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In vitro assembly of polymorphic virus-like particles from the capsid protein of a nodavirus

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ABSTRACT

Viral capsid proteins are programmed to assemble into homogeneous structures in native environments; but the molecular details of these assembly pathways are seldom clearly understood. In order to define the chain of events in the construction of a minimal system, we attempted controlled assembly of the capsid protein of a small insect nodavirus, Flock House Virus (FHV). Bacterial expression of the FHV capsid protein, and subsequent *in vitro* assembly, generated a heterogeneous population of closed particles. We show that in spite of the altered structure, these particles are capable of membrane disruption, like native viruses, and of incorporating and delivering foreign cargo to specific locations. The unique structure and characteristics of these particles extends our understanding of nodavirus assembly. Additionally, the establishment of a bacterial production system, and methods for *in vitro* assembly and packaging are of considerable benefit for biotechnological applications of FHV.

1. Introduction

The self-assembly of highly symmetric virus capsids exhibits remarkable physical homogeneity *in vivo*. Multiple copies of capsid protein(s) and genome associate spontaneously and precisely to form regular capsid shells, rarely generating unusual or defective particles. In spite of innumerable efforts to understand the pathways of association by mutational studies, heterologous expression and *in vitro* assembly of viral capsid proteins, the molecular details of these processes remain to be elucidated for most viruses. In addition, developing methodologies for directed or controlled assembly of capsids *in vitro* has become essential due to the current applications of virus-based nanostructures in the fields of nanomedicine and nanotechnology.

Expression of virus structural proteins in heterologous systems or *in vitro* assembly has frequently led to the formation of "alternative" forms of capsids, which indicates that under non-native conditions, capsid proteins have the inherent ability to assume diverse modes of interactions. Studies have shown that assembly reactions, *in vivo* or *in vitro*, could be led off-path and the final product substantially modulated by – (a) mutations or truncations in capsid proteins (Barcena et al., 2004; Calhoun et al., 2007), (b) variations in charge composition through alteration in pH or availability of divalent cations (Barcena et al., 2004; Calhoun et al., 2007; Erickson and Rossmann, 1982; Erickson et al., 1985; Kanesashi et al., 2003; Lokesh et al., 2002; Salunke et al., 1989; Sangita et al., 2004; Sastri et al., 1997, 1998; Satheshkumar et al., 2005; Savithri and Erickson, 1983), and, (c) variations in the nature of the nucleic acid scaffold (Kler et al., 2013; Krol et al., 1999). Assembly of virus-like particles (VLPs), can be affected *in vitro* by factors such as resistance markers and osmotic pressure (Brown et al., 2009). Coarse-grained molecular dynamics simulation has shown that assembly of regular icosahedral structures can be subverted by varying parameters such as capsid protein concentration and temperature, leading to a gamut of non-icosahedral, but enclosed and stable structures (Nguyen and Brooks, 2008). These studies have underlined the requirement for uniformity in parameters for correct assembly, and have also indicated that the presence of regulatory events or partners under *in vivo* conditions might be essential to prevent formation of mis-assemblies, thus ensuring fidelity and guaranteeing the production of native particles (Annamalai and Rao, 2005; Seo et al., 2012). Assembling viral capsids *in vitro* requires a thorough understanding of the factors required to promote alternative interactions under heterologous conditions.

Flock House Virus (FHV), an insect nodavirus utilized as an antigen display platform and antitoxin (Manayani et al., 2007; Schneemann et al., 2012), is an effective model for studying self-assembly of viral capsids. FHV is a structurally simple virus, with a T=3 icosahedral capsid constructed from 180 copies of a single capsid protein, alpha (Fig. 1A), encapsulating a single stranded,

