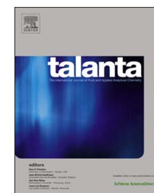
Talanta 176 (2018) 308–317

Contents lists available at ScienceDirect

Talanta

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



Facile synthesis of copper(II)-decorated functional mesoporous material for specific adsorption of histidine-rich proteins

Jiaojiao Wang, Rui Zhang, Xiaxia Yang, Xiaoyan Liu*, Haixia Zhang

State Key Laboratory of Applied Organic Chemistry, Key Laboratory of Nonferrous Metals Chemistry and Resources Utilization of Gansu Province and College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China

A R T I C L E I N F O

Keywords:
Mesoporous material
PPOSS-IDA-Cu^{2+}
Copper ions
Histidine (His)-rich protein
Hemoglobin

A B S T R A C T

The Cu^{2+}-decorated functional mesoporous material was fabricated by thermally initiated free-radical polymerization of octavinyl polyhedral oligomeric silsesquioxane. It was used as adsorbent for highly specific separation of histidine (His)-rich proteins in blood and cell lysate based on immobilized metal affinity chromatography. The functional mesoporous material (named as PPOSS-IDA-Cu^{2+}) was characterized in detail and its selectivity and binding capacity were evaluated using a His-rich protein (bovine hemoglobin, BHb) and other proteins (bovine serum albumin, myoglobin, lysozyme and horseradish peroxidase) containing fewer surface-exposed His residues as model proteins. The results indicated that PPOSS-IDA-Cu^{2+} exhibited large specific surface area and good selective adsorption ability and the maximum adsorption capacity for BHb was 3150 mg g^{-1}. Moreover, PPOSS-IDA-Cu^{2+} had excellent recyclability and the adsorption capacity of the reused material for BHb remained almost unchanged after six cycles. In addition, PPOSS-IDA-Cu^{2+} not only showed excellent performance for the removal of highly abundant hemoglobin in human blood, but also can be a good adsorbent for the enrichment of proteins in cell lysate. It was the first time to explore the application of Cu^{2+}-decorated functional material as an adsorbant for the separation of proteins in cell lysate. This approach can be combined with other techniques which can remove or deplete highly abundant proteins from real biological samples to obtain more comprehensive data about low abundant His-rich proteins in proteomic analysis.

1. Introduction

The development of efficient methods for the selective isolation and detection of protein targets from complex samples is of great significance in proteomics [1–3]. Among the existing separation techniques, immobilized metal affinity chromatography (IMAC) is the most frequently used method since its first introduction by Porath et al. [4,5]. And it selectively capture of specific proteins based on the interaction between the immobilized metal ions and electron donor groups such as histidine (His) residues located on the surface of proteins [6].

The application of immobilized metal affinity materials in protein separation includes the following three aspects: (1) immobilized metal affinity materials as one of the main methods of protein purification, can selectively separate His-tagged recombinant proteins [7,8]. (2) It can be used to remove highly abundant His-rich proteins from real biologic samples prior to analysis to reduce the complexity of samples and facilitate the identification of the low abundant biomarkers. For example, hemoglobin (Hb), a His-rich protein, is one of the most highly abundant proteins (HAP) for human and animal in red blood cells. In some diseasea, such as sickle cell disease, free Hb is found as high as 0.9 mg mL^{-1} in plasma. The predominance of free Hb is unfavourable for proteome research due to the suppressing of the information on low abundant proteins (LAP). However, these LAP may be the potential biomarkers for disease [9]. (3) It can be used for the enrichment of low abundant His-rich proteins which play vital roles in some disease. For example, the prion protein, extremely His-rich protein in neurons, is crucial for prion pathogenesis [10], and the plasmodium falciparum His-rich protein 2 has been proved to be related to malaria [11].

