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Three-dimensional structure and stoichiometry of *Helmintosporium victoriae190S* totivirus

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Abstract

Most double-stranded RNA viruses have a characteristic capsid consisting of 60 asymmetric coat protein dimers in a so-called T = 2 organization, a feature probably related to their unique life cycle. These capsids organize the replicative complex(es) that is actively involved in genome transcription and replication. Available structural data indicate that their RNA-dependent RNA polymerase (RDRP) is packaged as an integral capsid component, either as a replicative complex at the pentameric vertex (as in reovirus capsids) or as a fusion protein with the coat protein (as in some totivirus). In contrast with members of the family Reoviridae, there are two well-established capsid arrangements for dsRNA fungal viruses, exemplified by the totiviruses L-A and UmV and the chrysovirus PcV. Whereas L-A and UmV have a canonical T = 2 capsid, the PcV capsid is based on a T = 1 lattice composed of 60 capsid proteins. We used cryo-electron microscopy combined with three-dimensional reconstruction techniques and hydrodynamic analysis to determine the structure at 13.8 Å resolution of *Helminthosporium victoriae 190S virus* (Hv190SV), a totivirus isolated from a filamentous fungus. The Hv190SV capsid has a smooth surface and is based on a T = 2 lattice with 60 equivalent dimers. Unlike the RDRP of some other totiviruses, which are expressed as a capsid protein-RDRP fusion protein, the Hv190SV RDRP is incorporated into the capsid as a separate, nonfused protein, free or non-covalently associated to the capsid interior.

Keywords: Hv190SV; Double-stranded RNA; Virus capsid; Cryo-electron microscopy; Three-dimensional structure

Introduction

Double-stranded (ds)RNA viruses share numerous general architectural and functional principles (Lawton et al., 2000; Mertens, 2004). With the exception of birnaviruses (Böttcher et al., 1997; Castón et al., 2001) and chrysoviruses (Castón et al., 2003), all dsRNA viruses from the mammalian reoviruses to the bacteriophage ϕ6, including plant and fungal viruses, share a specialized capsid consisting of 120 protein subunits in a so-called T = 2 organization. T = 2 capsids are used as a template to prime the assembly of surrounding capsids (Grimes et al., 1998; Johnson and Reddy, 1998), as organizers of the genome and replicative complex within them (Lawton et al., 2000; Patton et al., 1997), and as molecular sieves to isolate dsRNA molecules or replicative intermediates (Diprose et al., 2001; Lawton et al., 1997). This tendency is followed by numerous well-studied members of the family *Reoviridae*. There are nonetheless many fungal and protozoal dsRNA viruses that remain to be characterized structurally. L-A virus (Wickner, 2001), the type species of the genus *Totivirus* in the family *Totiviridae*, which infects the yeast *Saccharomyces cerevisiae*, is the best molecularly and structurally characterized totivirus, and has a canonical T = 2 layer (Castón et al., 1997). The

Abbreviations: 3DR, three-dimensional reconstruction; CP, capsid protein; cryo-EM, cryo-electron microscopy; ds, double-stranded; ctf, contrast transfer function; Hv190SV, *Helminthosporium victoriae 190S virus*; RDRP, RNA-dependent RNA polymerase.

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totivirus UmV, which infects the corn pathogen *Ustilago maydis*, also has a T = 2 layer (Cheng et al., 1994). Furthermore, *Penicillium chrysogenum virus* (PcV), a symptomless fungal virus of the family *Chrysoviridae* (Ghabrial et al., 2005; Jiang and Ghabrial, 2004), has an authentic T = 1 capsid with 60 equivalent protein subunits (Castón et al., 2003).

This study addresses the capsid structure of the totivirus *Helminthosporium victoriae 190S virus* (Hv190SV). Hv190SV infects the phytopathogenic filamentous fungus *Helminthosporium victoriae* (teleomorph*: Cochliobolus victoriae*), the causal agent of Victoria blight of oats (Sanderlin and Ghabrial, 1978). The Hv190SV genome is a single-segment dsRNA molecule (5178 bp; (Huang and Ghabrial, 1996)) that encodes the capsid protein (CP, 772 residues; M_r 81,200; apparent M_r ~88,000), and a minor virion-associated protein, the RNA-dependent RNA polymerase (RDRP, 770 residues; M_r 84,300; apparent M_r ~92,000). In a major difference from L-A virus, however, Hv190SV RDRP is expressed as a separate nonfused protein rather than as a CP-RDRP fusion protein, even though CP and RDRP open reading frames (ORF) have overlapping stop and start codons (Soldevila and Ghabrial, 2000). This implies that the RDRP is incorporated into the capsid interior as a free protein, thus sharing certain analogies to the PcV particle (Castón et al., 2003). Although the Hv190SV capsid is encoded by a single gene, it contains three closely related CP, p78, p83 and p88 (Ghabrial et al., 1987). p88 is the primary translation product, and p83 and p78 represent posttranslational proteolytic processing products of p88 at its C-terminal region (Huang and Ghabrial, 1996; Huang et al., 1997). In addition, whereas p88 and p83 are phosphoproteins, p78 is nonphosphorylated (Ghabrial and Havens, 1992). Purified Hv190SV virion preparations contain two types of particles, 190S-1 and 190S-2, that differ slightly in sedimentation rates and in capsid composition, and probably represent different stages in the virus life cycle (Ghabrial, 1994). The 190S-1 capsids contain p88 and p83 in equimolar amounts, and the 190S-2 capsids are composed of similar amounts of p88 and p78.

