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Evaluation of human interferon adsorption performance of Cibacron Blue F3GA attached cryogels and interferon purification by using FPLC system

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ABSTRACT

In this study, we have focused our attention on preparing supermacroporous cryogels as a potential dye-affinity adsorbent for interferon purification. For this purpose, 2-hydroxyethyl methacrylate (HEMA) and Cibacron Blue F3GA (CB) were selected as main monomer and dye–ligand. Cibacron Blue F3GA attached supermacroporous poly(2-hydroxyethyl methacrylate) [poly(HEMA)/CB] cryogels were prepared and characterized by swelling test, scanning electron microscopy, elemental analysis, and FTIR. After that, the effecting factors such as pH, concentration, interaction time, and ionic strength on the interferon separation were evaluated. The maximum adsorption capacity of poly(HEMA)/CB cryogels was obtained as 38.2 mg/g at pH 6.0. Fast protein liquid chromatography (FPLC) system was used for interferon purification from human gingival fibroblast extract. The chromatography parameters, capacity and selectivity factors, resolution and theoretical plate number were found as 7.79, 9.62, 4.23 and 554, respectively. Although some decreases in total protein content, from 320 μg to 18 μg, and interferon activity, from 2.6×10^3 IU to 2.2×10^3 IU, were determined, specific antiviral activity increased from 7.19 IU/μg to 122.2 IU/μg. The purified interferon samples have 97.6% purity determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. After repeated ten adsorption–desorption cycles, no significant decrease was determined in adsorption capacity of cryogel. In result, poly(HEMA)/CB cryogels have an application potential for rapid, cheap and specific purification of interferon.

1. Introduction

Over the last decades, there has been increasing interest and innovations in the field of biotechnology, biomedicine and applied biochemistry including research and development of vital biochemicals and biopharmaceutical compounds [1]. In proportion to the ascending scientific treatment, extraordinary development and expanding use of biomolecules, especially proteins, in the medical, food, drug and cosmetic industries, commercial values of treated biomolecules also increased [2]. Many of the biomolecules must be separated and purified before use and it is urgently needed to meet requirements for high purity and yield. Because of some difficulties and limitations, numerous separation procedures mainly based on the physicochemical and biological properties of the interested molecule such as antibodies, cytokines, enzymes, therapeutic DNA, and plasmids have been under investigation [3]. Among the numerous studied techniques, adsorption based separation and purification methodologies are widely investigated phenomenon having its origin in several different disciplines of science [4].

Cryogels are considered as one of the new types of polymeric hydrogels with a considerable potential use in biotechnology [5]. Beside the general advantages, i.e. high chemical and physical stability, easy protein adsorption and elution capability, low-cost and reusability, of mostly used polymeric supports, cryogels are very good alternatives for protein purification with additional advantages such as large pores, short diffusion path, and low pressure drop [6]. For example, short diffusion path allow optimal utilization of the immobilized ligand on the pore walls [7]. Osmotic stability can be also listed as one of the main advantages of cryogels [8]. They are gel matrices formed in moderately frozen solutions of monomeric or polymeric precursors and typically have interconnected macropores (or supermacropores), allowing unhindered diffusion of solutes of practically any size such as nano- and even microparticles [9]. Cryogels can be used as a monolithic form as well as in a membrane form to combine the advantage of membrane chromatography, in terms of high flow-rates and high productivity [5]. They allow high flow-rates enabling the processing of large volumes within short process times for both adsorption and elution processes. They are cheap materials and they can be used as

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disposable avoiding cross-contamination between batches [10]. Hence, it is possible to use cryogels in chromatographic separation of biological nanoparticles (plasmids, proteins, viruses, cell organelles) and even all of cells (*Escherichia coli*) from natural sources [11].

In affinity chromatography, numerous molecules, including enzymes, coenzymes, cofactors, antibodies, amino acids, oligopeptides, proteins, nucleic acids, and oligonucleotides may be used as bioligands for designing the novel and specific adsorbents [12]. Because of their restrictions and precautions in the adsorption and elution studies, difficulties in retention of their original biological activity, storage stability, their needing to extensive purification processes, high cost of production and complexity in the immobilization of these bioaffinity ligands, the numbers of the attempts and interest in developing biomimetic ligand are getting more. The synthetic dye–ligands have been considered as important alternatives for affinity chromatography; hence, dye-affinity chromatography is an effective and widely used method for the purification of biomolecules [13]. Dye–ligands are commercially available, inexpensive, and can be easily immobilized, especially on solid matrices bearing hydroxyl groups and able to bind most types of proteins in a remarkably specific manner [14]. It should be especially pointed out that, although dyes are all synthetic in nature, they are still classified as affinity ligands because they can interact with the active sites of many proteins by mimicking the structure of the substrates, cofactors, or binding agents for those proteins. Therefore, a number of textile dyes, also known as reactive dyes, have been used for protein purification [15]. Dye–ligands can exhibit selectivity resulting from the cumulative effects of multiple weak binding such as electrostatic, hydrophobic, hydrogen binding and van der Waals interactions with fast kinetics; in spite of having low binding constants (10^{-4} to 10^{-6} M^{-1}) [16,17].