Nitrilotriacetic acid (NTA) and iminodiacetic acid (IDA) are the most common chelating ligands used in immobilized metal ion affinity chromatography techniques. For example, Altıntas et al. synthesized IDA immobilized poly(glycidyl methacrylate) beads and charged with Cu^{2+} for affinity binding of Hb from human blood hemolysate [12]. Xie and coworkers used mercaptopropionic acid as a stable anchor to attach NTA ligands on the surface of Fe_3O_4/Au core/shell nanoparticles, and the resulting products reacted with Ni^{2+} to obtain Fe_3O_4/Au-NTA-Ni^{2+} nanoparticles for selective enrichment and separation of His-tagged maltose-binding protein [7]. Nevertheless, a major disad-

* Corresponding author.
E-mail address: liuxiaoy@lzu.edu.cn (X. Liu).

http://dx.doi.org/10.1016/j.talanta.2017.08.016
Received 15 May 2017; Received in revised form 30 July 2017; Accepted 5 August 2017
Available online 09 August 2017
0039-9140/ © 2017 Elsevier B.V. All rights reserved.

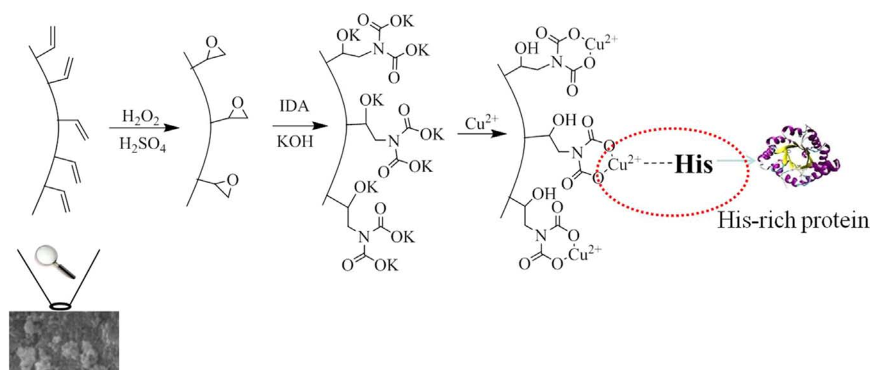


Fig. 1. Synthesis protocol of PPOSS-IDA-Cu^{2+} and adsorption mechanism of PPOSS-IDA-Cu^{2+}.

vantage of the above systems is a limited surface area as well as low functional group density, which greatly hampers their practical application in protein separation. Therefore, the invention of new adsorbents which possess large specific surface area, high loading density of metal ion and excellent reusability is of great significance.

Polyhedral oligomeric silsesquioxanes (POSS) consisting of 8, 10, or 12 silicon atoms, which can be thought of as the smallest possible particles of silica, have a cage like molecular structure with sizes of from 1 to 3 nm in diameter [13,14]. Each POSS contains organic substituents on its outer surface that make POSS an ideal type of cross-link agents or functional monomers for nanocomposite materials [15,16]. It has been proved that POSS moieties can significantly enhance hydrophobicity [17,18], biological compatibility [19,20], permeability [21] and mass transfer rate [22] of the matrices.

In this work, we report the design and synthesis of a mesoporous matrix with large specific surface area prepared by thermally initiated free-radical polymerization of octavinyl polyhedral oligomeric silsesquioxane (ovPOSS) and then modified with epoxy group, followed by conjugating IDA and subsequently chelating Cu^{2+}. The adsorption selectivity of the as-obtained adsorbent (named as PPOSS-IDA-Cu^{2+}) for proteins was evaluated by proteins containing different amounts of surface-exposed His residues (bovine hemoglobin, bovine serum albumin, myoglobin, lysozyme and horseradish peroxidase). Finally, the practical application of this approach was also validated by the selective removal of highly abundant His-rich proteins from human blood and specific enrichment of His-rich proteins from cell lysate.

2. Experimental

2.1. Chemicals

All the chemicals were listed in the Electronic Supplementary information (ESI).

