Journal of Biotechnology 163 (2013) 233–242

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

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

Discontinuous and continuous purification of single-chain antibody fragments using immobilized metal ion affinity chromatography

Carlos Andrés Martínez Cristancho[a], Florian David[b], Ezequiel Franco-Lara[b], Andreas Seidel-Morgenstern[a,c,*]

[a] Max Planck Institute for Dynamics of Complex Technical Systems, Sandtorstr. 1, 39106 Magdeburg, Germany
[b] Institute of Biochemical Engineering, Technische Universität Braunschweig, Gaussstr. 17, 38106 Braunschweig, Germany
[c] Institute of Process Engineering, Otto-von-Guericke University, P.O. Box 4120, 39106 Magdeburg, Germany

ARTICLE INFO

Article history:
Received 15 March 2012
Received in revised form 28 August 2012
Accepted 30 August 2012
Available online 14 September 2012

Keywords:
Single-chain Fragment variable antibody
Immobilized metal ion affinity chromatography
Simulated moving bed
Histidine-tagged recombinant protein purification
Bacterial expression
Adsorption equilibrium constants

ABSTRACT

This work describes the adsorption–desorption behavior of a histidine-tagged single-chain Fragment variable antibody (D1.3 scFv) on a commercial immobilized metal ion affinity chromatography (IMAC) column. A clarified cell culture supernatant originating from *Bacillus megaterium* was characterized using single column experiments in a pH-gradient elution mode. The cell culture supernatant containing the antibody fragment D1.3 scFv could be treated in the chromatographic separation process as a pseudo-binary mixture. Adsorption equilibrium constants of the antibody fragment fraction (ABF) and the non-specifically retained protein impurity fraction (IMP) were determined experimentally at constant pH by reinjecting pulses of pooled fractions collected in preliminary batch gradient elution runs. Based on the estimated adsorption equilibrium constants a possible multicolumn open-loop three-zone two-step pH-gradient simulated moving bed (SMB) process is suggested and designed, which possesses the potential to isolate continuously the antibody fragment fraction (ABF) containing the single-chain antibody fragment D1.3 scFv.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

In recent years, several therapeutic antibody fragments have been approved by the US Food and Drug Administration (FDA) and are already in the market (Scott and Clarke, 2009). Among the novel therapeutic recombinant antibody fragments under development, small-sized and engineered fragments such as the single-chain Fragment variable formats (scFv) offer new alternatives to the full-length monoclonal antibodies (Brereton et al., 2007). Single-chain antibody fragments are becoming essential tools for research, diagnostic and therapy due to their improved tissue perfusion or increased specificity for a defined antigen (Hust and Dübel, 2004; Weisser and Hall, 2009).

Novel high-yield and low cost bacterial expression systems are being developed for the production of recombinant proteins like antibody fragments (David et al., 2010). In addition to that, gram positive bacteria like *Bacillus megaterium* are able to secret proteins in high amount into the culture supernatant making the cell disruption step unnecessary and increasing the target protein yield (Jordan et al., 2007; David et al., 2011). Expression technologies allow the fusion of polyhistidine tags to recombinant proteins, without affecting the structure or function of the tagged protein but facilitating its purification (Hochuli et al., 1988; Clemmitt and Chase, 2000). The optimization and success of the purification of therapeutic recombinant proteins depend strongly on the development and implementation of downstream processes consisting of a minimum number of steps. In particular, efficient continuous operation modes are attractive (Gottschalk, 2009; Carta and Jungbauer, 2010).

In immobilized metal ion affinity chromatography (IMAC) the selected ligands attached to the solid stationary phase are divalent transition metal ions, usually copper, nickel, zinc or cobalt (Knecht et al., 2009). The corresponding technique was developed by Porath in the mid-1970s and exploits the ability of some exposed amino acid residues on the protein surface, such as the imidazole group of histidine, to form chelates with the immobilized metal ions (Porath et al., 1975). The elution of the adsorbed proteins is performed under gentle, non-denaturating conditions and by two different

* Corresponding author at: Max Planck Institute for Dynamics of Complex Technical Systems, Sandtorstr. 1, 39106 Magdeburg, Germany.
E-mail address: seidel-morgenstern@mpi-magdeburg.mpg.de (A. Seidel-Morgenstern).

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2012.08.022

mechanisms: by lowering the pH (protonation of the imidazole group of histidine) or by the introduction of a competing agent (pure imidazole) (Porath, 1992). IMAC columns have high capacity, stability, and reproducibility at similar costs as ion exchange columns (Chaga, 2001). A possible drawback of IMAC columns is the leakage of metal ions during elution.

For the purification of histidine-tagged single-chain antibody fragments using IMAC currently discontinuous single column processes are dominant (Skerra et al., 1991; Essen and Skerra, 1993). However, there is an increasing interest in using more productive multicolumn configurations, in which biological multi-component mixtures are fed uninterruptedly and the target biomolecules are withdrawn continuously at two or more characteristic outlet ports. Various alternative configurations have been suggested and were successfully applied for protein purification, such as the multicolumn countercurrent solvent gradient purification (MCSGP) process (Müller-Späth et al., 2008), size exclusion simulated moving bed (SE-SMB) chromatography (Ottens et al., 2006; Buhlert et al., 2009), and the open-loop simulated moving bed (SMB) chromatography (Kessler et al., 2007; Gueorguieva et al., 2011) as an adaptation of the classical SMB principle (Broughton and Gerhold, 1961). In contrast to SMB, the MCSGP process offers the possibility to isolate more than two fractions. It is an attractive concept to realize purifications requiring solvent gradients exploiting an elegant but complex combination between continuous steps connections of all columns and batch-wise elution steps (Krättli et al., 2011).

