Journal of Immunological Methods 372 (2011) 30–41

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Research paper

Stable expression and purification of a functional processed Fab′ fragment from a single nascent polypeptide in CHO cells expressing the mCAT-1 retroviral receptor

Nicolas Camper[a], Teresa Byrne[b], Roberta E. Burden[c], Jenny Lowry[b], Breena Gray[b], James A. Johnston[a], Marie E. Migaud[c], Shane A. Olwill[b], Richard J. Buick[b,*], Christopher J. Scott[c]

[a] Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Science, Queen's University of Belfast, 97 Lisburn Rd, Belfast, BT9 7BL, Northern Ireland, United Kingdom
[b] Fusion Antibodies Ltd, Springbank Industrial Estate, Pembroke Loop Rd, Belfast, BT17 0QL, Northern Ireland, United Kingdom
[c] School of Pharmacy, Queen's University of Belfast, 97 Lisburn Rd, Belfast, BT9 7BL, Northern Ireland, United Kingdom

ARTICLE INFO

Article history:
Received 28 February 2011
Received in revised form 24 June 2011
Accepted 24 June 2011
Available online 19 July 2011

Keywords:
Antibody Fab′ fragment
CHO cells expression
Retroviral infection
FMDV 2A peptide
Furin cleavage site
Fas receptor

ABSTRACT

Monoclonal antibodies and derivative formats such as Fab′ fragments are used in a broad range of therapeutic, diagnostic and research applications. New systems and methodologies that can improve the production of these proteins are consequently of much interest. Here we present a novel approach for the rapid production of processed Fab′ fragments in a CHO cell line that has been engineered to express the mouse cationic amino acid transporter receptor 1 (mCAT-1). This facilitated the introduction of the target antibody gene through retroviral transfection, rapidly producing stable expression. Using this system, we designed a single retroviral vector construct for the expression of a target Fab′ fragment as a single polypeptide with a furin cleavage site and a FMDV 2A self-cleaving peptide introduced to bridge the light and truncated heavy chain regions. The introduction of these cleavage motifs ensured equimolar expression and processing of the heavy and light domains as exemplified by the production of an active chimeric Fab′ fragment against the Fas receptor, routinely expressed in 1–2 mg/L yield in spinner-flask cell cultures. These results demonstrate that this method could have application in the facile production of bioactive Fab′ fragments.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Antibodies play increasing roles in the fields of therapy, diagnostics and research owing to their ability to specifically recognise their target molecule (Reichert, 2008; Borrebaeck, 2000). The most predominantly used antibody format is the full length IgG, but other smaller recombinant forms are increasingly being found advantageous in specific circumstances. For example, in both cancer treatments and diagnostics, smaller antibody formats such as Fab′ fragments have improved tissue penetration improving bioavailability and detection of the tumour respectively (Beckman et al., 2007; Wu and Yazaki, 2000; Yokota et al., 1992; Olafsen et al., 2004; Todorovska et al., 2001). Similarly, in research applications, the use of smaller antibody fragments devoid of Fc domains can produce higher sensitivity and reliability in assays such as surface plasmon resonance (SPR) assays and ELISA (Ylikotila et al., 2006; Peluso et al., 2003).

One major difficulty in the generation of antibody fragments is due to their heterodimeric nature; it is not only important to express both heavy and light chains at high levels, but also to express them at equimolar levels which can be challenging in

Abbreviations: CHO cells, Chinese hamster ovary cells; CDS, coding sequence; Fab, antigen-binding fragment; Fab′, Fab with hinge region on the heavy chain; Fd′, truncated antibody heavy chain; FMDV, foot and mouth disease virus; mCAT-1, mouse cationic amino acid transporter receptor 1; V_H, antibody heavy chain variable domain; V_L, antibody light chain variable domain.

* Corresponding author. Tel.: +44 2890 432800; fax: +44 2890 020446.
E-mail address: richard.buick@fusionantibodies.com (R.J. Buick).

0022-1759/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2011.06.029

respect to differences in transcription and translation rates (Li et al., 2007a, 2007b; Schlatter et al., 2005; Humphreys et al., 2002). One possible approach to achieve equimolar expression of the heavy and light chains is to express the chains together as a single polypeptide incorporating a linker region that is cleaved in the ER/Golgi allowing the separation, folding and assembly of the heterodimeric antibody (Fang et al., 2005; Reed et al., 2007).

Here we demonstrate the application of this methodology for the production of active Fab′ fragments using a cleavable linker incorporating a furin cleavage site (Reed et al., 2007; Thomas, 2002) and a FMDV 2A self-cleaving peptide (Fang et al., 2005; Ryan et al., 1991; de Felipe et al., 2003). We demonstrate the generation of a chimeric Fab′ fragment against the Fas receptor, a receptor involved in promotion of apoptosis and a potential anti-tumour target (Trauth et al., 1989; Yonehara et al., 1989; Rokhlin et al., 1998; Tillman et al., 1999). The recombinant construct was stably expressed in a genetically engineered CHO cell line expressing the mouse cationic amino acid transporter 1 (mCAT-1) receptor and providing an efficacious way of generating cells stably expressing recombinant proteins by ecotropic retrovirus-mediated gene transfer (Albritton et al., 1989; Closs et al., 1993; Scholz and Beato, 1996; Suzuki et al., 2006). The processed and functional anti-Fas Fab′ fragment was expressed in 1–2 mg/L of culture in this expression system, demonstrating the potential for the facile production of other Fab′ fragments.