2.2. Instruments

The obtained products were characterized by Fourier transform infrared spectroscopy (FT-IR, VEREEX 70V FTIR, Bruker, USA), VarioEl element analyzer (Hanau, Germany), scanning electron microscope (SEM, MIRA 3, TESCAN, Chech), energy dispersive X-ray spectroscopy (EDS, TEAM™, EDAX, USA), atomic absorption spectroscopy (AAS, AA240, Varian, USA) and nitrogen adsorption-desorption measurements at 77 K on a Tristar 3000 Surface Area and Porosimetry analyzer (Micromeritics Instrument Corp., USA). The surface-area measurement was based on the Brunauer-Emmett-Teller (BET) method, and the pore-size distribution was calculated from the Barrett-Joyner-Halenda (BJH) formula. In addition, contact angle measurement was carried out at room temperature by taking images of sessile drops of 3.0 μL of ultrapure water at the testing materials fixed on slides and calculating the contact angle with a contact angle plugin for ImageJ (http://rsbweb.nih.gov/ij/plugins/contact-angle.htm) [23].

The analytical system was performed on a TU-1800 spectrophotometer (Beijing, China) and a Varian 210 high-performance liquid chromatography (HPLC) (California, USA) equipped with two high pressure gradient pumps, a 325 UV–Vis detector and Varian Star Chromatographic workstation. All separations were carried out on a TSK-GEL G3000SWxL column (300 × 7.8 mm, 5 μm) at room temperature. The UV–Vis detector was operated at 280 nm and the flow-rate of the mobile phase (0.1 mol L^{-1} Na_2SO_4 + 0.05% NaN_3 in 0.1 mol L^{-1} PBS (composition in mmol L^{-1}: 43.5 Na_2HPO_4 and 56.5 NaH_2PO_4, pH 6.7) was maintained at 0.8 mL min^{-1}. All solutions (including sample solutions and mobile phase) were filtered through a 0.22-μm nylon membrane filter.

Electrophoretic analysis of proteins in biological samples was performed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) with 10% running gel and the gels were stained with Coomasie Brilliant Blue R-250. After destaining, the gels were imaged and processed using Image Processing and Analysis in Java software (ImageJ, downloaded from http://imagej.net/Downloads).

2.3. Synthesis of PPOSS-IDA-Cu^{2+} materials

The functionalized mesoporous material PPOSS-IDA-Cu^{2+} was prepared as shown in Fig. 1. Details of these procedures were given below.

2.3.1. Preparation of polymeric octavinyl polyhedral oligomeric silsesquioxane (PPOSS) by thermally initiated free-radical polymerization of ovPOSS

OvPOSS was synthesized according to the method described in the literature [24]. Briefly, trichlorovinylsilane (20 mL) was added to 200 mL of acetone in a 500 mL flask and nitrogen gas was bubbled through the reaction to expel oxygen. Then ultrapure water (70 mL) was added dropwise and the mixture was stirred at 40 °C for 72 h under a nitrogen atmosphere. After cooling to room temperature, the precipitates were separated by filtration and washed with acetone. The white solid was dried under vacuum at 30 °C overnight.

The preparation of PPOSS with mesoporous structure was based on the reference [25]. First, ovPOSS (1.20 g) was dispersed in porogenic solvent THF (4.05 mL), and porogen PEG 200 (1.06 mL) was added. Then AIBN (0.19 g) was added to the above mixture with ultrasonic agitation for 10 min to promote dissolution of the mixture. Poured the homogeneous precursor into a 20 mL glass vial and deoxygenated by bubbling through nitrogen for 2 min, followed by polymerization in the sealed vial at 60 °C for 24 h in oven. After polymerization, the polymers were washed with THF for 24 h in a Soxhlet apparatus, and dried in a vacuum oven at 50 °C overnight. Finally, the product was ground up for subsequent modification and named as PPOSS.

Download English Version:

https://daneshyari.com/en/article/5140629

Download Persian Version:

https://daneshyari.com/article/5140629

Daneshyari.com