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Report: The bacteriophages MS2 and Qβ have an icosahedral protein shell composed of 180 identical subunits surrounding an RNA molecule of about 3500 nucleotides. We have earlier determined and refined the structure of MS2 at 2.8 Å resolution (Valegård et al., 1990; Golmohammadi et al., 1993), the structure of fr at 3.5 Å resolution (Liljas et al., 1994), and the structure of Qβ at 3.5 Å resolution (Golmohammadi et al., 1996).

The coat protein of these viruses has at least two important roles in the life cycle of the virus. By binding to a specific stem-loop structure on the viral RNA it represses the translation of the viral replicase gene. This binding of coat protein the RNA is also the initiation of virus assembly. Recombinant MS2 coat protein forms virus-like capsids. Crystals of these capsids are isomorphous with crystals of the native virus. We have been able to soak these crystals with RNA fragments corresponding to the binding site in the viral RNA, and determine the structure of the complex. The structure of a 19-nucleotide long stem-loop fragment showed that strictly conserved regions on the inside of the protein shell were binding two exposed adenine bases, which had been shown to be essential for binding (Valegard et al., 1994).

During the visit, data was collected for a number of crystals. Most of the data was collected for complexes of specific RNA fragments and MS2 protein capsids.

Data was collected for four such complexes. The capsids were formed both with coat protein having the native sequence and a T45A mutant, where one of the residues involved in specific RNA interaction was mutated.

All this data has been successfully processed. The two native complexes gave 90 000 independent reflections to 2.6 Å resolution and 95 000 independent reflections to 2.75 Å resolution, respectively. The scaling R-factor was 14.2 and 14.3 %. One of the mutant complexes gave 130 000 independent reflections to 2.7 Å resolution with a scaling R-value of 16.4 %. All these complexes have been solved using molecular replacement with the recombinant capsid structure as the model. The electron density maps showed that the RNA fragments used did not bind strongly to the protein capsid. Although this is a negative result, it is of great interest, since together with earlier studies of other RNA fragments it shows the importance of certain features of the RNA to achieve binding under these circumstances.

Some data was also collected from crystals of phage Qβ treated with 2-mercaptoethanol, in an attempt to reduce the disulphide bonds which link the coat protein molecules in these capsids. Some of these crystals were of space group R32, in contrast to the $C222_1$ space group of the normal Qβ crystals. A total of 39 000 reflections to 4.0 Å resolution were collected. The structure was solved using the model from the $C222_1$ crystal form. The R-factor was 30.1 % for all reflections. The resulting map was averaged using the non-crystallographic symmetry. The map was strikingly similar to the map of the $C222_1$ crystal form, confirming our interpretation that some of the loops in the Qβ capsid is disordered, The disulphide bonds, however, were still present, indicating that these bonds are very difficult to break or, alternatively, reforms very easily in the capsid.

References

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