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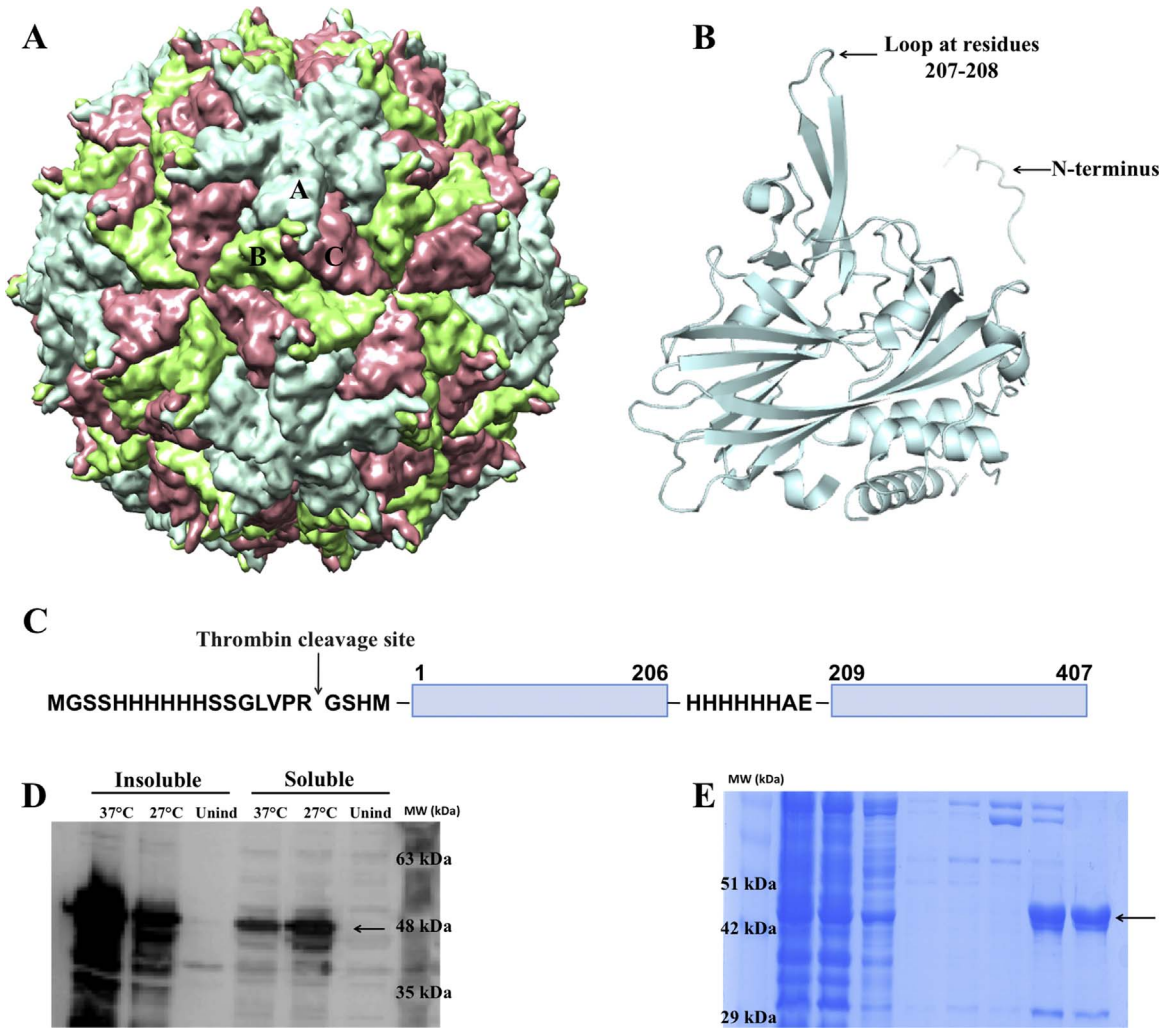

Fig. 1. (A) A representation of Flock House Virus based on its X-ray crystal structure (PDB ID: 4FTB). (B) A ribbon representation of the tertiary structure of FHV alpha, with the N-terminus (beginning from residue 20 in the crystal structure) and the loop at residues 207–208 indicated by arrows. (C) Schematic showing the modified version of alpha with two 6x-his-tags, one at the N-terminus and another at residues 207–208. (D) Bacterial expression of modified FHV alpha. Western blot with an anti-His monoclonal antibody, showing soluble and insoluble fractions of protein induced at 27 °C (8 h) and 37 °C (4 h). (E) SDS-PAGE showing FHV alpha purified using Nickel affinity chromatography. The position of alpha is indicated by arrows.

bipartite, positive sense RNA genome (Fisher and Johnson, 1993). Milligram quantities of FHV capsids, 30 nm in diameter, are usually generated by infection or transfection of DL-1 cells (Gallagher and Rueckert, 1988; Schneemann and Marshall, 1998). VLPs of FHV, structurally indistinguishable from native virus, and containing an equivalent amount of non-genomic, insect cell derived nucleic acid, are produced by infecting Sf21 cells with recombinant baculovirus expressing alpha (Schneemann et al., 1993). Native or non-genomic nucleic acid is presumably required for capsid assembly, since empty capsids or free capsid protein have not been isolated from either of the expression systems so far. No auxiliary virus-derived or host-cell factors required for assembly have been identified either, suggesting that all the information needed for the formation of higher-order assemblies is inherent in the capsid protein. The N-terminus of alpha has been found to be particularly essential, since truncating residues 1–50 completely inhibits assembly, while deleting residues 1–31 yields highly heterogeneous structures, including wild-type T=3 particles, small bacilliform-like and irregular particles (Dong et al., 1998). The preponderance of positively charged residues in this region indicates that it might initiate assembly by interacting with cognate or non-cognate RNA (Marshall and Schneemann, 2001; Venter et al., 2009).

In this study, we attempted *in vitro* assembly of FHV capsids from alpha expressed in an *E. coli* expression system. A removable hexahistidine tag (6x-his-tag) was introduced at the N-terminus of alpha in order to sterically hinder assembly-related associations, and thus preclude formation of particles in cells. An additional 6x-his-tag was introduced between residues 207–208 of alpha, a region exposed as loops on the capsid, and capable of accommodating insertions without compromising any quaternary interactions (Manayani et al., 2007) (Fig. 1B). We found that removal of the N-terminal tag from purified alpha triggers productive assembly *in vitro*, however, the particles generated were morphologically distinct from native virus or VLPs, being circular, closed and polydisperse. These alternative particles were capable of encapsulating extraneous material included in the assembly reaction, and could be directed to specific locations by a small, targeting peptide incorporated in the capsid protein. Taken together, our data demonstrates that the capsid protein of FHV has the potential to assemble into diverse structures, with the polymorphism being manifested under non-ideal conditions, and that these unusual assembly pathways can be harnessed for biomedical applications.