Biomaterials 32 (2011) 619–627

Contents lists available at ScienceDirect

Biomaterials

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

Thermally-modulated on/off-adsorption materials for pharmaceutical protein purification

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ARTICLE INFO

Article history:
Received 22 July 2010
Accepted 6 September 2010
Available online 2 October 2010

Keywords:
Adsorption
Albumin
Silica
Plasma proteins
Protein adsorption
Surface modification

ABSTRACT

For the development of temperature-responsive adsorption materials for pharmaceutical protein purification, poly(*N*-isopropylacrylamide-co-*N*,*N*-dimethylaminopropylacrylamide-co-*N*-tert-butylacrylamide) (P(IPAAm-*co*-DMAPAAm-*co*-tBAAm) brush grafted silica beads were prepared through a surface-initiated atom transfer radical polymerization (ATRP). The prepared silica beads as a chromatographic stationary phase were evaluated by observing their thermo-responsive elution profiles of plasma proteins including human serum albumin (HSA) and γ-globulin. Chromatograms of two proteins indicated that negatively-charged HSA was adsorbed on the cationic copolymer brush modified silica beads at higher temperatures with low concentration of phosphate buffer (PB) (pH 7.0) as a mobile phase. The HSA adsorption was attributed to (1) an enhanced electrostatic interaction with the cationic copolymer brush at low concentration of PB and (2) an increased hydrophobic interaction from the dehydrated copolymer at high temperature. Step-temperature gradient enabled HSA and γ-globulin to be separated by the modulation of HSA adsorption/desorption onto the copolymer brush grafted silica beads. These results suggested that the prepared copolymer brush grafted silica beads adsorbed negatively-charged proteins both through electrostatic and hydrophobic interactions by the modulation of column temperature and gave attractive adsorption materials for protein purification process.

1. Introduction

Surfaces which are resistant to the adsorption of proteins or cells, have been extensively investigated for using as a separation tool, biosensor, implants and other blood contacting devices [1–4]. These surfaces were developed for preventing disappointed results such as a non-specific protein adsorption leading to reduced detection sensitivity or platelets adhesion accelerating thrombosis. By the large number of researchers, the design of the bioinert surfaces were attempted to be performed through the modification of functional groups or polymers using these hydrophilicity, conformational flexibility, excluded volume effect, or repulsive interaction [5–7]. On the contrary, functional surfaces adsorb proteins have been also investigated for analytical devices or protein purification process. These surfaces were developed by the modification of hydrophobic or electrostatic functional groups to surfaces, and the adsorption/desorption of proteins are modulated by organic solvents and the excessive amount of salt [8,9]. However, these processes induced some possible damages to protein structure, resulting in the increase of the production cost. If these adhesive surfaces can be switched instantly to non-adhesive surface by external-stimuli, adsorbed proteins could be controlled freely, leading to protein purification process without organic solvents or the excessive amount of salt without any possible damages to protein structure. For developing an ideal adsorption materials, which adsorb/desorb proteins freely, the modification of stimuli-responsive polymer like poly(*N*-isopropylacrylamide) (PIPAAm) to substrate is supposed to be the best way, because PIPAAm can alter its hydrophobic and structural properties with external temperature change at its lower critical solution temperature (LCST) of 32 °C [10]. This intrinsic thermo-responsive property is widely used in biomedical applications, such as drug and gene delivery systems [11–13], bioconjugates [14,15], and cell culture substrates [16–19], and tissue engineering for regenerative medicine [20–24], because the phase transition point is near the body

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doi:10.1016/j.biomaterials.2010.09.012

temperature. Utilization of surface property change of PIPAAm modified materials, some kinds of proteins were adsorbed onto the materials' surfaces. However, the adsorption process has less selectivity. Additionally, only hydrophobic interaction between PIPAAm and proteins was weak for protein adsorption [25].

Thus, our research group designed adsorption materials which can be switch their protein adsorption/desorption properties with a high selectivity. Thermo-responsive copolymer brush with ionic group was prepared on silica beads surface using surface-initiated atom transfer radical polymerization (ATRP) as shown in Fig. 1(A). Densely packed polymer brush structure on solid base prepared surface-initiated ATRP has significantly strong electrostatic and hydrophobic interactions with proteins due to the large amount of grafted copolymer per a specific surface area [26–29]. Additionally, as shown in Fig. 1(B), the thermally-modulated conformational change of the grafted charged copolymer brush increase gives its net charge density per a specific area, because the fixed charge density of expanded molecules increases its net charge density by the shrinking of molecule size, and the change in charge density will be applicable to the adsorption of the aimed proteins (Fig. 1(B)). Characterization of the dense cationic copolymer brush on silica beads as adsorption materials was investigated by chromatographic analysis using hydrophobic steroids, adenosine nucleotides, and two plasma proteins, human serum albumin (HSA), and γ-globulin.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (IPAAm) and *N*,*N*-dimethylaminopropylacrylamide (DMAPAAm) were kindly provided by Kohjin (Tokyo, Japan). IPAAm was recrystallized from n-hexane. DMAPAAm was purified by distillation under reduced pressure at 113 °C (1 mmHg). *N*-*tert*-butylacrylamide (tBAAm) was obtained from Wako Pure Chemicals (Osaka) and recrystallized from acetone. CuCl and $CuCl_2$ were purchased from Wako Pure Chemicals. Tris(2-aminoethyl)amine (TREN) was purchased from Acros Organics (Pittsburg, PA, USA). Formaldehyde, formic acid, and sodium hydroxide were purchased from Wako Pure Chemicals. Tris(2-(*N*,*N*-dimethylamino) ethyl)amine (Me_6TREN) was synthesized from TREN, according to a previous report [30]. Silica beads (the average diameter: 5 μm, the pore size: 300 Å, the specific surface area: 100 m^2/g) were purchased from Chemco Scientific (Osaka). Hydrochloric acid, hydrofluoric acid, and ethylenediamine-*N*,*N*,*N*′, *N*′-tetraacetic acid disodium salt dehydrate (EDTA · 2Na) were purchased from Wako Pure Chemicals. 2-(*m*/*p*-chloromethylphenyl)ethyltrichlorosilane was obtained from ShinEtsu Chemical Industry (Tokyo). 2-Propanol (HPLC grade), dichloromethane, and toluene (dehydrate) were purchased from Wako Pure Chemicals. Adenosine nucleotides and steroids were purchased from Wako Pure Chemicals, and human serum albumin and γ-globulin were purchased from Sigma Chemicals (St. Louis, MO). Water used in this study was Milli-Q water prepared by an ultrapure water purification system, synthesis A10, Millipore, Billerica, MA) unless otherwise mentioned.

