International Journal of Biological Macromolecules 111 (2018) 178–185

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: https://www.journals.elsevier.com/biomac

Protein A and protein A/G coupled magnetic SiO_2 microspheres for affinity purification of immunoglobulin G

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ARTICLE INFO

Article history:
Received 1 December 2017
Received in revised form 30 December 2017
Accepted 4 January 2018
Available online 06 January 2018

Keywords:
Protein A
Protein G
Immunoglobulin G
Affinity chromatography
Magnetic beads

ABSTRACT

Protein A carrying magnetic, monodisperse SiO_2 microspheres [Mag(SiO_2)] with bimodal pore size distribution including both mesoporous and macroporous compartments were proposed as an affinity sorbent for IgG purification. Protein A was tightly bound onto the aldehyde functionalized-Mag(SiO_2) microspheres. The mesoporous compartment provided high surface area for protein A binding and IgG adsorption while the macropores made easier the intraparticular diffusion of protein A and IgG. The selection of relatively larger microspheres with high saturation magnetization allowed faster magnetic separation of affinity sorbent from the IgG isolation medium, less than 1 min. With these properties, the proposed sorbent is an alternative to the common sorbents in the form of core-shell type, magnetic silica nanoparticles with more limited surface area and slower magnetic response. By using protein A attached-Mag(SiO_2) microspheres with the concentrations lower than 50 mg/mL, IgG isolation from rabbit serum was performed with a purity higher than 95%, with an isolation yield comparable to commercial magnetic resins, and in shorter isolation periods. IgG could be also quantitatively isolated from rabbit serum with the sorbent concentrations higher than 50 mg/mL. Successive IgG isolation runs indicated that no significant protein A leaching occurred from the magnetic matrix.

1. Introduction

Therapeutic monoclonal antibodies, a class of biotechnological and biopharmaceutical products have attracted significant interest for the production of extremely purified antibodies [1,2]. Since the first approval for Orthoclone OKT3 (the first therapeutic monoclonal antibody for prevention of kidney transplant rejection) in 1986, ~70 monoclonal antibody products including Fc-fusion proteins, antibody fragments, and antibody-drug conjugates will be the dominant products of the biopharmaceutical market by 2020 [2]. These products have been used in both immunodiagnosis and immunotherapy for the treatment of various diseases and the scientific research applications [2–7]. The demand for large-scale production of this class of biomolecules requires not only the reduction of manufacturing costs, but also rapid progression to give extremely pure products by applying an effective purification process with high yields and reliability [8].

Affinity chromatography, one of the traditional techniques for protein purification, relies on the purification of proteins from complex feed stocks via reduction in the number of processing steps and reduced costs in the downstream separation steps involved [9,10]. Protein A affinity chromatography, an IgG-Fc receptor with high selectivity to IgG, is commonly utilized for the specific purification of IgG (high purity, >99% possible) from different complex sources [10–13]. Additionally, protein G, a bacterial cell wall protein with two IgG binding domains, is also used for selective IgG purification [8]. Usually, protein A is immobilized onto various adsorbents including cross-linked agarose, porous glass, silica gel, porous polystyrene/divinylbenzene, ceramic material, polymethacrylate, cross-linked cellulose, and polyvinylether as commercially available matrices [13,14]. These kinds of affinity materials are used for the purification of various antibodies [9,11,13–19]. Furthermore, other typical adsorbents including three-dimentional (3D) monolithic structures as novel separation media were developed to achieve a higher throughput and low cost purification of biomolecules [15,20–23]. However, there is an urgent demand for the development of stable/suitable small molecule ligands (i.e., ligand stability) to match the selectivity of the immobilized protein A to monoclonal antibodies with a high level of purity [11,24].

