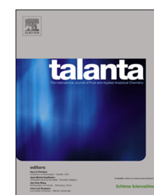
Talanta 119 (2014) 452–457

Contents lists available at ScienceDirect

Talanta

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



Highly selective enrichment of phosphopeptides with high-index facets exposed octahedral tin dioxide nanoparticles for mass spectrometric analysis

Rongna Ma [a], Junjie Hu [a], Zongwei Cai [b], Huangxian Ju [a,*]

[a] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China
[b] Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Kowloon, Hong Kong, PR China

ARTICLE INFO

Article history:
Received 21 September 2013
Received in revised form
14 November 2013
Accepted 16 November 2013
Available online 22 November 2013

Keywords:
Tin dioxide
Matrix-assisted laser desorption/ionization mass spectrometry
Phosphopeptide
Enrichment

ABSTRACT

High-index facets exposed octahedral tin dioxide (SnO_2) nanoparticles were successfully synthesized and applied to selectively enrich phosphopeptides for mass spectrometric analysis. The high selectivity and capacity of the octahedral SnO_2 nanoparticles were demonstrated by effectively enriching phosphopeptides from digests of phosphoprotein (α- or β-casein), protein mixtures of β-casein and bovine serum albumin, milk, and human serum samples. The unique octahedral SnO_2 with abundant unsaturated coordination Sn atoms exhibited enhanced affinity and selective coordination ability with phosphopeptides due to their high chemical activity. The strong affinity led to highly selective capture and enrichment of phosphopeptides for sensitive detection through the bidentate bonds formed between surface atoms and phosphate. The phosphopeptides could be detected in β-casein down to 4×10^{-9} M or in the mixture of β-casein and BSA with a molar ratio of even 1:100. The performance in selective enrichment of phosphopeptides from drinking milk and human serum showed powerful evidence of high selectivity and efficiency in identifying the low-abundant phosphopeptides from complicated biological samples. This work provided a way to improve the physical and chemical properties of materials by tailoring their exposed facets for selective enrichment of phosphopeptides.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Protein phosphorylation, one of the most important protein post-translational modifications, plays the key roles in regulating complex biological processes, such as cellular growth, division, and signaling transduction. Thus, the identification and localization of phosphorylation sites in proteins are of important significance for understanding biochemical pathways and disease states [1–4]. Mass spectrometry (MS) has emerged as the most powerful technique for examining the phosphorylation process due to its ultrahigh sensitivity, wide dynamic range and superior speed in analyzing complex samples. Unfortunately, owing to the low abundance of phosphorylated peptides, the substoichiometry of phosphorylation and the signal suppression of non-phosphorylated peptides, phosphorylation analysis still remains a great challenge for comprehensive understanding of protein phosphorylation [5,6]. Therefore, selective capture and enrichment of phosphorylated peptides from the complex mixtures prior to the interrogation of phosphorylation by MS has become an interesting research topic in bioanalysis.

A variety of enrichment strategies such as immobilized metal ion affinity chromatography (IMAC) [7–11], immunoprecipitation [12,13], and metal oxide affinity chromatography (MOAC) [14–17] have been developed to selectively capture and enrich the targeted phosphopeptides. Although IMAC is the most widely used technology, MOAC has shown higher capacity for selective enrichment of phosphopeptides and phosphoproteins [18]. Recently, various metal oxides such as titanium dioxide (TiO_2) [19–21], zirconium dioxide [18], alumina [22], tin dioxide (SnO_2) [23–28] and some composites [29–32] have been demonstrated to be specific in the trapping of phosphoproteins/phosphopeptides due to their specific and reversible interaction with phosphate groups through Lewis acid–base and bidentate bonds [14]. Especially, SnO_2 has been identified as an affinity probe for phosphopeptide enrichment and shown more excellent selectivity and less nonspecific binding than TiO_2 [23].

