Available online at www.sciencedirect.com
SCIENCE DIRECT®
Protein Expression and Purification 42 (2005) 255–267
Protein Expression & Purification
www.elsevier.com/locate/yprep

Production, purification, and characterization of human scFv antibodies expressed in *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Escherichia coli*

Keith D. Miller, Jane Weaver-Feldhaus, Sean A. Gray, Robert W. Siegel [1], Michael J. Feldhaus *

Pacific Northwest National Laboratory, 902 Battelle Boulevard, P.O. Box 999, Richland, WA 99352, USA

Received 16 December 2004, and in revised form 21 April 2005
Available online 17 May 2005

Abstract

Single chain (scFv) antibodies are used as affinity reagents for diagnostics, therapeutics, and proteomic analyses. The antibody discovery platform we use to identify novel antigen binders involves discovery, characterization, and production. The discovery and characterization components have previously been characterized but in order to fully utilize the capabilities of affinity reagents from our yeast surface display library, efforts were focused on developing a production component to obtain purified, soluble, and active scFvs. Instead of optimizing conditions to achieve maximum yield, efforts were focused on using a system that could quickly and easily produce and process hundreds of scFv antibodies. Heterologous protein expression in *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Escherichia coli* were evaluated for their ability to rapidly, efficaciously, and consistently produce scFv antibodies for use in downstream proteomic applications. Following purification, the binding activity of several scFv antibodies were quantified using a novel Biacore assay. All three systems produced soluble scFv antibodies which ranged in activity from 0 to 99%. scFv antibody yields from *Saccharomyces*, *Pichia*, and *E. coli* were 1.5–4.2, 0.4–7.3, and 0.63–16.4 mg L^{-1} culture, respectively. For our purposes, expression in *E. coli* proved to be the quickest and most consistent way to obtain and characterize purified scFv for downstream applications. The *E. coli* expression system was subsequently used to study three scFv variants engineered to determine structure–function relationships.

Keywords: scFv; Expression; Activity; Purification

Antibodies are used for many research, diagnostic, and therapeutic applications. Regardless of the antibody format, scFv,[2] Fab, or IgG, the ability to produce the affinity reagent easily in an active purified form is essential. Feldhaus et al. [1] described the identification of novel antigen binding clones from a human single chain antibody library expressed on the surface of *Saccharomyces cerevisiae*. Solution-based magnetic column

* Corresponding author. Present address: Merrimack Pharmaceuticals, Inc., Cambridge, MA 02142, USA.
E-mail address: mfeldhaus@merrimackpharma.com (M.J. Feldhaus).
[1] Present address: ADD Hybridoma Group, Abbott Laboratories, Abbott Park, IL 60064, USA.

[2] *Abbreviations used:* scFv, single chain fragment variable; TFF, tangential flow filtration; GaM, goat anti-mouse IgG; K_D, equilibrium affinity constant; ELISA, enzyme-linked immunosorbent assay; V_H, variable heavy chain; V_L, variable light chain; dAb, domain antibody; kDa, kilodalton; FACS, fluorescence activated cell sorting; mAb, monoclonal antibody; hEGF, human epidermal growth factor; Ni–NTA, nickel–nitrolotriacetic acid; P_i, inorganic phosphate; HRP, horseradish peroxidase; His, histidine; *Sc*scFv, scFv expressed or purified from *Saccharomyces cerevisiae*; *Pp*scFv, scFv expressed or purified from *Pichia pastoris*; *Ec*scFv, scFv expressed or purified from *Escherichia coli*; scFvs, single chain antibodies; Arg, arginine; Trp, tryptophan; SO, *Shewanella oneidensis*; IMAC, immobilized metal affinity chromatography; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; EDTA, ethylenediamine tetraacetic acid; PMSF, phenylmethylsulfonylfluoride.

1046-5928/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pep.2005.04.015

enrichment and FACS-based selection of antigen binding populations identified clones encoding antigen-specific scFv. This approach exploits the quantitative properties and high resolution of FACS to identify and characterize binding properties such as epitope specificity and affinity [2]. For many applications, such as steady-state affinity determinations, off rate measurements, or characterization of biophysical properties [3], analyses using the surface displayed scFv provide a straightforward alternative to performing these assays with purified scFv. However, downstream scFv applications such as ELISA, scFv immobilization, or fluorescent labeling are more practical using purified soluble scFv.

