Characterization of Mycoplasmatales virus-laidlawii 3

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SUMMARY

Purified preparations of Mycoplasmatales virus-laidlawii 3 were negatively stained and studied by electron microscopy. They were seen to consist of uniform sized particles having a polyhedral head, 57 nm by 61 nm, and a short tail, 25 nm long, joined to the head at one vertex by a collar. The particles were shown to have buoyant densities of 1.477 g/ml in CsCl, 1.32 g/ml and 1.26 g/ml in potassium tartrate and a sedimentation coefficient, s20, w, of 290 ± 13. They are composed of 35.2% double-stranded DNA and five structural polypeptides with approximate mol. wt. of 172,000, 81,000, 73,000, 68,000 and 43,000. The classification of the virus from its morphology and chemical properties is discussed.

INTRODUCTION

In a recent publication, Gourlay & Wyld (1973) described the isolation of Mycoplasmatales virus-laidlawii 3 (MV-L3), a new virus capable of replicating in Acholeplasma laidlawii. It is serologically different from the other viruses of A. laidlawii, MV-L1 (Gourlay, 1970) and MV-L2 (Gourlay, 1971), and is able to grow on host strains that are resistant to MV-L1 and MV-L2. The morphology of MV-L3 was shown to be dissimilar to that of MV-L1, which has a rod-shaped particle 16 nm wide and 90 nm long (Bruce et al. 1972), and MV-L2, which is spherical with a mean diam. of 80 nm (Gourlay et al. 1973). Both MV-L1 and MV-L2 contain DNA (Gourlay, Bruce & Garwes, 1971; Gourlay et al. 1973).

This paper reports the purification of MV-L3, some aspects of its morphology and the identification of its nucleic acid type and structural polypeptides.

METHODS

Virus culture. MV-L3 was grown in GS broth cultures of Acholeplasma laidlawii M1305/68 (Gourlay & Wyld, 1972) for 48 h at 37 °C and infectivity was titrated by plaque assay on lawns of M1305/68 as previously described (Gourlay & Wyld, 1973).

Purification of virus. Infected broth cultures were filtered through Millipore membranes type GS, 220 nm pore size, and the filtrate chilled to 2 °C in melting ice. Finely ground ammonium sulphate was added and dissolved to give 55% saturation and the resulting precipitate was allowed to flocculate at 4 °C for one hour before being sedimented at 15000 g for 20 min at 4 °C. The pellet was dissolved in isotonic saline buffered with 10 mM-tris, pH 7.2 (T-S), to one tenth of the volume of the original culture, Nonidet-P40 was added
to a concentration of 0.4% and the suspension was layered over 5 ml volumes of 10% sucrose in T-S contained in 25 ml polycarbonate tubes. The virus was sedimented at 250,000 g_\text{av} for 90 min at 4 °C in the 8 × 25 ml Ti rotor of the MSE Superspeed 65 ultracentrifuge. The pellet was resuspended in T-S and solid cesium chloride added at the rate of 0.79 g/ml of virus suspension, producing a solution of density 1.5 g/ml. The density gradient, formed by centrifuging at 150,000 g_\text{av} for 18 h at 4 °C in the 8 × 25 ml Ti rotor, was fractionated by siphon into 0.5 ml samples and the virus detected as infectivity, extinction or radioactivity, as noted in the text. CsCl was removed by extensive dialysis at 4 °C against T-S or, when the virus was required for DNA isolation, against SSC (0.15 M-NaCl-0.015 M-citrate, pH 7.0).

**Isopycnic sedimentation in potassium tartrate gradients.** Linear 20% to 50% gradients were constructed using aqueous solutions of recrystallized potassium tartrate. Unpurified and partly purified virus was introduced on the top or throughout the gradient during its formation. After centrifuging in swing-out rotors at 70,000 g_\text{av} for 16 to 24 h the gradients were fractionated by siphon and the virus was located by measurement of \(E_{260}\) and \(E_{280}\) and by infectivity assay. The refractive index of each fraction was measured and the solution density calculated with the equation \(\rho = an_\text{D} + b\), where \(\rho\) is the density of solution at 25 °C, \(n_\text{D}\) is the refractive index of solution and the coefficients \(a\) and \(b\) have values of 5.459 and 6.2951 respectively. These values had been derived experimentally using solutions of purified potassium tartrate.

**Electron microscopy.** Purified virus preparations were applied to carbon-collodion coated grids, negatively stained for a few seconds with 2% uranyl acetate, pH 4.5, or 2% potassium phosphotungstate, pH 6.5, and examined using a Philips EM300 electron microscope. Measurements of virus particles were made using the photographic plates whose magnification had been calibrated by reference to the lattice spacing of beef liver catalase.

