Journal of Chromatography A, 1393 (2015) 57–64

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Purification process of recombinant monoclonal antibodies with mixed mode chromatography

Sophie Maria[a], Gilles Joucla[a], Bertrand Garbay[a], Wilfrid Dieryck[a], Anne-Marie Lomenech[b], Xavier Santarelli[a], Charlotte Cabanne[a,*]

[a] Univ Bordeaux, BPRVS, EA 4135, F-33000, Bordeaux, France, Bordeaux INP, BPRVS, EA 4135, F-33000, Bordeaux, France
[b] Univ Bordeaux, Centre de Génomique Fonctionnelle, Plateforme Protéome, Bordeaux F-33000, France

ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form 6 March 2015
Accepted 7 March 2015
Available online 14 March 2015

Keywords:
Mixed mode chromatography
Antibody
Host cell proteins
Purification process

ABSTRACT

An innovative process to purify mAb from CHO cell culture supernatant was developed. This three-step process involved two mixed mode resins and an anion exchange membrane. We used a human IgG mixture to determine the optimal conditions for each purification step. Thereafter, the whole process was evaluated and improved for the purification of a recombinant mAb produced in the supernatant of CHO cells. Once optimized, yield and purity of 88% and 99.9%, respectively were comparable to those obtained in a conventional process based on a capture step using protein A. In addition, aggregates, HCPs and DNA levels in the purified fraction were below regulatory specifications. Then we used mass spectrometry to identify contaminating proteins in the antibody fraction in order to highlight the behavior of HCPs.

1. Introduction

Industrial processes for the purification of recombinant monoclonal antibodies (mAbs) produced in CHO cells are usually based on three successive chromatography steps. The first consists in the capture of the recombinant antibody from the culture supernatant by affinity chromatography using protein A. This technique has proved to be very efficient in terms of selectivity and purity, and has become the standard in pharmaceutical companies. However, a survey indicated that 60% of biopharmaceutical companies would like to replace the "protein A" step [1]. Indeed, there are several technical reasons for this such as protein A leakage, the formation of protein aggregates during the elution step which is performed at acidic pH, and the financial aspect linked to the high cost of the protein A resins. These problems explain why numerous studies have attempted to find alternative technologies to capture antibodies. Multimodal or mixed-mode chromatography has recently emerged as a candidate for innovative methods of antibody purification. Mixed-mode chromatography was developed in the late 1950s with hydroxyapatite [2,3]. The following generations of mixed-mode ligands were developed after 1970 and were used in numerous applications [4–7]. In the 80s, these resins were widely used for the purification of nucleic acids but were curiously neglected in the protein purification field. It was not before the pioneering work of Burton and Harding, who developed a variant called "Hydrophobic Charge Induction Chromatography", that interest for mixed mode chromatography to purify proteins was rekindled [8]. They tested numerous ligands having heterocycles known for their hydrophobicity and demonstrated that the combination of hydrophobic and ionic interactions led to the emergence of new selectivity. Since then, mixed-mode chromatography has been used to evaluate its performances for mAb purification that does not involve protein A [9–14].

The second and third chromatography steps used to purify recombinant antibodies should provide orthogonal modes of interaction when compared to the capture step to enable effective separation of any remaining contaminants (Host Cell Proteins, DNA, viruses) or unwanted forms of the antibody (aggregates). While the process uses Protein A for capture, the second and third steps are respectively cation-exchange chromatography (CEX) and anion-exchange chromatography (AEX) operated in a flowthrough mode. The CEX step eliminates host cell proteins, aggregates and leached Protein A, whereas the AEX flowthrough step removes contaminating DNA from the producing cells and achieves further reduction in host cell protein impurities. This sequence of steps has been widely adopted as a generic purification scheme for a number of recombinant mAbs [15,16]. Concerning the AEX step, it is generally accepted that the flowthrough mode is the most effective. Indeed, at neutral pH, the positively charged antibody does not bind to the matrix, whereas negatively charged species do [17,18]. However,

* Corresponding author.
E-mail address: charlotte.cabanne@bordeaux-inp.fr (C. Cabanne).

http://dx.doi.org/10.1016/j.chroma.2015.03.018
0021-9673/

owing to the limitations of the classical chromatography column, i.e. flow distribution, flow rate limitations and cleaning validation, membrane chromatography was developed, and anion-exchange membrane adsorbers are already being used as an alternative to column chromatography in some mAb manufacturing processes [19]. In light of this advantage, protocols have been developed for the use of membrane adsorbers for all purification steps [20].

Our goal was to benefit from these recent improvements in order to develop an innovative process to purify recombinant mAbs produced by CHO cells. Our strategy was to evaluate a three-step process based on two successive mixed mode chromatography steps, followed by an anion-exchange membrane step. Preliminary experiments to validate the three steps individually were first performed using a human antibody. Thereafter, the whole process was evaluated for the purification of a recombinant mAb produced by CHO cells. We paid special attention to the monitoring of contaminating proteins and DNA. At each step of the process, DNA concentration was measured, the global HCPs content was quantified using an ELISA test, and the identity of the contaminating proteins was determined by mass spectrometry analyses.

