Electron-Microscopic Study of a Mycoplasmatales Virus, Strain MV-Lg-pS2-L 172

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ABSTRACT. Morphology and adsorption of a globular virus, lysing Acholeplasma laidlawii were studied in ultrathin sections of plaques in a lawn of the host strain. The virus was globular, about 50 to 90 nm in diameter, with a clearly defined membrane, 6.5 to 8 nm thick. A protuberance about 25 to 35 nm long and 12 to 20 nm thick was observed on numerous virus particles. The evenly granulated, electron-optically dense content of the cells became clearer in cells affected by the viruses. Fibrillar structures of different thickness and small dense areas appeared in cells assumed to be in the preliminary stages of lysis. The interactions in the virus-host system and possible development stages of the virus are discussed.

A globular virus affecting Acholeplasma laidlawii was isolated (Liška, 1972) and its properties briefly described (Tkadlecček and Liška, 1973; Hufková et al., 1973). Since no closer morphological information was available about adsorption, injection and multiplication of this type of viruses, the vital functions of the isolated strain were studied by electron microscopy. Mycoplasmas are the most primitive microorganisms and the virus described is a relatively simple particle. This leads to anticipating that the virus-host system would also limit its interactions to the most primitive ones, which could help to reveal some phylogenetically original forms of the development of the virus-host relations. The results of this paper may throw light on this subject.

MATERIALS AND METHODS

Microorganisms. The virus MV-Lg-PS2-L 172 was grown on Acholeplasma laidlawii, strain S2 as follows from its coding (Liška, 1972).

Media. Liquid media No. 74 or 81 were used for growing the host Acholeplasma strain. Culture medium No. 74 consisted of 150 ml of concentrated Tryptone (Bioveta Terezin, Czechoslovakia), 10 g dehydrated yeast autolysate (Imuna, Šarišské Michaňy, Czechoslovakia), 3.5 g NaCl, 5 g sodium acetate, 10 g dehydrated Brain Heart Infusion (Oxoid), 10 mg phenol red, 5 g glucose, 70 ml horse serum (Bioveta, Ivanovice na Hané, Czechoslovakia) per litre. Medium No. 81 contained 21 g dehydrated PPLO Broth (Difco), 10 g dehydrated Yeast autolysate (Imuna), 5 g Na acetate, 10 mg phenol red, 5 g glucose, 70 ml horse serum per litre. Medium No. 75, used for virus plating consisted of 150 ml of concentrated Tryptone (Bioveta), 10 g dehydrated Tryptone (Bioveta), 10 g dehydrated Yeast autolysate (Imuna), 7 g dehydrated Nutrient Broth (Léčiva, Czechoslovakia), 3.3 g NaCl, 5 g sodium acetate, 10 mg phenol red, 10 g agar, 5 g glucose, 70 ml horse serum (Bioveta) per litre. Culture media were autoclaved for 20 min at 137°C without glucose (25% solution).
and Yeast autolysate (10%) which were autoclaved separately and, as well as serum, mixed with the main solution before pouring the plates or distributing the liquid media. A solution of Nutrient Broth (21 g/litre) was prepared, autoclaved and after filtration used in an equivalent amount for the preparation of medium 75 before it was autoclaved. Final pH of the media was adjusted to 7.8 with 1M or 10M NaOH.

**Virus-plating.** *Acholeplasma laidlawii* strain S2 was grown in liquid medium No. 74 or 81 for about 12 h, until it reached the middle or late logarithmic phase of growth and about 10⁷ or 10⁸ cells/ml. One ml of this culture was mixed with 0.1 ml of the virus suspension in a slightly alkaline (pH about 7.3) phosphate (0.03M) saline (5.5 g NaCl/litre) buffer, containing about 10⁴ virus particles/ml. From the resulting 10 × dilution in the indicator culture, 0.2 ml were spread on the surface of a pre-dried agar plate (10 cm diameter) of medium No. 75.

**Electron microscopy.** After about one day of incubation, when numerous plaques appeared, several drops of 1.5% glutaraldehyde in buffered saline (Morel *et al.* 1971) were placed on the agar. Then, parts of plaques with the adjoining lawn of the host cells were cut out with a glass tube and the blocks were dipped into the glutaraldehyde solution. After two hours, the upper layer of the agar with the lawn and plaques was cut off from the blocks with a razor blade, divided into small pieces, washed in buffered saline and postfixed with 1% OsO₄. After dehydration in graded acetone, the specimens were embedded in Durcupan ACM (Fluka) and sectioned. Semi-thin sections were stained with brilliant-cresyl blue and examined in a light microscope. Suitable areas with different stages of lysis were selected for ultrathin sections, which were stained with uranylacetate and lead citrate and examined in an electron microscope (Tesla BS 513).

**RESULTS**

Three main types of biological unit were observed in the ultrathin sections of plaques in the lawn of the host strain: (1) Virus particles with a diameter of 50 to 90 nm, a majority of them 50—65 nm; (2) cells of *Acholeplasma laidlawii*, of which the majority had the diameter of 0.6 to 1.2 μm; (3) small, electron-dense, roughly spherical bodies with a diameter of approximately 100—200 nm.

The virus particles, observed in large numbers, were globular units, of which the content was electron-optically relatively dense and homogenous, with a clearly defined membrane, composed of at least two layers — a dense outside layer (1.8—2.5 nm) and a more transparent medium layer, about 3.0—3.6 nm thick. It is very probable that there is a third, dense, inner layer (again 1.8—2.5 nm thick) forming the inner side of the membrane, and this fuses more or less with the content of the particle, having approximately the same electron-optical density. This assumption was supported by the observation of a membrane in the particles of which the content was partly separated from the membrane (Plate 2, fig. 8) and of empty ghost particles of virus size (Plate 2, fig 8), showing only the empty membrane. The thickness of this virus-enclosing membrane was about 6.5—8.5 nm. The structure and thickness of the membrane and its layers suggest the characteristics of biological “unit membranes”.

In many cases a protuberance about 25—35 nm long and 12—20 nm thick was seen on the virus particles (Plate 1, fig. 1—5). As can be seen from the figures, the protuberance had the structure of a cylinder consisting of a membrane-like sheath with a denser content which may be either a core, or a hollow with the stain accumulated inside. The particles and their protuberances seemed to have a rather soft, pliant consistency and, although the majority of them were about 50—65 nm in