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Supporting Material

Backbone Trace of Partitivirus Capsid Protein from Electron Cryomicroscopy and Homology Modeling

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SUPPORTING MATERIAL

The Supporting Material includes Materials and Methods, Fig. S1 legend, and Fig. S1. See published article for references corresponding to the numbered citations.

MATERIALS AND METHODS

Viruses

PsV-S and -F virions were grown and purified as previously described (8,20).

CryoTEM

Small aliquots (~2 to 3 μl) of purified virions were vitrified, and cryoTEM image data were recorded, essentially as described for earlier work on PsV-S (20). For PsV-F a total of 193 micrographs were recorded in an FEI Polara microscope on a $4K^2$ Gatan Ultrascan CCD camera at a nominal magnification of 104,300, yielding an effective pixel size of 1.44 Å. All PsV-F images were acquired with the automated LEGINON system (27). The PsV-S image data (16 micrographs) were recorded on Kodak SO-163 electron-image film in an FEI Sphera microscope operated at a nominal magnification of 50,000. These micrographs were digitized at 6.35-μm intervals on a Nikon Coolscan 8000 microdensitometer (pixel size = 1.27 Å). Both sets of image data were acquired under low-dose conditions (~15 e/$Å^2$) at liquid nitrogen temperatures with 200 keV electrons, and they exhibited minimal specimen drift and astigmatism. Images of PsV-S and -F were recorded at underfocus settings of 1.4–2.2 μm and 0.9–3.3 μm, respectively.

3D image reconstructions

The program RobEM (http://cryoem.ucsd.edu/programDocs/runRobem.txt) was used to extract individual particle images and to estimate the defocus level of each micrograph. AUTO3DEM (28) (version 3.12) was used to perform all initial steps in determining particle origins and orientations, 3D reconstruction, and resolution estimation. A PsV-F density map was computed from 8,199 particle images, each 315^2 pixels in size, and made use of a previously determined 8.0-Å cryo-reconstruction of PsV-F (8) as a starting model. Similarly, a PsV-S map was computed from 14,252 particle images, each 381^2 pixels in size, starting from a previously determined 7.3-Å cryo-reconstruction of PsV-S (20). Corrections to compensate in part for the effects of the microscope contrast-transfer function were applied to Fourier-transformed image data as described previously (29,30). Once the quality of the maps as measured by Fourier-shell-correlation (FSC) criteria (31) showed no further improvement via automated processing with AUTO3DEM, additional steps were taken to improve each reconstruction. First, all particle images were reboxed to ensure that the refined center of each particle was centered within the box window. Also, the assigned value of the objective lens defocus for each micrograph was redetermined, and more stringent criteria were used to include in the data set only micrographs whose power spectra exhibited clear Thon rings beyond a spatial frequency of ~1/6 Å. Next, the subroutines P3DR and PO^2R were used as stand-alone applications to refine and improve the two cryo-reconstruction maps manually. This primarily involved close monitoring of all iterations of refinement while different combinations of inverse temperature factor (32) and amplitude-weighting schemes (29) were employed. Inverse temperature factors, generally ranging between about 1/100 and 1/400 $Å^2$ during early refinement cycles (~6-7 Å resolution limits) and about 1/50 and 1/200 $Å^2$ towards the end of refinement, were applied during processing to enhance high-spatial-frequency Fourier terms in the reconstructed maps as well as to refine particle origins and orientations. Rather than relying strictly on FSC criteria to gauge the success of refinement, we found it more reliable to inspect features in the newly reconstructed maps to note signs of noise reduction and hence map improvement, especially in well-defined and

recognizable α-helical segments. The existence of a 3.3-Å-resolution PsV-F crystal structure (8) served as an excellent, quality control guide, which allowed us to assess the success or failure of the previous refinement cycle in improving map quality.

When no further improvement to the reconstructed density maps could be made by means of the above strategies, we computed final, sharpened maps of PsV-F and -S. The PsV-F atomic model provided a rational means to select an optimal inverse temperature factor (1/50 $Å^2$) and amplitude weighting factors (d1 = 1.0 and d2 = 0.1, as defined in ref. 29) to compute the final cryoTEM map of PsV-F. These same optimized parameters were subsequently used to compute the final PsV-S map. The resolution of the PsV-F cryo-reconstruction was estimated to be 6.6 Å based on a conservative Fourier-shell correlation of 0.5 31). However, direct comparison of the PsV-F reconstruction to filtered versions of the crystal structure indicated that the actual resolution of the map was slightly better than 5.0 Å (see Fig. 2 and following section). The final PsV-F map was computed from structure factor data out to a spatial frequency limit of 1/4.5 Å, but with data beyond 1/5.0 Å multiplied with a Gaussian function to attenuate the structure factor amplitudes smoothly to zero between 1/5.0 and 1/4.5 Å. The resolution of the PsV-S cryoTEM map was similarly estimated to be 6.6 Å based on FSC analysis, but better than 5.0 Å based on the appearance of secondary-structure features and comparisons with the PsV-F data. Such analysis suggested that the quality of the PsV-S map was at least as good as and perhaps a little better than the PsV-F map as described in more detail below. Hence, the final PsV-S map was also computed with all structure factor data out to 1/4.5 Å with the same Gaussian damping function applied as with PsV-F. The nominal pixel size of the PsV-F cryoEM map was 1.44 Å, but radial scaling of the map against the crystal structure showed the actual pixel size to be 1.42 Å, and thus the calibrated image magnification to be 105,600. Final cryoTEM maps have been deposited in the EMBL-EBI Electron Microscopy Database (ID codes EMD-5161 for PsV-F and EMD-5162 for PsV-S).

