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Enhanced assembly and colloidal stabilization of primate erythroparvovirus 1 virus-like particles for improved surface engineering

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ABSTRACT

Virus-like particles (VLPs) are the product of the self-assembly, either *in vivo* or *in vitro*, of structural components of viral capsids. These particles are excellent scaffolds for surface display of biomolecules that can be used in vaccine development and tissue-specific drug delivery. Surface engineering of VLPs requires structural stability and chemical reactivity. Herein, we report the enhanced assembly, colloidal stabilization and fluorescent labeling of primate erythroparvovirus 1 (PE1V), generally referred to as parvovirus B19. *In vitro* assembly of the VP2 protein of PE1V produces VLPs, which are prone to flocculate and hence undergo limited chemical modification by thiol-specific reagents like the fluorogenic monobromobimane (mBBr). We determined that the addition of 0.2 M L-arginine during the assembly process produced an increased yield of soluble VLPs with good dispersion stability. Fluorescent labeling of VLPs suspended in phosphate buffered saline (PBS) added with 0.2 M L-Arg was achieved in significantly shorter times than the flocculated VLPs assembled in only PBS buffer. Finally, to demonstrate the potential application of this approach, mBBr-labeled VLPs were successfully used to tag human hepatoma HepG2 cells. This new method for assembly and labeling PE1V VLPs eases its applications and provides insights on the manipulation of this biomaterial for further developments.

Statement of Significance

Application of virus-derived biomaterials sometimes requires surface modification for diverse purposes, including enhanced cell-specific interaction, the inclusion of luminescent probes for bioimaging, or the incorporation of catalytic properties for the production of enzyme nanocarriers. In this research, we reported for the first time the colloidal stabilization of the primate erythroparvovirus 1 (PE1V) virus-like particles (VLPs). Also, we report the chemical modification of the natural Cys residues located on the surface of these VLPs with a fluorescent probe, as well as its application for tagging hepatoma cells *in vitro*. Keeping in mind that PE1V is a human pathogen, virus–host interactions already exist in human cells, and they can be exploited for therapeutic and research aims. This study will impact on the speed in which the scientific community will be able to manipulate PE1V VLPs for diverse purposes. Additionally, this study may provide insights on the colloidal properties of these VLPs as well as in the effect of different protein additives used for protein stabilization.

1. Introduction

Virus-like particles (VLPs) are excellent candidates for the production of vaccines and tissue-specific drug delivery [1,2]. These particles as well as viral capsids have been successfully used as

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scaffolds for the construction of novel biomaterials [3–7]. VLPs are constructed, either *in vivo* or *in vitro*, from proteins that constitute the virus capsid. The resulting particles are therefore very similar in structure and antigenic properties to natural virions, but they are non-infective.

Many types of viruses have been employed for the construction of VLPs, including parvovirus [8–12]. Several groups have constructed VLPs from primate erythroparvovirus 1 (PE1V), formerly known as parvovirus B19 (B19V) [11]. PE1V belongs to the Erythroparvovirus genus in the Parvovirinae subfamily of the Parvoviridae family. This virus has been associated with diseases such as aplastic crisis in chronic hemolytic anemia, arthritis, chronic anemia in immunodeficiency syndromes, and hydrops fetalis. However, PE1V is most commonly related with the so-called "fifth disease" or erythema infectiosum [13]. PE1V presents a narrow tropism and the host restricts its replicative ability. This virus targets erythroid progenitor cells in the bone marrow, cells that are permissive to PE1V infection [12]. PE1V is internalized through clathrin-dependent endocytosis and spreads throughout the endocytic pathway to the lysosome. This entry pathway suggests that these VLPs could be used to carry therapeutic molecules to the lysosome, perhaps to treat lysosomal diseases.

PE1V is icosahedral with a diameter of 18–25 nm. The capsid harbors a single molecule of ssDNA of about 5600 bp. The capsids of PE1V virions are composed of 60 subunits of 2 structural proteins – VP1 (5%) and VP2 (95%) – with molecular weights of 84 and 58 kDa, respectively. These proteins are identical at their C-termini and differ by the presence of 227 additional residues at the N-terminus of VP1, the commonly designated VP1 unique region (VP1u) [12]. Nevertheless, PE1V VLPs can be obtained *in vivo* from expression of VP1 and VP2 or only VP2 in heterologous systems [14]. The *in vitro* self-assembly of PE1V VP2 into VLPs has also been reported [10,15]. Inclusion bodies (IBs) of the PE1V VP2 can be produced in large scale from *Escherichia coli*. These IBs can be solubilized with guanidinium hydrochloride (GdnHCl) and purified. The unfolded VP2 can then be reassembled into VLPs through a dialysis step against PBS for the removal of GdnHCl. During assembly, the particles flocculate producing high turbidity with a concomitant low yield of soluble particles (13–16%). This flocculation results in downstream problems for the manipulation of these particles for industrial or pharmaceutical purposes. In particular, flocculation decreases the reactivity of chemical groups located on the surface of VLPs, which can be used for surface engineering of the particles.

