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Exploring the use of heparin as a first capture step in the purification of monoclonal antibodies from cell culture supernatants

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ABSTRACT

In this work, an innovative and cost effective alternative to Protein A based on a commercially available HiTrap™ Heparin resin was evaluated as a first capture step for the purification of a monoclonal antibody from different CHO cell culture supernatants. Preliminary studies using pure human serum antibodies and fetal bovine serum showed that heparin can be used as a cation exchanger for the capture of antibodies. Different adsorption and elution buffers were screened and the most promising results were obtained using 20 mM phosphate buffer at pH 8.0 as binding buffer and 1 M NaCl in 20 mM phosphate buffer at pH 8.0 as elution buffer. The performance of the capture step was evaluated in terms of yield, purity, and purification factor of the elution pool obtained. The studies revealed that heparin is capable of an impressive performance, with recoveries higher than 96% from a diafiltered serum-free CHO cell supernatant for all tested conditions (pH 7.0–8.0), and surpassing 99% at pH 8.0. This is especially important considering that the separation did not rely on a highly selective bioaffinity step. The purification factor was found to increase with the pH value, with the highest value (representing a 3.1-fold increase) also obtained at pH 8.0.

1. Introduction

Monoclonal antibodies (mAbs) are currently the most prevalent class of recombinant protein therapeutics, providing valuable new treatment options for several diseases, including cancer and autoimmune disorders [1]. The commercial potential of these compounds is clearly demonstrated by the increasing number of mAb-based drugs that have been approved or are under clinical trials. This growing demand is expected to be maintained in the foreseeable future, in a market that ascended to nearly $75 billion in 2013 and is predicted to reach around $125 billion by 2020, considering the current approval rate [2]. Despite the effectiveness and safety of mAbs the general access to these therapeutics is barred by their high manufacturing costs [3,4], thus, making the development of more economic processes a pressing need.

Mammalian cells are currently the expression system of choice for large-scale production of mAbs with Chinese hamster ovary (CHO) cells being the most widely used cell line [5]. Advances in cell culture technology have resulted in increased expression levels and higher cell densities. This improvement in the upstream processes has shifted the focus toward the downstream processes, which are now considered as the bottleneck in the manufacturing of mAb therapeutics [6,7], not only in terms of cost but also of processing capacity. For these reasons, the purification of mAbs has been receiving more and more attention, particularly in an attempt to re-design, optimize, and intensify the different steps employed downstream. These currently follow a platform approach, comprising a first capture step with Protein A affinity chromatography, and two subsequent polishing steps that are required to meet the purity criteria of the final product [8,9]. Although Protein A is currently the industry golden standard for a highly efficient and selective capture of mAbs there are some problems arising from this chromatography step, being the most pressing arguments for replacing it based on process economics, and intrinsic limitations of the ligand itself. These limitations include the possibility of ligand leaching and co-elution along with the target antibody, poor stability to harsh sanitization conditions, and consequences in the integrity of the mAb in the elution pool, since the acidic elution that is employed may cause inactivation and/or aggregation of the product [10].

In an attempt to overcome these problems, alternative procedures to capture the target mAb from different feedstocks have been explored, and the possibilities range from non-chromatographic strategies (aqueous two-phase extraction, precipitation, membrane filtration) to non-Protein A chromatographic steps, involving for instance multimodal or ion exchange

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chromatography [11,12]. In particular, the use of cation exchange chromatography (CEX) has shown great potential as a primary capture step in antibody purification [13–15], and it has effectively been implemented in some processes at industrial scale, namely in the production of adalimumab, commercialized under the trade name of Humira® [16].

There are a multitude of compounds that hold the potential to be employed as cation exchangers, either because they were synthetically produced to serve this purpose, or because they naturally possess the ability to perform like so. Heparin is a naturally occurring sulfated glycosaminoglycan that is characterized by the highest density and distribution of negative charges among any known biological macromolecule [17]. One of the widespread applications of pharmaceutical-grade heparin is in medicine, where it is used to passivate the surfaces of indwelling devices, such as catheters, and it is also administered as anticoagulant drug [18]. Immobilized heparin has been mostly used as an affinity ligand to bind a wide range of biomolecules [19–21], particularly blood proteins like antithrombin and other coagulation factors [22], and less often as a cation exchanger ligand [23]. The demand for synthetic heparin has motivated several studies to prepare heparin-like polysaccharides and oligosaccharides [24,25], with recent efforts being centered in a chemoenzymatic approach to synthesize the naturally-occurring form of the compound [26]. The scale of the synthesis has experienced a 100 000-fold increase in a few years [27], and the elimination of animal-sourced materials may be guaranteed by the establishment of feasible methods to prepare heparins in accordance with FDA's guidelines.

In this work, an innovative application for the heparin chromatography was evaluated, deriving from its strong cation exchanger potential, namely the ability to capture a basic mAb from CHO cell culture supernatants. The salt tolerance of the medium was assessed performing a direct capture of mAbs at the native supernatant's conductivity conditions and also at a lower conductivity, after a diafiltration step. The performance of the proposed capture step was estimated in terms of yield, protein purity and purification factor, and compared to the outcome of Protein A affinity chromatography.

