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Research paper

Parameters affecting the display of antibodies on phage

M. Kirsch, M. Zaman, D. Meier, S. Dübel*, M. Hust

Institut für Biochemie und Biotechnologie, Abteilung Biotechnologie, Technische Universität Braunschweig, Spielmannstr.7, 38106 Braunschweig, Germany

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Abstract

Despite the fact that a multitude of antibody phage display libraries has been built, systematic comparisons of critical design parameters are rare. Here we analysed the impact of various factors on the performance of the phage display system. First, we compared several vector designs for the display of Fab fragments of antibodies. Bicistronic as well as monocistronic expression of the antibody/pIII operon and vectors using fd–pIII as well as LC–pIII fusions were tested. Further, we evaluated the influence of glucose on the promoter induction. We compared monovalent versus oligovalent display of the antibody fragments and we used antibody fragments with different folding efficiency to assess the influence of the individual antibody sequences on the performance of the system. Finally, both phage display efficiency and yield of soluble Fab fragments were analysed. The significant differences found for phage yield, display of Fabs on the phage and expression of soluble Fabs suggest to use a bicistronic vector with an fd-fragment–pIII fusion for the construction of future Fab phage display libraries.

Keywords: Fab; Phage display; Recombinant antibody; scFv; Vector design

1. Introduction

Within the past decade, antibody phage display has proven its capability to supply human antibody fragments, binders to toxic or non-immunogenic substances and even fragile conformational variants.

However, a broad distribution of this technology as a routine method has been hindered by the still delicate handling of very large antibody repertoires and the extreme sensitivity of the method. It has to be kept in mind that every *E. coli* clone rescued from a phage panning experiment results from a discrete interaction of a single antibody fragment with a single antigen molecule. As a consequence, the use of antibody phage display, particularly in the companies dominating the applications, has been mainly focused to the development of therapeutics, where extensive optimizations in respect of every single application are feasible. In this respect, the vast majority of reports utilise scFv libraries (Hust and

* Corresponding author.
E-mail address: s.duebel@tu-bs.de (S. Dübel).

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Dübel, 2004), as the smaller size and the composition from a single polypeptide facilitate production and folding in *E. coli*, in turn allowing a more efficient presentation of the antibody repertoire on the phage surface. Recently, however, novel applications in research, in particular for proteomics and antibody microarrays, induced a re-thinking of this strategy. Here, a robust method adapted to require minimal effort per antigen is necessary. Individual optimizations of clones or antibody format changes would neutralise the benefits of the possible automation. As a result, Fab fragments experience a renaissance as they are much more compatible to established immunological protocols than scFv fragments. However, due to the necessity to produce two different polypeptide chains, Fab expression vectors are more complex than the scFv systems (for review see Hust and Dübel, 2005).

First, the expression of two antibody chains must be well balanced for optimal Fab phage display. Several parameters could affect the optimal expression and display of Fab fragments. Various genetic designs for the expression of two polypeptides have been tried. A monocistronic arrangement of the antibody genes results in two mRNAs. This design was used, e.g., in pComb3 (Barbas et al., 1991), pDH188 (Garrard et al., 1991), pFAB4 and pFAB5c (Ørum et al., 1993). In contrast, in a bicistronic arrangement, both antibody chains will be translated from one mRNA. Examples for bicistronic designs are pEXmide3 (Söderlind et al., 1993), pFAB60 (Johansen et al., 1995), pFAB73h (Engberg et al., 1996) and pCES (de Haard et al., 1999).

Second, either the light chain or the heavy chain can be fused to pIII. Ørum et al. (1993) and Engberg et al. (1996) used an LC–pIII fusion. The HC–pIII fusion was employed by Barbas et al. (1991) and de Haard et al. (1999), among others. As we demonstrate in this study, this orientation can strongly affect the display efficiency.

Third, most phagemids use the lacZ promoter for the antibody expression cassettes. This promoter can be down regulated by glucose in the medium. For example, for the human single fold scFv libraries I+J (Tomlinson I+J), the presence of 0,1% glucose (5.5 mM) gave optimal results (Tomlinson, 2004), whereas removal of glucose was used for induction in other systems (Welschof et al., 1997).

Fourth, the amount of antibody-fragment–pIII fusion protein incorporated into the phage particle, generated with different helper phage, varies by more than two orders of magnitude. The use of helper phage which enforce oligovalent display is frequently used to improve the panning efficiency, but their effect on overall performance in relation to the other factors has not yet been systematically assessed. Several systems for enforced oligovalent display have been reported recently (Rondot et al., 2001; Baek et al., 2002; Soltes et al., 2003). Here we analysed the interference of a well established member of each helper phage type (monovalent VCSM13, oligovalent Hyperphage) with the above factors. Some vectors, e.g., pComb3 (Barbas et al., 1991), utilised an amino terminally truncated form of pIII as a fusion partner for the antibody chain(s). These systems, however, do not allow to benefit from the systems enforcing oligovalent display, as the amino terminal deletion of the pIII would result in phage which are not infective anymore. Therefore, these variants were not included in this study.

Fifth, for convenience, many phage display vectors allow the expression of soluble antibody fragments once the panning process has been finished. Typically, the switch from *E. coli* SupE strains to non-suppressor strains activates a stop codon between the antibody and phage coat protein domains. A desirable feature of a phage display vector optimized for proteomic antibody generation would be a combination of good display yields and compatibility with the oligovalent and monovalent display with a good yield of soluble Fabs without subcloning into an expression vector. For this reason, we have chosen to include the SupE codon into the vectors analysed here.

Sixth, different antibody sequences are known to exhibit large differences in production yield in the *E. coli* periplasm ranging from mg/L to almost marginal yields. We acknowledged a respective influence on the comparison by using antibodies with “bad” and “good” folding abilities. As a representative of the “good” folders we used the D1.3 antibody, binding to lysozyme (Ward et al., 1989). The RNA polymerase II binding antibody ‘215’ represents the “bad” folding species (Liu et al., 1999). This study allowed to conclude on the significance of all these factors and their interactions and resulted in the suggestion of an “optimized” phage display vector.