In this study, magnetic monodisperse-porous SiO_2 microspheres [Mag(SiO_2)] with bimodal pore-size distribution synthesized by a developed staged-shape template hydrolysis and condensation protocol

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https://doi.org/10.1016/j.ijbiomac.2018.01.019
0141-8130/

were utilized as backbone matrix for stable coupling of protein A or (protein A + protein G) with high stability. Protein A or (protein A + protein G) was coupled covalently onto the magnetic sorbent by means of glutaraldehyde activation using appropriate spacer arms. The effects of covalent linking of ligands [i.e. protein A or (protein A + protein G)] on the selectivity and isolation capacity of new magnetic sorbents through IgG purification were investigated.

2. Experimental

2.1. Materials

The chemicals used for the preparation of magnetic poly(methacrylic acid-*co*-ethylene dimethacrylate), poly(MAA-*co*-EDMA) microspheres were purchased from Sigma Chemical Co., St. Louis, MO, USA, as reported in our earlier study [25]. 2,2′-azobisisobutyronitrile (AIBN), hydrochloric acid (HCl), and benzoyl peroxide (BPO) were obtained from Merck AG (Darmstadt, Germany). Tetraethyl orthosilicate (TEOS), ammonium hydroxide solution (25%–28% NH_3 basis), acetonitrile (HPLC grade), 2-propanol (IPA), (3-glycidyloxypropyl) trimethoxysilane (GLYMO), sodium carbonate (Na_2CO_3), ethylenediamine (EDA), and glutaraldehyde (GA, 50% aqueous solution), Tris-HCl, dipotassium phosphate (K_2HPO_4), glycine, Tween-20, and sodium chloride (NaCl) were also purchased from Aldrich Chem. Co. Protein A and protein G were supplied from Thermo-Fisher Scientific, U.S.A. Rabbit IgG and rabbit serum were acquired from Sigma-Aldrich Co., U.S.A.

2.2. Preparation of protein A and protein A/G coupled Mag(SiO_2) microspheres

Magnetic, monodisperse-porous SiO_2 microspheres [Mag(SiO_2)] were used as starting material for the synthesis of protein A or (protein A + protein G)-carrying sorbent. Mag-SiO_2 microspheres were obtained by a new staged-shape template hydrolysis-condensation protocol using magnetic poly(MAA-*co*-EDMA) microbeads as template according to the method reported previously [25]. For the preparation of GA activated microspheres, Mag(SiO_2) (200 mg) was reacted with GLYMO (4 mL) within toluene (10 mL) under reflux (110 °C) for 24 h. GLYMO attached-Mag-SiO_2 microspheres were washed with ethanol, distilled water, and Na_2CO_3 solution (200 mM) using magnetic separation. The resulting microspheres were reacted with EDA (2 M) at 60 °C for 6 h in an aqueous Na_2CO_3 solution (15 mL, 0.25 M) and subsequently washed with phosphate buffer (20 mL, 100 mM, pH 7.2) three times. Finally, a solution of GA (10% v/v) in phosphate buffer (20 mL, 100 mM, pH 7.2) was utilized for the preparation of aldehyde groups functionalized microspheres [GA-Mag(SiO_2)] at room temperature for 8 h. The as-prepared microspheres were separated by a magnet and washed with phosphate buffer (20 mL, 100 mM, pH 7.2) three times. GA-Mag(SiO_2) microspheres were used as starting material for covalent binding of protein A or protein A + protein G. Typically, the solution of protein A (5 mg/mL, 2.5 mL) in phosphate buffer (100 mM, pH 8.0) was added onto GA-Mag(SiO_2) microspheres (200 mg) and the microspheres were dispersed in the solution by vortexing. Then, $NaBH_4$ (2.5 mg/mL) was added and the aqueous dispersion was incubated at room temperature for 24 h with shaking [26]. Protein A bound microspheres [pA-Mag (SiO_2)] were collected by a magnet and washed with phosphate buffer (100 mM, pH 8.0) three times to remove the non-specifically adsorbed protein. Protein A/G ligands were also directly treated with GA-Mag(SiO_2) using an almost identical process to that mentioned above, with a constant protein A/G concentration (including protein A: 2.5 mg/mL and protein G: 2.5 mg/mL) in phosphate buffer (2.5 mL, 100 mM, pH 8.0). Hence protein A + protein G bound microspheres [pAG-Mag(SiO_2)] were obtained.

