

## 03-Crystallography of Biological Macromolecules

therefore progressed in PG432 ( $R_{merge}$  0.11 and 0.19 for two data sets). Difference maps were calculated using "pseudo" 432 data sets produced using I23 virus data folded into the 432 asymmetric unit with one of two models for the disordering:

1. Random arrangement of the the 2-folds throughout the crystals (vector mean of phased I23 structure factors models the observed data). 2. Mosaic blocks of the crystal contain similarly oriented particles, but arrangement of 2-folds between different mosaic block is random (arithmetic mean of I23 intensities provides model).

Model 2 provided an excellent match to the observed G67 data. The pseudo 432 data for the parent virus were scaled in resolution shells to the G67 data then the I23 asymmetric unit regenerated by duplicating the records to write h,k,l and k,h,l. Difference maps were calculated a set of accurate phases for the native virus in space group I23. Averaging the difference maps to impose icosahedral symmetry then revealed the structural differences between the parent and mutant viruses. However the nature of the disordering suggested a possible route for deconvolution of the data by back-transformation of a map obtained in the way described above to provide estimates of the true I23 structure factors. Our observed data provide a series of constraints in reciprocal space:

$$2(F_{OBS})^2 = (F_{23,hkl})^2 + (F_{23,khl})^2$$

These constraints are applied to improve our estimates for the intensities. We have constructed an iterative process of map calculation and constraint application. Using synthetic test data the correlation coefficient between the reconstructed  $F_{23}$  and the true values rose from 0.70 to 0.97 over 6 cycles. A two cycle application improved the G67 maps. Since this procedure provides a rather general method for convoluting entirely overlapped reflections we intend to investigate its general use in deconvoluting wavelength overlaps that occur using the Laue technique.

**PS-03.02.09** ICOSAHEDRAL SYMMETRY OF A DOUBLE-SHELLED RICE DWARF VIRUS BY THE ROTATION FUNCTION. By Y. Morimoto<sup>1</sup>, K. Tomizaki<sup>1</sup>, T. Tsukihara<sup>1</sup>, T. Omura<sup>2</sup>, M. Koizumi<sup>3</sup>, H. Mizuno<sup>4</sup> and H. Kano<sup>4</sup>. <sup>1</sup>The University of Tokushima, Japan, <sup>2</sup>National Agriculture Research Center, Japan, <sup>3</sup>National Institute of Sericultural and Entomological Science, Japan, <sup>4</sup>National Institute of Agrobiological Resources, Japan.

Rice Dwarf Virus (RDV), a phytoreovirus, from *Nephotettix* species infects systemically rice and wheat plant and causes stunting or dwarfing of plant bodies. The virus being a double-shelled particle is approximately 700 Å in diameter and the molecular weight of  $6.52 \times 10^7$ . A total of 540 identical protein subunits are on the particle surface, suggesting  $T=9$  icosahedral symmetry of the capsid. It is of great interest to study the large and complicated virus in structure of double-shell arrangement and in the function of virus. Structure determination may permit improvement of immunization efficiency and enhancement of immunological activity by modifying the structure of the antigen, the surface of the virus coat.

The virus has been crystallized in the cubic space group I23 with  $a=789\text{Å}$ . Two particles are included in a unit cell. Diffraction experiments were carried out with a macromolecular Weissenberg camera at Photon Factory, Japan. Camera length was 860mm, wavelength 1.04Å, Oscillation angle 1.5°. Intensity data were obtained from three native crystals with an index searching procedure and merged into an independent data set ( $\sim 10\text{Å}$ ).

Self-rotation function was calculated at 20Å resolution with the integration radius of 100Å using Crowther's program (The Molecular Replacement Method, 1977, 173-178). The rotation function showed icosahedral symmetries such as 2-, 3- and 5-fold

axes with significant peaks higher than 60% of the ideal height. Some of 2- and 3-fold axes are coincident to those of the crystallographic symmetry. The RDV particles are located on the special positions of the Wyckoff notation "a" in the cell. From the particle size ( $\sim 700\text{Å}$ ) and the cell dimension, it suggests that the viruses are packed with the closest distances in the I23 lattice.

**PS-03.02.10** CRYSTALLIZATION AND CHARACTERIZATION OF COXSACKIEVIRUS B3 (CVB3). By Jodi K. Bibler\*, Marcia Kremer, Liang Tong and Michael G. Rossmann. Department of Biological Sciences, Purdue University, West Lafayette, IN 47906.

Coxsackievirus B3 (CVB3) is a small, icosahedral, single-stranded RNA virus belonging to the enterovirus genus within the picornavirus family. The enteroviruses include poliovirus, the coxsackie A and B viruses, hepatitis A virus and the echoviruses. The coxsackie B viruses are causative agents of a wide variety of mild to severe diseases and are the most common agents known to cause viral myocarditis. CVB3-Gauntt causes myocarditis in mice and provides an excellent animal model in which to study virus structure-function and drug therapy.

CVB3 crystallizes in a primitive monoclinic space group ( $a = 574.6\text{Å}$ ,  $b = 302.1\text{Å}$ ,  $c = 521.6\text{Å}$ ,  $\beta = 107.7^\circ$ ) with two virions in the asymmetric unit. The crystals diffract well to 2.7Å resolution and 66% of the X-ray diffraction data has been collected to 3.0Å. Systematically weak reflections and the self-rotation function confirms pseudo R32 symmetry. This pseudo R32 symmetry orients and positions each particle in the monoclinic cell near face-centered positions. The resultant packing arrangement is equally consistent with space groups P2 or P2<sub>1</sub>. Currently, work is underway to establish the deviations from R32 packing for both independent particles in the monoclinic asymmetric unit and to determine the true space group (P2 or P2<sub>1</sub>). These parameters must be accurately known in order to determine and extend phases to the resolution limits of the available data and to successfully utilize the high order of non-crystallographic redundancy (120 fold) during molecular replacement real space averaging.

**PS-03.02.11** PURIFICATION AND CRYSTALLOGRAPHIC STUDY OF AFRICAN HORSE SICKNESS VIRUS CORE PROTEIN VP7. By A. K. Basak\*, J. Grimes, P. Roy and D. I. Stuart. Laboratory of Molecular Biophysics, Oxford University, U. K.

African horsesickness (AHS) is a disease of caused by a dsRNA Orbivirus transmitted by gnats. Nine different serotypes of the virus have been identified. AHS virions, in common with their paradigm, bluetongue virus (BTV), consist of seven structural proteins (VP1-VP7) and three non-structural proteins (NS1-NS3). AHSV is very similar to BTV both in morphology and biochemical properties. The full length cDNA coding for core protein VP7 of the (AHSV-4) virus has been cloned and expressed in insect cells using a baculovirus expression system. This protein is the major component of the inner capsid of the virion and is thought to be present in 780 copies in the intact virus.

We have purified the protein and crystallized it in 2.5M urea. The protein is very hydrophobic in nature and has been crystallized at 20°C by vapour diffusion using the sitting drop method. The crystals exhibit tetragonal symmetry with unit cell dimensions  $a=b=157.10\text{Å}$ ,  $c=57.90\text{Å}$ ,  $\alpha=\beta=\gamma=90^\circ$ , and belong to space group I4. A complete native data set to 3.0Å spacings has been collected inhouse on a Marresearch imaging plate. We hope to solve the structure by the molecular replacement method using the homologous structure of BTV-VP7 (Basak, A.K.; Grimes, J.M.; Roy, P. and Stuart, D.I.; this meeting).