These functional and molecular features make characterization of the Hv190SV capsid structure an attractive prospect. We used cryo-electron microscopy (cryo-EM) combined with three-dimensional reconstruction (3DR) and complementary biophysical techniques to determine the structure at 13.8 Å resolution and the protein stoichiometry of the Hv190SV capsid. We found that Hv190SV, as a representative of totiviruses that infect filamentous fungi (forming a separate cluster by phylogenetic analysis), shares the canonical T = 2 capsid with totiviruses that infect yeast and possibly those that infect parasitic protozoa. Comparative analyses of L-A and Hv190SV CP nonetheless suggest a new arrangement of the Hv190SV capsid protein domains.

Results

Virions and virus-like particle purification

Hv190SV virions and empty capsids were purified after two cycles of differential centrifugation and linear sucrose density gradient centrifugation (see Materials and methods). Whereas full virions were purified from a naturally infected fungal isolate, Hv190SV empty capsids were expressed in a virus-free fungal host transformed with a CP recombinant vector (pCB190S-mt; Annamalai and Ghabrial, unpublished results). In both cases, homogeneous populations of particles were obtained, as tested in Coomasssie blue stained SDS-PAGE gels (Fig. 1A). Fractions enriched in Hv190SV particles were identical and consisted of two major polypeptides (p88 and p78) in equimolar amounts and a minor polypeptide (p83). As predicted, no other minor proteins were detected in full virion preparations, although viral RDRP is known to be present. The final yield of purified 190SV virions from the naturally infected isolate is generally low, since two cycles of density gradient centrifugation are required to remove the chrysovirus Hv145S, commonly associated with Hv190SV (Sanderlin and Ghabrial, 1978). A substantially higher yield (20–50 fold) of

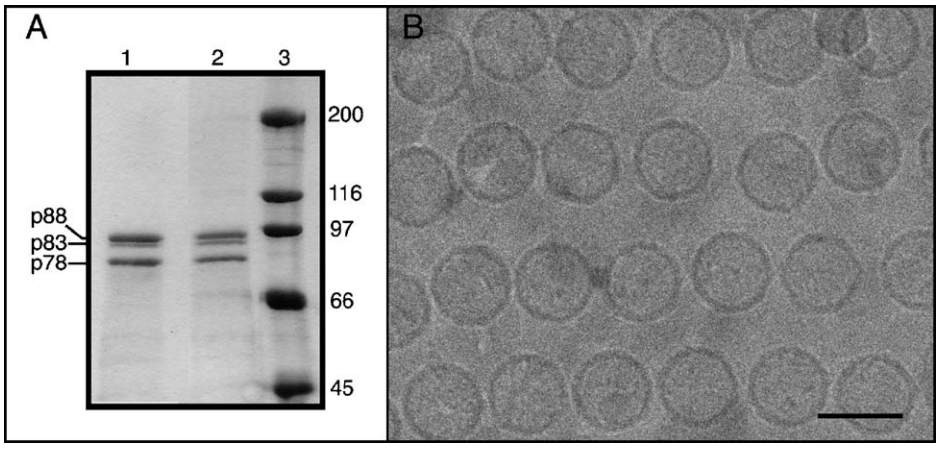

Fig. 1. Analysis of Hv190SV empty particles by SDS-PAGE and cryo-electron microscopy. (A) Purified empty particles from a virus-free isolate transformed with a vector containing CP coding region (lane 1), and purified Hv190S virions from a naturally infected fungal isolate (lane 2) were analyzed for protein content by 7.5% SDS-PAGE and stained with Coomassie brilliant blue. Positions of p88, p83 and p78 are indicated. The apparent molecular weights of protein standards are indicated (lane 3). (B) Cryoelectron micrograph of Hv190SV empty capsids. Scale bar, 50 nm.