Journal of Immunological Methods 378 (2012) 95–101

Contents lists available at SciVerse ScienceDirect

Journal of Immunological Methods

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

Research paper

Recombinant IgA production: Single step affinity purification using camelid ligands and product characterization

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ARTICLE INFO

Article history:
Received 11 November 2011
Received in revised form 3 February 2012
Accepted 10 February 2012
Available online 20 February 2012

Keywords:
IgA purification
Bioaffinity
VHH
Jacalin
Recombinant expression

ABSTRACT

Immunoglobulins of isotype A (IgA) mediate a key role in mucosal immunity and are promising new immunotherapeutic candidates, but difficulties in obtaining enough material often hamper their *in vivo* exploration. We established recombinant Chinese hamster ovary (CHO) cells which stably expressed two IgA1 antibodies under serum-free conditions. The two cell lines achieved significantly different specific productivities of 16 pg per cell and day and 100 times less, a common phenomenon in recombinant antibody expression which challenges the production and purification process. Polymeric IgA in crude culture supernatants was assembled with J chain and showed expected specificity. We employed an immobilized camelid anti-human alpha-chain VHH ligand and isolated both recombinant IgAs at high purity and yield in a single chromatographic step. The described method was irrespective of the light chain and specificity and may be used as a generic capture step for the isolation of any IgA. Results were compared with a multistep purification process consisting of an affinity step based on immobilized jacalin followed by anion exchange and hydrophobic interaction chromatography.

1. Introduction

The estimated market value of recombinant therapeutic antibodies rapidly approached $43 billion in 2010 (Elvin et al., 2011), but not one of those is an IgA. This is remarkable because IgAs have a unique role in mediating mucosal immunity and could foster the treatment or prevention of a variety of diseases. This highlights an important bias in the way the field of immunotherapy has developed: clinical candidates are chosen more on the basis of enabling purification technology than on the potential clinical merits of the antibody.

To date, IgA has lacked the enabling bioaffinity capture method that permitted the commercial success of more than two dozen IgGs. Protein A has been documented to bind human VH3 encoded IgAs via the light chain variable region (Grov, 1975, 1976), but in the 35 years since that discovery, there has been no report of Protein A being seriously considered as a general IgA purification tool. IgA purification is sometimes performed using immobilized jacalin, an α-D-galactose binding lectin, which only binds IgA1 but not IgA2. The usefulness of immobilized jacalin for IgA1 purification is limited, because lectins are additionally accessible to similarly glycosylated proteins which co-elute as impurities with the product (Roque-Barreira and Campos-Neto, 1985). Alternative strategies rely on Protein L which specifically recognizes the framework region 1 of certain kappa light chains, but does not bind human kappa light chains of subtype II and antibodies with lambda light chains (Boes et al., 2011). More recently, camelid VHH ligands have been promoted as affinity ligands for a variety of challenging purification applications. VHH ligands are 12–15 kDa single-domain ligands that lack any light chain and the CH1 domain of conventional antibodies. Due to

Abbreviations: CHO, Chinese hamster ovary;DHFR, dihydrofolate reductase;IgA, immunoglobulin A;MTX, methotrexate;TFF, tangential flow filtration

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doi:10.1016/j.jim.2012.02.010

their unique stability and specificity, VHH ligands have been successfully used for the chromatographic purification of different molecules (Detmers et al., 2010).

Here we describe the development of recombinant CHO production cell lines which secrete two different human IgA molecules together with the human J chain. The antibodies are directed against the gp41 envelope protein of HIV-1 and were originally of human IgG1 subtype (Grunow et al., 1988; Buchacher et al., 1994). A camelid anti-human alpha-chain specific ligand was employed in VHH chromatography to isolate IgAs with kappa (3D6) or lambda (4B3) light chains from cell culture supernatants at high purity and recovery yield in a single step. Results were compared to an alternative IgA purification strategy based on immobilized jacalin, anion exchange (AEX) and hydrophobic interaction chromatography (HIC).

2. Materials and methods

2.1. Generation of expression plasmids

3D6 and 4B3 cDNAs of heavy and light chain variable regions were isolated from hybridoma cell lines (Felgenhauer et al., 1990; Kunert et al., 1998). The cDNA sequence for human IgA1 heavy chain (J00220), kappa light chain (J00241) and lambda light chain (J00253) constant regions as well as J chain (NP653247) can be retrieved online via GenBank. All cDNAs were codon optimized for expression in CHO cells and synthesized by GeneArt AG (Germany). Coding regions were under control of SV40 promoters and expression vectors were constructed to combine heavy chain with the dhfr selection/amplification marker (Wolbank et al., 2003) and J chain with neomycin (Fig. 1).

