Journal of Chromatography B 1060 (2017) 53–62

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Secretory immunoglobulin purification from whey by chromatographic techniques

Alexander Matlschweiger[a], Hannah Engelmaier[a], Gottfried Himmler[b], Rainer Hahn[a,*]

[a] Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, A-1190 Vienna, Austria
[b] Angothera GmbH, Donau-Oder Kanal IV SW 80, A-2301 Gross-Enzersdorf, Austria

ARTICLE INFO

Keywords:
Anion exchange chromatography
Negative chromatography
Small pore resin
Giga-porous resin
Bacterial toxin

ABSTRACT

Secretory immunoglobulins (SIg) are a major fraction of the mucosal immune system and represent potential drug candidates. So far, platform technologies for their purification do not exist. SIg from animal whey was used as a model to develop a simple, efficient and potentially generic chromatographic purification process. Several chromatographic stationary phases were tested. A combination of two anion-exchange steps resulted in the highest purity. The key step was the use of a small-porous anion exchanger operated in flow-through mode. Diffusion of SIg into the resin particles was significantly hindered, while the main impurities, IgG and serum albumin, were bound. In this step, initial purity was increased from 66% to 89% with a step yield of 88%. In a second anion-exchange step using giga-porous material, SIg was captured and purified by step or linear gradient elution to obtain fractions with purities > 95%. For the step gradient elution step yield of highly pure SIg was 54%. Elution of SIgA and SIgM with a linear gradient resulted in a step yield of 56% and 35%, respectively. Overall yields for both anion exchange steps were 43% for the combination of flow-through and step elution mode. Combination of flow-through and linear gradient elution mode resulted in a yield of 44% for SIgA and 39% for SIgM. The proposed process allows the purification of biologically active SIg from animal whey in preparative scale. For future applications, the process can easily be adopted for purification of recombinant secretory immunoglobulin species.

1. Introduction

Immunoglobulin A (IgA) is the most abundant antibody class in the human body [1]. In its secretory form (SIgA) it is the main effector of the mucosal immune system [2,3]. With its high therapeutic potential it is a promising candidate for prophylaxis or therapy against various diseases associated with the respiratory, gastrointestinal or urogenital tract [2,4]. SIgA consists of two IgA monomers connected through a J-chain via disulfide linkage of their α-chains. The J-chain is associated with the secretory component (SC) [5]. The SC is derived from the polymeric immunoglobulin receptor that mediates transport of IgA across the epithelium to the luminal side, where the complex is released by proteolytic cleavage [6]. The SC has a protective function making IgA highly stable at acidic pH and resistant to proteases [7]. So far, medical application of SIgA has been hampered by difficulties in producing and purifying large quantities [8].

In order to obtain highly pure SIgA, as required for potential therapeutic use, chromatographic purification techniques are essential. A highly selective bio-affinity resin, such as Protein A for IgG purification, is not available for IgA, though weak interaction between Protein A and IgA have been reported [9]. Jacalin, a plant derived lectin that binds IgA, has been immobilized onto chromatographic resins and used for affinity purification [10–12]. However, the binding capacity is low and other glycoproteins are also bound [10,11]. Furthermore, Jacalin only binds to IgA1 [13]. A significant advancement in IgA purification was the introduction of a camelid VHH ligand enabling single step purification at purities of 95% [14]. Binding capacities were not reported in these papers, but have been specified with 8 mg/mL by the manufacturer [15]. Despite its high selectivity, the high cost and low capacity of this resin may prove prohibitive in large scale biotherapeutic manufacturing. Considering the large molecular weight of SIgA [16,17], size exclusion chromatography (SEC) is an obvious purification strategy [18,19], which is highlighted by several early studies on the isolation and characterization of SIgA, which used SEC as the main separation method [19–22]. In contrast, for preparative and industrial applications SEC is restricted by the low sample volume that can be loaded.

Considering the aforementioned shortcomings, we performed a study to develop a purification scheme based on non-affinity chromatography

* Corresponding author.
E-mail address: rainer.hahn@boku.ac.at (R. Hahn).

http://dx.doi.org/10.1016/j.jchromb.2017.05.028
Received 4 April 2017; Received in revised form 23 May 2017; Accepted 25 May 2017
Available online 26 May 2017

media capable to deliver pure SIgA at large quantities. Due to lack of expression systems that can provide sufficient recombinant SIgA, we used animal whey as feed stock for our development. Animal whey, and in particular caprine whey which was our preferred material, is a comparably rich source, with secretory immunoglobulins (SIg) representing 2–4% of total protein [16,17]. Aside from SIgA, whey also contains considerable amounts of secretory immunoglobulin M (SIgM) [16,17].

We have recently developed a method based on ultra/diafiltration and PEG precipitation that can deliver SIg at a concentration of 2–4 mg/mL and a purity of around 70% [23]. This product was used as a starting material for our investigations to establish a chromatographic purification platform. The goal of our study was two-fold: the first aim was to develop a simple and efficient purification platform for SIg molecules, while enabling high throughput and considerable capacity. Secondly, the method developed here should be generally applicable to recombinant SIg without major modifications. Consequently, the actual separation principles applied must be based on the large molecular size as well as the general molecular properties such as hydrophobicity and isoelectric point. The second intention was to provide a sufficient amount of either SIgA and/or SIgM from caprine whey for antigen binding assays.