The focus of this work was the purification of histidine-tagged single-chain antibody fragments using immobilized metal ion affinity chromatography (IMAC). For this reason, gradient elution chromatography was studied using stepwise pH-gradients. The antibody fragment fraction (ABF) was the most retained component in the cell culture supernatant due to the presence of a histidine affinity tail. The clarified cell culture supernatant can be considered as a pseudo-binary mixture by lumping of all undesirable proteins eluting before the target fraction as impurities. It is rather clear that the mobile phase pH will dominate the elution of the antibody fragment fraction (ABF) and the closest eluting protein impurity fraction (IMP). The influence of the mobile phase pH on the adsorption equilibrium constants for both components (ABF and IMP) was determined in this work carrying out single batch pulse experiments under isocratic pH elution conditions. Based on the dependence of the adsorption equilibrium constants on the mobile phase pH, an open-loop three-zone two-step pH-gradient simulated moving bed (SMB) process will be suggested for the continuous chromatographic purification of the antibody fragment fraction (ABF). The process was investigated theoretically through the simulation of an equivalent true moving bed (TMB) process using an equilibrium stage model. The theoretical study of the continuous multicolumn countercurrent process delivered a region of applicable operating parameters. Additionally, concentration profiles were simulated within a cascade of three columns for selected operating points. Finally, the productivity, the specific buffer consumption and final product concentration of collecting the antibody fragment fraction (ABF) continuously at the extract port of the theoretical SMB unit is evaluated and compared with the corresponding performance of a standard experimental batch process.

2. Materials and methods

2.1. Bacterial expression of an antibody fragment

The lysozyme specific histidine-tagged single-chain Fragment variable antibody format (D1.3 scFv) was expressed in *B. megaterium* (David et al., 2011).

The *B. megaterium* strain YYBm1 carrying the plasmid pEJBMD1.3scFv was used for the bacterial expression (Yang et al., 2007). The preculture were carried out with fructose concentration of 5 g/L in shaking flasks. Lab scale fermentations were carried out in a 5-L Bioreactor using an adapted oscillating fed-batch strategy. It is worth noting that *B. megaterium* allows the production of functional single-chain antibody fragments by direct secretion of proteins into the culture medium. More details regarding the production of the single-chain antibody fragment D1.3 scFv are given in the literature (David et al., 2011).

The single-chain antibody fragment D1.3 scFv, a 27 kDa recombinant protein, has a concentration around 0.025 mg/ml in the supernatant.

2.2. Preparation of samples prior to chromatography

The non-clarified cell culture supernatant coming from the bioreactor were pretreated and clarified prior to chromatography. First, the supernatant was centrifuged for 30 min at 4 °C and 4000 rpm, in order to remove the biomass present in the harvested cell culture fluid (Thermo Scientific Heraeus® Biofuge Stratos Centrifuge, Heraeus Holding GmbH, Hanau, Germany). Next the supernatant was separated carefully from the precipitate. The particulate matter still present was removed by filtration (Pall® Acrodisc® 32 mm syringe filter with 0.2 μm Supor® Membrane, Pall Life Sciences, Ann Arbor, MI, USA). The clarified cell culture supernatant was aliquoted, stored frozen at −30 °C, in order to preserve completely the protein structure and activity. Aliquoted samples were thawed before the chromatographic runs.

2.3. Selected stationary phase

Prepacked and ready-to-use chromatographic commercial columns were used (HisTrap™ FF crude, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Column volumes were 1 ml (2.5 cm × 0.7 cm i.d.). The columns were precharged with Ni Sepharose™ 6 Fast Flow (highly cross-linked agarose beads with nickel as metal ion). Average particle size was 90 μm. After use the columns were always washed, first with water and then with a solution of 20% ethanol. The columns were stored at 4 °C.

2.4. Loading and elution buffers

In order to generate a descending stepwise pH-gradient in the IMAC columns, loading and elution buffers are needed. The loading and elution buffers were prepared by dissolving sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$, Merck KGaA, Darmstadt, Germany), disodium monohydrogen phosphate anhydrous (Na_2HPO_4, Merck KGaA, Darmstadt, Germany) and sodium chloride (NaCl, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) in water. The pH values were adjusted either to 6.8 (loading buffer) or 4.2 (elution buffer) with either concentrated solutions of sodium hydroxide or hydrochloric acid.

Buffers were filtered prior to the chromatographic runs (cellulose nitrate filters 0.20 mm, Sartorius Stedim Biotech, Göttingen, Germany). The water used in all experiments was purified in a Milli-Q® ultrapure water purification system (Milli-Q® gradient, Millipore SAS, Molsheim, France).

2.5. Instruments

Batch chromatographic experiments were carried out in an ÄKTA Purifier controlled by Unicorn software (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The pH-gradient was monitored online at the column outlet using an 88 μl flow cell equipped with

Download English Version:

https://daneshyari.com/en/article/6491926

Download Persian Version:

https://daneshyari.com/article/6491926

Daneshyari.com