2.2. Preparation of ATRP-initiator immobilized silica beads

2-(*m*/*p*-chloromethylphenyl) ethyltrichlorosilane as an ATRP-initiator modified silica beads was prepared as shown in the first step in Fig. 1(A), according to previous reports [31,32]. First, silica beads were washed with concentrated hydrochloric acid

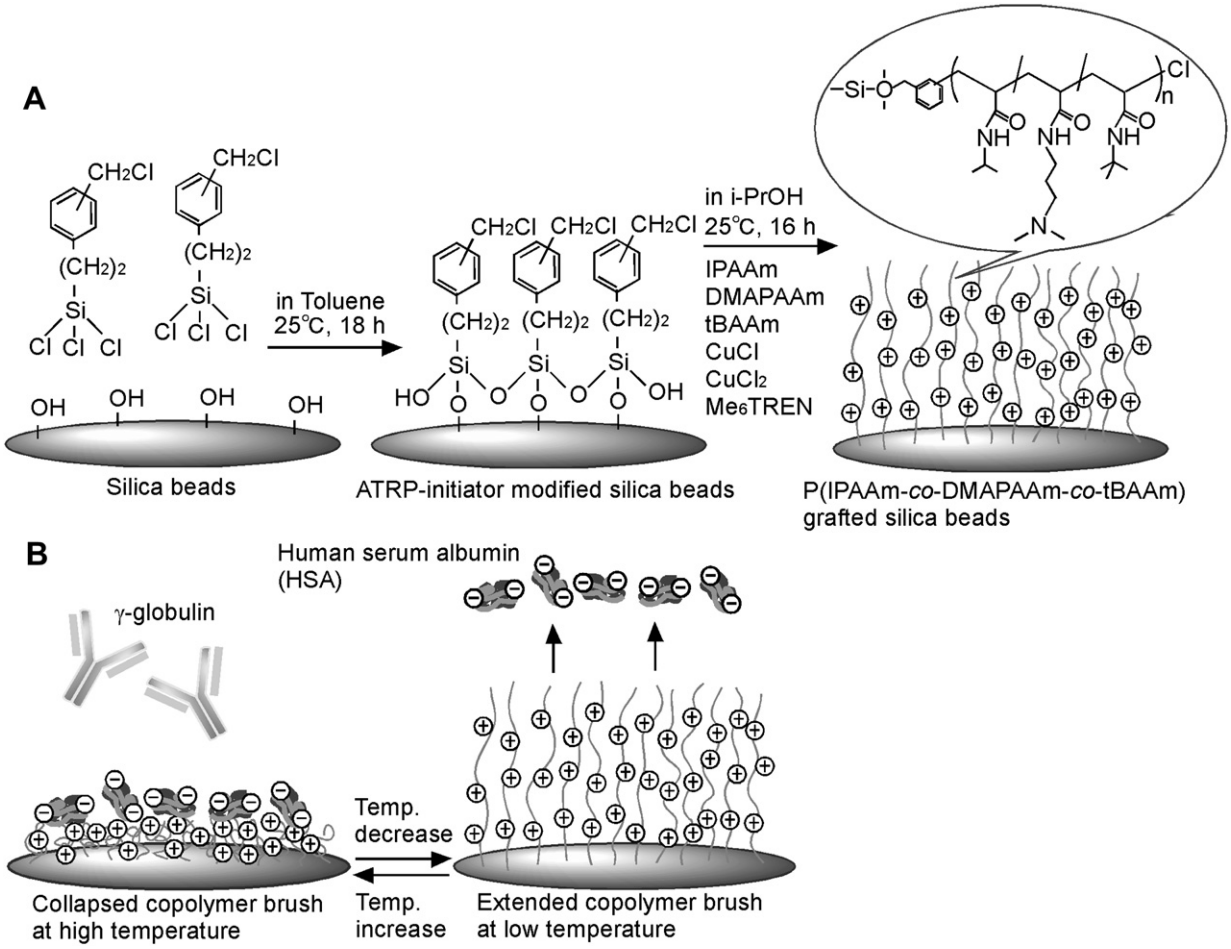

Fig. 1. (A) Scheme for the preparation of thermo-responsive cationic copolymer modified adsorption material using a surface-initiated atom transfer radical polymerization (ATRP). (B) Schematic illustration of the thermally-modulated adsorption/desorption of proteins.