As an affinity probe, SnO_2 has been used for preparation of gas sensors [33,34], photocatalysis [35] and fabrication of lithium rechargeable batteries [36]. Recently, some surface scientists have demonstrated that the performance of SnO_2 in gas sensing and catalysis is greatly affected by the surface structure. In principle, phosphopeptides enrichment by metal-oxide like SnO_2 occurring on the surface metal-oxide, considering the fact that high-energy

* Corresponding author. Tel/fax.: +86 25 83593593.
E-mail address: hxju@nju.edu.cn (H. Ju).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.049

or high-index facets with abundant unsaturated coordination atoms usually exhibit high surface activity [37–43], the morphological control of metal oxides may provide a way to further tailor the physical and chemical properties of nanomaterials. Thus it is possible to use high-index facets tin oxide nanoparticles for efficiently improving sensitivity and selectivity of phosphopeptides enrichment.

This work investigated the high-index facets of octahedral SnO_2 nanoparticles and observed the strong affinity of the nanoparticles to phosphopeptides due to the formation of bidentate bonds between high-density surface Sn atoms and phosphate [14]. The high surface activity made the nanoparticles become a novel metal oxide material for selective enrichment of phosphopeptides. By coupling with the enrichment process, a mass spectrometric method was thus proposed for simultaneous detection of three phosphopeptides in the tryptic digest of β-casein down to 4×10^{-9} M. The performance of octahedral SnO_2 nanoparticles in selective enrichment of low abundance phosphopeptides from the tryptic digests of drinking milk and human serum clearly demonstrated its great capability in phosphoproteome analysis for real biological samples. This is the first example using the high-index facets exposed metal oxide materials for phosphopeptide capture, and provides the promising application in proteomics.

2. Experimental

2.1. Materials and reagents

Tin (IV) chloride pentahydrate ($SnCl_4 \cdot 5\,H_2O$), trifluoroacetic acid (TFA, ≥90%), ammonium bicarbonate, DL-dithiothreitol (DTT), iodoacetamide (IAA), phosphoric acid (≥85%), β-casein (Mw ~24 kDa), bovine serum albumin (BSA, Mw ~66 kDa), α-casein (Mw ~24 kDa) and 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sequencing grade modified trypsin was obtained from Promega (Madison, USA). Acetonitrile (ACN) was obtained from Merck (Darmstadt, Germany). Hydrochloric acid (36.0–38.0%), polyvinylpyrrolidone (PVP, K-30) and ethanol (≥99.7%) were purchased from Nanjing Chemical Reagent CO., Ltd. (China). All these reagents were used as received without further purification. Drinking milk was purchased from a local grocery store. The clinical serum samples were from Jiangsu Institute of Cancer Prevention and Cure. All aqueous solutions were prepared using ultra-pure water (≥18 MΩ, Milli-Q, Millipore).

2.2. Synthesis of octahedral tin dioxide nanoparticles

Octahedral tin dioxide nanoparticles with high-index {221} facets were synthesized through a hydrothermal route at 200 °C for 12 h according to previous report with some modifications [44]. About 1.2 mL of concentrated hydrochloric acid (mass fraction 36.5–38%) was added to 12 mL ethanol/distilled water (1/1, v/v) under intense ultrasonic treatment. About 0.5 g of $SnCl_4 \cdot 5\,H_2O$ was added into the mixture, sonicated for 15 min at a frequency of 40 kHz, and stirred for 60 min. Then 0.315 g PVP was added, sonicated for 15 min, and stirred for 60 min. The obtained solution was transferred to a Teflon-lined stainless steel autoclave and kept at 200 °C for 12 h. The product was further underwent centrifugation and washed with deionized water and ethanol several times, and then dried in air at 80 °C for 24 h.

2.3. Characterization of octahedral tin dioxide nanoparticles

Scanning electron microscopic images were obtained with a Hitachi S-3000 N scanning electron microscope at an acceleration voltage of 10 kV. The transmission electron microscopic (TEM) images were gained on a JEM-2100 TEM (JEOL, Japan). Powder X-ray diffraction patterns were recorded on Rigaku Dmax 2200 X-ray diffractometer with Cu Kα radiation (λ=1.5416 A).The dynamic light-scattering measurement was carried out using a Brookhaven BI-9000AT instrument (Brookhaven Instruments Corporation).