2. Materials and methods

2.1. Materials

The hybridoma cell line (clone 2A6) expressing a monoclonal antibody raised against the extracellular domain of the human Fas receptor was a kind gift from Dr. S. Olwill (Fusion Antibodies Ltd, Belfast, UK). Multiple choice human spleen cDNA was supplied by Origene (Rockville, Maryland). RNA-STAT60™ reagent was purchased from Biogenesis (Poole, UK). The Im-Prom II™ Reverse Transcription System was supplied by Promega (Madison, USA). The 3′-RACE System™ was purchased from Invitrogen (Paisley, UK). RNAse was obtained from Qiagen (Crawley, UK). All primers were ordered from MWG (Ebersberg, Germany). BioMix Red Taq polymerase was supplied by Bioline (London, UK). Agarose was ordered from Cambrex (Wokingham, UK). The TOPO® Cloning kit is a product of Invitrogen (Paisley, UK). The plasmid MiniPrep kit from Qiagen (Crawley, UK) was used for plasmid preparation. DNA sequencing was done by Fusion Antibodies Ltd (Belfast, UK) on an ABI3100 DNA analyser. *Eco*RI and *Not*I restriction enzymes and buffers were supplied by Invitrogen (Paisley, UK). Calf intestinal phosphatase and T4 DNA Ligase were purchased from New England Biolabs (Ipswich, Massachusetts). pcDNA5 and pOG44 vectors were supplied by Invitrogen (Paisley, UK).

Flp-In CHO cells, Dulbecco's modified Eagle medium (DMEM) and CHO-S-SFM II media, L-glutamine, Pen/Strep, Zeocin and Hygromycin were supplied by Invitrogen (Paisley, UK). Ham's F12 and foetal calf serum were ordered from PAA Laboratories GmbH (Pasching, Austria). Fugene 6 transfection reagent was supplied by Roche Diagnostics GmbH (Mannheim, Germany). Puromycin, Polybrene, Trypan Blue and Trypsin/EDTA were ordered from Sigma-Aldrich (Poole, UK).

Spinner flasks were purchased from Bellco (Bohemia, New York). Haemocytometers were purchased from Immune Systems Ltd (Paignton, UK). Vivaflow 200 crossflow concentrator was supplied by Sartorius AG (Göttingen, Germany). Immobilised metal-ion affinity chromatography (IMAC) AKTA systems and Hi-Trap Ni-NTA IMAC columns (1 mL) were purchased from GE Healthcare (Little Chalfont, UK). MicroBCA kits were purchased from Pierce (Rockford, USA). 4–20% (w/v) Criterion polyacrylamide gels were supplied by BioRad (Hemel Hampstead, UK). The recombinant human Fas receptor extracellular domain antigen and the Grb2-associating protein (Gasp) antigen were kind gifts from Dr. Richard Buick (Fusion Antibodies Ltd, Belfast, UK). Mouse anti-human Vκ light chain antibody was a product of BD Pharmingen (San Diego, USA), mouse anti-His_5 antibody a product of Qiagen (Crawley, UK). Goat α-mouse-HRP, goat anti-human Fab-HRP and diaminobenzidine (DAB) were ordered from Sigma-Aldrich (Poole, UK). TMB was supplied by Bio Panda (Belfast, UK). All other reagents were purchased from Sigma-Aldrich (Poole, UK).

2.2. Development of a CHO cell line expressing the mCAT-1 receptor

The mCAT-1 open reading frame was amplified from murine cDNA using forward 5′ TTTTTTAAGCTTACCATGGGCTG-CAAAAACCTGCTCGGTC-3′ and reverse 5′ TTTTTTGGATCCT-CATTTGCACTGGTCCAAGTTGCTG-3′ primers incorporating *Hind*III and *Bam*HI restriction sites respectively (as underlined), and subsequently sub-cloned into pcDNA5/FRT using restriction enzymes *Hind*III and *Bam*HI. Flp-In CHO cells growing in complete Ham's F12 medium and 100 μg/mL zeocin were plated at a density of 2.0×10^5 cells per 35 mm dish. Cells were co-transfected 24 h later with pcDNA5/FRT-mCAT-1 and pOG44 vectors at a ratio of 1:4 respectively, using Fugene at a 1:7 ratio (DNA : Fugene) as per manufacturer's instructions. The media was removed and replaced at 48 h with complete Ham's F12 medium containing 500 μg/mL hygromycin. Cells were routinely passaged in 500 μg/mL hygromycin until a heterogeneous pool of hygromycin resistant cells expressing the mCAT-1 receptor was selected.

Expression of the mCAT-1 receptor by pcDNA5/FRT-mCAT-1 transfected, hygromycin resistant cells was confirmed by RT-PCR. Briefly, total RNA was extracted from 2×10^6 pcDNA5/FRT-mCAT-1 transfected, hygromycin resistant cells as well as from untransfected Flp-In CHO cells used as a control using RNA-STAT60™ according to the manufacturer's instructions. For both cell lines, extracted RNA (0.5 μg) was used for the synthesis of cDNA with the Im-Prom II™ Reverse Transcription System following the manufacturer's instructions. Contaminating RNA was then removed from the cDNA by incubation with RNase A (1 μL) at 37 °C for 30 min. For both cell lines, the sequence encoding the mCAT-1 receptor gene was amplified from the cDNA using the forward primer 5′-ATGGGCTG-CAAAAACCTGCTCGGTC-3′ in combination with reverse primer 5′-TCATTTGCACTGGTCCAAGTTGCTG-3′. Briefly, 20 ng of cDNA was added to a standard PCR mix (25 μl Green Master Mix, 21 μl PCR water) containing the mCAT-1 specific forward and reverse primers (5 pmol each) and amplified using a programme of 10 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 2 min 30 s at 72 °C; and a final elongation of 10 min at

Download English Version:

https://daneshyari.com/en/article/2088422

Download Persian Version:

https://daneshyari.com/article/2088422

Daneshyari.com