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Short communication

Use of bacteriophage particles displaying influenza virus hemagglutinin for the detection of hemagglutination-inhibition antibodies

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SUMMARY

Bacteriophage lambda capsids provide a flexible molecular scaffold that can be engineered to display a wide range of exogenous proteins, including full-length viral glycoproteins produced in eukaryotic cells. One application for such particles lies in the detection of virus-specific antibodies, since they may obviate the need to work with infectious stocks of highly pathogenic or emerging viruses that can pose significant biosafety and biocontainment challenges. Bacteriophage lambda capsids were produced that displayed an insect-cell derived, recombinant H5 influenza virus hemagglutinin (HA) on their surface. The particles agglutinated red blood cells efficiently, in a manner that could be blocked using H5 HA-specific monoclonal antibodies. The particles were then used to develop a modified hemagglutininationinhibition (HAI) assay, which successfully identified human sera with H5 HA-specific HAI activity. These results demonstrate the utility of HA-displaying bacteriophage capsids for the detection of influenza virus-specific HAI antibodies.

1. Introduction

Bacteriophage lambda capsids can provide a highly flexible scaffold for the high density display of foreign peptides and proteins (Gupta et al., 2003; Mikawa et al., 1996; Santini et al., 1998; Sternberg and Hoess, 1995; Yang et al., 2000). One commonly used method is to fuse the protein or peptide of interest to the major phage capsid protein, gpD, thereby producing phage particles that incorporate the desired protein into their capsids when propagated in *E. coli* host cells (Mikawa et al., 1996; Santini et al., 1998; Sternberg and Hoess, 1995). However, this approach is suboptimal for glycosylated proteins such as the HIV-1 envelope glycoprotein or the influenza virus hemagglutinin (HA). Consequently, an alternative display approach has therefore been developed which allows one to "decorate" preformed, gpD-deficient phage with exogenously supplied gpD or recombinant gpD-fusion proteins, produced in eukaryotic cells (Mattiacio et al., 2011; Sternberg and Hoess, 1995). This approach permits the decoration of lambda phage capsids with glycoproteins produced in eukaryotic cells, such as the HIV-1 envelope protein (Mattiacio et al., 2011).

To display the influenza virus HA on the surface of lambda phage, a soluble, recombinant gpD:HA protein was produced in insect cells, since these cells have been shown to support the production of biologically functional, glycosylated HA (Treanor, 2009). For this purpose, the HA from a well-characterized H5N1 influenza virus (A/Vietnam/1203/04) was used, and the gpD:H5HA fusion protein was then purified using a C-terminal hexahistidine tag and a nickel-affinity column (Fig. S1).

Conditions for the decoration of gpD-deficient phage capsids with the purified, recombinant gpD:H5HA proteins were optimized. Attempts to decorate gpD-deficient phage particles with gpD:H5HA protein alone resulted in phage capsids that were unstable in the presence of EDTA, reflecting incomplete occupancy of available gpD binding sites (Fig. S2). This is presumably because the large gpD:H5HA protein is sterically hindered from binding all 420 of the gpD binding sites on the capsid (Yang et al., 2000), much like the large gpD:HIV-Env fusion proteins described previously (Mattiacio et al., 2011). To address this problem, "mosaic" phage particles were produced by decorating gpD-deficient capsids with a combination of both gpD:H5HA and wild-type gpD (Mattiacio

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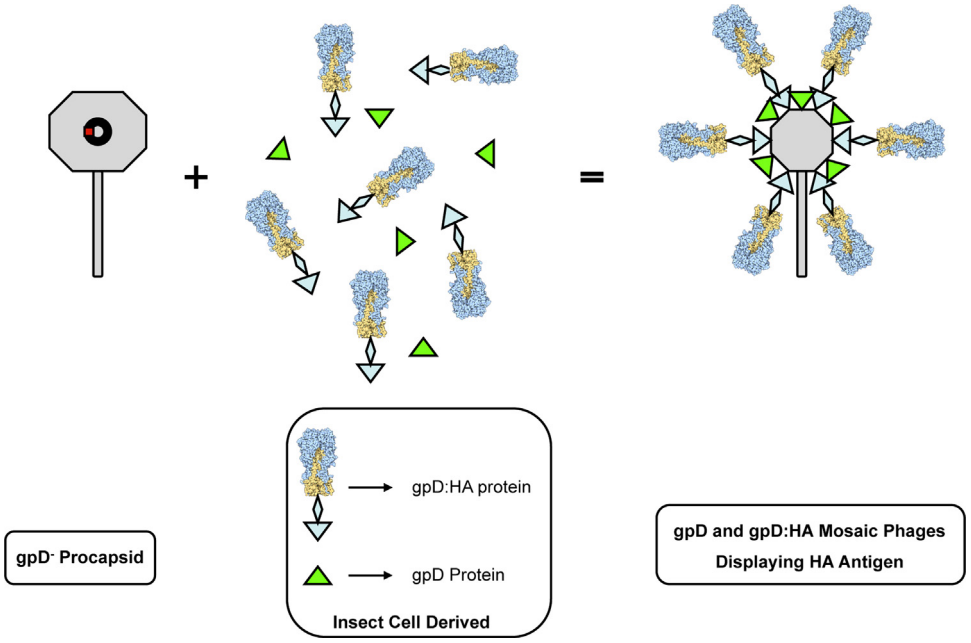