2.3. Immunoglobulin G isolation using pA-Mag(SiO_2) and pAG-Mag(SiO_2) sorbents

Herein, pA-Mag(SiO_2) microspheres (10 mg) were suspended in 500 μL of adsorption buffer (1× TBS: containing 25 mM Tris, 0.15 M NaCl, 0.05% Tween-20) and washed three times by vortexing. For a typical isolation, 500 μL of IgG solution (1 mg/mL) was added onto the magnetic sorbent and incubated at room temperature for 1 h. IgG loaded pA-Mag(SiO_2) microspheres were collected using an external magnet and washed with adsorption buffer. Elution of IgG from magnetic sorbent was carried out using 200 μL of glycine buffer (100 mM, pH 2.2) three times for 15 min. Subsequently, the eluted proteins were neutralized with 30 μL of Tris-HCl buffer (1 M, pH 8.5) in each step (including supernatant and eluate solutions) and stored at 4 °C for further analyses. The reusability of pA-Mag(SiO_2) microspheres was investigated by five successive IgG isolations under the same conditions. In a typical isolation process, sorbent was washed with K_2HPO_4 buffer (500 μL, 50 mM, pH 9.3) and adsorption buffer between successive isolations for 1 min.

2.4. IgG isolation from rabbit serum using pA-Mag(SiO_2) and pAG-Mag (SiO_2) sorbents

Magnetic microspheres (10 mg) were dispersed in 500 μL of adsorption buffer (1× TBS: containing 25 mM Tris, 0.15 M NaCl, 0.05% Tween-20) and washed three times followed by magnetic separation. The rabbit serum solution (containing 10 μL rabbit serum and 490 μL adsorption buffer) was added onto the microspheres and the resulting dispersion was incubated at room temperature for 1 h. IgG loaded microspheres were collected by an external magnet and washed with adsorption buffer three times. The elution and neutralization of isolated IgG from magnetic microspheres were carried out with the same process as mentioned in section 2.3.

2.5. Determination of protein concentration and yields

Protein concentration of the eluate solution as well as the protein purification parameters (i.e. equilibrium IgG adsorption (mg IgG/g sorbent), desorption yield (% w/w), isolation yield (% w/w) and target protein purity) were determined as reported earlier [27]. The error bars indicating standard deviations for each mean value of equilibrium IgG adsorption, desorption yield or isolation yield were calculated based on three replicate runs.

3. Results and discussion

Magnetic monodisperse-porous silica [Mag(SiO_2)] microspheres 6.5 μm in size were selected as the base matrix for the synthesis of protein A affinity sorbent for IgG purification. Typical SEM images showing the size distribution and surface morphology of Mag(SiO_2) microspheres are given in Fig. 1A. As seen here, the microspheres were synthesized with a narrow size distribution. The SEM image with higher magnification (17.000×) showed that the microspheres had a porous surface. The selection of relatively larger microspheres with a high saturation magnetization (i.e. 25 emu/g) allowed the fast magnetic separation of protein A affinity sorbent from the IgG isolation medium within the time periods less than 1 min. Mag(SiO_2) microspheres had a bimodal pore size distribution including both mesoporous and macroporous compartments lying between 6 and 140 nm with a median pore size of 54 nm [25,27]. The mesoporous compartment provided necessary surface area (i.e. 45 m^2/g) for protein A binding and IgG adsorption, while the macropores made easier the intraparticular diffusion of large molecules like protein A and IgG. Hence, the intraparticular mass transfer resistance during both ligand binding and target molecule purification should be lower due to the presence of macroporous compartment. With these properties, the proposed silica based sorbent is a serious alternative to