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Authors

Olson, Norman H
Smith, Thomas J
Kolatkar, Prasanna R
et al.

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CRYOELECTRON MICROSCOPY OF COMPLEXES OF HUMAN RHINOVIRUS WITH A MONOCLONAL F_{AB} AND THE VIRAL CELLULAR RECEPTOR

Norman H. Olson, Thomas J. Smith, Prasanna R. Kolatkar, Marcos A. Oliveira, Roland R. Rueckert,* Jeffrey M. Greve,** Michael G. Rossmann, and Timothy S. Baker

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907; *Institute for Molecular Virology and Department of Biochemistry, University of Wisconsin, Madison, WI 53706; **Molecular Therapeutics, Inc., Miles Research Center, 400 Morgan Lane, West Haven, CT 06516

Cryoelectron microscopy and image analysis techniques make it possible to study structural and functional relationships of macromolecular complexes that currently are not easily examined with crystallographic techniques. We have recorded images of frozen-hydrated human rhinovirus serotype-14 (HRV-14) complexed with a neutralizing, monoclonal, antibody fragment (F_{ab}-17Ia; Fig. 1A); and HRV-16 complexed with the amino-terminal, two-domain fragment (D1D2) of its cellular receptor (intercellular adhesion molecule-1, ICAM-1; Fig. 1B). Three-dimensional reconstructions (Figs. 2A,B) were calculated to ~3nm resolution from 35 and 44 images of each complex, respectively. The HRV-14/F_{ab} structure clearly identified the footprint of the F_{ab} on the surface of the virion. The HRV-16/D1D2 reconstruction presents, for the first time, the three-dimensional structure of a complete virus complexed with its cellular receptor.

Human rhinovirus is the major cause of the common cold. The icosahedral virus consists of 60 copies each of four proteins (VP1-VP4) and a single copy of a single-stranded RNA genome[1]. Four areas on the surface of the virion have been identified as neutralizing immunogenic sites (NIm-Ia, NIm-Ib, NIm-II and NIm-III)[2]. A surface depression, or canyon, that is 2nm deep and 1.2-1.5nm wide encircles each of the twelve, fivefold vertices and lies approximately half-way between the fivefold and threefold icosahedral axes. This canyon was initially identified as the site of cellular recognition with structural, mutational, and drug-binding studies[2]. ICAM-1 normally functions as a cell surface ligand for the lymphocyte function-associated antigen-1 adhesion receptor and in leukocyte adhesion during inflammation. The extracellular portion of ICAM-1 consists of five, immunoglobulin-like domains[3]. The N-terminal portion of the ICAM-1 molecule is narrow enough to fit into the canyon and bind to viral residues on the canyon floor, however a F_{ab}, which has twofold-related heavy and light chains, is thought to be too large to fit inside the canyon[1].

Comparisons of the two reconstructions (Fig. 2) confirmed key hypotheses about structural and functional aspects of HRV which were based on previous studies. Both the F_{ab} and D1D2 molecules fully saturated HRV (~60 copies/virion). The F_{ab} molecule is narrow at its base and widens into a globular head (Fig. 2A). A central depression in the head corresponds to the switch region between the constant and variable domains. The F_{ab}s surrounding adjoining vertices are twofold related and lie in position as expected for the formation of a $(F_{ab})_2$ structure[4]. The D1D2 molecule is approximately 7.5nm long and is bilobed as expected for the two-domain ICAM-1 fragment (Fig. 2B). Mutational analysis indicated that F_{ab}-17Ia is bound to NIm-Ia on VP1[5] which is located about one-third the distance from the fivefold vertex along a line connecting the icosahedral fivefold and threefold axes. This location is confirmed in the reconstruction; however, the F_{ab} footprint also straddles the canyon. The footprint of D1D2 is centered over the canyon, as expected, but it also contacts both rims of the canyon. Consequently, binding of F_{ab}-17Ia to the virion precludes binding of the cellular receptor, as was previously suggested[6], since the two footprints overlap.

These reconstructions, in conjunction with model building experiments based on known atomic structures, are currently being used to investigate the precise interactions of the HRV virion to the bound molecules[7].