2. Materials and methods

2.1. Chemicals

Potassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Panreac Quimica Sau (Barcelona, Spain). Phosphate buffered saline (PBS), sodium chloride (NaCl), and sodium thiosulfate ($Na_2S_2O_3$) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium dihydrogenocitrate anhydrous ($C_6H_7NaO_7$) and hydrochloric acid (HCl) were purchased from Fluka (Buchs, Switzerland). Sodium acetate (CH_3COONa), formaldehyde (CH_2O) 37%, sodium carbonate (Na_2CO_3), and silver nitrate ($AgNO_3$) were obtained from Merck (Darmstadt, Germany). Acetic acid (CH_3COOH) 100% (AnalaR Normapur®) was purchased from VWR BDH Prolabo (Radnor, Pennsylvania, USA). All other chemicals were of analytical grade. Water used in all experiments was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Biologicals

Human normal immunoglobulin was obtained from Octapharma (Lachen, Switzerland), as a commercially available solution for therapeutic administration (Gammanorm®), in which the active substance has a concentration of 165 mg/mL, and at least 95% corresponds to immunoglobulin G (IgG). Ultra-low (UL) IgG Fetal Bovine Serum (FBS) was purchased from Life Technologies (Grand Island, NY, USA). Anti-human interleukin-8 (anti-IL-8) antibody-producing CHO DP-12 clone#1934 (ATCC CRL-12445), containing a DHFR expression system, was obtained from the American Type Culture Collection (LGC Standards, Middlesex, UK).

2.2.1. Chinese hamster ovary cell cultures

CHO DP-12 cells were grown in a serum-free medium, ProCHO™ 5 (Lonza Group Ltd Belgium), which was supplemented with 4 mM L-glutamine (Gibco, Life Technologies), 2.1 g/L $NaHCO_3$ (Sigma–Aldrich), 200 nM Methotrexate, MTX (Sigma–Aldrich), 10 mg/L recombinant human insulin (Sigma–Aldrich), 0.07% (v/v) lipids (Lonza), and 1% (v/v) antibiotics (50 U/mL penicillin and 50 μg/mL streptomycin, both from Gibco). CHO DP-12 cells were also grown using a mixture of 75% (v/v) of ProCHO™ 5, and 25% (v/v) of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) of UL IgG FBS. DMEM was formulated to contain 4 mM L-glutamine, 4.5 g/L D-glucose, 1.5 g/L $NaHCO_3$, 200 nM MTX, 2 mg/L recombinant human insulin, 35 mg/L L-proline (all from Sigma–Aldrich), 0.1% (v/v) of a trace element A, 0.1% (v/v) of a trace element B (both from Cellgro®, Manassas, VA, USA), and 1% (v/v) of antibiotics (50 U/mL penicillin and 50 μg/mL streptomycin). The composition of trace element A includes 1.60 mg/L $CuSO_4{\cdot}5H_2O$, 863.00 mg/L $ZnSO_4{\cdot}7H_2O$, 17.30 mg/L selenite. 2Na, and 1155.10 mg/L ferric citrate, while the trace element B is composed by 0.17 mg/L $MnSO_4{\cdot}H_2O$, 140.00 mg/L $Na_2SiO_3{\cdot}9H_2O$, 1.24 mg/L molybdic acid, ammonium salt, 0.65 mg/L NH_4VO_3, 0.13 mg/L $NiSO_4{\cdot}6H_2O$, and 0.12 mg/L $SnCl_2$. The cultures were carried out as described by dos Santos et al. [28]. The produced anti-IL-8 mAb had an isoelectric point (pI) of 9.3.

2.3. Pre-conditioning of cell culture supernatants

CHO cell culture supernatants were diafiltered via tangential flow ultrafiltration using a QuixStand benchtop system (GE Healthcare, Uppsala, Sweden), equipped with an ultrafiltration hollow fiber cartridge (Xampler Ultrafiltration Cartridge UFP-10-E-3MA from GE Healthcare). The cartridge was composed by 13 polysulfone hollow fiber membranes of 1 mm inner diameter and 10 kDa nominal molecular weight cut-off, providing an effective filtration area of 110 cm^2 and 3 mL void volume. An initial volume of 200 mL of cell culture supernatant was continuously processed for 4 diafiltration volumes, and samples from permeate and concentrate were collected throughout the process to monitor conductivity and IgG concentration. The supernatants were buffer exchanged into 20 mM phosphate buffer at pH 7.0, 7.5, and 8.0, and the conductivity was decreased from ~8 mS/cm to ~2 mS/cm (ECTestr Low conductivity tester, OAKTON Instruments, Vernon Hills, IL/USA). The diafiltered serum-free supernatant had a mAb concentration in the range of 90–110 mg/L, while the analogue serum-containing supernatant had a mAb concentration around 60 mg/L.

2.4. Purification of monoclonal antibodies

Purification of anti-IL-8 was performed with two different resins: (i) HiTrap Heparin HP pre-packed 1 mL column (GE Healthcare), and (ii) HiTrap MabSelect SuRe pre-packed 1 mL column (GE Healthcare). Purification assays were performed in an ÄKTA™ Purifier 100 system from GE Healthcare. Data were acquired and processed using Unicorn 5.11 software. UV absorbance at 280 nm, conductivity, and pH of the outlet stream were continuously monitored. Flowthrough and eluate pools were collected using a Frac-920 fraction collector from GE Healthcare, and further analyzed with regard to IgG concentration and total protein content.