Microporous and Mesoporous Materials 200 (2014) 165–173

Contents lists available at ScienceDirect

Microporous and Mesoporous Materials

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

Atom transfer radical polymerization of diverse functional SBA-15 for selective separation of proteins

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ARTICLE INFO

Article history:
Received 1 June 2014
Received in revised form 23 July 2014
Accepted 15 August 2014
Available online 4 September 2014

Keywords:
Functionalized SBA-15
Atom transfer radical polymerization
Proteins separation
High-abundant proteins

ABSTRACT

Poor detection of low-abundant proteins is a common problem in most current protein identification schemes. This is attributed partially to the existence of high-abundant protein. Therefore, selective removal of specific proteins prior to analysis is in great demand. In response these needs, we previously described two kinds of ion exchange groups functionalized SBA-15 synthesized by direct silylanization reaction. Herein, we designed and synthesized strong anion, cation and zwitterion functionalized SBA-15 via atom transfer radical polymerization (ATRP). The previous and current materials were characterized and theirs adsorption abilities for some model proteins (bovine serum albumin (BSA), hemoglobin (Hb), myoglobin (Mb) and lysozyme (Lys)) were evaluated. The results demonstrated that different functionalized SBA-15 is capable to selective removal of certain proteins in protein mixture. It also indicated that this sample preparation technique could be potentially applied to reduce the complexity on protein identification for biological samples with a wide dynamic range.

1. Introduction

In protein detection, low-abundant proteins (LAP) are often not detectable in the presence of high-abundant proteins (HAP) in protein mixtures. For enabling detection and analysis of LAP, specific capture and removal of highly-abundant proteins (HAP) from highly complex protein mixtures are appealing in the present [1].

So far, various sample clean-up procedures have been developed for more efficient protein identification. For example, albumin can be removed by iso-electric trapping [2,3] and affinity columns chromatography [4–7]. Immunoglobulin G (IgG) can be removed by affinity chromatography using immobilized protein A or protein G [8–11]. Strong anion exchange (SAX) functionalized SBA-15 can be used to remove HAP and enrich LAP [12]. Likewise, reversed phase (C18) and strong cation exchange (SCX) materials can be stacked and they significantly improved protein identification by liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) for protein digest [13]. In addition, some synthesized adsorbent such as in-situ polymerized stationary phase [14] and modified alumina nanofiber membranes [15] have also been developed for removal or separation of proteins. For these approaches, sample extraction and preparation are critical for the analysis of proteins over a wide range of abundance levels and extraction of protein is highly sample dependent. Therefore, flexible protein separation materials are promising to achieve the best fractionation and analysis conditions.

SBA-15 with highly ordered large pores attracted many biological analysts' attention. It has a more stable structure, more tolerant of a wider range of environments, greater resistance to organic solvents properties and has been explored extensively for the size selective immobilization and adsorption of large biomolecules [16,17]. However, an extra surface modification was usually needed to enhance the adsorption selectivity of biomolecules [18–24].

In our previous works, sulfonic acid and quaternary ammonium modified SBA-15 materials were successfully fabricated with selective proteins adsorption properties [12,25]. We found that the adsorption behavior of SBA-15 was different using different functionalization method. Therefore, we modified sulfonic acid or quaternary ammonium groups onto the surface of SBA-15 using a "grafting through" approach by atom transfer radical polymerization (ATRP) in this work. One attractive feature of ATRP is the synthesized polymer containing a terminal radically transferable atom, which can participate in initiating various post-polymerization reactions [26]. This advantage can be utilized in the multifunctional material preparation. Herein, ATRP was applied for dual-layer or multi-layer zwitterionic functionalized SBA-15 preparation. The SBA-15 synthesized here and from our previous reported method [12,25] was used to investigate the adsorption behavior for different proteins (bovine serum albumin (BSA), hemoglobin (Hb), myoglobin (Mb) and lysozyme (Lys)).

