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In vitro binding of anthrax protective antigen on bacteriophage T4 capsid surface through Hoc–capsid interactions: A strategy for efficient display of large full-length proteins

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Abstract

An in vitro binding system is described to display large full-length proteins on bacteriophage T4 capsid surface at high density. The phage T4 icosahedral capsid features 155 copies of a nonessential highly antigenic outer capsid protein, Hoc, at the center of each major capsid protein hexon. Gene fusions were engineered to express the 83-kDa protective antigen (PA) from *Bacillus anthracis* fused to the N-terminus of Hoc and the 130-kDa PA-Hoc protein was expressed in *Escherichia coli* and purified. The purified PA-Hoc was assembled in vitro on *hoc*$^-$ phage particles. Binding was specific, stable, and of high affinity. This defined in vitro system allowed manipulation of the copy number of displayed PA and imposed no significant limitation on the size of the displayed antigen. In contrast to in vivo display systems, the in vitro approach allows all the capsid binding sites to be occupied by the 130-kDa PA-Hoc fusion protein. The PA-T4 particles were immunogenic in mice in the absence of an adjuvant, eliciting strong PA-specific antibodies and anthrax lethal toxin neutralizing antibodies. The in vitro display on phage T4 offers a novel platform for potential construction of customized vaccines against anthrax and other infectious diseases.

Keywords: Bacteriophage T4; Virus assembly; Hoc; Phage display; Protective antigen; Vaccine

Introduction

In phage display, a foreign peptide, domain, or protein, is fused to a virus structural protein and exposed on the outer surface of the virus particle (Smith and Petrenko, 1997). The coat proteins of filamentous phages (M13, fd, and f1), the minor coat protein pIII (4–5 copies) and the major coat protein pVIII (2700 copies), have been extensively used primarily to display libraries of six to eight amino acid (aa) peptides (Smith, 1985; Markland et al., 1991). Other display systems using icosahedral phages λ [gene product (gp) D, the capsid decoration protein, 420 copies; gpV, the major tail protein, 192 copies] (Sternberg and Hoess, 1995; Maruyama et al., 1994) and T7 (gp10, the major capsid protein, 415 copies) (Rosenberg et al., 1996; Castillo et al., 2001) can display larger peptides and domains derived from targeted genes or c-DNA libraries.

While these systems have been enormously successful for displaying combinatorial libraries of short peptides, they also possess certain inherent limitations (see Smith and Petrenko, 1997; Manoutcharian et al., 2001; Castagnoli et al., 2001; Hoess, 2002). With the filamentous phages, display of certain peptides is restricted, or not possible, since the fused peptide may not be able to successfully pass through the *E. coli* envelope where the coat protein assembly occurs. Since the phage structural proteins used in the above display systems are normally essential or conditionally lethal, display is inefficient because the fusion proteins, particularly those fused to large peptides and domains, interfere with the virion assembly. It is often necessary to express wild-type protein along with the recombinant fusion protein in order to generate viable phage (Maruyama et al., 1994; Mikawa et al., 1996).

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Phage T4 (Fig. 1A) exhibits unique features that can be exploited for phage display. The prolate icosahedral capsid shell (T = 20; width, 86 nm; length, 119.5 nm; Fokine et al., 2004) consists of three essential proteins: 930 copies (155 hexamers) of the major capsid protein, gp23* (49 kDa; blue subunits; "*" represents the cleaved mature form), 55 copies (11 pentamers) of the minor capsid protein gp24* (46 kDa; purple subunits) at eleven of the twelve vertices, and 12 copies (1 dodecamer) of the portal protein gp20 (61 kDa; capsid-proximal ring as part of the green structure at the base) at the twelfth vertex through which DNA enters and exits (Fokine et al., 2004; Black et al., 1994).

Two outer capsid proteins, Hoc (*h*ighly antigenic *o*uter *c*apsid protein; 39 kDa, yellow subunits) and Soc (*s*mall *o*uter *c*apsid protein; 10 kDa, white subunits), decorate the phage T4 outer capsid surface (Fig. 1A; Ishii and Yanagida, 1975, 1977). Hoc is present up to 155 copies per capsid with each monomer occupying the center of the gp23* hexon, and Soc is present up to 810 copies per capsid with each monomer bridging two gp23* monomers of adjacent hexons (Fokine et al., 2004; Iwasaki et al., 2000). The Soc subunits form an outer "cage" providing additional stability to the capsid. The functional significance of Hoc is unknown. Sequence alignments suggest that Hoc encodes IgG-like domains (Bateman et al., 1977), which may be involved in interactions with the bacterial surface.

