

Electron Cryo-Microscopy Studies of *Helminthosporium victoriae* Virus 190S

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The parasitic nature of viruses is a source of intense interest owing to the wide variety of potential hosts, which include humans, livestock, fungi, agricultural crops, as well as many others [1]. Viruses are differentiated by variations in properties such as size, capsid morphology, genomic composition, replication strategy, and host target [1, 2]. The family *Totiviridae* of dsRNA viruses is characterized by virions with a non-segmented dsRNA genome that infect yeast, smut, filamentous fungi, protozoa and shrimp [2-4]. The subject of this study, *Helminthosporium victoriae* virus 190S (HvV190S) was discovered in 1978 in the filamentous fungus *Helminthosporium victoriae* (*H. victoriae*) [2,4]. Virions have a capsid with a smooth outer surface and a maximum diameter of ~400 Å. The single dsRNA genome segment codes for two viral proteins: coat protein (CP, 81 kDa) and RNA dependent RNA polymerase (RdRp, 92 kDa) [2-4].

We used the methods of electron cryo-microscopy (cryoEM) and 3D image reconstruction to examine the native structure of three types of HvV190S particles. These included (i) virions, (ii) capsids+RdRp (lacks the dsRNA genome but contains the RdRp), and (iii) empty capsids (devoid of dsRNA and RdRp). Small aliquots (3.5 µl) of purified samples were applied to support films made of continuous carbon that had been glow-discharged for 20 sec. in an Emitech K950X evaporator and the grids were then plunge vitrified in an FEI Mark III Vitrobot. Micrographs were recorded on Kodak SO-163 electron image film using an FEI Polara Microscope operated at 200kV under minimal dose conditions (24 e-/Å²) and at nominal magnifications of 39,000X and 59,000X (Fig. 1A-C). Micrographs were digitized on a Nikon 8100 microdensitometer at step size of 6.35 µm (corresponds to 1.076 and 1.628 Å/pixel at the specimen for the two respective magnifications). Program ctfind3 was used to estimate the defocus of each micrograph and all other image processing and reconstruction steps were carried out using the programs AUTO3DEM and RobEM [6 - 8].

Preliminary cryo-reconstructions of the three different types of HvV190S particle, computed at respective resolutions of 7.4Å, 9.8Å, and 7.8Å, reveal closely similar capsid structures (Fig. 2) and four well-defined, concentric rings of density attributed to the dsRNA genome in the virion (Fig. 2B). Difference map analysis reveals that although most of the CP subunit remains rigid in the three particles, small portions of the subunit do change conformation in response to the presence or absence of genome and/or RdRp.

References

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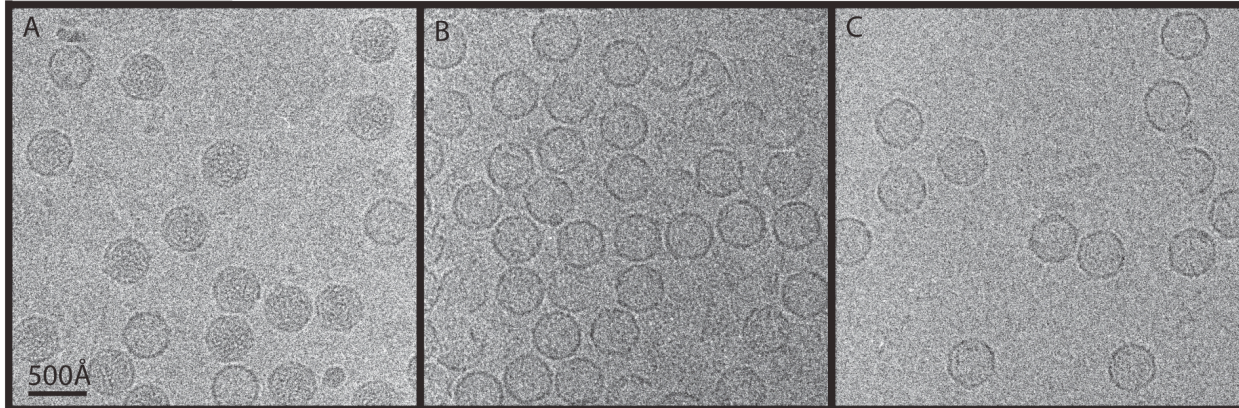


Fig. 1. Cryoelectron micrographs of unstained, vitreous HvV190S (a) virions, (b) capsid+RdRp, and (c) empty capsids. Cryoelectron micrographs show particles to have a smooth outer surface and a relatively thin capsid shell.

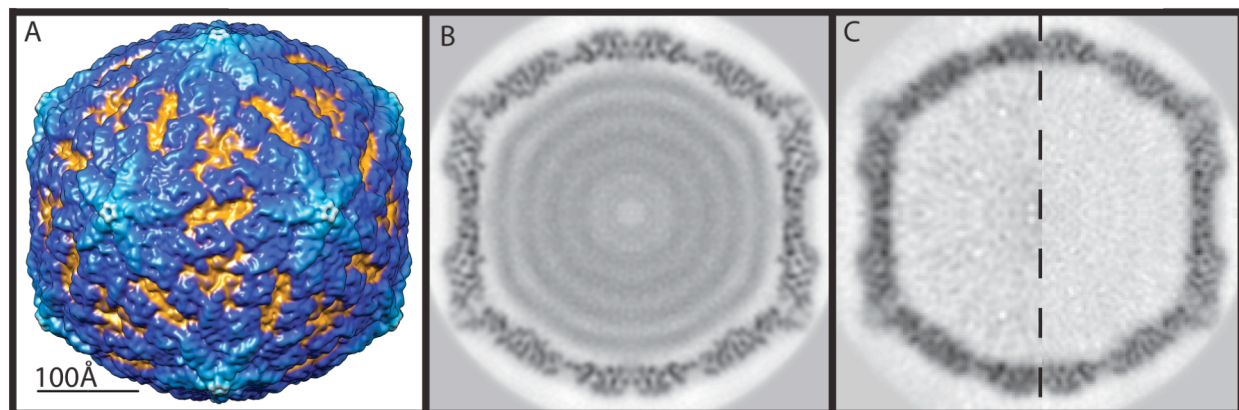


Fig. 2. (A) Radially color-cued surface representation of the HvV190S virion with the inner most portion of the outer capsid surface rendered orange and higher radial features transitioning from dark blue to light blue and then white. Surface features on the capsid are similar on the other two particle types (data not shown). Planar, 1-Å-thick equatorial section through an icosahedrally-averaged reconstruction of HvV190S virion (B), capsid+RdRp (C, left half) and the empty capsid (C, right half) at 7.4, 9.8, and 7.8 Å resolutions, respectively (darkest regions correspond to densest regions in the density map).