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http://dx.doi.org/10.1016/j.micromeso.2014.08.027
1387-1811/

Finally, the functionalized materials with selective adsorption ability for specific protein were picked to achieve the aim of sequential fractionation of the investigated proteins.

2. Experimental

2.1. Materials

Mesoporous SBA-15 silica molecular sieve used in this study has a specific surface area (S_{BET}) of 379 m^2/g and a mean adsorption average pore width of 5.3 nm, which were obtained from Shanghai Boyle Chemical Co. Ltd. (Shanghai, China). Quaternary ammonium *n*-octadecyldimethyl [3-(trimethoxysilyl)propyl] ammonium chloride (ODTPAC, 50% in methanol) was purchased from Fluorochem Ltd., Old Glossop (U.K.). (3-mercapto) propyltrimethoxysilane (MPTMS) (⩾95%), 3-aminopropyltriethoxysilane (APTES) (⩾97%) and 2-acrylamido-2-methylpropanesulfonic acid (⩾98%) (AMPS) were purchased from Alfa Aesar (a Johnson Mathey Company). 3-(methacryloylamino)propyl trimethylammonium chloride, 50 wt.% solution in water (MAPTAC) was purchased from J&K Chemical Ltd. (Beijing, China). α-Bromoisobutyrylbromide (BIBB) (⩾97%) was purchased from TCI Chemical Reagent Co. Ltd. (Shanghai, China).

Dimethylformamide (DMF) and triethylamine (Shanghai Chemical Reagent, 99%) was vacuum distillated after dried with magnesium sulfate and barium oxide respectively. Toluene (Tianjin Chemical Co., 99%) was dried by refluxing over CaH_2 and distilled just before use. Copper (I) bromide (A.R. grade, Shanghai Chemical Reagent) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried. N,N,N′,N″,N‴-pentamethyldiethylenetriamine (PMDETA) were purchased from Aladdin Reagent Co. with the highest purity and used without further purification. Acetic acid was purchased from Tianjin Guangfu Chemical Reagent Co. Ltd. (Tianjin, China). Boric acid, phosphoric acid, potassium dihydrogen phosphate, sodium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium azide, sodium sulfate and sodium hydroxide were obtained from Tianjin Chemical Reagent Co. Ltd. (Tianjin, China). All these reagents were analytical-reagent grades and used without further purification. Ultrapure water was prepared from Millipore (Bedford, MA, USA). BSA (fatty acid free), Hb, Mb and Lys were purchased from Sigma–Aldrich (Vienna, Austria) and used without further purification.

100 mg of Coomassie brilliant blue G-250 (Jing Ke Hong Da Biotechnology Co., Ltd. Beijing, China) was dissolved in 50 mL ethanol (95%) and 100 mL H_3PO_4 (85%). The solution was then diluted to 1000 mL with deionized water. It was used as color agent for protein determination.

2.2. Instrumentation

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, 325 UV–Vis detector and Varian Star Chromatographic workstation. Chromatographic conditions included TSK-GEL $G3000SW_{xL}$ column at 20 °C (300 × 7.8 mm) and isocratic elution (0.1 mol L^{-1} Na_2SO_4 + 0.05% NaN_3 in 0.1 mol L^{-1} Phosphate Buffer (pH 6.7)) with a flow-rate of 0.8 mL min^{-1} and the UV wavelength was 280 nm. Both solutions were filtered through a 0.22-μm nylon membrane filter.

2.3. Synthesis of functionalized SBA-15

SBA-15 was refluxed in 3 mol L^{-1} HCl for 8 h. After that it was filtered and washed with deionized water until Cl^- detection is negative. Then it was dried under reduced pressure at 100 °C for 8 h and stored in a desiccator before use.

