Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602102741

Cucumber Mosaic Virus-Fab Complex Examined by Electron Cryo-Microscopy and Three-Dimensional Image Reconstruction

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Cucumber mosaic virus (CMV) infects a wide variety of plants worldwide and causes economically important diseases of many agricultural crop and ornamental species.[1] The type member of the genus *Cucumovirus*, and a member of the *Bromoviridae* family, this ssRNA virus is spread nonpersistently by aphids from plant to plant during feeding.[2,3]

The 305Å diameter virion is a truncated icosahedron with T=3 quasi-symmetry[4]. Each asymmetric unit is made up of three capsid proteins (subunits A, B, C) with identical amino acid sequences but arranged with quasi-equivalence[4]. The N-termini of the B and C subunits interact to form a hexameric bundle of amphipathic helices below the quasi-six fold axes. However, these same amino acids in the A subunit do not interact with one another in the pentamers and are disordered in the X-ray crystal structure.[4]

In our efforts to gain insight about transmission of CMV by insects, we used electron cryo-microscopy and three-dimensional image reconstruction methods [5-8] to study the interaction of CMV complexed with monoclonal Fabs. Point mutations in the capsid protein can result in mutants that prevent aphid transmission of virus without eliminating infectivity.[4,9-10] Transmission mutants of the virus were used to screen a series of monoclonal antibodies to CMV. The Fab generated for this study bound to CMV, but not to two of eight H-I loop transmission mutants. Within the tertiary structure of the CMV capsid subunit, the β H-I loop contributes to the only acidic patch on the surface of each subunit and is involved in aphid transmission [4]. CMV was incubated with a large excess of Fab to ensure saturation and then prepared for electron cryo-microscopy and imaged as described.[5] Images were recorded with ~24e^-/Å^2 in a Philips CM300 FEG transmission electron microscope at a nominal magnification of 47,000x. Eight micrographs recorded over a range of defocus levels were digitized at 14 μm intervals and analyzed by three-dimensional reconstruction methods [8]. A final reconstruction was computed to 15Å resolution from 1,369 particle images (FIG.1). The effects of the microscope contrast transfer function were partially corrected in the Fourier transform of each image [8] and an inverse temperature factor of 1000Å^2 was applied to the model used for refinement.

Our results show that the Fabs bind differently to the quasi-equivalent capsid subunits despite their having identical sequences. Fabs only bind to the A subunits which form the twelve pentamers at the icosahedral fivefold axes (FIG.1A-E). Also, only one Fab binds to each pentamer because the binding site embraces two A subunits in each pentamer and steric hinderance precludes additional Fabs from binding to a given pentamer. No Fabs bind to the B and C subunits which comprise the hexamers and occupy quasi-sixfold sites. We conclude that the A-A and B-C epitopes must be conformationally distinct such that antibodies are unable to bind to hexamers.[11]

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[11] Work supported in part by grants from the NIH to TSB (GM-33050), TJS (GM10704 and AI45976) and the USDA to KLP (NRI 1999-0251and 2002-00647) and awards to the Purdue Structural Biology Group from the Keck Foundation and from Purdue University. We gratefully acknowledge for assistance and training.

