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Peptide presentation on primate erythroparvovirus 1 virus-like particles: In vitro assembly, stability and immunological properties

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ABSTRACT

Virus-like particles (VLPs) have demonstrated to be valuable scaffolds for the display of heterologous peptides for vaccine development and other specific interactions. VLPs of primate erythroparvovirus 1, generally referred as parvovirus B19 (B19V), have already been produced in-vivo and in-vitro from the recombinant VP2 protein of this virus. In this study, chimeric forms of B19V VP2 were constructed, and their ability to assemble into VLPs was evaluated. Chimeras were composed of the VP2 protein fused, at its N-terminus, with two peptides derived from the fusion glycoprotein (F) of the respiratory syncytial virus (RSV). The chimeric proteins self-assembled into VLPs morphologically similar to B19V virions. Stability of these VLPs was analyzed under denaturation conditions with guanidinium chloride (GdnHCl). Our results indicate that the presence of the heterologous fragments increased the stability of VLPs assembled by any of the VP2 chimeras. Specific proteolysis assays shown that a fraction of the N-termini of the chimeric proteins is located on the outer surface of the VLPs. Immunogenicity of VLPs against RSV was evaluated and the results indicate that the particles can elicit a humoral immune response, although these antibodies did not cross-react with RSV in ELISA tests. These results provide novel insights into the localization of the N-termini of B19V VP2 protein after in vitro assembly into VLPs, and point them to be attractive sites to display peptides or proteins without compromise the assembly or stability of VLPs.

1. Introduction

Virus-like particles (VLPs) are composed of the structural components of virions except for their genetic material. VLPs mimic the natural structure of virions and retain most of its structural properties, but are not infective. These particles are also an excellent model to study the mechanisms of assembly and disassembly of viral capsids and have called attention to their potential applications in nanotechnology and medicine. In the latter field, VLPs are an excellent platform for the presentation of antigenic peptides and other heterologous molecules with biological functions (Herbst-Kralovetz et al., 2010; Santillán-Uribe et al., 2015).

There are several reports on the in vivo production of VLPs in mammalian, insect and yeast cells (Zeltins, 2013). In contrast, a limited number of works are available on the in vitro production of VLPs (Cuillel et al., 1983; Salunke et al., 1986 Finnen et al., 2003; Yin et al., 2010; Hadravová et al., 2012; Sánchez-Rodríguez et al., 2012). An advantage of the in vitro assembly is the possibility to include chimeric forms of the virus proteins in well-defined proportions during the assembly (Santillán-Uribe et al., 2015). One of the well-characterized in vitro assembly systems is the Primate erythroparvovirus 1, generally referred as parvovirus B19 (B19V) (Sánchez-Rodríguez et al., 2016). B19V is a non-enveloped member of the *Parvoviridae* family, and its virions show icosahedral symmetry with a diameter of 20–24 nm (Heegaard and Brown, 2002). The capsid is assembled from 60 subunits of the structural proteins VP1 and VP2 (5% VP1 and 95% VP2). VP1 is identical to VP2 except for 227 additional amino acid residues located at the N-terminus of VP1, the so-called unique region (uVP1) (Kawase et al., 1995).

B19V VLPs can be produced either in vitro or in vivo from VP2 (Sánchez-Rodríguez et al., 2012). B19V virions, naturally produced empty capsids, and VP2 VLPs have been analyzed by cryoelectron microscopy (cryoEM) (Kaufmann et al., 2008). The main difference between empty capsids and VP2 VLPs is an increased density around the icosahedral 5-fold axis for the empty capsids. On the other hand, VP2 VLPs show an increased density inside the particle

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and close to the same icosahedral symmetry axis. This is congruent with the lack of electronic density found for residues 1–17 by X-ray diffraction experiments in VP2 VLPs (Kaufmann et al., 2004). In despite of this information, chimeric forms of VP1 and VP2, whose N-termini were modified, produced VLPs and the N-termini were presented on the surface of these particles as deduced from experimental data (Miyamura et al., 1994; Gilbert et al., 2005; Amexis and Young, 2006; Ogasawara et al., 2006).

The localization of VP2 N-termini is undetermined for in vitro-assembled B19V VLPs. As previous reports indicate that the N-termini of VP2 may be exposed on the surface of particles assembled in vivo, we hypothesized, first, that for in vitro assembled VLPs is possible that some of its N-termini are exposed on the surface of the particles, and second, that if the N-termini are exposed on surface, this region should be a candidate to present peptides by generating chimeric forms of VP2 in which the heterologous peptides were fused to the N-termini of the chimeras. To test our first hypothesis, we selected 2 peptides containing antigenic sites of the Respiratory Syncytial Virus (RSV) as models for the generation of chimeras. To test the second hypothesis, we included sites for the Factor Xa restriction protease between the heterologous peptides and the VP2 protein. Proteolytic release of the heterologous peptides from the VLPs would indicate its external localization. We evaluated the individual ability of the chimeras to form VLPs, either alone or in combination with selected amounts of VP2. The resulting particles were analyzed to demonstrate the presence of the chimeric protein in VLPs and its effect on the stability of the produced particles. Finally, we tested the antigenic properties of VLPs assembled by VP2 and the chimeric proteins.

2. Materials and methods

2.1. Chemicals and biochemicals

Chemicals and culture media were purchased from Sigma. Restriction enzymes and Factor Xa protease were purchased from New England Biolabs. T4 DNA ligase was purchased from Roche.