Several reports are available on the use of additives to aid in the refolding of proteins expressed as IBs [16,17]. Herein, we report the use of L-Arg during the assembly process of PE1V VP2 into VLPs. The resulting particles were characterized for size and stability. Furthermore, VLPs were labeled with monobromobimane (mBBr) to measure the reactivity of surface thiol groups in the presence of L-Arg. Finally, the biotechnological potential of the labeled particles was tested by tagging human hepatoma HepG2 cells *in vitro*.

2. Materials and methods

2.1. Chemicals and biochemicals

All chemicals were purchased from Sigma–Aldrich (USA and Mexico). Culture media were purchased from Life Technologies (USA). SYTOX-Green was purchased from Thermo Fisher Scientific (USA). Protino Ni-TED resin was purchased from Macherey Nagel (Germany).

2.2. Computational methods

The structure of PE1V-VP2 VLPs (PDB 1S58) was visually analyzed with PyMOL 1.7 (Schrödinger, LLC). Surface area exposed to solvent was calculated according to Gerstein's method [18] with a probe radius of 1.4 Å.

2.3. Expression and purification of PE1V VP2

VP2 protein was expressed in *E. coli* BL21(DE3) cells harboring a synthetic VP2 gene inserted in the pET22b(+) vector, as previously described [10]. This recombinant protein has a 6xHis tag at its C-terminus to simplify the purification procedure by IMAC using Protino Ni-TED resin in denaturing conditions [10]. The resulting protein is kept in solubilization buffer (5 M GdnHCl, 20 mM Tris–HCl, 0.15 M NaCl, 1 mM EDTA, 1 mM DTT, pH 8) until use. The protein concentration was estimated by the bicinchoninic acid assay (BCA) and 280 nm absorbance.

2.4. In vitro assembly of VLPs

Assembly of VP2-VLPs was carried out by dialysis of 1 mL of the recombinant protein (0.75 mg/mL in solubilization buffer) three times against 50 mL of the adequate buffer supplemented with different amounts of L-Arg. Solubilization buffer was also used for storing the protein for few days at room temperature or for up to 3 months at 4 °C. For pH studies, dialysis was performed against 50 mM sodium acetate buffer for pH 4.0, 50 mM sodium phosphate for pH 7.4 and 50 mM Tris for pH 8.5. For further analyzes, dialysis buffer consisted of PBS, pH 7.4 or PBS supplemented with L-Arg, (0.05–0.40 M), either in the presence or the absence of 10% Glycerol. All dialyses were carried out at 4 °C for 36 h.

2.5. Characterization of VLPs

VLP particle size was determined using dynamic light scattering (DLS) in a Zetasizer μV (Malvern) equipped with a photodiode laser (830 nm). Dispersant refractive index was measured (1.33) in a refractometer (Atago Abbe, NAR-3T), and viscosity was assumed to be 1.003 cP [10]. Samples were filtered first through 2.0 μm and then 0.22 μm polyvinylidenefluoride (PVDF) syringe filters (Millipore). Protein concentrations were adjusted between 0.1 and 0.3 mg/mL. Measurements for each sample were averaged over 10 runs of 10 measurements per run at 20 °C.

Samples were also analyzed by isopycnic centrifugation in continuous CsCl gradients (1.33 g cm^{-3}) in 50 mM of the same buffer used during the assembly, with 10 mM EDTA and 0.5% Triton X-100 [10]. To assess the effect of SDS on the stability of particles, SDS was added either to the CsCl solution or only to the protein sample. Ultracentrifugation was carried out in a Beckman Optima XL90 Ultracentrifuge using an SW 60 Ti rotor at 483,750×*g* for 9 h at 20 °C.

For TEM analyses, samples were adsorbed onto Formvar-coated copper grids for 5 min. Excess sample was then removed by blotting with filter paper. The grid was negatively stained with phosphotungstic acid (3%, pH 7.0) for 5 min and the excess of colorant was removed. The grids were examined in a transmission electron microscope (JEOL JEM-2010).

Thermal stability of soluble VLPs was determined by following the turbidity of the samples at 450 nm. Soluble particles (200 μL), assembled in different solutions of PBS with or without L-Arg and Glycerol, were incubated at 37 and 50 °C for 60 min. At lower temperatures, only the control without L-Arg produced turbidity at long times.

2.6. Thiol labeling of VLPs with monobromobimane (mBBr)

Particle labeling was performed by adding 100 μL of 10 mM monobromobimane (4-bromomethyl-3,6,7-trimethyl-l,5-diazabicy