Journal of Immunological Methods 360 (2010) 39–46

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Research paper

Antibody Fab display and selection through fusion to the pIX coat protein of filamentous phage

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ARTICLE INFO

Article history:
Received 7 January 2010
Received in revised form 24 May 2010
Accepted 2 June 2010
Available online 17 June 2010

Keywords:
Phage display
pIX
Fab
Antibody engineering
Respiratory syncytia virus
RSV

ABSTRACT

Fab antibody display on filamentous phage is widely applied to de novo antibody discovery and engineering. Here we describe a phagemid system for the efficient display and affinity selection of Fabs through linkage to the minor coat protein pIX. Display was successful by fusion of either Fd or Lc through a short linker to the amino terminus of pIX and co-expression of the counter Lc or Fd as a secreted, soluble fragment. Assembly of functional Fab was confirmed by demonstration of antigen-specific binding using antibodies of known specificity. Phage displaying a Fab specific for RSV-F protein with Fd linked to pIX showed efficient, antigen-specific enrichment when mixed with phage displaying a different specificity. The functionality of this system for antibody engineering was evaluated in an optimization study. A RSV-F protein specific antibody with an affinity of about 2 nM was randomized at 4 positions in light chain CDR1. Three rounds of selection with decreasing antigen concentration yielded Fabs with an affinity improvement up to 70-fold and showed a general correlation between enrichment frequency and affinity. We conclude that the pIX coat protein complements other display systems in filamentous phage as an efficient vehicle for low copy display and selection of Fab proteins.

1. Introduction

Filamentous phage display is widely used for protein engineering, most notably for antibody discovery and maturation (Hoogenboom, 2005). The external surface of the phage is dominated by the major coat protein, pVIII, that encapsulates the positive single-stranded DNA genome or surrogate phagemid. The termini of the core are capped by pairs of minor coat proteins, pIII and pVI at one end and pVII and pIX at the other (Clackson and Lowman, 2000). For antibody engineering, the pIII protein, or an N-terminal truncated variant, has been the preferred fusion protein, in part because its low copy number is suited for affinity selection (Garrard et al., 1991; Bradbury and Marks, 2004). However, the pIII protein is critically involved in the attachment and entry process and proteins displayed on pIII may interfere with the efficiency of infection (Krebber et al., 1995; Marzari et al., 1997; Malmborg et al., 1997 and Spada and Plückthun, 1997). Counter to prior conclusions (Endemann and Model, 1995), the pIX protein was found to localize on the phage surface in an amino-terminal exposed orientation suitable for protein and peptide display (Gao et al., 1999). Like pIII, pIX is present at low copy number on the phage and it has been applied in a phagemid format for selection of specific antibodies from a scFv fusion library (Gao et al., 2002). While scFv are useful in their own right, the unnatural Vh–Vl configuration sometimes confounds conversion to a full antibody format, a process more readily accomplished with Fabs. Here we describe the efficient display of Fab proteins of known specificity through fusion of either the Fd or Lc to pIX coupled with soluble expression of the complementary chain, and demonstrate the utility of this system in affinity maturation from libraries of Fab variants.

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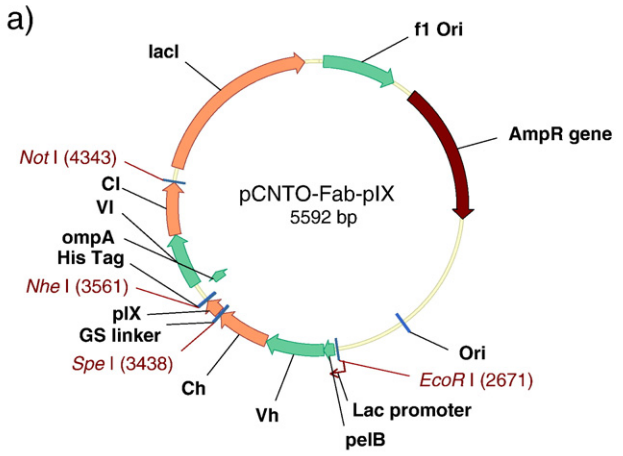
doi:10.1016/j.jim.2010.06.001



b)

	Ch/Cl	Linker	pIX	Tag
HcpIX	CDKTH	TSGGGGS	MSVLVYSFASFVLGWCLRSGITYFTRLME TSS	**ASHHHHHH**
λLcpIX	PTECS	TSGGGGS	MSVLVYSFASFVLGWCLRSGITYFTRLME TSS	**ASHHHHHH**
κLcpIX	NRGEC	TSGGGGS	MSVLVYSFASFVLGWCLRSGITYFTRLME TSS	**ASHHHHHH**

Fig. 1. The pCNTO phagemid vector. (a) A vector map for the construct with Ch (CH1) fused to pIX. (b) Amino acid sequences showing the linkage of either Hc (Fd) or Lc (λ or κ) to pIX starting on the left with the C-terminal sequence of Ch or Cl. Stop codon is represented by an *.

