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Immunoglobulin G purification from bovine serum with pseudo-specific supermacroporous cryogels

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ABSTRACT

Supermacroporous cryogels were synthesized and used for the purification of immunoglobulin G (IgG) from bovine serum. Imidazole functional groups were incorporated into basic polymeric backbone to gain pseudo-specificity to cryogels by using n-vinylimidazole as comonomer. The cryogels were prepared in aqueous solution of monomers partially frozen inside plastic syringe column by cryogelation. Poly (2-hydroxyethyl methacrylate-n-vinylimidazole) [poly(HEMA-VIM)] cryogels were prepared by polymerization of water-soluble functional monomer, n-vinylimidazole, with basic monomer HEMA. The characterization methods including swelling test, Fourier transform infrared spectroscopy (FTIR), elemental analysis, and scanning electron microscopy (SEM) were performed to evaluate physical and chemical properties of cryogels prepared. Bovine IgG adsorption on plain and composite cryogels was studied with respect to different parameters such as, pH, IgG concentration, flow rate, ionic strengths, and adsorption time. The best adsorption of bovine IgG was observed at pH 7.4 up to 21.1 mg per unit mass of poly (HEMA-VIM) cryogel. Elution of IgG adsorbed from the cryogels was easily achieved with 0.1 M acetate buffer containing 1 M NaCl at pH 4.0. In order to describe the adsorption process, we applied some equilibrium and kinetic adsorption models to the data. The results best fitted to Langmuir model showing monolayer protein adsorption and surface homogeneity of cryogel. Finally, we evaluated IgG purification from bovine serum under optimal condition determined. The purification efficiency and IgG purity were investigated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) study.

1. Introduction

Vertebrates produce five isotypes of immunoglobulin classified as IgG, IgA, IgM, IgD, and IgE. Immunoglobulin G is the main immunoglobulin isotype in humans as well as in animal, because of its high level in blood serum. Three isotypes of immunoglobulin have been identified in bovine: IgG, IgA, and IgM. Bovine IgG contains two subclasses IgG1 and IgG2. IgG1 is the main component of milk and saliva secretion [1]. In serum, both the subclasses of IgG are found in about equal concentrations (IgG1: 11.2 mg/mL, and IgG2: 9.2 mg/mL) [2]. Similarly to other animal species, bovine IgG has a multitude of immunological functions, including binding invading microbes such as bacteria and virus leading to their destruction [3]. Bovine IgG was widely utilized in the immunological supplementation of foods, specifically in infants formulates [4]. Available evidences suggest various beneficial effects of colostrum-based IgG products in improving exercise performance for trained athletes and cyclists, in oral immunotherapy and as a cholesterol-lowering remedy for patients with hypercholesterolemia [5–8]. Numerous chemically based, solid-phase chromatography methods have been developed and optimized to achieve antibody purification in particular applications [9]. Bovine immunoglobulin G has been purified using various methods, mainly chromatographic techniques including ion exchange chromatography (IEC), and pseudobioaffinity chromatography [10–12].

Traditional packed-bed chromatography with immobilized stationary phase, despite its high resolving power, has certain limitations such as a high-pressure drop and low flow-rates difficulties in efficient scale up [13]. The most obvious progress has been made in the development of alternative chromatography tools such as membrane adsorbents, monoliths and cryogels [14–16]. Cryogel technology has progressed rapidly and is being applied in several major research fields. Cryogels as supermacroporous adsorbents are a class of monolithic compounds obtained by cryogelation process, which allows the production of macroporous polymers with controlled porosities. The large pores and short diffusion property allows a high flow rate and consequently increase mass transfer thus making cryogels suitable matrices for use in environmental, biotechnological and medical applications [17–20].

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In the present study, cryogels containing imidazole functional groups were synthesized and characterized by swelling test, FTIR, elemental analysis and SEM. These cryogel were conducted to performance evaluation for immunoglobulin G adsorption from aqueous solution and purification from bovine serum. Then, some kinetic and equilibrium mathematical models were also applied to data determined to describe interaction between cryogel and analyte molecules. After purification of IgG molecules from bovine serum, we evaluated their purity and purification efficiency by SDS–PAGE study.

2. Experimental

2.1. Materials

For the synthesis of adsorbents, 2-Hydroxyethyl methacrylate (HEMA), N,N′-methylene-bis(acrylamide) (MBAAm), ammonium persulfate (APS) and N,N,N′,N′-tetramethylenediamine (TEMED) were supplied by Sigma Chemical Co. (St. Louis, MO). Functional co-monomer n-vinylimidazole (VIM) was purchased from Aldrich (Steinheim, Germany). Whole bovine serum (sterile filtered) and bovine gamma globulin (fraction II) were obtained from MP Biomedical LLC (France). Bovine immunoglobulin G (IgG) solutions were obtained by dissolving bovine gamma globulin in different types of inorganic buffers according to each experiment. Barnstead (Dubuque, IA, USA) ROpure LP® reverse osmosis unit of deionized water was used in the all experiments.