2.2. Selection of peptides derived from protein F of SRV

The fusion protein F of the RSV envelope is required for infection and also is the primary target of neutralizing antibodies, therefore is the leading candidate for vaccine development (Collins and Melero, 2011; Vaughan et al., 2015). Several antigenic sites have been reported for protein F. The antigenic site II has been mapped to residues 254–277, while antigenic sites IV–VI have been mapped to residues 429–437 (Smith et al., 2002; McLellan et al., 2011). Based on the sequence of the RSV F protein (GenBank Accession number AAB59858), two peptides containing the antigenic sites II and IV–VI were chosen, the first included residues 215–278, and the second included residues 400–457, from now called FII and FIV. According to the crystallographic structure of protein F (PDB 3RRR), FII corresponds to an α-helix followed by a large loop (240–253), which in turn is followed by an antiparallel 2 α-helix bundle separated by a small loop (262–267). This 2-helix bundle and loop appears to be the center of the antigenic site II. Peptide FIV, in the structure of protein F, corresponds to a β-sandwich, in which the strand composed of residues 430–434 is the center of the antigenic sites IV–VI (429–437) (McLellan et al., 2011).

2.3. Construction of expression vectors for recombinant B19V VP2, VP2-FII, and VP2-FIV genes

Expression vector for VP2 has been previously constructed (Sánchez-Rodríguez et al., 2012). Protein sequences for FII and FIV peptides preceded by a Met residue and followed by the Factor Xa cutting sequence were fused at the N-terminus of VP2 protein (Supplementary data 1). The protein sequences were traduced to DNA and optimized for expression in *E. coli*. The chimeric genes *VP2-FII* and *VP2-FIV* were chemically synthesized (Epoch Life Sciences Inc.) and inserted into the *Nde*I and *Xho*I sites of pET22b+ (Novagen). The sequences of the final constructs were verified by DNA sequencing (ABI Prism 3100). The resulting plasmids were named pETVP2-FII and pETVP2-FIV. All the genes are under the control of phage T7 operator and code for 6×His tags fused to the C-termini of the proteins, to simplify protein purification.

2.4. Expression and purification of recombinant VP2, VP2-FII, and VP2-FIV

Plasmids pETVP2, pETVP2-FII, and pETVP2-FIV were used to transform *E. coli* BL21(DE3) cells. Purification protocol for the chimeras is the same previously described for VP2 (Sánchez-Rodríguez et al., 2012). In short, the transformed cells were grown in LB containing ampicillin (50 μg/mL) and induced with 0.2 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 16 h at 26 °C, with orbital shaking of 250 rpm. Cells were harvested by centrifugation, and the pellet was suspended in lysis buffer (0.3 M NaCl, 50 mM sodium phosphate, pH 7). Cells were disrupted by ultrasonication, and then centrifuged at 5000 x *g* at 4 °C. VP2 and the chimeras remain in the insoluble fraction. The pellets were washed washing buffer (0.3 M NaCl, 50 mM sodium phosphate, 2%Triton X-100, 3 M urea, pH 7) and with lysis buffer. The pellets were solubilized in 60 mL of column buffer (5 M guanidinium chloride (GdnHCl), 0.3 M NaCl and 50 mM sodium phosphate, pH 7). Proteins were purified by IMAC under denaturing conditions, using Protino Ni-TED resin (Macherey Nagel). Pure proteins were concentrated by filtration in 50-kDa MWCO centrifugal filter devices (Millipore). At this step the elution buffer was changed to solubilization buffer (5 M GdnHCl, 20 mM TrisHCl, 0.15 M NaCl, 1 mM EDTA, 1 mM DTT, pH 8). Purity was assessed by SDS-PAGE analysis. Protein concentration was estimated by the bicinchoninic acid assay (BCA).

2.5. in vitro assembly of VLPs

VLPs were assembled by dialysis of 1.5 mL of the pure proteins VP2, VP2-FII or VP2-FIV, or mixtures of them with a total protein concentration of 0.75 mg/mL, against 3 × 50 mL of phosphate buffered saline (PBS) solution (NaCl 137 mM, KCl 2.7 mM, Na_2HPO_4 10 mM, KH_2PO_4 1.8 mM, pH 7.4) at 4 °C for 36 h. After dialysis samples were microfiltered (first 2.0 μm and then 0.22 μm) with polyvinylidene fluoride (PVDF) syringe filters (Millipore). The in vitro assembly approach for the production of hybrid VLPs composed by VP2 and its chimeras allows testing different ratios of concentration just by changing the amounts of each protein during the assembly process.

2.6. VLPs characterization

DLS measurements were performed using a Zetasizer μV (Malvern) equipped with a photodiode laser (830 nm). Refractive index was measured in a refractometer and viscosity was assumed according to the molarity of GdnHCl solutions. Protein concentration was adjusted to 0.3 mg/mL. DLS measurements for each sample were run at 20 °C. Samples were also analyzed by isopycnic centrifugation in continuous CsCl gradients (1.33*g* cm^{-3} in PBS added with 10 mM EDTA and 0.5% Triton X-100) (Sánchez-Rodríguez et al., 2012). Ultracentrifugation was carried out in a Beckman Optima XL90 Ultracentrifuge equipped with an SW 60 Ti rotor, at 483,750 × *g* for 9 h at 20 °C.

For the analysis of VLPs by TEM, samples were applied to Formvar-coated copper grids and negatively stained with 3% phos-