The Surface Structure of Polyoma Virus

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SUMMARY

Characteristic 'two-side' electron microscope images and three-dimensional image reconstructions confirm that the surface structure of polyoma virus is based on the icosahedral $T = 7$ lattice. Tilting experiments show that the lattice is right handed ($T = 7d$).

INTRODUCTION

Electron micrographs of negatively stained polyoma virus were first published by Wildy et al. (1960). The surface was shown to consist of close-packed, large morphological units some of which were 6 co-ordinated with their neighbours and some 5 co-ordinated. From this latter observation it was concluded that the virus particles have icosahedral symmetry and a 42 morphological unit model of the surface structure was proposed. The proposal of this model was criticized by Caspar & Klug (1962) since it was not based on a detailed analysis of the electron microscope images. The model was also questioned by Mattern (1962) and another model with 92 morphological units was proposed by Mattern, Allison & Rowe (1963) for the similar K-virus. However, following the demonstration that the surface structure of human papilloma virus was composed of 72 morphological units (Klug & Finch, 1965) in an arrangement corresponding to the clustering of 420 structure units in hexamers and pentamers at the vertices of the $T = 7d$ icosahedral surface lattice (Caspar & Klug, 1962), Klug (1965) showed that the published electron micrographs of Wildy et al. (1960) and of Crawford, Crawford & Watson (1962) were inconsistent with the 42 structure, but was able to identify in them the 72 unit structure. Klug also pointed out that the images of K-virus showed it to be of the same structural type but could not unequivocally identify the 72 unit pattern in the actual images published by Mattern et al. (1963).

In this paper are presented characteristic two-side electron microscope images of negatively stained polyoma virus (i.e. images of particles completely enveloped in stain and which are therefore superposition patterns of detail from both near and far sides of the virus particles). These images and three dimensional image reconstructions confirm that the surface structure consists of 72 morphological units at the vertices of a $T = 7$ icosahedral surface lattice. Tilting experiments show that it is the right-handed version of the lattice, $T = 7d$.

METHODS

Negatively stained specimens of polyoma virus were prepared, following the method of Huxley & Zubay (1960), on holey carbon films attached to specimen grids. The stain used was a 1% solution of sodium phosphotungstate (pH 7). The grids were examined in a Phillips EM 300 electron microscope operating at 80 kV and at a magnification of × 51000.
Fig. 1. Electron micrograph of polyoma virus particles suspended in stain over a hole in the carbon substrate. This method ensures ‘two-side’ images, i.e. superposition patterns of detail from the near and far sides of the virus particles. The film of stain was broken and contracted and although the particles are somewhat compressed, the distortion in this field is fairly isometric. Recognizable characteristic images from fields such as this (Fig. 2) were tested for preservation of symmetry and the best preserved were included in reconstructing the three dimensional image shown in Fig. 4.

For photography, fields were chosen over holes in the carbon where the stain had broken and contracted isometrically yielding fairly circular images and where the particle density was sufficient to give twenty to thirty such images but not so great that particles were closely packed together and distorting each other. In the tilting work the grids were tilted through a nominal $\pm 6^\circ$ in the high resolution stage of the microscope.

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

RESULTS

A typical electron micrograph of a field of negatively stained polyoma virus particles photographed over a hole in the carbon film is shown in Fig. 1. In the absence of the support film the particles are equally stained on the near and far sides and the resulting images are patterns arising from the superposition of surface detail from both sides of the particles.
Many of the images patterns can be immediately recognized by their correspondence to members of the gallery of superposition patterns computed to simulate the images of human wart virus (Klug & Finch, 1968); some examples are shown in Fig. 2. Since the model used in computing this gallery was based on the hexamer and pentamer clustering of structure units on a $T = 7$ icosahedral surface lattice it is evident that polyoma virus has a similar structure.

While these characteristic two-side images show that polyoma has a 72 morphological unit surface structure, and this is confirmed by the results of three-dimensional image reconstruction presented later, single images are insufficient to determine whether the lattice has left or right handedness. The view parameters given in Fig. 2 correspond to the right-handed structure since this was the hand of human wart virus for which the simulated image gallery was calculated, but identical images would occur with the enantiomorphically related structure for different view parameters (Klug & Finch, 1968, Fig. 1). It is however possible to determine the hand of the polyoma structure by following the changes that occur in the images of particles as they are rotated in a known sense about a known axis in the electron microscope. This was the method used by Klug & Finch (1968) to confirm the handedness of the human wart virus structure. Pairs of images of the same particles of polyoma obtained with the specimen stage tilted by a nominal $+6^\circ$ and $-6^\circ$ are shown in
Fig. 3. (b) Images of polyoma virus particles. (c) Images of the same particles tilted through a nominal 12° about a tilt axis parallel to the arrow. The direction of rotation is anticlockwise looking in the direction of the arrow. (a) and (d) Computed superposition patterns from the gallery published by Klug & Finch (1965) which correspond most closely to the images in (b) and (c). Using their co-ordinate system, in each case θ = 90° and the values of φ are given. The values of φ increase in each case from (a) to (d) corresponding to an anticlockwise rotation of the model structure looking in the direction of the arrow. Thus the structure of the virus like that of the model is based on the right-handed version of the lattice, $T = 7d$.