2.2. Synthesis of poly(HEMA-VIM) cryogels

The Poly(HEMA-VIM) cryogels were prepared as follows: 1.3 mL (10.8 mmol) of HEMA and 0.1625 mL (1.8 mmol) of VIM were dissolved in 4.5375 mL of deionized water whereas 0.283 g of MBAAm was separately dissolved in 10 mL of deionized water. Then, two aqueous solution were mixed together to obtain total concentration of monomers around 10.9% and functional monomer ratio as 1:6 (VIM:HEMA) by mole. The cryogel was produced by free radical polymerization initiated by TEMED and APS. After adding APS (20 mg) the solution was cooled in an ice bath for 20 min to inhibit direct gelation of monomers at room temperature. After addition of TEMED (25 μL), the reaction mixture was stirred for 1 min, then, poured into plastic syringe column. The solution was frozen at −12 °C and allowed to polymerize for 24 h at same temperature and then thawed at room temperature. The plain cryogels were also synthesized for comparison purpose with same recipe except VIM comonomer. The cryogels were extensively washed with ethanol and water to remove any unreacted monomer or initiator and then stored in sodium azide 0.02% at 4 °C.

2.3. Characterization of cryogels

2.3.1. Swelling test

The swelling ratios (SR%) of cryogels were determined in distilled water. The experiment was conducted as follows: the cryogel sample initially dried until constant weight was carefully weighed ($W_{initial}$, ±0.0001 g) before being placed in a 50 mL vial containing distilled water. The vial was put into an isothermal water bath with a fixed temperature (25.0 ± 0.5 °C) for 2 h. The sample was taken out from the medium, wiped by a filter paper to remove water attached on the surface, and weighed (W_{final}). The weight ratio of dry and wet cryogel was recorded. The swelling ratio was calculated by applying the following formula: $SR\% = [(W_{final} - W_{initial})/W_{initial}] \times 100\%$; where units of the weights before and after swelling in g, respectively.

2.3.2. Surface morphology

The surface morphology of the cryogels was examined using scanning electron microscopy (SEM, Jeol, JSM 5600, Tokyo, Japan) technique. The samples were initially dried in air at 25 °C for 7 days prior to analysis. A fragment of the dried cryogel was mounted on a SEM sample holder and was coated with a thin gold layer by a sputter for 2 min. The sample was then attached into the microscope. Finally, the surface of the sample was scanned at the desired magnification to study the morphology of the cryogels.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectroscopy was used to evaluate chemical structure of cryogels in the solid state (Perkin Elmer, Spectrum one, USA). The sample of 98 mg cryogel was prepared by dispersing in dried IR-grade and pressed it into pellet forms while spectra were recorded in the wavenumber range of 4000–450 cm^{-1}.

2.3.4. Elemental analysis

In order to evaluate the amount of VIM incorporated into the cryogel structure, the synthesized cryogels were subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932, USA).

2.4. Optimization of bovine IgG adsorption condition from aqueous solutions

All adsorption procedures were carried out at room temperature in continuous separation set-up. The cryogel was washed with 10 mL of water and then equilibrated with 10 mL of the appropriate buffer solution. Bovine IgG solution was passed through the cryogel, by a peristaltic pump, and the adsorption capacity was calculated from mass balance determined in respect to IgG amount in the solution before/after adsorption. The adsorption was followed by monitoring the decrease in UV absorbance at 280 nm. The effect of pH, flow-rate, ionic strength, initial concentration of bovine IgG solution on the adsorption capacity of the cryogels were studied. The effective factors such pH, flow-rate, ionic strength, initial IgG concentration were varied in the appropriate ranges of 4.0–9.0, 0.5–2.0 mL/min, 0.0–1.0 M NaCl, and 0.1–3.0 mg/mL IgG, respectively whereas keeping other factors at a constant value determined. For kinetic analysis, the samples were collected from circulating system in desired time intervals between 0 and 120 min. The experiments were performed as triplicate as measurements have been done for statistical purpose. The instrument response was periodically checked with standard solutions of immunoglobulin G (IgG).

2.5. IgG adsorption from whole bovine serum

Bovine serum sample was diluted with 0.1 M phosphate buffer (pH 7.4) at the ratios of 1/10 and 1/20. Diluted bovine serum (10 mL) was pumped through the cryogel at a flow-rate of 1.0 mL/min for 35 min. The diluted bovine serum, non-retained fractions, and the retained eluted fractions obtained after separation from each cryogel, were studied by SDS–PAGE analysis under reducing conditions.

3. Results and discussion

3.1. Synthesis and characterization of cryogels

The poly(HEMA-VIM) cryogels were prepared by the cryo-copolymerization of HEMA with VIM at a 1:6 by mole ratio, in which VIM was selected as the pseudo-specific functional co-monomer for the adsorption of bovine IgG. As known VIM monomer has an