The Structure of *Nudaurelia capensis* β Virus: the First Example of a Capsid with Icosahedral Surface Symmetry \( T = 4 \)

By J. T. FINCH and R. A. CROWTHER

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

AND D. A. HENDRY and J. K. STRUTHERS

Department of Botany and Microbiology, Rhodes University, P.O. Box 94, Grahamstown, South Africa

(Accepted 5 March 1974)

SUMMARY

The three-dimensional reconstruction from electron micrographs of a virus isolated from larvae of the pine emperor moth, *Nudaurelia capensis* virus, shows that its surface structure is based on the \( T = 4 \) icosahedral surface lattice. The 240 protein subunits are clustered in trimers with the four trimers per icosahedral face approximately planar but with deep grooves between adjacent faces.

INTRODUCTION

The quasi-equivalence theory proposed by Caspar & Klug (1962) provides a basis for the classification of the protein shells of isometric viruses in terms of the triangulation number \( T \), which specifies an icosahedral surface lattice accommodating 60\( T \) equal protein subunits. According to this theory, \( T \) can take the values 1, 3, 4, 7, etc., of which \( T = 1 \) denotes the shell built from 60 structurally equivalent subunits, while for \( T > 1 \) the subunits are structurally quasi-equivalent. Several examples of virus shells with the symmetry of the lattices \( T = 3 \) or \( T = 7 \) have been established but, until now, none with that of the lattice \( T = 4 \).

It is of considerable interest that the insect virus *Nudaurelia capensis* β (NβV) has been found to have a structure based on a \( T = 4 \) icosahedral surface lattice, since the absence of this class of capsid would have indicated further limitations on capsid formation in addition to those presented by Caspar & Klug (see Discussion). Three-dimensional image reconstructions from electron micrographs presented here show that NβV is built from 240 structure units clustered in trimers and arranged with the symmetry of the \( T = 4 \) lattice.

NβV is the most extensively characterized member of a group of five non-occluded viruses known to infect the Pine Emperor moth, *Nudaurelia cytherea capensis* (Lepidoptera: Saturniidae), in South Africa (Juckes, 1970). NβV has been shown to be isometric with a diam. of about 35 nm (Hendry, Bekker & van Regenmortel, 1968; Tripconey, 1970). Sedimentation coefficients of 210 S (Tripconey, 1970) and 220 S (Hendry et al. 1968) have been reported for NβV at infinite dilution. Polson, Stannard & Tripconey (1970) obtained particle weight values ranging between \( 15.4 \times 10^6 \) and \( 16.3 \times 10^6 \) for this virus. Struthers & Hendry (1974) determined a nucleic acid content of 11% for NβV, which corresponded to a mol. wt. for the RNA of about \( 1.8 \times 10^6 \), and thus ascribed to NβV the cryptogram \( R/1:1:8/11:S/S:1/0 \). They showed by electrophoresis on SDS-polyacrylamide gels that the
METHODS

NβV was purified from infected larvae of the Pine Emperor moth using the procedure of Struthers & Hendry (1974). Negatively stained specimens of virus particles were prepared following the method of Huxley & Zubay (1960): a drop of virus solution was applied to a holey carbon film attached to a specimen grid, washed with 0·1 M-KCl and a few drops of a 1% aqueous solution of uranyl acetate and the excess solution was withdrawn on to filter paper. Fields of virus particles were photographed both on the carbon film and over holes in the film. In the latter case the fields were chosen to provide areas where the stain had broken and contracted isometrically, yielding fairly circular images, and where the particle density was sufficient to give 20 to 30 such images but not so great that particles were jammed together and distorted. Electron micrographs were taken with a Philips EM301 operating at 80 kV with a magnification of ×45000.

The three-dimensional image reconstructions were produced using the system described by Crowther, DeRosier & Klug (1970) and Crowther (1971).