For examination of less-ordered components in the particle interiors, including the RNA genome, the maps of PsV-S and -F were also computed at reduced resolution cutoffs. While the general nature of the interior features remained very similar from high to lower resolutions, a resolution of 8–10 Å appeared best for highlighting these features (see Figs. 6 and S1). For all maps, density-contour levels were adjusted between 0.8 and 2.0 σ to optimize display of particular features.

Resolution estimation for final PsV-F map

We estimated the effective resolution of the PsV-F cryo-reconstruction based on its comparison to the PsV-F atomic model (8). First, the program RAVE (33) was used to place the PsV-F atomic model at the center of a cubic unit cell whose size (763.3 Å) matched the cryo-reconstruction and with the icosahedral-symmetry axes of the model and cryo-reconstruction oriented identically. Similarly, and for reference purposes, a series of six crystallographically averaged maps, which were generated at resolutions of 5.97–3.30 Å during the noncrystallographic symmetry averaging and phase-extension steps of the crystal-structure determination, were each placed in the center of an identical cubic cell. Next, structure factors were computed from the PsV-F cryo-reconstruction, the atomic model, and all six averaged maps. The program CCP4 (34) was then used to compute correlation coefficients (CCs) between the structure factors derived from the model vs. those from each of the averaged maps. The CCs remain uniformly high at low spatial frequencies (up to ~1 Å below the resolution limit of the map) but sharply decline to baseline noise levels at the resolution limit of each map (see Fig. 2 *A*). Inspection of the correlation plots indicated that the resolution cutoff (where the the PsV-F crystal structure began to correlate poorly with crystallographic maps calculated at a similar resolution limit) coincided with the point at which the CC drops to about 0.2. Thus, using this CC=0.2 threshold as a criterion, we estimated the effective resolution of our final PsV-F cryoTEM map to be ~4.7 Å.

It is important to note that the CC in this report is not the same as FSC. The CC was calculated by comparing the cryoTEM density map to the X-ray crystal structure, not (as for FSC) by comparing reconstructions computed from two evenly split portions of the cryoTEM

image data. We chose CC=0.2 as the proper threshold not arbitrarily, but rather because it yielded a resolution consistent with the known resolutions of the crystal-derived maps. Our conclusion is thus not that CC=0.2 should always be the proper threshold, but rather that the proper threshold should be defined by the comparative result when both types of maps are available.

Molecular modeling of PsV-S CP subunits

The PsV-F crystal structure was initially fitted into the PsV-S map using the program O (33). The subunit A of the PsV-F CP was subsequently modified based on PsV-S density features to count for differences in length and orientation of secondary structure elements and conformation of various loops. To confirm the polypeptide tracing, secondary structure predictions were performed for PsV-S using the JPred programs (35), and the pattern of predicted secondary structures was used to compared with that of our initial model. To facilitate sequence assignment, primary sequence alignment between PsV-F and PsV-S was calculated using the program T-Coffee (17), with default settings except for the Blosum matrix, as implemented at http://www.ebi.ac.uk/Tools/t-coffee/. The size and shape of the side chain "bumps" were also used to guide sequence determination. Model stereochemistry features were regularized using the program O. The subunit B of our PsV-S model was obtained by applying a local 2f symmetry, which was initially copied from the PsV-F CP dimer and subsequently refined using the program CNS (36), to generate a PsV-S CP arch dimer. Subunit B was then manually adjusted to model any conformational differences between the subunits A and B. In addition, both subunits were fine-tuned to remove any possible close contacts between symmetry-related molecules within the icosahedral capsid. The 2f-symmetry operation and the generation of a complete icosahedral capsid were performed using CCP4 programs and the RAVE program suite (33), respectively. At the end, a final round of real space refinement of the overall model against the cryoEM map was performed using the program RSRef (37,38). The quality of the final PsV-S model, which contains only main chain atoms, was evaluated using the program Moleman2 (39) by calculating bond angles between three consecutive Cα atoms and dihedral angles between four consecutive Cα atoms. About 93% of the aa residues were found in the most favored, additional allowed and generously allowed regions, compared to about 97% observed for refined models determined at 2 Å resolution or higher. Coordinates of the PsV-S main-chain trace have been deposited in the RCSB Protein Data Bank (ID code 3IYM).

Preparation of figures

Graphical representations were prepared using Molscript (40), PyMOL (http://www.pymol.org), and the Chimera visualization software packages (41).

FIGURE S1 Density-deficient features in the RNA region of the PsV-S cryo-reconstruction. The density map computed at 8-Å resolution is shown in a slab view including densities within 6 Å on either side of an equatorial (2f) section. The map was displayed at a low density-contour level (0.8 σ) to include low-density features and colored by radial depth-cueing from red (*low radii*) to blue (*high radii*). The density-deficient gap between the capsid shell and RNA is spanned by rod-like structures tentatively assigned to the N-terminal peptides of CPA and CPB (see Fig. 6). Other density-deficient features are located within the central RNA region at the 5f (5) and 3f (3) symmetry axes as labeled. The different RNA layers as seen in Fig. 6 *A* are not visible in this display due to the lower contour level, which has allowed the layers to merge.