2. Experimental

2.1. Materials

Adsorbents Q Sepharose Fast Flow, Capto Core 700 and empty Tricorn 5/50, 5/100 and 10/100 columns were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Adsorbents Poros 50HQ and Poros 50HS were purchased from ThermoFisher Scientific (Life Technologies Corporation, Grand Island, NY, USA). CIMmultus SO3 and CIMmultus QA advanced composite column (bed volume 1.0 mL) were purchased from BIA Separations (Ljubljana, Slovenia). Chemicals were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). The chromatographic system used was an ÄKTA-Explorer 100 (Amersham Biosciences, Uppsala, Sweden) and the used software was Unicorn, version 5.11. All preparative chromatograms show a normalized y-axis of C/C_0. Normalization was performed by dividing the UV-signal of the runs with the maximum UV-signal of the sample.

2.2. Preparation of pre-purified SIg concentrate

The preparation of SIg from caprine whey is described in detail in Matlschweiger et al. [23]. Briefly summarized the experimental procedure is as follows: Crude whey containing small amounts of residual casein and fat micelles was clarified by a combination of centrifugation and depth/sterile filtration. Subsequently whey was concentrated 30–40 fold on a 500 kDa hollow fiber membrane. After this a fractionated polyethylene glycol (PEG) precipitation at 3 and 7% (w/v) was performed. The secretory immunoglobulin fraction (7% fraction) was re-dissolved and diafiltrated on a 300 kDa hollow fiber membrane at a volume exchange factor of 10. Pre-purified SIg concentrate was diafiltrated with the corresponding running buffer at a volume exchange factor of 4.

2.3. Chromatographic purification on Capto Core 700

The resin was packed into a Tricorn 10/100 column by flow packing according to the user manual. The final bed height was 9.7 cm and the bed volume was 7.4 mL. Running buffer was 20 mM Tris 150 mM NaCl pH 7.2 with a conductivity of 17.5 mS/cm. The applied linear flow rates were 250 cm/h, 500 cm/h and 900 cm/h corresponding to residence times of 2.26 min, 1.13 min and 0.63 min, respectively. Fractions of the flow-through were collected and further analyzed. Regeneration of the resin was performed with 30% Isopropanol 1 M NaOH according to the manual.

2.4. Cation-exchange chromatography

The cation-exchange processes were carried out using a 20 mM Acetate pH 5.0 as the running buffer A and 20 mM Acetate 2 M NaCl pH 5.0 as the elution buffer B. For CIMmultus SO3, the flow rate was 5 mL/min and step elutions in the range of 15–25% buffer B corresponding to 0.3–0.5 M NaCl was performed. Poros 50HS was packed into a Tricorn 5/50 column with a final bed height of 4.6 cm and a bed volume of 0.9 mL. The applied linear flow rate was 137.5 cm/h (residence time 2 min). Step gradient elution was performed with a percentage of buffer B of 10–20% corresponding to a salt concentration of 0.2–0.4 M NaCl. For both columns a final elution step at 100% buffer B (2 M NaCl) was performed to remove remaining bound proteins. Fractions of the flow-through as well as the eluates were collected and further analyzed.

2.5. Anion-exchange chromatography

The anion-exchange processes were carried out using 20 mM Tris pH 7.2 as the running buffer A and 20 mM Tris 1 M NaCl pH 7.2 as the elution buffer B. For experiments with 20 mM Tris 63 mM NaCl pH 7.2, samples were diafiltrated with 20 mM Tris pH 7.2 afterwards 63 mM NaCl was added and pH was adjusted. For CIMmultus QA, the flow rate was 5 mL/min and linear gradient elutions to 100% buffer B with a gradient length of 10 CV were performed. Q Sepharose Fast Flow was packed into a Tricorn 10/100 column with a final bed height of 8.4 cm and a bed volume of 6.6 mL. The linear flow rate was varied between 100 and 500 cm/h corresponding to a residence time of 5.0–1.0 min. Poros 50HQ was packed into a Tricorn 5/100 column with a final bed height of 9.9 cm and a bed volume of 1.94 mL. The residence time was kept constant at 2 min for both column dimensions. Linear gradient elution was performed with a gradient length of 20 column volumes (CV) to 15% buffer B followed by a step gradient to 100% buffer B. Step gradient elution was performed with 15% buffer B followed by a step gradient to 100% buffer B. Fractions of the flow-through as well as the eluates were collected and further analyzed.

2.6. Analytical size exclusion chromatography

Waters Alliance e2695 separation module was used for analytical SEC with a UV/VIS Detector 2489 (both Waters, Milford, MA, USA). The columns used were an Agilent BioSEC 5 500 Å 4.6 × 300 mm and an Agilent BioSEC 5 500 Å guard 4.6 × 50 mm (both Agilent Technologies, Santa Clara, CA, USA). The flow rate was 0.4 mL/min and the injection volume was 20 µL. The equilibration and running buffer were 1x PBS. For quantitative analysis a mixture of human IgM from plasma (Sigma-Aldrich, St. Louis, MO, USA) and human IgA from colostrum (Sigma-Aldrich, St. Louis, MO, USA) with mass concentrations of 100; 75; 50; 25; 10; 5 and 1 µg/mL of IgM and IgA was injected and a calibration curve was made. Same procedure was performed with a mixture of polyclonal IgG (kind gift of Octapharma, Vienna, Austria), bovine serum albumin, β-lactoglobulin and α-lactalbumin (all Sigma-Aldrich, St. Louis, MO, USA) to establish a calibration curve for the impurity fraction. UV signals were recorded at 214 and 280 nm.

2.7. SDS-PAGE analysis

Samples were added with NuPAGE LDS Sample Buffer (4x) (Invitrogen, Waltham, MA, USA) and were boiled for 10 min. Samples were loaded onto Bis-Tris 4–12% gels, the running buffer was 1x MES-SDS running buffer. Running conditions were 200 V, max ampere, 50 min. The marker was Invitrogen SeeBlue 2 Pre-Stained Protein Standard (Invitrogen, Waltham, MA, USA). Protein staining was performed with Coomassie Blue and Bismark Brown [24].