RESULTS

Visual analysis of images

An electron micrograph of negatively stained NβV particles suspended in stain over a hole in the carbon substrate is shown in Fig. 1. The film of stain over the hole had broken and contracted in the electron beam, and in the area shown the contraction was fairly isometric in the plane of the film, leading to images with a circular rather than elliptical boundary.
Fig. 2. Analysis of the image marked in Fig. 1. (a) Enlargement of the image. (b) The boundaries of the lightest regions in image (a). (c) The superposition on (b) of a projection of an icosahedron down a twofold axis (full lines): the dotted lines subtriangle the icosahedral faces into the $T = 4$ icosahedral surface lattice. The characteristics of this type of image are that the light areas avoid the locations of the vertices and the centres of the edges of the $T = 4$ lattice and tend to form Y-shaped regions within the small triangular facets of the lattice. (d) Idealized image of this type summarizing the above characteristics: it is the trimer clustering pattern for the $T = 4$ lattice. (e) One of the triangular facets of the $T = 4$ lattice in (d) showing that although the arms of the Y-shaped unit seen in the superposition pattern point to the vertices, the arms of the individual trimers may be somewhat skew. If the full lines indicate the orientation of the trimer arms on one side of the structure, then the arms on the opposite side will be in the orientation indicated by the dotted lines, but when seen in this projection the summed density from the two sides will be mirror symmetric about the medians.

The diam. of these particles were about 30 nm and their images show considerably more detail than those of isolated particles on the carbon substrate whose diam. were about 50 nm. This difference in diam. is similar to that observed for other virus particles and, in those cases where the spherically averaged diam. is available from low angle X-ray diffraction work, it is evident that in broken stain films over holes the particles are often considerably compressed. Isolated particles on the carbon substrate appear too large, presumably as a consequence of flattening.

The images show quite fine detail which is fairly uniformly distributed, and this indicates that the protein subunits are not grossly clustered, for example into hexamers and pentamers. No images with exact threefold or fivefold symmetry were observed, although some images had features which indicated that they were close to threefold or fivefold views. However, one particularly striking type of image can be interpreted as the projection down a twofold axis of an arrangement with icosahedral symmetry and with approximately the
shape of an icosahedron. An example of this type of image is marked 1 in Fig. 1 and is shown enlarged in Fig. 2(a). It has the hexagonal shape of an icosahedron viewed down a twofold axis and two sets of five bright spokes can be seen surrounding the locations of the proximal fivefold axes. These spikes point in directions midway between neighbouring fivefold axes. The lines joining neighbouring fivefold axes are relatively dark and break up the image into triangular facets of intensity corresponding to the projected faces of the circumscribing icosahedron. Within the innermost two of these facets in particular, the intensity, apart from random variations from image to image, follows the symmetry of the $T = 4$ icosahedral surface lattice (Caspar & Klug, 1962) obtained by subdividing each face of the icosahedron symmetrically into four triangles. This is illustrated in Fig. 2 where the bright areas of the image in (a) are marked in (b) and the projection of the $T = 4$ lattice shown superposed in (c). These bright areas conspicuously avoid the local twofold axes of the lattice (the centres of the edges of the triangles) and also the lattice points (the vertices of the triangles) but tend to occur at the centres of the triangles with spokes radiating towards the lattice points. A drawing of an image idealised on this basis is shown in Fig. 2(d), in which equiangular Y-shaped trimers are drawn within the lattice triangles. The disposition of the arms of the trimers deduced from this one view is necessarily mirror-symmetrical with respect to the surface lattice, as a consequence of the $mm$ mirror symmetry of the two-fold projection of an icosahedral arrangement. However, the actual arrangement of matter in the capsid could well be slightly skew as indicated in Fig. 2(e): if the full lines indicate a skew orientation of a trimer on say the near-side of the structure then the dotted lines indicate the lie of the subunits on the far side, and the resultant superposition in projection will be symmetrical.

Most of the other recognizable types of image were consistent with the structure just described. However, rather than trying at this stage to approach a detailed model of the surface structure by comparing simulated images from trial models with the electron micrographs, we applied the method of three-dimensional reconstruction, using the above model as a basis for identifying the approximate orientations of the virus particles giving rise to the various images.

**Three-dimensional image reconstruction**

Images identified in this way and showing no obvious distortion were digitized and their two-dimensional Fourier transforms calculated. The residual computed from the 'common lines' (Crowther et al. 1970) was used to check the extent to which the transforms were consistent with their having arisen from a structure with icosahedral symmetry. Typical plots of the mean phase residual as a function of spatial frequency for three images are shown in Fig. 3. For the images 1 and 2, the residual remained below $90^\circ$ (the value corresponding to random correlation along the common transform lines) out to spatial frequencies of 2 to 2.5 nm. On this basis the particles giving rise to these images were judged as better preserved compared, for example, with image 3, for which the residuals were consistently greater than those of 1 and 2, and exceeded $90^\circ$ at a spatial frequency of about 3 nm. The plots for images 1 and 2 were typical of the best preserved images that were obtained in N/IV and confirmed the icosahedral symmetry of the virus particles at least to spatial frequencies of about 2 nm. The results of a common lines search also showed that the direct interpretation of the images as described in the first section of the results was substantially correct since this provided the basis for assigning values to the view parameters for the various images. These values were optimized by searching locally for that combination which gave a pattern of common lines with minimal residual differences, and the
resulting optimum coordinates did not differ usually by more than a few degrees from those proposed initially.