2. Material and methods

2.1. Equipment

An Akta Explorer 100 workstation monitored with Unicorn 5.0 (GE Healthcare, Uppsala, Sweden) was used for chromatographic experiments. Protein detection was performed by measuring UV absorbance at 280 nm. A HANNA HI8820N conductivity meter and a HANNA HI2210 pH meter (Tanneries, France) were used to prepare buffers.

An ACQUITY UPLC system (Waters Corporation, Milford, USA) was used for analysis.

The buffers and cell culture supernatant were filtered through 0.45 μm cellulose membranes (Sartorius Stedim Biotech, Goettingen, Germany) before use.

2.2. Biological material

Two types of samples were used for purification: (i) a human immunoglobulin G partially purified from Cohn fractions II and III, which was purchased from Sigma (St Louis, MO, USA) and (ii) a crude cell culture supernatant from an antibody-producing CHO cell line (CHO-DP12, ATCC CRL-12445). These cells produce a humanized anti-IL-8 antibody and have been adapted to suspension culture in serum-free medium CD CHO (GIBCO Invitrogen SARL, Cergy-Pontoise, France) supplemented with 8 mM L-glutamine [21]. Cell cultures were performed in a 2 l stirred tank bioreactor with a supply and control tower (DCU, DFC4, Biostat® B plus, Sartorius Stedim Biotech, Aubagne, France) and a supervision system (MFCS, Sartorius Stedim Biotech, Aubagne, France). Dissolved oxygen was set at 50%, pH value at 7.4 and culture was performed at 37 °C. Supernatant was harvested from extended cultures until viability dropped below 92%. Cell counting and viability were determined with a hemocytometer (Malassez type) by using the trypan blue exclusion method at 0.2% (w/v) final dye concentration [22].

2.3. Chemicals

Buffers and solutions were prepared using chemicals of analytical grade from Sigma (St Louis, MO, USA). Tris-HCl, sodium phosphate, sodium citrate, sodium acetate and citric acid were prepared at desired pH and conductivity.

2.4. Liquid chromatographic resins and columns

We used three types of chromatographic resin: (i) HEA HyperCel (Pall Life Science, Saint Germain en Laye, France); (ii) Capto MMC ImpRes (GE Healthcare, Uppsala, Sweden) packed in columns Tricorn GE Healthcare (4 ml, 1 cm × 5.5 cm) and (iii) HiScreen Capto MMC column (4.7 ml, 0.77 cm × 10 cm) prepacked (GE Healthcare, Uppsala, Sweden). Height Equivalent to a Theoretical Plate (HETP) and asymmetry were assessed by injection of 5% acetone (v/v) at 1% of the volume of the column in order to validate the column package.

We used two types of membrane chromatography: (i) a Sartobind Q 75 and (ii) a Sartobind STIC PA Nano (Sartorius Stedim Biotech, Aubagne, France). A cleaning protocol was performed after each purification step according to the manufacturer's instructions.

The SEC-UPLC column ACQUITY UPLC BEH200 SEC was purchased from Waters (Mildford, USA).

2.5. Analysis

2.5.1. Antibody aggregates and quantification analysis by SEC-UPLC

Antibody concentration in the collected fractions was determined by Size Exclusion Ultra Performance Liquid Chromatography (SEC-UPLC). Samples of 10 μl were injected onto the SEC column regulated at 30 °C. The mobile phase consisted in 0.1 M sodium sulphate in 0.1 M phosphate buffer pH 6.8. Elution was performed isocratically at 0.3 ml/min with a run time of 10 min. Antibody peak areas were determined by integration of the 215 nm signal and compared to a calibration curve generated with diluted samples of a known concentration of human polyclonal antibody SLH66 from Equitech-Bio (Kerrville, Texas, USA).

2.5.2. Host cell protein determination by ELISA

HCPs were quantified with the CHO HCP ELISA kit (#CM015) from Cygnus Technologies (Southport, NC, USA) according to the high sensitivity protocol recommended by the manufacturer.

2.5.3. Host cell DNA

The Quant-iTTM dsDNA HS assay kit with the Qubit® apparatus (Invitrogen, Carlsbad, USA) was used to measure DNA concentration.

2.5.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Fractions showing absorbance peaks at 280 nm were analyed by SDS-PAGE using TGX the Stain-Free FastCast Acrylamide Kit. Molecular masses were estimated using standard markers (Bio-Rad Precision Plus Protein Standard). SDS-PAGE gels were revealed by Stain-Free technology (Bio-Rad).

2.5.5. HCP identification

For each sample, the peptide mixture generated from trypsin digestion was analyzed by online capillary high-performance liquid chromatography (Dionex, Amsterdam, Netherlands) coupled to a nanospray LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, Illkirch, France). Data were acquired in a data-dependent mode alternating, a high-resolution mass spectrometry (MS) scan survey over the range m/z 300–1700, and 10 MS/MS scans in an exclusion dynamic mode. Identification was carried out by database search against the Uniprot entries from *Cricetulus griseus*.