Viruses of *Entamoeba histolytica*

V. Ultrastructure of the Polyhedral Virus V₃₀₁

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The polyhedral amoebal virus V₃₀₁ presents sectioned profiles consistent with its having icosahedral (532) symmetry. The DNA appears to be packed in an orderly, folded arrangement of lesser symmetry.

We have previously demonstrated the polyhedral contour of a virus isolated from the HB-301 strain of *Entamoeba histolytica* (4) and suggested that it may be an icosahedral virus, on the basis of six- and five-sided profiles (13). In this note, we will provide additional evidence of its icosahedral symmetry and some unusual observations on the packing of its nucleic acid, now known to be DNA (9).

This virus isolate, V₃₀₁, has been characterized in detail (8), and the methods employed in this study by the electron microscope have been described (13).

In the upper line of Fig. 1, icosahedra are presented on their five-, three-, and twofold axes of rotational symmetry, and profiles which might result from such orientations are presented in the second line. It should be noted that the fivefold profile will result only from a "superficial" section of the icosahedral model. A section centered about the midportion of the model would present either a 10-sided image or, at low resolution, a nearly circular profile. Viruses presenting pentagonal profiles actually do appear "thin," i.e., poorly staining, and of small diameter relative to viruses seen in other identifiable orientations. In the lower two lines are profiles of viruses which are compatible with their being icosahedra viewed on five-, three- and twofold axes of rotational symmetry.

Additional observations of note have been made on the organization of the virus core. Occasional sections of viruses oriented so as to present a six-sided profile reveal an ordered stacking of fine filaments (Fig. 2h, i). Each filament is about 8 nm in diameter and spaced at intervals of about 13 to 14 nm center to center. In cross sections of the filaments (normal to x axis, Fig. 2h, i), there appear to be 19 filaments in a hexagonal array: one filament in the center surrounded by a ring of six and an

outer ring of 12 (cf. Fig. 2b, e). In other orientations, we have observed what appear to be curved connections between longitudinally sectioned core filaments (Fig. 2; cf. c, d, f, g and

FIG. 1. Demonstration that V₃₀₁ has icosahedral symmetry. In the upper line, icosahedral models are seen normal to their five-, three-, and twofold axes of rotational symmetry. The second line represents profiles of the models in the upper line. In the lower lines are seen, at the same magnification, electron microscopic sections of the V₃₀₁ virus which are compatible with the icosahedral models. Virus particles are approximately 80 nm in diameter (edge to edge as seen on a threefold axis).

j, k, l, m). In these sections, three to five linear filaments can be observed in one of two orientations: either they are perpendicular to opposite edges of the hexagonal profile (Fig. 2f, j, k), or they are parallel to a line joining two points of the hexagonal profile (Fig. 2g, l, m). All of these

observations are compatible with the virus containing one long strand of DNA folded as in the model presented in Fig. 2a. The circularity or linearity of the molecule and the number of double strands of DNA within each observed filament cannot be determined at this time. It

FIG. 2. A hypothetical model of a folded linear structure such as DNA which could account for the electron microscopy sections of the V_{301} virus seen in (h) through (m). Orthogonal axes of the model are designated in (a), which is viewed nearly parallel to its z axis. (b-d), "Sectioned" views of the model seen on x, y, and z axes, respectively. Filaments depicted normal to the y axis (c) are not truly co-planar. (e-g), Sectioned views of the model with orthogonal axes of the model coincident with orthogonal twofold axes of an icosahedron. (h-m), Sectioned viruses seen in different orientations: (h) and (i) present cross-sectioned filaments with an arrangement compatible with (b) and (e), whereas (j) and (k) resemble (c) and (l) and (m) show s-shaped core images resembling (d) and (g). (h-m), Printed so as to resolve the core filaments. The viral coat, by which the orientation is established, is more clearly resolved in darker prints ($\times 120,000$).

should be noted that the core model has a point group symmetry which is substantially lower than 532 (icosahedral) symmetry. The model possesses three orthogonal twofold axes and therefore has 222 (orthorhombic) symmetry. Such a model, oriented within an icosahedral shell such that its three axes of twofold symmetry were coincident with three orthogonal twofold axes of the icosahedron (Fig. 2e, f, g), would account for all of the observations previously noted.

Additional packing arrangements of the core model within an icosahedron might also be compatible with the electron micrographs. For example, the core model's twofold x axis, which reveals a hexagonal stacking of filaments on cross section (Fig. 2b), might be coincident with a threefold axis of the icosahedron. In this case, only one of the other two twofold core axes could coincide with a twofold axis of the icosahedron, and the symmetry of the combined models would be 2 (monoclinic). Alternatively, if none of the core model's twofold axes were truly coincident with the icosahedron's twofold axes, the combined model would have no symmetry.

Among the multitude of known viruses, there is but one in which the arrangement of its nucleic acid has been determined in detail from the X-ray diffraction studies of Caspar (1) and Franklin (5). That is tobacco mosaic virus in which the single-stranded RNA is helically arranged so as to follow the overall helical plan of the "cylindrical" aggregate of identical protein molecules [reviewed by Caspar (2)]. In a limited number of other viruses, it is known that the RNA exists in the core in some sort of helical arrangement: myxo- and paramyxoviruses, and bullet-shaped viruses of the vesicular stomatitis type (12, 15). On the basis of X-ray diffraction studies on turnip yellow mosaic virus, the packaging of its RNA appears to be related to the icosahedral (532) symmetry of its protein coat (10).

Of the DNA viruses, perhaps even less is known about the organization of their DNA within the viral core. Some of them, including the adenoviruses (7) and papovaviruses (16), are known to contain basic core proteins intimately associated with the DNA and perhaps functioning, among other things, in the assembly of the virion. The core of some of the pox viruses has been depicted as a stout filament folded into an s-shaped structure (12, 14). A recent report suggests that the DNA of a herpes virus may form a toroidal structure, as though the DNA were spooled around an electron-lucent, central, cylindrical mass (6). Certain of

the bacteriophages appear to have a DNA-free region within the central part of the core (3, 11). However, in none of these instances was the folding pattern of the DNA molecule established.

The icosahedral amoebal virus V_{301} presents an arrangement of its nucleic acid which, to the best of our knowledge, is unique among viruses and clearly distinguishes this amoebal virus from the bacterial and algal viruses which it otherwise closely resembles (13).

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