Two independent reconstructions were performed, each using data from four of the best images as judged by minimal common residuals, and with values of the view parameters sufficiently differing that, together with the icosahedral symmetry, the complete three dimensional transform of the specimen could be evaluated by interpolation to spatial frequencies of 2.1 nm.

The two reconstructions yielded by Fourier synthesis were essentially identical and a stereoview of sections of a contour plot of the top half of one of these is shown in Fig. 4. Here the view is close to the direction of a threefold axis of the reconstruction and shows how the outer protein density is confined within the triangular faces of the circumscribing icosahedron, leaving markedly empty grooves about 3 to 4 nm wide and about 3 nm deep along the icosahedral edges. One of these grooves can be seen almost end-on at the top of this view. Within the icosahedral face, the protein appears as four regular trimers which follow the local symmetry of the T = 4 icosahedral surface lattice. The centres of the individual units are slightly skewly disposed on the lattice (see also Fig. 6). The same degree of skewness was obtained in both reconstructions but since no tilting work was done, the absolute sense of the skewness is not known. In Fig. 5, which is a stereoview of the same contour plot in a direction close to the twofold axis, black discs at the peaks make evident the near planarity of the large triangular faces and the sharp break in the surface lattice along the groove (the icosahedral edge). There is as a result a considerable distortion of the local sixfold axis of the T = 4 lattice which coincides with the icosahedral twofold axis in the centre of the marked region.

The arrangement on one of the icosahedral faces is most clear in Fig. 6 which is a view down a threefold axis of the outermost sections of the reconstruction cut parallel to an icosahedral face. The outermost three trimers protrude about 0.7 nm more than the inner ones and extend to a radius of about 18 nm. All the trimers then form Y-shaped columns
Fig. 4. A stereoview of a contour plot of the three-dimensional image reconstruction of N/V, seen in a direction close to a threefold axis. The icosahedral faces are separated by deep grooves, one of which can be seen end-on at the top of the reconstruction. Within an icosahedral face, the high density regions form four Y-shaped units centred on the local threefold axes of the $T = 4$ icosahedral surface lattice. The black column in the centre of the reconstruction was a support for the sections and is of no structural significance.

Fig. 5. A stereoview of the reconstruction seen close to a twofold axis, with the density peaks corresponding to individual structure units marked by black discs. Within an icosahedral face, the discs are fairly evenly spaced and are related by the local symmetry elements of the $T = 4$ lattice. Larger distances separate the structure units in adjacent faces resulting in the grooves along the icosahedral edges.

normal to the icosahedral face which can be followed inwards to a radius of about 10.5 nm where the density distribution becomes fairly uniform. This columnar appearance is most clear in the oblique view of these sections shown in Fig. 7. These results are summarized diagrammatically in Fig. 8. This arrangement of subunits gives rise to 162 holes into which stain penetrates, comprising 120 on the local twofold axes, 30 on the strict twofold axes and 12 on the fivefold axes. It is perhaps these features which led Tripconey (1970) to conclude that the capsid contained 162 subunits.

A gallery of projections of the reconstructed image was computed at 5° intervals of rotation about a twofold axis. Because of the fairly even distribution of surface detail, the number of striking patterns obtained was comparatively small. However, as shown in
Fig. 6. Stereoview normal to the top few sections of a plot of the reconstruction down a threefold axis showing the clustering of the structure units into four columnar trimers within an icosahedral face.

Fig. 7. An oblique stereoview of the plot in Fig. 6, showing that the arrangement of units in an icosahedral face is not quite planar. The central trimer lies about 0.7 nm (one section) below the plane containing the other three.

Fig. 9, there is a very good correspondence between those that do occur and the characteristic images of NβV. This is particularly striking for the projection down the twofold axis (φ = 0), but for other projections the skew disposition of the subunits resulted in patterns of m (left–right) mirror symmetry rather than mm, and the corresponding top–bottom asymmetry seen in the accompanying electron microscope images.

