Ultrastructure of Mycoplasmatales Virus laidlawii 1

By JUDY BRUCE, R. N. GOURLAY, R. HULL* AND D. J. GARWES

Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, England

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SUMMARY

The morphology and ultrastructure of Mycoplasmatales virus laidlawii 1, a new helical DNA virus, were studied by electron microscopy and optical diffractometry. Numerous small unenveloped rod-like particles and a few longer forms were seen in purified, concentrated preparations of infective virus, and associated with virus-infected mycoplasmal cells in culture. Observations on these virus particles are discussed in relation to their structure.

INTRODUCTION

A recent report (Gourlay, 1970) described the isolation of a virus, subsequently named Mycoplasmatales virus laidlawii 1 (MV-L1), which infects Acholeplasma laidlawii, a member of the Mycoplasmatales. A later communication (Gourlay, Bruce & Garwes, 1971) described the purification of the virus which was found to be a short rod probably containing DNA. This paper presents further morphological studies on the virus both in purified preparations and in association with mycoplasmal cells.

METHODS

The host, A. laidlawii strain BNI, was grown in modified glucose serum broth or agar (Gourlay & Wyld, 1972). To recover and purify virus, solid medium cultures of infected cells were incubated at 37°C for 24 hr and then washed with phosphate buffered saline. The suspension obtained was treated with Nonidet P40 to dissolve host membranes before concentration through two caesium chloride density gradients (Gourlay et al. 1971).

Mycoplasma cells were grown in broth. They were infected during the early log phase of growth and incubated at 37°C for up to 10 hr. Cells were harvested by centrifuging at low speed and, to prevent lysis, were fixed for 3 to 5 min. with 3% glutaraldehyde in 0.1 M-sodium cacodylate buffer solution at pH 7.0.

For electron microscopy, purified virus and infected cells were applied to carbon-collodion coated grids, washed thoroughly with 0.1 M-sodium cacodylate or 2% ammonium acetate at pH 7.0 before negatively staining for a few seconds with either 2% potassium phosphotungstate at pH 6.5 or 2% uranyl acetate at approximately pH 4.5. A 1% solution of uranyl formate at pH 3.5 was sometimes used for studies at high resolution. The dimensions of the virus particle were measured from photographic enlargements of accurate magnification, calibrated with reference to the lattice spacing of crystalline beef liver catalase (Koch Light Laboratories Ltd), fixed in 3% glutaraldehyde and applied to the grid with the specimen.

* John Innes Institute, Colney Lane, Norwich.
Fig. 1. Purified preparations of MV-L1, negatively stained with 2% uranyl acetate. (a) showing degradation at one end of some particles. (b) arrow indicates particle penetrated by stain. (c) upright particles, some showing stain penetration into centre.
RESULTS

Negatively stained preparations of purified, concentrated virus showed numerous straight or slightly curved rods (Fig. 1 a, b, c). Nearly all such particles were rounded at one end; usually the other end was rounded also but was sometimes flat or visibly degraded to one or two short protuberances. Measurements of particles showed two modal lengths: the average length of complete rods was 90 nm. and of the others 80 nm. (Fig. 2). The mean end-on diameter of particles was 16 nm. as measured from upright particles (Fig. 1 c). However, rods lying flat on the mounting film showed a mean diameter of 14.5 nm. This discrepancy is probably due to overlapping stain which masked the diameter of flat particles. Available
concentrations of virus were insufficiently high for measurement of centre to centre spacings in close packed arrays of particles.

When stained with uranyl salts, especially uranyl formate, some particles were penetrated by stain (Fig. 1b), thus indicating hollow centres and ends normally sealed; wall thickness measurements were from 5.2 to 6.2 nm.

Difficulties were experienced in obtaining clear optical diffraction patterns from electron micrographs of particles. This was probably due to the small size of the particles and the limited repetition of structure. However, certain intense spots were found consistently (Fig. 3) and indicated diffracting elements arranged in a hexagonal lattice forming a helix of pitch angle about 20°. Using catalase as a standard for measurement, the lattice spacing was about 4.8 nm.

Long curved rods, exceeding 500 nm. in length, appeared occasionally in purified cultures (Fig. 4a). Their diameters and optical diffraction patterns were similar to those of the short
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Fig. 5. (a) Cells of *Acholeplasma laidlawii* from broth culture with associated long forms. Negatively stained with 2 % potassium phosphotungstate. (b) A hollow form of greater diameter, that is 30 nm.

particles; it is presumed that they are nucleoproteins since their buoyant density was the same as that for particles in infective preparations.

Similar long rods were observed attached to mycoplasmal cells in centrifuged deposits of infected broth cultures. Numerous particles of length between 100 and 500 nm. were attached by one end to cellular membranes (Fig. 4b) and one of these rods is penetrated by
stain. Other samples of centrifuged deposits yielded numerous apparently hollow long rods (Fig. 5a) of diameter similar to that of normal rods. These long rods appeared most frequently in cultures of cells inoculated with virus late in log phase and in those incubated for over 4 hr after infection. Less frequently we found rods with greater diameter (about 30 nm.) and wall thickness about 7.5 nm. (Fig. 5b).

Deposits from cultures of A. laidlawii which had not been inoculated with virus showed no rods of the types described.

DISCUSSION

Our results suggest that MV-L1 is an unenveloped and helically symmetrical DNA virus which is probably the first of this type to be described. The normally occurring particle in infective preparations is a slightly sinuous rod of 16 nm. diameter and 90 nm. length. Optical diffraction studies indicate that the tubular portion of the particle consists of subunits arranged in hexagons to form a helix. Under normal staining conditions the lattice spacing from optical diffraction patterns of hexagonally arranged protein subunits has been shown to be half the centre-to-centre spacing of the hexagons (Bancroft, Hills & Markham, 1967; Hitchborn & Hills, 1968); on this basis the centre-to-centre spacing of the protein hexagons of MV-L1 particles is approximately 9.6 nm. Hull, Hills & Markham (1969) demonstrated that it was possible to form the hemispherical ends of bacilliform particles by using half icosahedra and that the structure of the tubular part depended upon the axis across which the icosahedron was cut. The structure deduced from the optical diffraction patterns of MV-L1 is consistent with one based upon a 12 morphological subunit icosahedron (T = 1) cut across its two-fold axis. This gives a particle of 16 nm. diameter and a tubular portion consisting of morphological subunits arranged in a two-start helix with 5.6 subunits per turn on a pitch angle of about 20° (Fig. 6).

The two preferred lengths of virus particles (Fig. 2) correspond to particles with either
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both ends rounded (90 nm. long) or with one end rounded (80 nm.) and the other showing disintegration. The difference in preferred lengths is consistent with the loss of a half icosahedron (8 nm.) from one end. Virus particles appeared to attach by one end only to host membranes. No particles were observed to be attached at both ends. As the unattached end was always rounded it is likely that degradation occurs at the end normally involved in attachment.

The long rods described may not be infective although some are nucleoproteins. Failure in the mechanism which normally closes the protein tube into a particle of standard length may be due to the inclusion of either a multiple length of virus nucleic acid or possibly of host DNA. The long rods of different morphology were seen only in broth cultures and may be polymers of virus or host protein lacking nucleic acid.

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REFERENCES


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