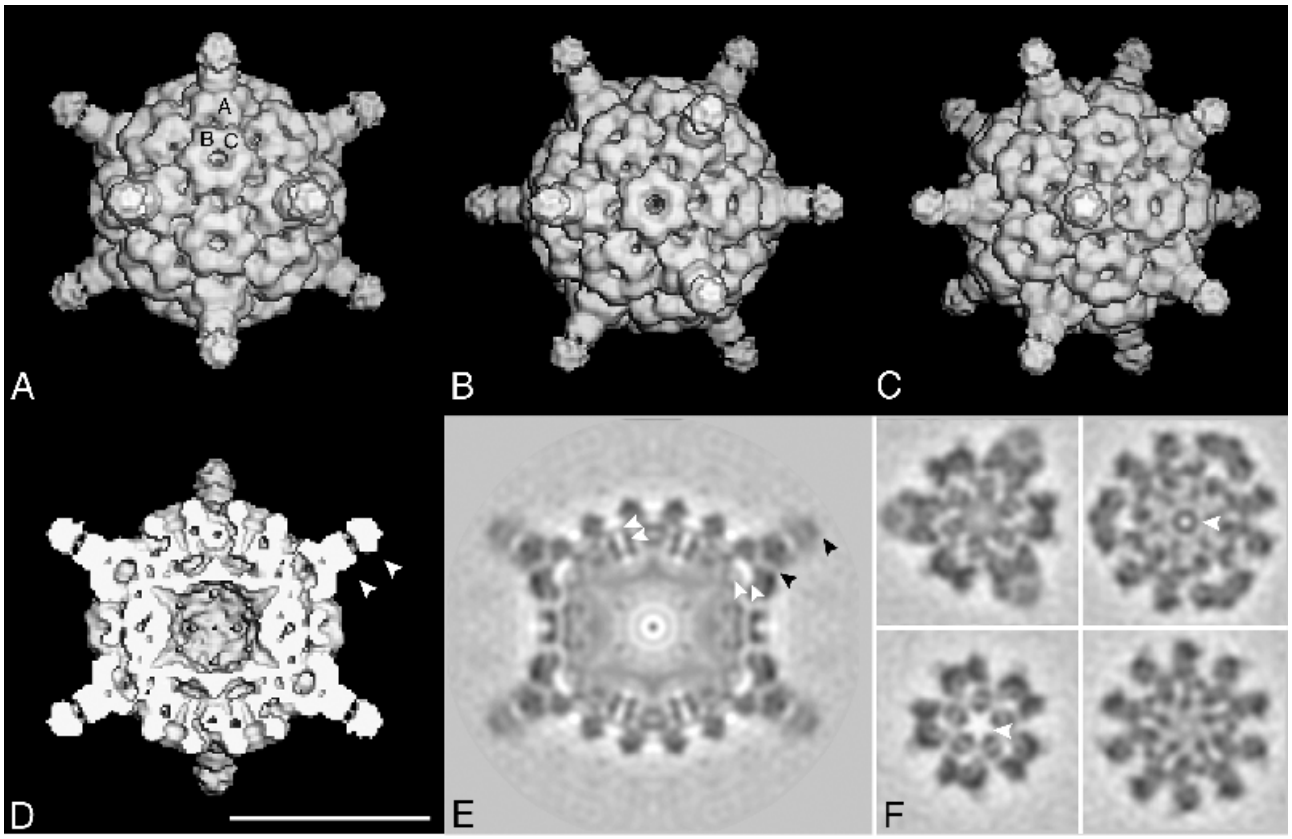

FIG.1. Shaded-surface views of CMV + Fab reconstruction viewed along twofold (A), threefold (B) and fivefold (C) axes of symmetry. The front half of the reconstruction viewed as in (A) was removed to expose internal features of the structure (D). White arrowheads identify the Fab constant and hypervariable domains, which are located at high and low radius, respectively. An equatorial section of the electron density projection (E), viewed as in (D), more clearly shows the Fab domains (black arrowheads) at the fivefold axes and the radially-directed rods of density below each hexamer (white arrowheads at left). Tangential sections of electron density projections (F) compare interactions within hexamers (top panels) and pentamers (bottom panels). Upper and lower panels show sections approximately at the level of the left and right sets of white arrowheads (E), respectively. A ring of density ascribed to the CMV bundle of helices beneath the hexamer is clearly visible in cross section (upper right; white arrowhead). The large cavity at the base of the pentamer is also visible (lower left; white arrowhead). Scale bar: 250Å.

https://doi.org/10.1017/S1431927602102741 Published online by Cambridge University Press