Interferons are biologically active and very important proteins for the immune system of mammalian species. They can be classified as a member of large group of glycoproteins known as cytokines and were named as "interferon" because of their ability to interfere with viral proliferation [18]. Interferons are most rapidly synthesized by peripheral blood leukocytes, lymphoblastoid and myeloblastoid cell lines on somatic cells in response to an appropriate stimulus in the presence of pathogens such as viruses, bacteria, or parasites, or other antigens or tumor cells [19]. Then, they are secreted into the surrounding medium, bind to receptor on target cells and induce transcription of some genes and these results in an anti-viral state in the target cells [20]. Although they modulate specific cellular functions in some cases, the main function of these proteins in the mammalian bodies is preventing viral replication in newly infected cells as gain a resistance to infection especially in the first line of defense against viral infections [21]. Beside the uses of interferons in treatments of kidney cancer, multiple myeloma, carsinoid, lymphoma and leukemia, especially recombinant human interferon-α, that comprises a family of extracellular signaling proteins with antiviral, antiproliferating and immunomodulatory activities, is especially used for the treatment of AIDS-related Kaposi's sarcoma, hairy cell leukemia, and chronic hepatitis B and C [22]. Therapeutic value of interferons against certain types of tumors such as brain tumors and malignant melanomas caused both increasing interest in these proteins [23] and more focusing on investigations aimed to obtain treated and purified interferons [24]. The purification of human interferons from various sources has been attempted by a variety of methods including metal-chelation, precipitation, cation or anion-exchange, gel filtration, hydrophobic and immunoaffinity chromatography over many years and some protocols have been proposed that yield homogenous protein [25–29].

According to increase in demand of interferon market, there is a necessity to develop simple, fast and effective techniques for the purification of them. Hence, the design and application of novel affinity sorbent for the adsorption of recombinant human interferon-α (rHuIFN-α) from aqueous solution and the purification from human gingival fibroblast were studied and reported here. Firstly, poly(HEMA) cryogels were synthesized by cryopolymerization of HEMA monomer. Then, Cibacron Blue F3GA was covalently attached on them through substitution reaction between triazine groups of the dye molecules and hydroxyl groups of HEMA. After that, Cibacron Blue F3GA attached poly(HEMA) [poly(HEMA)/CB] cryogels were characterized using swelling test, FTIR, elemental analysis, and SEM. After the characterization step, system parameters such as adsorption rate, pH, rHuIFN-α concentration and ionic strength were investigated to evaluate their effects on the rHuIFN-α adsorption performances of dye-affinity cryogels. The reusability of the cryogels was also tested. Finally, poly(HEMA)/CB cryogels were used for interferon purification from natural source, human gingival fibroblast.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA), N,N′-methylene bisacrylamide (MBAAm) and ammonium persulfate (APS) were obtained from Sigma (St. Louis, USA). N,N,N,N′′-tetramethylene ethylenediamine (TEMED) was purchased from Fluka A.G. (Buchs, Switzerland). Recombinant human interferon-α (rHuIFN-α) (freeze-dried powder; 99% pure by RP-HPLC) was supplied from ProSpec-Tany TechnoGene Ltd. (Rehovot, Israel). Cibacron Blue F3GA was purchased from Polyscience (Warrington, USA) and used as received. All other chemicals were of reagent grade and purchased from Merck A.G. (Darmstadt, Germany). All water used in the experiments was purified using a Barnstead (Dubuque, IA, USA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion-exchange packed-bed system.

2.2. Preparation of poly(2-hydroxyethyl methacrylate) [poly(HEMA)] cryogels

A typical preparation procedure is as follows: HEMA monomer (0.5 ml) and crosslinker N,N′-methylene bisacrylamide (100 mg) were dissolved in 10.0 ml deionized water and; then, nitrogen gas was passed through the solutions under the vacuum (100 mmHg) for removal of dissolved oxygen gases for 5 min. The total monomer concentration is 6.0%. The cryogels were then produced by free radical polymerization initiated by TEMED (120 μl) and APS (10 mg). After adding APS (1% of the total monomers, w/v) the solution was cooled in an ice bath for 2–3 min. TEMED (1% of the total monomers, w/v) was added and the reaction mixture was stirred for 1 min. Then, the reaction mixture was divided and poured into sealed glass tubes (i.d. 5 mm × 20 mm). The polymerization solutions in the glass tubes were frozen at −12 °C for 24 h and then thawed at room temperature. After that, cryogels were washed with water and ethanol three times; then, stored in buffer containing 0.02% sodium azide at 4 °C until use.

2.3. Cibacron Blue F3GA attachment onto poly(HEMA) cryogels

Dye attached cryogels were obtained by immobilization of dye–ligand onto poly(HEMA) cryogels [30]. Immobilization studies were performed in the shaking water bath for controlling the reaction temperature. Cibacron Blue F3GA was dissolved in 50 ml of water (dye concentration: 5 mg/ml). Then, this aqueous dye solution was transferred to 50 ml of distilled water containing