Fig. 4. Stereoview of a reconstruction of polyoma virus including data to a Fourier cut-off of 2.5 nm. The view is close to a twofold symmetry axis and only the top half of the reconstruction is shown. Two 5 co-ordinated morphological units can be seen clear of the periphery, towards the upper right and lower left, and the path between them is two unit steps out and one step to the right, corresponding to the icosahedral $T = 7d$ lattice.

Fig. 3, together with the closest simulated images from the human wart virus gallery. The absolute sense of rotation of the virus particles in going from +6° to −6° was determined by inspection of the specimen stage, allowance being made for the rotation about the microscope axis of the image relative to the specimen. This sense was found to be the same as that required for the rotation of the model structure to change the simulated image accordingly (i.e. a tilt from +6° to −6° corresponds to an increase in the φ co-ordinate). Thus the hand of the polyoma structure is the same as that of the model of human wart virus and it is thus based on a $T = 7d$ lattice.
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For three-dimensional reconstruction, images were tested for the extent to which they were consistent with the icosahedral symmetry of undistorted virus particles, and their view parameters refined using the method of minimizing the common line residuals (see for example Crowther, Geelen & Mellema, 1974). The best images showed some correlation with icosahedral symmetry to spatial frequencies of about 2.5 nm. Three reconstructions were made, two using independent sets of five images and one using the six best of those ten images. A plot of the latter reconstruction is shown in Fig. 4, and appropriate projections of this are shown alongside the various characteristic images in Fig. 2.

All three reconstructions showed virtually the same features, 72 morphological units over the capsid surface, centred approximately at the lattice points of a \( T = 7 \) icosahedral surface lattice, and all lying at about the same radius. They are first distinguished at a radius of about 18 nm and extend to about 21 nm. At a radius of 19 nm, the distances between the morphological units are all about 7.5 to 8.0 nm except for the distances between the hexamers immediately neighbouring pentamers which are about 9.3 nm. This opening up of the lattice around the fivefold vertices is similar to that found in the reconstruction of human papilloma virus where the corresponding distances are 10.4 and 11.8 nm, respectively (Crowther & Amos, 1971). The morphological units are hollow and slightly conical in shape with a diam. of about 5 nm, although this is very sensitive to the choice of contour level chosen as the boundary between protein and stain. The pentamers appear very slightly smaller than the hexamers, but only if this were a consistent feature of many reconstructions could one regard it as structurally significant. The reconstruction shows little indication of regular substructure within the morphological units.

DISCUSSION

On the basis of the quasi-equivalence theory of Caspar & Klug (1962), a \( T = 7 \) icosahedral shell would be built from 420 structure units which are chemically identical or sufficiently similar that each had essentially the same pattern of bonds with its neighbours. Polyoma virus is known to contain at least seven proteins (Roblin, Härle & Dulbecco, 1971; Friedmann & David, 1972) of which three, VP4, 5, 6 are cell histone proteins (Frearson & Crawford, 1972). Of the virus specific proteins the major component, VP1 of mol. wt. 45,000, is present to the extent of about 380 copies per capsid and two others, VP2, VP3 of mol. wt. 34,000 and 24,000, could be present to the extent of 60 copies per capsid (Friedmann & David 1972). Thus it is numerically possible that VP1 could account for the 60 capsid hexamers (360 structure units) and either VP2 or VP3 or both for the 12 pentamers (60 structure units).

From the electron microscope results above there is apparently no gross difference between the sizes of the structure units making up the pentamers and hexamers. A similar observation was made by Kiselev & Klug (1969) who showed that the wide and narrow tubular polymers associated with papilloma and polyoma virus in unfractionated preparations were built from hexamers and pentamers respectively of units of about the same size. Both results are consistent with the hexamers and pentamers being made up from the same or similarly sized subunits. If the subunits are the same, both being built from VP1, there is no indication in the reconstruction, of extra density that could correspond to VP2 and VP3. It seems more likely that VP1 accounts for hexamers only and that pentamers are built from VP2 and VP3.
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REFERENCES


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