

**02.7-2** PROGRESS IN THE STRUCTURE DETERMINATION OF A COMMON COLD VIRUS. By Edward Arnold, John W. Erickson, Elizabeth A. Frankenberger, Hans-Jürgen Hecht, Ming Luo, Michael G. Rossmann and Gerrit Vriend, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA.

The rhinoviruses are small animal viruses which are best known for their ability to cause the common cold. Rhinoviruses constitute a subgroup of the picornaviruses, a family of viruses which cause a variety of infectious diseases in man and other mammals. Picornaviruses are isometric viruses with an external diameter of 300 Å and a mass of 8.4 million daltons. They contain a single strand of RNA encapsidated by an icosahedral protein coat consisting of 60 copies of each of four proteins ranging in size from 7,000 to 37,000 daltons.

We have obtained cubic crystals of human rhinovirus 14 (HRV14) from PEG-tris solutions at pH 7.2. These crystals have the symmetry of space group  $P2_3$ ,  $a=445.1$  Å, contain four particles per cell, and diffract to at least 2.8 Å resolution. Using a 5 Å native data set collected via the oscillation photography method, we have determined the orientation of the four HRV14 particles in the cubic cell. A prominent peak at  $0, \frac{1}{2}, \frac{1}{2}$  in a Patterson function calculated at low resolution, and extinctions of a face-centered nature below 30 Å resolution, both suggested a pseudo face-centered arrangement for the particles. A rotation function calculated with 5 Å native data indicated that the particles are tilted away from a special arrangement by a six degree rotation about the threefold axis or body diagonal of the cubic cell. A locked rotation function was calculated which gave an accurate value of -6.0 degrees for the tilt angle. Knowledge of the particle orientation will be crucial for ensuing stages of the structure determination, including heavy atom location and refinement in isomorphous derivatives, and phasing by the molecular replacement method.

At DESY in Hamburg, we have collected high resolution (3 Å) native and  $UO_2F_5$  derivative data sets of the cubic HRV14 crystals using 7x7 inch film cassettes. We are currently (February) analyzing the results and difference Pattersons.

We have also collected low resolution (7 Å) X-ray diffraction data from crystals of mengovirus, another human picorna (cardio) virus. These data were used to find the orientation of this virus in its  $P2_3$  cell ( $a=421$  Å). A cross-rotation function shows that human rhinovirus 14 and mengovirus have similar structures.

**02.7-3** TOBACCO MOSAIC VIRUS: INTERACTIONS BETWEEN RNA AND PROTEIN DETERMINED BY REFINEMENT AGAINST FIBRE DIFFRACTION DATA\*EQUILIBRIUM BINDING STUDIES. By A.C. Bloomer and A. Mondragon, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

The coat protein of tobacco mosaic virus (TMV) crystallises only in the form of the two-layer disk, a sub-assembly of 34 molecules arranged in two rings. The protein conformation has been refined at 2.8 Å by a procedure which maintains strict 17-fold non-crystallographic symmetry. These coordinates, after transposition into the helical lattice of the intact virus, have been fitted to the RNA coordinates (three nucleotides per protein subunit) of Stubbs & Stauffacher (J. Mol. Biol. (1981) 152, 387-96) and subsequently refined against the fibre diffraction intensities from ordered gels of the intact virus (Stubbs *et al.* (1977) Nature 267, 216-221).

The axial interactions between protein molecules in adjacent turns of the basic viral helix are necessarily quite different from those seen in the protein disk, whereas the lateral interactions are very similar. The protein-nucleic acid interactions will be presented together with a comparison of the different protein-protein interactions seen in various aggregates of the coat protein molecule.

Assembly of the virus is initiated by insertion of a hairpin loop of RNA with specific sequence (the origin of assembly) into the central hole of a protein disk. The initial interaction between RNA and disk is being investigated by studying the binding of specific oligonucleotides (e.g. the hexanucleotide AAGAAG) which bind very tightly to disk preparations.

**02.8-1** CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION STUDIES ON COLICIN  $E_3$  IMMUNITY PROTEIN. By M. Shoham, B.L. Levinson(\*) and F.M. Richards(\*) Dept. of Structural Chemistry, The Weizmann Institute of Science, Rehovot, Israel, and (\*) Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, U. S. A. Colicin  $E_3$  is an antibiotic-like protein of MW = 63,000 produced by *E. Coli* strains harboring the Col  $E_3$  plasmid. It penetrates sensitive *E. coli* cells and kills them by inactivating their protein biosynthetic machinery. The target on the ribosome is 16S rRNA, which colicin  $E_3$  cleaves enzymatically 49 bases in from the 3'-terminus. Colicin  $E_3$  is secreted from cells and isolated as an equimolar complex with its 9,800-dalton immunity protein, also plasmid-encoded, which protects the producing strain from endonucleolytic attack by colicin on its own ribosomes.

We have crystallized immunity protein in the orthorhombic space group C222 with cell dimensions  $a = 78.7$  Å,  $b = 54.1$  Å,  $c = 36.1$  Å, and one molecule of MW 9,800 per asymmetric unit. Crystals were grown by vapor diffusion in hanging drops at 4 °C from 55% saturated ammonium sulfate, 0.1 M sodium chloride, 60 mM acetate, pH 5.5. The value of  $V_m = 1.96$  Å<sup>3</sup>/dalton indicates a solvent content of 37% (v/v). The crystals diffract to a resolution of 2 Å, and thus are suitable for high resolution X-ray analysis.

We have also crystallized the complex of colicin  $E_3$  with immunity protein, from ammonium sulfate solutions. These crystals diffract to 7 Å resolution.