2. Results

2.1. Fab display through the pIX protein

The pCGMT9 phagemid (Gao et al., 1999) served as the backbone for development of a phagemid vector suitable for insertion of heavy (Vh) and light chain (Vl) variable region segments in a Fab format for display through fusion of the CH1 or CL fragments to pIX. Fig. 1 shows the features of the dual display and expression vector pCNTO-Fab-pIX, in the format for display of CH1 (Ch) linked to pIX and Lc (Vl–Cl) as a secreted fragment. For display through light chain, the pIX gene was fused to the C-terminus of the CL region through a short linker and Fd (Vh–CH1 region) was expressed as the soluble secreted fragment. Both vectors can be modified to secrete soluble Fab upon excision of the pIX gene via restriction digest with SpeI and NheI followed by self-ligation (see Materials and methods).

For initial studies, we constructed a Fab derivative of T56, a scFv specific for TNFα. The Vh and Vl genes were placed into the pCNTO phagemid vector via conventional cloning to create the Lambda light chain (λ Lc) Fab. This Fab was used to evaluate display through linkage of Fd or Lc to the N-terminus of pIX. Phage particles for both constructs were generated by transforming the pCNTO-T56 phagemid vector into TG1 cells and subsequent infection with helper phage. The recovered phage were evaluated by phage ELISA for surface display of the Fd or λ Lc via the pIX protein and for co-association of the corresponding soluble Lc or Fd fragment, respectively (Fig. 2a). As shown in Fig. 2b, serial dilutions of phage with the Fab linked through either Fd or Lc showed dose response binding to both anti-Fd or anti-Lambda antibody coated wells, indicating the display of both chains. The capture appeared to be specific because helper phage alone gave no signal in these assays.

To further evaluate display via Fd or Lc, two different Fabs specific for the respiratory syncytial virus (RSV) F protein, Fab T40 (Tsui et al., 2002) and Fab B23 (Tsui, et al. manuscript in preparation), were cloned into the pCNTO vectors. Both Fabs have Kappa light chains (κ Lc).

Phage ELISA results (Fig. 2c), show both T40 and B23 phage were captured by anti-Fd or anti-Kappa antibodies in either format of Fd or Lc fusion to pIX. These results further demonstrate that either Fd or Lc can be displayed by linkage to pIX and that the respective soluble Lc or Fd fragment co-associates with the displayed chain on the surface of the phagemid particle. Both the T40 and B23 Fab phage specifically bound to the RSV-F antigen in either Fd or Lc

Fig. 2. Fab display through pIX. a) Schematic showing the format of the Phage ELISA. The phage pictured on the left has Fd (Hc) linked to pIX and on the right has Lc linked to pIX. Display of the Fd or the Lc on the phage was measured by capture of the phage with a Fd or Lc specific antibody, respectively, coated on the ELISA well and detection with a HRP labeled M13 antibody. Display of functional Fab was detected by capture of the phage on plate wells coated with antigen followed by detection with the M13 antibody. b) Fd and Lc display for the T56 TNF Fab. The T56 Fab was displayed by linking either Fd (T56pCNTO Hc-pIX) or Lc (T56pCNTO Lc-pIX) to pIX and expressing the counter chain as a secreted, soluble fragment. Phage produced from both constructs were captured on the anti-Fd (Hc specific) or anti-Lambda (Lc specific) coated wells. The graph bars show the luminescence signal (RLU) for serial 1/5 dilutions of phage (left to right), starting with a neat concentration. Helper phage ("M13") was used as a negative control. c) Display and antigen-specific binding for the T40 and B23 RSV Fabs. Both Fabs were displayed by linkage of Fd ("HcpIX") or Lc ("LcpIX") to pIX. Serial dilutions of phage were captured on wells coated with anti-Fd antibody, anti-Kappa antibody, RSV-F protein, or TNF protein (negative control) and detected as in b. In b and c, serial dilution samples were measured in single wells.