Most important, both Hoc and Soc, unlike the structural proteins used in other phage display systems, are nonessential and assembled on the capsid lattice following the completion of assembly (Ishii and Yanagida, 1975; Black et al., 1994). Null (amber or deletion) mutations in either or both the genes do not affect phage production, phage viability, or phage infectivity. Foreign domains/proteins can be fused to the N- and C-termini of Hoc or Soc and displayed on the capsid (Ren et al., 1996; Jiang et al., 1997; Ren and Black, 1998).

All the phage display systems previously reported, including the T4 system, are in vivo-based, i.e., expression and assembly of the foreign peptide/protein occurs during phage infection. This is problematic for the display of large domains and full-length proteins because variations in intracellular expression, aggregation of the expressed protein, nonspecific proteolysis, and interference with phage assembly often result in inefficient display, lack of structural homogeneity, and large differences in the copy number of the displayed protein. In phage λ display, of the 192 copies of gpV per virion, only ~1 copy of gpV-β-galactosidase fusion (116 kDa) was incorporated into phage (Maruyama et al., 1994), and only ~34 copies of the 420 copies of gpD were present as an equivalent fusion protein (Mikawa et al., 1996; Hoess, 2002). In the T7Select display system, of the 415 copies of gp10, only 0.1–1 copy of gp10-β-galactosidase fusion was assembled (Rosenberg et al., 1996). In phage T4 display, about 25–100 copies of Soc-VP1 (VP1; a 33-kDa fragment of poliovirus capsid protein) were displayed of the possible 810 copies (Ren et al., 1996), and 20–40 copies of Hoc-CD4 (CD4; a 20-kDa fragment of the HIV receptor) were displayed of the possible 155 copies (Ren and Black, 1998). In our own published work, the copy number of displayed PorA-Soc (PorA; a 4-kDa porin peptide from *Neisseria meningitidis*) varied between 10 and >100% of the expected copy number in different preparations (Jiang et al., 1997). In addition, nonspecific proteolysis of both the displayed Hoc and Soc fusion proteins and display of aggregated protein were evident (Ren et al., 1996; Jiang et al., 1997). Although Brenner and colleagues developed an in vivo suppression strategy, which can control the fusion protein expression to some extent (Maruyama et al., 1994; Mikawa et al., 1996), very little, if any, control can be exerted on other in vivo display parameters in order to generate particles of desired composition.

The above limitations are quite serious for applications such as vaccine development, structural biology, and quantitative biochemistry, wherein particles displaying a defined copy number of biologically active molecules are needed. An attractive strategy would be to perform the binding reaction in vitro using purified and functionally

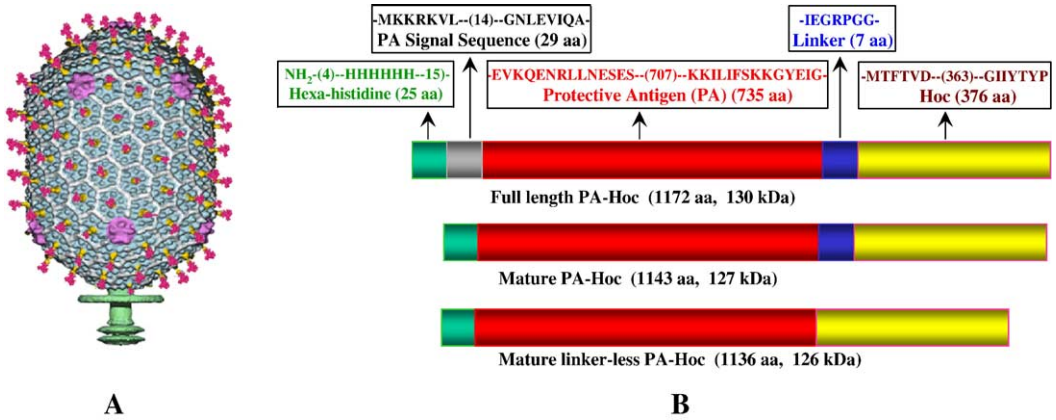

Fig. 1. Schematics of the recombinants. (A) Anthrax PA displayed on bacteriophage T4. The hypothetical structure is generated by merging the X-ray structure of PA (Petosa et al., 1997) to the Hoc monomer (yellow spikes) of phage T4 cryo-EM reconstruction reported by Fokine et al. (2004). The gp23* is shown in blue, gp24* in purple, Soc in white, PA in red, portal, neck, and part of the tail in green. (B) Schematic of PA-Hoc fusion constructs. The PCR-based SOE strategy (Horton et al., 1989; Rao and Mitchell, 2001) was used to fuse PA and Hoc genes via the linker as shown.