Virus structure determination

Recently, K. Valegard, L. Liljas, K. Fridborg and T. Unge of the University of Uppsala, Sweden, have published the structure of bacteriophage MS2 (Nature, 345, 36, 1990). Two aspects of this study are truly remarkable.

Bacteriophage MS2 is an isometric virus. The virus capsid, composed of 180 copies of the coat-protein subunits of molecular weight 13,700 daltons, encapsidates a single-stranded, positive-sense RNA genome of 3569 nucleotides. The genomic and coat-protein sequences are known.

During the past decade, the structures of six plant, four animal and one insect viruses have been determined. Although these viruses infect very different organisms, they display a remarkable similarity in their coat protein structure. The principal domain of the coat proteins of these viruses consists of an eight-stranded, antiparallel, β-barrel motif with a complex connectivity or topology, which has been described as 'jelly roll' or 'swiss roll' topology. The subunits are wedge-shaped and have a thickness of 40 Å. Their shape allows formation of hexameric and pentameric clusters without leaving large holes and hence leads to tightly packed icosahedral capsids. It was therefore assumed that other icosahedral viruses would also be built from protein domains of similar three-dimensional structure. The structure of MS2 coat protein, contrary to these expectations, is totally different.

Each icosahedral asymmetric unit of MS2 coat consists of three chemically identical protein subunits designated A, B and C. Each monomer has the shape of a triangle, with base of 60 Å, height of 20 Å and a thickness of 20 Å. The RNA-facing side consists of five β-strands with a meander (antiparallel strands connected by a short loop) topology. The exposed part of the sub unit consists of two more β-strands and two helices at the carboxy terminus. The structure resembles those of bovine platelet factor 4 and a domain of the class I histocompatibility antigen HLA-A2.

Another intriguing aspect of this work is the method used for structure determination. Protein structures are usually determined by the method of isomorphous replacement. However, due to the complexity of structure, this method, by itself, does not provide an interpretable electron density map for viruses. A technique known as molecular replacement is used for further improvement of electron density maps. Icosahedral viruses have 5-fold axes of symmetry, which are incompatible with translationally periodic lattices. Hence the crystallographic asymmetric units of virus crystals invariably contain multiple copies of chemically identical units. Molecular replacement refines the phases of X-ray reflections by imposing strict equivalence of electron density values in these crystallographically independent regions of the crystal. The molecular replacement method is also useful for determining an unknown structure starting from a known, similar structure.

On the basis of the assumption that the structure of MS2 will be similar to that of other isometric viruses, the authors attempted structure determination starting from a model based on the structure of southern bean mosaic virus. As the structures are totally different, it might be anticipated that the final electron density map would be totally meaningless. In contrast, the authors obtained a final map which was found later to be the Fourier transform, with phases shifted by 180° from the true phases. Such a structure is known as the Babinet's opposite of the true structure and is internally consistent with the observed amplitudes of the X-ray reflections. Although the Babinet opposite structure could not obviously be interpreted, the authors have successfully used the resulting phases to solve two heavy-atom derivatives and obtain the structure at 8.8 Å resolution. Further extension of resolution is entirely on molecular replacement techniques. These remarkable aspects of crystallographic phase determination will be described by the authors in an independent publication.

M. R. N. Murthy
Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012