Fig. 1. Production of lambda phage capsids decorated with HA. Phage capsids were decorated with HA by adding a mixture of gpD:H5HA fusion protein and wild-type gpD protein to gpD-deficient lambda phage particles. This produced mosaic phage capsids displaying H5 HA at high copy number. Not drawn to scale.

et al., 2011). This resulted in phage capsids that were stable in the absence of EDTA, which is indicative of full occupancy of the available gpD binding sites on the capsid (Fig. S2). A large batch of these "mosaic" decorated particles was then prepared, purified by CsCl density gradient purification, and analyzed by immunoblot assay using a polyclonal anti-gpD antiserum. This confirmed that both the wild-type and the gpD:H5HA fusion proteins were incorporated into the lambda phage capsid (Fig. S3). The physical structure of these mosaic decorated capsids is shown schematically in Fig. 1.

Phage capsids decorated with gpD:H5HA should display HA on their surface in a multivalent array, allowing them to efficiently bind to sialic acid. An experiment was therefore conducted to determine whether phage decorated with recombinant H5 HA could agglutinate chicken red blood cells (cRBC). To do this, cRBC were incubated with serially diluted aliquots of H5HA-displaying phage particles, WT phage particles, a positive control H1N1 influenza virus (A/New Caledonia/20/99) or PBS. H5HA-displaying phage particles agglutinated cRBCs readily when added at concentrations >5×10^8 PFU/well (Fig. 2). The positive control H1N1 influenza virus also agglutinated the cRBCs to a dilution of 1:1024 (consistent with the titer of this virus stock), while WT phage particles and PBS alone did not agglutinate cRBCs (Fig. 2).

Two H5 HA-specific monoclonal antibodies (Mabs) (kindly provided by Dr. Gary Nabel, NIH VRC) were next used to determine whether HA-specific antibodies could block the hemagglutination activity of H5 HA-displaying phage particles. Mab 9E8 has high H5 specific neutralizing activity, while Mab 9B11 has lower H5-specific neutralizing activity (Yang et al., 2007). Mab 9E8 blocked hemagglutination by H5 HA-displaying phage particles at concentrations as low as 1 ng/well, but had no effect on hemagglutination by H1N1 influenza virus (A/New Caledonia/20/99) (Fig. 3). Mab 9B11 also inhibited hemagglutination by the H5 HA-displaying phage, though only at higher concentrations (100 ng/well) (Fig. 3).

These findings suggested the H5 HA-displaying phage particles could have utility in hemagglutination-inhibition (HAI) assays, which are widely used in seroepidemiologic studies of influenza

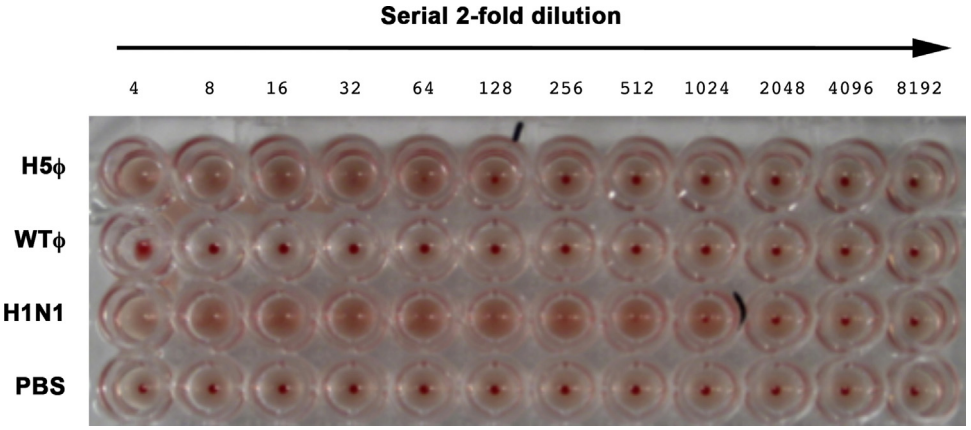

Fig. 2. Phage particles decorated with H5 HA efficiently agglutinate RBCs. Phage particles (either decorated with H5 HA or not; respectively, H5ϕ and WTϕ), or influenza virus virions (H1N1) were serially diluted in PBS in a 96-well V-bottom plate (Costar); negative control wells contained PBS alone (PBS). 50 μl of a suspension of chicken red blood cells (RBCs) was then added to each well, and samples were incubated at 4 °C for 1 h, after which the plate was photographed. Agglutination titer was determined as the last dilution at which agglutination was detected.