20 h. 30 μL nonfat milk was mixed with 970 μL NH_4HCO_3 solution (50 mM), and the supernatant was obtained after centrifugation at the speed of 14,000 rpm for 25 min afterwards. The mixture was denatured at 100 °C for 5 min and then incubated with 30 μL trypsin solution (1 mg/mL) at 37 °C for 16 h. The obtained tryptic digested samples were used for enrichment for phosphopeptides.

2.5. Enrichment of phosphopeptides using ZnBLD particles or Fe_3O_4@ZnBLD composites

Typically, the tryptic digested samples were diluted to the appointed concentration, and added with 500 μg ZnBLD particles or Fe_3O_4@ZnBLD composites in the loading buffer containing 0.1 M HAC (ACN: H_2O = 1:1) followed by 5 min shaking. The phosphopeptides bound materials were separated using a magnet and then the supernatant was removed. The material was washed with the loading buffer for 3 times and subsequently eluted with ammonium hydroxide solution (10%). The obtained eluent was afterwards used for MALDI-ToF-MS analysis. After careful washing with loading buffer for 3 times, Fe_3O_4@ZnBLD particles were dried in the oven overnight and used for the next cycle.

2.6. Enrichment of phosphopeptides using Fe_3O_4@ZnBLD composites from human serum samples

The pristine human serum (10 μL) was dissolved in 200 μL loading buffer containing 0.1 M HAC (ACN:H_2O = 1:1), with the addition of 500 μg Fe_3O_4@ZnBLD composites. After 5 min shaking, the particles were separated and collected using a magnet and the supernatant was discarded. Phosphopeptides bound materials were washed with loading buffer for 3 times and eluted with ammonium hydroxide solution (10%). The obtained eluent was subsequently analyzed by MALDI-ToF-MS.

3. Results and discussion

3.1. Synthesis and characterization of Fe_3O_4@ZnBLD particles

A facile method was developed in our group for magnetization of $[Zn_2(bdc)(L\text{-}lac)(dmf)](DMF)$ (ZnBLD), using one-pot synthesis method [26]. In this work, Fe_3O_4@ZnBLD was prepared in a modified way with smaller size and higher surface area, which facilitated binding affinity properties. Meanwhile, the pores of ZnBLD were small, which may be beneficial to avoid the non-specific adsorption. Typically, 100 μg Fe_3O_4 nanoparticles were added into the mixture of $Zn(NO_3)_2{\cdot}6H_2O$ (10 mmol), 1,4-benzenedicarboxylic acid (5 mmol), and L-lactic acid (5 mmol) in DMF solution followed by sonication for 30 min, and then heated at 140 °C for 2 h with stirring at high speed of 1500 r/min. After careful washing with DMF and ethanol, the composites were dried overnight.

Download English Version:

https://daneshyari.com/en/article/5135864

Download Persian Version:

https://daneshyari.com/article/5135864

Daneshyari.com