The virus particle weight

The conclusion from the above reconstruction work that the capsid of NβV is built from 240 protein subunits, together with the results of Struthers & Hendry (1974) that the virus contains 11% by weight of RNA and that the protein subunits are of mol. wt. 61,000 ± 1000, establishes the virus particle weight as 16.5 × 10^6. For comparison, the calculated values of the particle weight from the hydrodynamic properties of the virus
measured by Polson et al. (1970) lie between 15.4 and 16.0 x 10^6, while a value of 17.5 x 10^6 was obtained from sedimentation equilibrium studies (Struthers, 1973). The closest estimate is 16.3 x 10^6 obtained by Polson et al. (1970) by the relatively simple technique of mixing known concentrations of NβV and haemocyanin and counting the numbers of the two types of particle in electron micrographs, although the standard particle weight of haemocyanin was itself determined from hydrodynamic data.

DISCUSSION

The reconstruction shows that at outer radii the protein subunits are clustered into Y-shaped trimers – the first example of a trimer clustering pattern (Caspar & Klug, 1963). At inner radii, of less than about 13 nm, the distance between adjacent protein subunits is approximately the same whether they lie in the same or adjacent icosahedral faces. The subunits are arranged in agreement with all the symmetry elements, strict and local, of the T = 4 icosahedral surface lattice, so that they are evidently quasi-equivalently bonded together at these inner radii. However, because the columnar shape of the trimers is normal to the icosahedral face rather than radial, the distance between the centres of adjacent subunits in adjacent icosahedral faces increases to about 5 nm at a radius of 17 nm, while that between subunits within a face remains about 3.5 nm. Thus, while the subunits within a face are still related by the local T = 4 symmetry, at this outer radius the lattices on adjacent faces are split apart leading, for example, to the uneven arrangement noted above of units around the local sixfold axes. It thus seems energetically favourable for the twelve subunits in one icosahedral face to remain packed tightly together at outer radii, at the expense of a considerably increased distance between subunits on adjacent faces, rather than maintain a slightly larger but equal distance between all subunits. It is possible that the observed effect is exaggerated by the stain during the microscopy, but it is unlikely that
such a marked, consistent and locally asymmetric feature can be wholly attributed to the interaction with stain and subsequent drying.

Although these are now well-established cases of virus protein shells which have the symmetries $T = 1$, 3 or 7, NβV is the first reported virus with a shell based on the $T = 4$ icosahedral surface lattice, and the question arises of possible paths of assembly. In $T = 1$ shells, all 60 subunits are structurally identical and the icosahedral shell results from the equivalent bonding between them. The many examples of $T = 3$ shells have suggested that this preference may have a structural basis. Indeed, the icosahedral asymmetric unit for the $T = 3$ lattice is a group of 3 subunits, related by a local threefold axis of the lattice, and it has been pointed out (A. Klug, personal communication) that this could well form the first stage of aggregation, and provide a set of 60 ‘units’ which bond together equivalently to form the spherical shell. Similarly for $T = 7$, a hexamer might form a natural...
unit of assembly leading to a shell containing 60 icosahedrally related hexamers, which is then completed by the addition of 12 pentamers. However, for $T = 4$ the icosahedral asymmetric unit, consisting of 4 subunits, does not form such a natural structural group and we must select, say, one of the outer trimers and one subunit of the inner trimer in an icosahedral face. This is a most unlikely grouping. For NfV, the morphology suggests that the natural sequence of assembly may be first into trimers, then into groups of four trimers (as in the icosahedral face) which then come together to form the icosahedral shell: there is, however, no evidence for such a scheme. It may be relevant to note that other insect viruses, for example, the considerably larger Tipula and Sericesthis iridescent viruses (Williams & Smith, 1958; Wrigley, 1969), also show planar icosahedral faces which maintain their coherence as the virus is broken down (Wrigley, 1969). It remains to be shown whether this is a coincidence or whether the insect viruses are more like surface crystals with sharp faces than simple quasi-equivalence theory would allow.

D.A.H. and J.K.S. thank Mr J. S. Hahn of the Department of Microbiology and Virology, University of Stellenbosch, South Africa, for taking preliminary electron micrographs of this virus.

REFERENCES


(Received 9 January 1974)