Talanta 159 (2016) 23–28

Contents lists available at ScienceDirect

Talanta

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

Selective adsorption of hemoglobin with polyoxometalate-derived hybrid by solidification of super-lacunary phosphotungstate polyoxoanions

Dan-Dan Zhang, Lin-Lin Hu, Qing Chen, Xu-Wei Chen, Jian-Hua Wang *

Research Center for Analytical Sciences, College of Sciences, Northeastern University, Box 332, Shenyang 110819, China

ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form
1 June 2016
Accepted 2 June 2016
Available online 3 June 2016

Keywords:
Super-lacunary phosphotungstate
Hemoglobin
Selective adsorption

ABSTRACT

A novel polyoxometalate (POM)-based hybrid P_8W_{48}-APTS is prepared by the solidification of super-lacunary $P_8W_{48}O_{184}^{40-}$ polyoxoanions with APTS in an acidic medium. The oxygen (O^-) atoms in $P_8W_{48}O_{184}^{40-}$ are bound to silicon atoms in APTS by the formation of Si-O linkage through dehydration condensation. The solidification is confirmed by characterizations with XRD, FT-IR, TGA, SEM and EDXS. Selective isolation of proteins of interest, hemoglobin (Hb) in this case, from complex sample matrices is achieved by using P_8W_{48}-APTS hybrid as adsorbent under controlled conditions. 5.0 mg of P_8W_{48}-APTS hybrid results in an adsorption efficiency of 93% for 100 mg L^{-1} hemoglobin in 1.0 mL sample solution at pH 7. The adsorption behavior of Hb onto P_8W_{48}-APTS hybrid fits Langmuir adsorption model, corresponding to an adsorption capacity of 355.0 mg g^{-1}. The retained Hb could be readily recovered with either a SDS solution (0.1 mol L^{-1}) or a Na_3PO_4 (0.1 mol L^{-1}) solution as stripping reagent, providing recoveries of 94.6% or 83.9%, respectively. The biological activity of Hb remains 96.7% after an adsorption/desorption process (with elution by SDS), which illustrates virtually no change on the conformation of hemoglobin. The P_8W_{48}-APTS hybrid has been applied for the selective adsorption of Hb from human whole blood, and the results are demonstrated by SDS-PAGE assay.

1. Introduction

As a unique class of metal-oxide clusters, polyoxometalates (POMs) have been a research focus for many years [1,2]. and gained extensive applications in various fields including catalysis, medicine, materials science, surface chemistry, biology, photochromism and electrochromism [3–8]. The size and structure of POMs within an enormous range provide the access to a huge library of readily available and controllable secondary building units, which can be interconnected by electrophilic linkers into interesting and functional architectures. The electrophilic linker could be organic or inorganic in nature, thus POMs-based materials are prime candidates for the design and construction of tailored molecular framework materials [9,10].

The super-lacunary $P_8W_{48}O_{184}^{40-}$ polyoxoanion is one of the most attractive and stable POMs with its first isolation in 1985 [11]. It exhibits glamorous features of excellent stability in aqueous medium within an unusually wide pH range (i.e., pH 1–8) and large central cavity (diameter of ca. 10 Å) [12–14]. This highly stable cluster is an oligomer consists of four subunits of the hexavacant $[H_2P_2W_{12}O_{48}]^{12-}$ polyoxoanion [15]. The incorporation of metal ions inside the P_8W_{48} wheel and the stability of $P_8W_{48}O_{184}^{40-}$ polyoxoanion make it an excellent build-block for the design of new functional materials. A series of derivatives, including $[Cu_{20}Cl(OH)_{24}(H_2O)_{12}(P_8W_{48}O_{184})]^{25-}$ [12], $[\{K(H_2O)\}_3\{Ru(p\text{-cymene})(H_2O)\}_4P_8W_{49}O_{186}(H_2O)_2]^{27-}$ [16], and $[P_8W_{48}O_{184}Fe_{16}(OH)_{28}(H_2O)_4]^{20-}$ [17], have been reported. These derivatives have demonstrated favorable performances in practical applications for gas storage, catalysis and medicinal chemistry. Because of many active oxygen atoms involved in the structure of the super-lacunary $P_8W_{48}O_{184}^{40-}$ polyoxoanions and their derivatives, they may behave as an attractive biocompatible medium for the potential adsorption/separation of biomacromolecules, by taking advantage of the fact that the active oxygen atoms provide reactive sites for binding with various moieties. In practice, however, it is impossible to recover the soluble polyoxoanions or their derivatives after adsorption of biomacromolecules/proteins from an aqueous medium. This makes the application of $P_8W_{48}O_{184}^{40-}$ polyoxoanions and their derivatives in sample pretreatment not feasible. In this respect, solidification of $P_8W_{48}O_{184}^{40-}$ polyoxoanions or linking them onto a suitable support should be useful for facilitating their practical applications in the adsorption of