Using hEGF-specific mAb, Siegel et al. [2] described a novel procedure for rapidly identifying and selecting epitope-specific scFv from a surface display yeast library. We have also selected scFv antibodies to human p53 peptide, *Shewanella oneidensis*, and *Botulinum* toxin antigens. The function of purified scFvs in vitro cannot be predicted a priori so a method was required to rapidly express and purify numerous scFv antibodies for use in various downstream applications.

Numerous reports describe high scFv yields in specific expression systems [4–12]. Yields obtained for specific scFvs in these systems are high, but significant optimization was usually required. For purposes of expressing and purifying numerous scFvs in a medium throughput approach, expression optimization for each selected scFv is not feasible. A system that produces consistent expression of active scFv in sufficient yields ($\geqslant$1 mg from a 1 L shaker flask) for downstream applications (e.g., immunoprecipitations, ELISA) was required for maximum utility of our antibody discovery platform.

For the current study, three production systems were interrogated for production yields, percent active material, and ease of use. The scFv antibodies were selected from a *S. cerevisiae* display library so we hypothesized the *S. cerevisiae* production system would be the most likely to produce correctly folded, active, and monomeric scFv. Complex eukaryotic protein expression, such as antibody fragments, is generally successful in yeast. Shusta et al. [13] described how the co-expression of a chaperonin (BiP) and the enzyme protein disulfide isomerase (PDI) increased the capacity of *S. cerevisiae* to secrete increased levels of scFv. The expression of soluble scFv in *Pichia pastoris* [5,6,8–11,14–16] and *Escherichia coli* [16–26] has also been described and we evaluated these two systems as well.

We describe the results from scFv expression in *S. cerevisiae*, *P. pastoris*, and *E. coli*. Instead of focusing on obtaining maximum yield, the goal was to rapidly obtain enough material (e.g., $\geqslant$1 mg) for downstream applications such as immunoprecipitations or ELISA (e.g., using epitope-specific hEGF scFv described by Siegel et al. [2]). In some cases, it was possible to make a side-by-side comparison of all three systems, whereas in other cases a particular scFv was expressed in only two systems.

The *E. coli* expression system was found to be best suited for our purposes and was thus used to conduct a more detailed analysis of the expression and stability of three variants of an scFv. Results from scFv refolding, V_L gene deletion, and an amino acid substitution are presented. The advantages of each system are discussed as well as the ease with which each fits into the platform we currently use for antibody discovery, characterization, and analysis.

Materials and methods

Reagents

Chemicals were obtained from Sigma–Aldrich (St. Louis, MO) or Fisher Scientific and were of the highest purity possible. Ni–NTA–Sepharose and anti-penta HIS monoclonal antibodies were from Qiagen (GmbH, Germany). Monoclonal antibody anti-c-myc 9E10 was purchased from Covance/BAbco (Princeton, NJ) and purified anti-V5 was kindly provided by Andrew Bradbury (Los Alamos National Laboratory, Los Alamos, NM). Anti-HSV monoclonal antibody, pET 27b plasmid, and Rosetta BL21(DE3) *E. coli* cells were from Novagen (Madison, WI). *Pichia* X-33 cells, the pPIC-ZαB plasmid, and all SDS–PAGE and immunoblotting supplies were purchased from Invitrogen (Carlsbad, CA).

Plasmids, strains, and scFv expression

Human single chain antibodies were selected from a *S. cerevisiae* naïve surface display library as previously described [1]. The following antigens were used in the selections: (1) human epidermal growth factor (hEGF); (2) *S. oneidensis* protein phosphotyrosine phosphatase (SO2208); (3) *S. oneidensis* Gfo/Idh/MocA family oxidoreductase (SO3120); (4) *S. oneidensis* α-subunit of DNA-directed RNA polymerase (SO0256); (5) peptides 20p and 378p derived from the human p53 protein; and (6) *Clostridium botulinum* neurotoxin subtype A. scFv genes were PCR-amplified from the pPNL6 surface display plasmid, sub-cloned into the pPNL9 expression plasmid, and transformed into *S. cerevisiae* YVH10 yeast as previously described [1].

Using a hEGF *S. cerevisiae* scFv (*Sc*scFv), an expression time course was conducted in four different media to determine the optimal time and media composition for maximum scFv expression. After overnight growth in buffered synthetic dextrose plus casein amino acids