

Cryo-electron microscopy and image reconstruction of PBCV-1, an algal virus with T=169 lattice symmetry

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Paramecium bursaria Chlorella virus 1 (PBCV-1), the type species of the *Phycodnaviridae* family, is a large, plaque-forming virus that replicates in certain unicellular, exsymbiotic, *Chlorella*-like green algae [1]. The virion ($\sim 1 \times 10^9$ daltons) has a 330,740 base pair dsDNA genome encapsulated within an icosahedral shell of ~ 190 nm diameter. Purified virions contain more than 50 different proteins, which account for 65% of the total virion mass and range in size from 10 to more than 200kDa. Vp54, the major capsid protein (40% of total virion protein) is a myristilated glycoprotein but it is not phosphorylated. Three other proteins are located on the virus surface. Virions contain 5-10% lipid, located inside the glycoprotein shell, which is required for virus infectivity [1,2]. Little is known about the detailed ultrastructure of PBCV-1 virions, though some information has been obtained with sectioned, negatively-stained, and metal-shadowed specimens [1,3]. We have used cryo-electron microscopy and three-dimensional (3D) image reconstruction methods [4-6] to examine the native morphology of PBCV-1.

Aliquots (3.5 μ l) of purified PBCV-1 samples [7] were applied to freshly-prepared, perforated carbon films. The grid was blotted with filter paper, rapidly plunged into liquid ethane, transferred to a cryo-holder and maintained at -186°C . Images were recorded on film at 38,000x nominal magnification in a Philips CM200 FEG microscope at 200kV, at $\sim 2.2\mu\text{m}$ underfocus, and with an electron dose of 2200e⁻/nm² (Fig. 1A). A total of 446 PBCV-1 particle images were extracted from 15 micrographs (digitized at 21 μm step size), and 356 of these images were used to compute a 3D image reconstruction to a resolution limit of 26Å. No corrections were made to compensate for the effects of the microscope contrast transfer function [8].

The image reconstruction reveals several features about PBCV-1 morphology (Fig. 1B-D). The capsid has a distinct icosahedral shape, with a maximum diameter of 190nm along the five-fold axes. The outer dimensions of the virion at the two- and three-fold axes are both ~ 165 nm. The capsid consists of 1692 doughnut-like capsomers arranged in a T=169, skew icosahedral lattice [9] (h=8, k=7; Fig. 1C). Twelve capsomers, each ~ 7 nm diameter, occur as pentamers at the five-fold vertices. The remaining 1680 capsomers are trimeric structures, also about 7nm in diameter and 7.5nm high. The prominent, cylinder portion of each trimeric capsomer extends 5nm above the surface of the capsid shell and most appear to have axial channels. The capsomers interconnect at their bases in a contiguous shell of density of 2-2.5nm thickness (seen in cross sectioned views of the reconstruction: not shown). The stoichiometry of trimers is consistent with the PBCV-1 capsid having 5040 copies of Vp54. The difference in oligomer state but similar size of the two types of capsomers suggests that the pentamers are composed of one of the minor capsid proteins. In addition, unlike the trimeric capsomers, each pentamer has a cone-shaped, axial cavity at its base.

Results at higher resolution will provide better correlation between structural and biochemical data for this complex virus. Tilt experiments [10] are also needed to distinguish the skew class (T=169 *laevo* or T=169 *dextro*) and thereby determine the absolute hand of the structure [11].

References

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11. We thank R. Ashmore for assistance with programming. Work supported in part by grants from the NIH (GM-33050) and NSF (MCB-9527131) to T.S.B.