* Corresponding author.
E-mail addresses: chenxuwei@mail.neu.edu.cn (X.-W. Chen), jianhuajrz@mail.neu.edu.cn (J.-H. Wang).

http://dx.doi.org/10.1016/j.talanta.2016.06.005
0039-9140/

biomacromolecules/proteins from biological sample matrices. Amino-functionalized silanes have been well demonstrated to be able to improve the adhesion on inorganic materials [18,19] and they are typically used to modify the surface of adsorbents to achieve enhanced adsorption [20]. Aminopropyltriethoxysilane (APTS) is a common silane agent [21] with excellent solubility, high branching capacity and flexibility. Most importantly, the functionalization processes using APTS as silane agent can be performed in both aqueous and anhydrous media [20].

In the present work, we developed an efficient strategy to solidify the $P_8W_{48}O_{184}^{40-}$ polyoxoanions with APTS as the solidification reagent, by linking the oxygen atoms in $P_8W_{48}O_{184}^{40-}$ with the silicon atoms in APTS via dehydration condensation. The obtained solid product/derivative, P_8W_{48}-APTS hybrid, exhibits excellent adsorption selectivity toward hemoglobin under controlled conditions. A solid-phase extraction protocol is thus developed by using the P_8W_{48}-APTS hybrid as adsorbent for the isolation of hemoglobin. The P_8W_{48}-APTS hybrid provides favorable biocompatibility during the adsorption/desorption process and selective isolation of hemoglobin from human blood is achieved.

2. Materials and methods

2.1. Materials and reagents

Hemoglobin from bovine blood (Hb, H2625, pI 6.8), bovine serum albumin (BSA, A3311, pI 4.9), cytochrome c (cyt-c, 30398, pI 10.1) are purchased from Sigma (St Louis, MO, USA) and used without further purification. The protein molecular weight marker (broad, D532A, Takara Biotechnology Company, Dalian, China) was a mixture of nine purified proteins (Mr in kDa: myosin, 200; β-galactosidase, 116; phosphorylase B, 97.2; serum albumin, 66.4; ovalbumin, 44.3; carbonic anhydrase, 29; trypsin inhibitor, 20.1; lysozyme, 14.3; aprotinin, 6.5). Coomassie Brilliant Blue G-250 and R-250, H_2O_2, H_3PO_4, CH_3COOH, H_3BO_3, NaOH, Na_2WO_3 and Na_3PO_4 are acquired from Sinopharm Chemical Reagent (Shanghai, China). Aminopropyltriethoxysilane (APTS, A107148, 98%) is purchased from Aladdin (Shanghai, China). Ammonium peroxydisulfate, hydrochloric acid, ethanol, glycerin (Bodi Chemical Holding, Tianjin, China) and KCl (Damao Chemical Holding, Tianjin, China) are used as received. These reagents are at least of analytical reagent grade unless otherwise specified. De-ionized water of 18 MΩ cm is used throughout the experiments.

Human blood samples from healthy volunteers are provided by the Hospital of Northeastern University.

2.2. Preparation and characterization of the P_8W_{48}-APTS hybrid

Polyoxometalate $K_{28}Li_5H_7[P_8W_{48}O_{184}]\cdot 92H_2O$ (P_8W_{48}) is produced firstly by following a previously reported approach [22], and P_8W_{48}-APTS hybrid is then prepared with the procedure as detailed in the following.