2.2. Host cell line, transfection, selection and gene amplification

Dihydrofolate reductase (dhfr) deficient CHO host cells, ATCC CRL-9096 (Urlaub and Chasin, 1980), were adapted to grow under protein-free conditions in a DMEM/Ham's F12 1:1 (PAA, Austria) formulation with 4 mM L-glutamine, 0.1 mM hypoxanthine, 0.016 mM thymidine, 0.25 g/l soy peptone, 0.1% Pluronic F-68 and a protein-free supplement (Polymun Scientific GmbH, Austria). DNA/PEI polyplexes were formed of 250 μg PEI (linear, MW: 25,000; Polysciences Inc., PA) with 25 μg plasmid DNA containing J chain, light chain and heavy chain in a ratio of 1:1:1 and used for transfection of 5×10^6 cells (Reisinger et al., 2009). After 24 h selection was started with ProCHO5 (Lonza, Switzerland) supplemented with 4 mM L-glutamine, 15 mg/l phenol red and 0.5 mg/ml G418. Transfectants were seeded at 1×10^5 cells/ml in five 96-well plates. In two transfection experiments a total of 960 wells were generated for each IgA variant. Gene amplification was initiated with 0.05 μM methotrexate (MTX) in selection medium and best clones were identified by ELISA screening. The best 5% producing wells were adapted to 0.1 μM MTX and one clone was selected for limited dilution subcloning to obtain a monoclonal cell line. Clones were fed with 0.4 μM MTX amplification medium and selected as described before. Finally, the best clone was subcloned a third time using the same approach.

2.3. Stability of cell lines

Antibody-containing supernatants were generated by cultivation of recombinant cell lines at 37 °C in spinner vessels at 50 rpm in 50 ml amplification medium. Cells were split every 3–4 days and re-seeded at 2.5×10^5 cells/ml. The total cell number was quantified using a Coulter Counter Z2 (Beckman Coulter, CA) and cell viability was determined by trypan blue dye exclusion. Recombinant cell lines were cultured for at least 10 passages to monitor growth rate and stability of product expression. Specific productivities (q_P) were calculated as picogram mAb per cell and day (pcd) and averaged from all passages in spinner flasks.

2.4. Characterization of IgAs

IgA was quantified by sandwich ELISA using a goat anti-human IgA α-chain specific antiserum (Sigma, MO) for coating and goat anti-human κ-chain (Sigma, MO) together with mouse anti-human λ-chain specific antiserum (Southern Biotech, AL), both peroxidase-conjugated, for detection. IgA standard from human colostrum (Sigma, MO) was adjusted to 400 ng/ml and diluted in 1:2 series together with samples.

Purity and molecule size distribution of purified IgA fractions was determined using NuPAGE Novex 3–8% Tris-

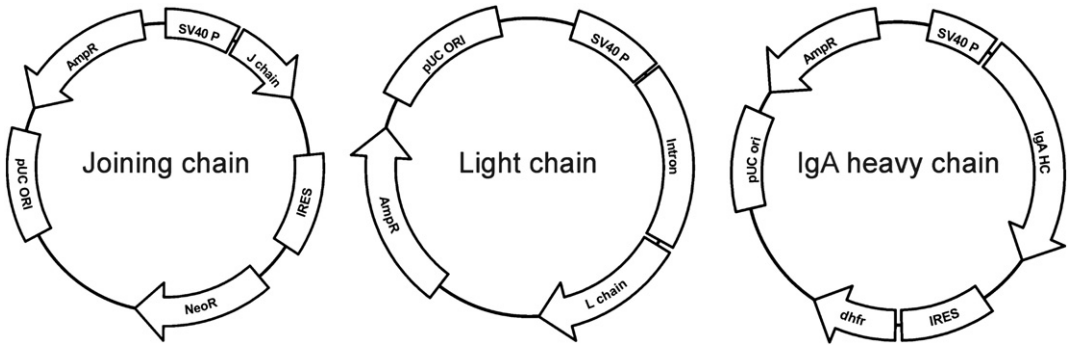

Fig. 1. Plasmids used for the co-transfection of dhfr-deficient CHO host cell line.