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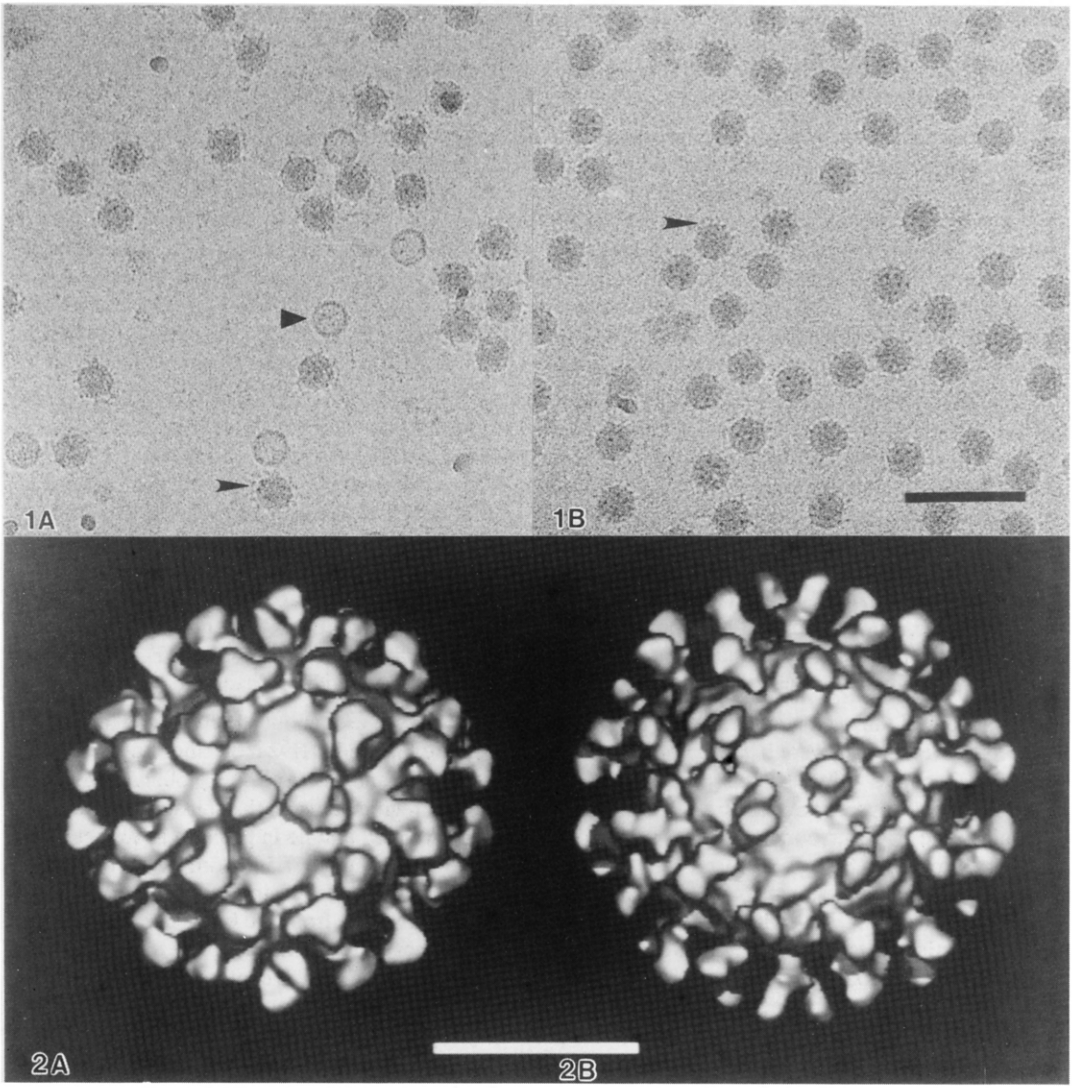

FIG. 1. Frozen-hydrated HRV-14/F_{ab}-17Ia (A), and HRV-16/D1D2 complexes (B). Arrows in each indicate F_{ab} or D1D2 molecules, respectively. Native virions have a smooth profile. Triangle (A) indicates an uncomplexed virion that has lost its ssRNA. Bar = 100 nm.

FIG. 2. Shaded, surface representations of three-dimensional reconstructions of HRV-14/F_{ab}-17Ia (A), and HRV-16/D1D2 complexes (B). Bar = 20 nm.