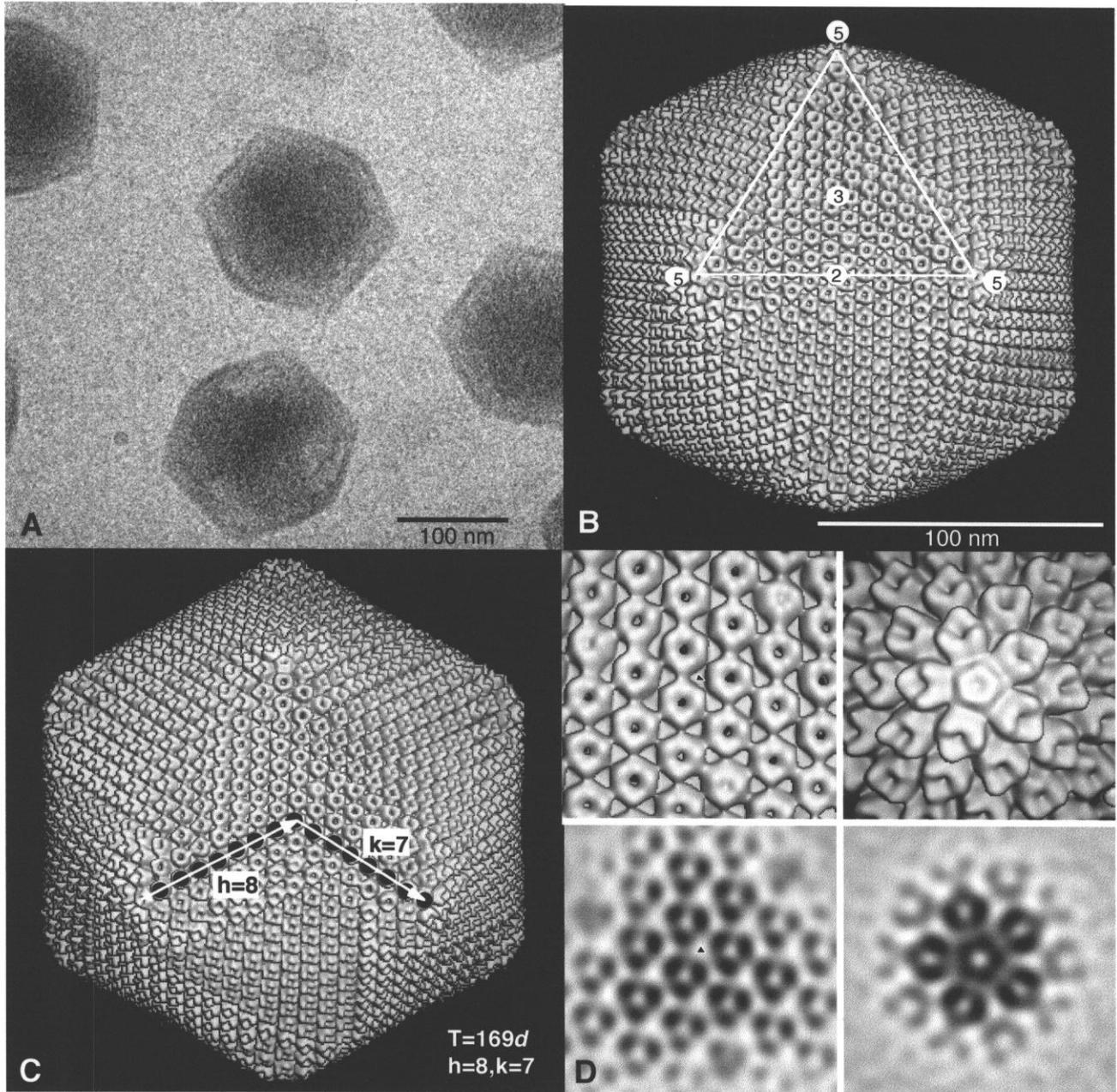


FIG. 1. A. Micrograph of vitrified of PBCV-1 sample, suspended over holes in a carbon substrate. This image, recorded on a $1K^2$ slow-scan CCD camera, shows a well-defined outer capsid and a non-uniformly distributed interior mass. B. Shaded-surface view of 3D reconstruction viewed along 2-fold axis. The white triangle outlines an icosahedral face and numbers identify icosahedral symmetry axes. C. View as in B, but along 3-fold direction. The $T=169d$ lattice symmetry is indicated by the arrangement of hexavalent and pentavalent capsomers (i.e. those surrounded by six or five other capsomers, respectively). Black circles highlight eight steps in the h direction followed by seven in the k direction that define the path between adjacent 5-fold vertices. Note: the true PBCV-1 structure may be the enantiomer of the one shown (i.e. be $T=169\ell$), because the absolute hand of the reconstruction remains to be determined. D. Close-up views along 3-fold (left; small triangles identify 3-fold positions) and 5-fold (right) axes of the PBCV-1 density map, represented as shaded-surfaces (top) or as density projections (bottom; darkest shades represent highest projected density). The trimeric nature of the hexavalent capsomers is clearly evident. Projection images include planar slabs of density, 3.3nm (left) and 4.4nm (right) thick, and include the most radially extended features in each view.