1.0 g $K_{28}Li_5H_7[P_8W_{48}O_{184}]\cdot 92H_2O$ is dissolved in 300 mL of de-ionized water, then 3 mL APTS (pH 7.0, adjusted by 1 mol L^{-1} HCl) is added into the polyoxometalate solution drop-wisely under vigorous stirring. After reaction for 40 min, the reaction system is adjusted to pH 1.0. The reaction mixture is then stirred vigorously for 24 h. The solid product is collected by filtration, washed with de-ionized water, followed by drying at 75 °C for 8 h. The final product is denoted as P_8W_{48}-APTS hybrid.

The morphology and energy-dispersive X-ray spectroscopy (EDXS) and SEM image of the obtained P_8W_{48}-APTS hybrid are recorded on a LEO1430VP scanning electron microscope (LEO, Germany). FT-IR spectra are recorded on a Nicolet 6700 spectrophotometer (Thermo Electron, USA) using a KBr disk from 400 to 3500 cm^{-1} with a resolution of 2.0 cm^{-1}. X-ray diffraction (XRD) patterns are taken on a Rigaku D/max-a X-ray diffractometer (Rigaku, Japan) with CuKα radiation (k=1.54056 Å) with a step size of 0.02°. The thermal stability of the product is analyzed by a TG-DSC simultaneous thermal analyzer (Mettler Toledo, Switzerland) within a temperature range of 25–800 °C. The granularity or particle size of the hybrid is measured by use of a Zetasizer Nano ZS90 (Malvern, UK).

2.3. Proteins adsorption with P_8W_{48}-APTS hybrid

In the present case, the adsorption performance of acidic, neutral and basic proteins, i.e., Hb, BSA and cyt-c, onto P_8W_{48}-APTS hybrid is evaluated. Britton-Robinson buffer solution within a range of pH 3–11 is used to investigate the effect of pH values on protein adsorption.

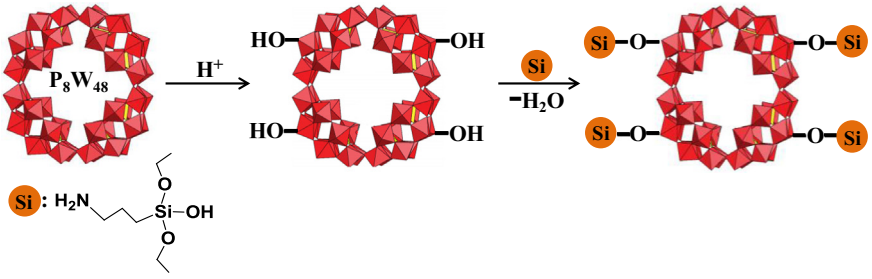
For protein adsorption, 5.0 mg of P_8W_{48}-APTS hybrid is mixed with 1.0 mL of protein solution, and the mixture is shaken vigorously for 40 min to facilitate the adsorption process. After centrifugation at 6000 rpm for 5 min, the supernatant is collected to quantify the residual protein content by monitoring the Soret band of protein species (406 nm for Hb, 409 nm for cyt-c and 595 nm for BSA) with a U-3900 UV–vis spectrophotometer (Hitachi, Japan).

The protein species adsorbed onto P_8W_{48}-APTS hybrid are then recovered by use of a 0.1 mol L^{-1} SDS solution as stripping reagent. For this purpose, 1.0 mL of SDS solution is mixed with the P_8W_{48}-APTS hybrid (after adsorption of protein) and the mixture is oscillated for 20 min to facilitate the recovery of the adsorbed protein from the P_8W_{48}-APTS hybrid. The supernatant after centrifugation at 6000 rpm for 5 min is collected for the ensuing analysis.

3. Results and discussion

3.1. Preparation and characterization of P_8W_{48}-APTS hybrid

The protocol for the preparation of P_8W_{48}-APTS hybrid is illustrated in Scheme 1. There are $40O^-$ atoms in the surface of $P_8W_{48}O_{184}^{40-}$ and all of them are protonated under acidic circumstance to produce



Scheme 1. Schematic illustration for the preparation of the P_8W_{48}-APTS hybrid.