2.4. Digestion of proteins

About 1 mg of the desired protein (β-casein or α-casein) was dissolved in 1 mL of NH_4HCO_3 solution (25 mM, pH~8), and digested with trypsin at an enzyme-to-protein ratio of 1:40 (w/w) for 16 h at 37 °C. The digestion of BSA was performed by adding 20 μL of 100 mM DTT in 100 μL of 4 mg mL^{-1} BSA in 25 mM NH_4HCO_3 solution (pH~8) to incubate at 100 °C for 10 min, followed with cooling, addition of 20 μL of 1.0 M IAA, incubation in the dark for 45 min at room temperature, and addition of 20 μL of DTT to react with the excessive IAA at room temperature for 1 h, and finally 10-fold diluting the mixture with 25 mM NH_4HCO_3 and adding trypsin at an enzyme-to-protein ratio of 1:40 (w/w) to incubate for 16 h at 37 °C. For real samples, 30 μL of drinking milk was added in 900 μL of NH_4HCO_3 solution (25 mM, pH~8) and centrifugated at 16,000 rpm for 15 min to obtain the supernatant for tryptic digestion. After the supernatant was denaturated at 100 °C for 5 min, 30 μg of trypsin was added in the solution to digest at 37 °C for 16 h, which was then diluted 25 times with 0.5% TFA in 50% ACN (v/v). All the tryptic peptide mixtures were stored at −20 °C until use.

2.5. Selective enrichment of phosphopeptides

After the digestion product was further diluted with 0.5% TFA in 50% ACN (v/v), 50 μL of octahedral SnO_2 (6 mg mL^{-1}) was added into 200 μL of the diluted peptide mixture (Fig. 1). The mixture was then vibrated at 25 °C for 30 min and centrifuged to obtain the precipitates, which was washed thrice with 0.1% TFA in 60% ACN (v/v). The human serum sample could be directly used for phosphopeptide capture by mixing 30 μL sample with 120 μL of 0.5% TFA in 50% ACN (v/v) and then treating the mixture with octahedral SnO_2 (6 mg mL^{-1}). After the trapped phosphopeptide was eluted using 5 μL 10% $NH_3 \cdot H_2O$ under sonication for 10 min, the eluate was analyzed by MALDI-TOF MS.

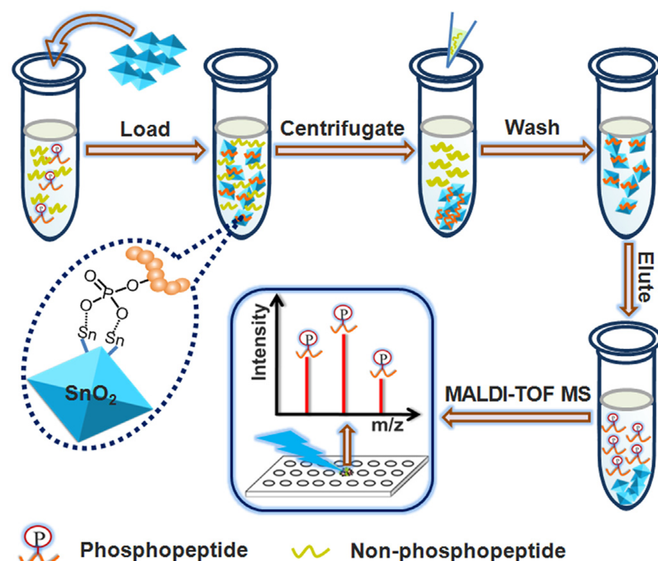


Fig. 1. Schematic illustration of selective enrichment of phosphopeptides using octahedral SnO_2 for mass spectrometric analysis.

Download English Version:

https://daneshyari.com/en/article/7681574

Download Persian Version:

https://daneshyari.com/article/7681574

Daneshyari.com