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Cryo-electron Microscopy, Antibody Labeling, and Image Analysis Reveals Macromolecular Interactions in Enveloped Alphaviruses

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Electron microscopy is an excellent tool for visualizing hydrated structures of biological specimens (Stewart and Vigers 1986), especially viruses and complexes of viral-cellular macromolecules that are too large to study with current X-ray crystallographic methods (Baker 1992). With low temperature, low dose, and image analysis procedures, cryo-electron microscopy (cryoEM) has proven to be an effective means to reveal both external and internal features of biological specimens (Cheng et al. 1994) including enveloped viruses (Cheng et al. 1995). Aid with monoclonal antibody labeling, two alphaviruses were used as a model system of enveloped viruses to study virus: host recognition.

Ross River virus (RR) and Sindbis virus (SIN), members from the SF and the SIN groups respectively, were chosen for antibody binding studies since both native structures were known (Cheng et al. 1995, Strauss and Strauss1994). The 27 closely-related, icosahedral, positive strand RNA viruses are subdivided into three major groups with an extremely wide host range. Three-dimensional structures of RR and SIN, complexed with antibody Fab fragments, were determined to a resolution of about 2.7nm with cryoEM and image reconstruction. Two structures clearly show that the Fab fragments bind to the extreme apexes of the trimeric glycoproteins (Fig. 1). This region of the spike appears to be composed of E2 glycoprotein since the epitopes that antibodies recognize are on E2. Moreover, the binding domains of the neutralizing antibodies were shown to be involved in virus; receptor binding (Vrati 1988, Stanley 1986). The overlap of the antibody foot-prints in two of the three type groups suggests that the tip region of the spikes is conserved for host recognition in alphaviruses.

Knowledge of the virus structure allows us not only to model the atomic structure of the capsid protein into a monomeric packing arrangement inside the viral envelope (Fig. 1), but also to map the host protein into a monomeric packing arrangement inside the viral envelope (Fig. 1), but also to map the host recognition sites on the surface of the glycoprotein (Chemg et al. 1995, Choi et al. 1991, Liu et al. 1994). On the basis of our model-building studies, the protein residues that are predicted to be involved in protein: protein, protein: RNA, and protein: glycoprotein contacts in the nucleocapsid, can now be probed with site-directed mutagenesis and X-ray crystallography to investigate viral assembly and uncoating.

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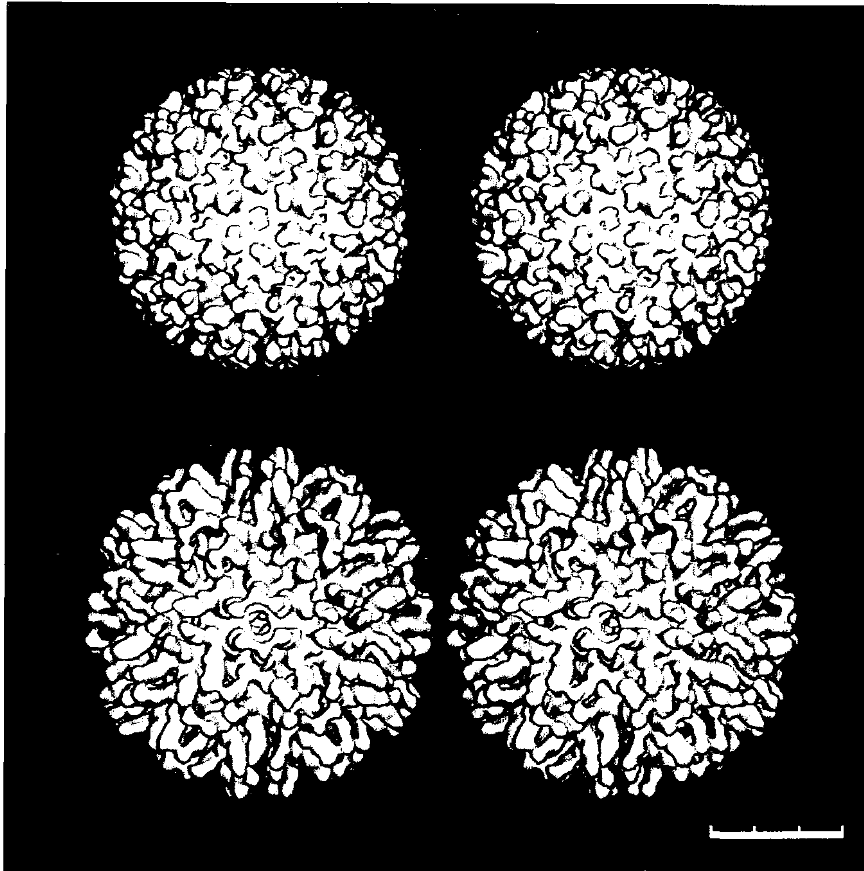

Fig. 1. Stereo pairs of surface rendered three-dimensional reconstructions reveal the structures of Ross River virus in a native and an antibody-bound conformation. The reconstruction was computed at 2.7 nm resolution with 68 and 42 independent particle surface-shaded representations are viewed along an icosahedral twofold axis. Comparison of the surface features of Ross River virus between its native particle and its complex form attached with monoclonal antibodies reveals the locations where the 240 Fab fragments binds the 80 copies of spikes. Binding domain of these antibodies also suggests to be a region in which the virus engages in the host recognition. The fact that the footprint of the antibody in Sindbis virus (data not shown) is nearby the one in Ross River virus suggests that the host recognition site may be conservative in alphaviruses. Bar=30 nm.