Quaternary ammonium functionalized SBA-15 (SBA-15-$C_{18}(CH_3)_2N^+Cl^-$, material I) and sulfonic acid-functionalized SBA-15 (SBA-15-SO_3H, material II) were prepared according to our previous work [12,25]. The synthesis procedures of other functionalized SBA-15 are given below (see Fig. 1).

2.3.1. Immobilization of atom-transfer radical polymerization (ATRP) initiators on SBA-15 surfaces

As shown in Fig. 1, aminopropyl modification of SBA-15 was prepared as follows: SBA-15 (4.0 g) was refluxed with 4.0 mL APTES in 50 mL freshly distilled toluene for 8 h. The solids were filtered and washed with toluene and ethanol. The resulting product (SBA-15-NH_2) was dried under reduced pressure at 80 °C for 12 h. The nitrogen content was 2.75 wt.% by elemental analysis. The immobilization of ATRP initiators on SBA-15 surface was performed as reported in literature [27]. Briefly, SBA-15-NH_2 (3.0 g) was dispersed in 50 mL of chloroform with 0.82 mL of triethylamine. The mixture was cooled to 0 °C in an ice water bath after which a solution of 0.73 mL BIBB and 10.0 mL of chloroform was added dropwise over 30 min and the mixture was stirred for 24 h at room temperature. Finally, the mixture was successively washed with chloroform and ethanol. The resulting product was collected and dried overnight under reduced pressure at 40 °C, affording 2.85 g of SBA-15-supported ATRP initiator (SBA-15-Br). Elemental analysis of C and N indicated that about 0.28 mmol initiator groups were immobilized per gram of SBA-15-Br (see Table 1).

2.3.2. Surface modification of SBA-15 with MAPTAC (or AMPS) by surface-initiated ATRP

SBA-15-Br (2.5 g) and dry toluene (30 mL) were added into a 100 mL dry flask. Oxygen was removed by subjecting the contents of the flask to three freeze–pump–thaw cycles. CuBr (0.040 g, 0.28 mmol) and PMDETA (0.058 mL, 0.28 mmol) were added under the nitrogen flow. Then degassed monomer MAPTAC (9.1 mL, 43.2 mmol) (or AMPS, 8.95 g, 43.2 mmol) was injected into above mixture solution. The mixture was magnetically stirred rapidly under a nitrogen environment and placed in a thermostatically controlled oil bath at the reaction temperature of 100 °C. After 40 min, the polymerization was stopped by opening the flask and exposing the solution to air. The reaction mixture was diluted with toluene and the polymer-grafted SBA-15 (SBA-15-$(MAPTAC)_n$-Br, or SBA-15-$(AMPS)_n$-Br) was separated by centrifugation. The resulting product was washed by toluene, acetone and ethanol, then dried overnight under vacuum. Thus SBA-15-$(MAPTAC)_n$-Br material (material III) (or SBA-15-$(AMPS)_n$-Br, material IV) was obtained.

2.3.3. Surface modification of SBA-15 with MAPTAC and AMPS by surface-initiated ATRP

Zwitterionic polymer brush functionalized SBA-15 materials were synthesized via ATRP polymerization as described above. Briefly, the surface of SBA-15 was modified with MAPTAC monomer (4.6 mL, 21.6 mmol) brush via ATRP approach. Then the resulting MAPTAC-grafted SBA-15 material was reacted with AMPS monomer (4.48 g, 21.6 mmol) via the aforementioned approach. This resulting material, SBA-15-$(MAPTAC)_n$-$(AMPS)_n$-Br (material V), was functionalized with MAPTAC (4.6 mL, 21.6 mmol) and AMPS (4.48 g, 21.6 mmol) again according to Section 2.3.2. Then SBA-15-$(MAPTAC)_n$-$(AMPS)_n$-$(MAPTAC)_n$-$(AMPS)_n$-Br (material VI) was obtained.

2.4. Characterization

XRD patterns were recorded with a D/max-2400 (Rigaku, Japan) powder X-ray diffractometer using CuKa radiation source (40 kV,