03-Crystallography of Biological Macromolecules

has been expressed in E. coli and purified to near homogeneity. An apparent octahedron in crystallization is that, even in high 
concentration (10 5 M) and high Mg 2+ concentration, the protein is soluble only 
to 2 mM. Crystallization experiments carried out in the presence 
and absence of oigomera of different lengths, using various 
methods and conditions, will be reported.

03.02 – Viruses

MS-03.02.01 STRUCTURAL STUDIES OF VIRAL CAPSIDS.
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The three-dimensional atomic structure of about a dozen 
different virus groups have now been determined. These 
include viruses that encapsidate single-stranded RNA, 
single-stranded DNA as well as double-stranded DNA. They 
also include viruses that infect plants, mammals, insects and 
bacteriophages. In the majority of cases the capsid proteins 
have an eight-stranded antiparallel β-barrel. The icosahedral 
organisation of the proteins follows the predictions of Caspar & 
Klug remarkably well. The adaptation to quasi-symmetrical 
environments is generally produced by different structures of 
flexible polypeptide ends that regulate the subunit contacts.

The combination of crystallographic and cryo-electron 
microscopic studies has become an important tool. It has been 
possible to determine the organization of scaffolding proteins 
in the assembly of bacteriophages such as phx174. These proteins, 
like chaperonins, are required for the assembly of empty 
capsids, but are not present in the mature infectious virions.

Other examples of viruses (e.g. paroviruses and picornaviruses) 
with neutralizing antibodies, of viruses complexed with their 
cellular receptors and in the analysis of recombinant cores 
of Sindbis virus.

MS-03.02.02 The Structure of Theiler's Virus Ming 
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Theiler's virus belongs to the picornavirus family and can be further divided into two subgroups. One is composed of highly virulent strains (e.g. FA and 
GDVII), which causes acute poliomyelitis in mice, and the other 
consists of less virulent strains (e.g. BeAn and DA), which creates demyelination in mice after the 
establishment of a persistent infection. The less 
virulent strains have been used as an animal model to study human 
demyelinating diseases such as multiple sclerosis. It has been shown that the 
virulence is related to the capsid protein, probably related to 
the mode of the virion attachment to the host receptor, host immune response and/or the 
capsid stability. The three dimensional structure of 
TMEV at 3.0 Å resolution reported here revealed the structural basis for the neural 
virulence of TMEV. Potential sites for virus attachment to the 
host receptor and immunogen determinants distinguishing the two subgroups were 
mapped on the capsid. The result helps us a great deal in understanding the host receptor recognition, 
virion stability, and viral pathogenesis of TMEV. We are also in the process of determining the structure of 
a highly virulent TMEV strain, GDVII. Our group has been successful to crystallize the GDVII virus 
and the crystals were brought to SSRL to collect X-ray
diffraction data. The data collection was performed at Station X7 using X-rays of 1.98 Å wavelength. The diffraction patterns were recorded on the newly installed image plate system made by Mar research. A total of about 50 useful images were processed and the space group of the crystals was determined as C2, a=675.4Å, b=235.7Å, c=58.4Å, β=90.6°. There is a whole virus particle in the asymmetric unit, which results in a 60-fold noncrystallographic averaging in phase determination by the molecular replacement method. About 50% unique data were collected to 3.5Å resolution and the Rmerge was 11.6% for data with 1/σ2>2.0. A locked rotation function was calculated using data to 4.0 Å resolution and the virus orientation was clearly identified in the unit cell. Work is now continued on the translation of the virus particles in the unit cell. The orientation of the virus particles is known, phases could be first derived from the BeAn coordinates and improved by electron density averaging over the 60-fold noncrystallographic symmetry. The final structure of GDVII will reveal the differences between the highly-virulent group and the persistent group on the capsid. We shall learn more about the relationship between virus infection and demyelination and persistence infection.


We have crystallized and determined the three-dimensional structure of A22 Iraq 24/67 foot-and-mouth disease virus (FMDV). We now have representative structures of at least one subtype from at least seven serotypes of A22 Iraq confirm and extend the findings from the comparison of O and C type viruses. A22 Iraq crystallized in space group P222, a data set 70% complete to 3Å was collected. Structures were phased using a partially refined model of A100 (Fry, E. et al unpublished) FMDV and a 2:1 Fo:Fc electron density map was calculated at 3Å resolution. The map, averaged over the 15 monomers in the asymmetric unit, was of high quality and enabled an initial model to be built. Refinement is in progress.

Preliminary analysis of the structure reveals that structural differences between A22 Iraq and other serotypes are confined to external loops. As observed for O1BFS (Acharya, R. et al. (1990), Nature (London), 347, 709-716) and O8-1 (Lea, S. et al. manuscript in preparation) FMDV, the longest surface loop which connects strands G and H of the β-barrel core of VP1 is largely disordered. This GH loop is an important antigenic feature and also implicated in binding to the cellular receptor. The mobility of this feature appears to be strongly conserved in FMDV. The implications of this finding will be discussed. Elsewhere on the surface of the virion there are shifts of several Angstroms in the positions of surface oriental loops. Moreover, there is evidence that structural alterations in one loop due to sequence variation can affect the conformation of adjacent loops. This structural information is being used to interpret the large amount of serological data available for serotype A FMDV.

EMpty capsids of FMDV lack the genomic RNA and are reported also to lack the cleavage of the precursor VP3 into VP2 and VP4 which is simultaneous with encapsidation and contributes to the stability of the virion. In order to probe both the mechanism of VP0 cleavage and the RNA-protein interactions on the interior of the capsid, we have determined the structure of empty capsids of A22 Iraq. Curiously, in these capsid VP0 has largely been cleaved into VP2 and VP4. Our investigation into the reasons for this finding will be presented. Empty capsids crystallized isoformously with the virus. A difference map revealed that the external surface structures of the virus and empty capsid are identical. However, on the interior surface the α-termini of VP1 and VP2 and the α-terminus of VP4 appear to be disordered in the empty capsid relative to the virus. These termini are all close to the interface between pentameric subunits which are assembly intermediates in FMDV. The presence of RNA in the virus appears to be necessary to stabilize these portions of the capsid polypeptides.


The structures of canine parvovirus (CPV) full and empty particles have been determined to atomic resolution. The structure of CPV empty capsid has also been refined recently.

Each subunit of the virus possesses an anti-parallel β-barrel motif that has been found in most viruses whose structures are known. On the viral surface, there are canyon around the fivefold axes and prominent spikes at the threefold axes. With analogy to Rhinovirus14, the canyons might be the receptor binding sites. Residues related to the antigenic properties as well as the host range determinants of the virus are found on the threefold spikes. Extensive interactions are observed among the threefold related subunits. Five β hair-pins at each fivefold axis make up a β-cylindrical structure. A substantial volume of the electron density is shown to be DNA, corresponding to about 13% of the virus genome. Different protein conformations are observed at the region where DNA binds.

MS-03.02.05 STRUCTURAL STUDIES OF TYPE B NEUROAMINIDASE. By Clinton L. White*, Musiri N. Janakraman, W. Graeme Laver, Gillian M. Air and Ming Luo; Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, Alabama, USA.