**Analytical ultracentrifugation.** Virus sedimentation coefficients were derived by the band-forming technique of Vinograd et al. (1963) in a Spinco Model E ultracentrifuge (Beckman-RIIC Ltd, London) equipped with a monochromator. Purified virus was diluted in distilled water and 25 μl samples were layered onto 1 M-NaCl in a type 2 band-forming centrepiece. All runs were carried out at 20 °C, at 12,000 rev/min and in an AN-H rotor. Photographs were taken on Ilford N5.31 film at 4 min intervals using light at 265 nm. After development, the negatives were scanned with a Joyce–Loebl Chromoscan microdensitometer and the sedimentation coefficients were determined using linear regression analysis of the data. These values were corrected to the density and viscosity of water at 20 °C. The partial specific volume of the virion, \(\bar{V}_{\text{virus}} = 0.668 \text{ ml/g}\), was calculated from the \(\bar{V}\) values of its constituent DNA and protein components according to the relation \(\bar{V}_{\text{virus}} = (\bar{V}_{\text{DNA}} \times W_{\text{DNA}}) + (\bar{V}_{\text{protein}} \times W_{\text{protein}})\) where \(\bar{V}_{\text{DNA}}\) and \(\bar{V}_{\text{protein}}\) are the assumed partial specific volume values for DNA (0.556 ml/g) and protein (0.75 ml/g). \(W_{\text{DNA}}\) and \(W_{\text{protein}}\) are the weight concentrations of the DNA and protein and are taken as 0.352 and 0.630 respectively.

**Carbohydrate analysis.** To distinguish between the presence of DNA or RNA in the virion, samples of purified virus were analysed using the diphenylamine reaction for deoxypentose (Burton, 1956) and the orcinol test for pentose (Mejbaum, 1939).

For the detection of other structural carbohydrates, purified virus was analysed for hexoses by the anthrone test (Yemm & Willis, 1954), for hexosamines (Rondle & Morgan, 1955), for fucose (Gibbons, 1955) and for sialic acid by the methods of Svennerholm (1957) and Warren (1959).

The quantitative analysis of DNA in the virion was conducted using the diphenylamine reaction and was calibrated with deoxyribose and with samples of purified DNA from calf.
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thymus, Escherichia coli and Micrococcus lysodeikticus, all obtained from Sigma London Chemical Co. Ltd, Surrey, England.

Incorporation of radioactive nucleosides. Two 50 ml lots of GS broth, one (T) containing 10 mm-[\textsuperscript{3}H]-methyl-thymidine (sp. act. 1 Ci/mm) and 10 mm-uridine, the other (U) containing 10 mm-[\textsuperscript{5}H]-uridine (sp. act. 1 Ci/mm) and 10 mm-thymidine, were inoculated with Acholeplasma laidlawii M13o5/68 and incubated at 37 °C for 7 h. They were then infected with MV-L3 and incubation was continued for 48 h. The virus was purified as described and radioactivity was determined in NE250 scintillant (Nuclear Enterprises Ltd, Edinburgh) using a Packard Model 2425 Tricarb spectrometer. [\textsuperscript{3}H]-methyl-thymidine (sp. act. 5 Ci/mm) was obtained from the Radiochemical Centre, Amersham, and [\textsuperscript{5}S]-uridine (sp. act. 26 Ci/mm) from NEN Chemicals GmbH, Dreieichenhain.

Isolation of virus DNA. Method 1, which is similar to that used by Shepherd, Bruening & Wakeman (1970) for cauliflower mosaic virus, is based upon the digestion of capsid protein by a proteolytic enzyme aided by sodium dodecyl sulphate (SDS; Joklik, 1962). Pronase and SDS were added to a sample of purified MV-L3 to final concentrations of 5 µg/ml and 0.25 % respectively, the mixture was incubated at 37 °C for 2 h and then the SDS concentration was increased to 1 % and incubation continued for 1 h. The digested sample was extracted twice at room temperature with equal volumes of freshly distilled phenol saturated with SSC and the final aqueous phase was extensively dialysed against SSC to remove phenol.

For method 2 purified virus preparations were made 1 % with respect to sodium lauroyl sarcosinate, heated at 60 °C for 60 min and diluted with 9 vol. of 59 % CsCl in SSC. The resulting solution was centrifuged in the MSE 3 × 6.5 ml Ti rotor at 150,000 g, at 20 °C and the density gradient was then fractionated into 0.2 ml samples by siphon. DNA was located by \(E_{260}\) or by radioactivity assay.

Method 3 was adapted from the technique that Bak & Black (1968) used to isolate DNA from mycoplasmas. To a purified virus preparation was added ethylenediaminetetra-acetic acid disodium salt to 10 mm and SDS to 2 %, the mixture was held at 60 °C for 10 min and then cooled in ice. One third volume of 5 m-sodium perchlorate was added and the suspension was shaken with chloroform at room temperature for 15 min. The aqueous phase was recovered, shaken with a second volume of chloroform and finally dialysed against SSC.

Some DNA preparations, as noted in the text, were further purified by isopycnic equilibrium centrifuging in solutions of CsCl in SSC, starting density 1.64 to 1.70 g/ml. The refractive index of the fractions from such gradients was measured and the solution density calculated using the equation of Vinograd & Hearst (1962).

Thermal denaturation of virus DNA. Solutions of virus DNA, prepared by methods 1 and 2, in 1 × SSC and 0.1 × SSC were heated at the rate of 1 °C increase per min in a Pye Unicam SP800 recording spectrophotometer fitted with a SP874 controlled temperature cell housing. The \(E_{260}\) was recorded and the temperature of the sample was measured by a thermocouple attached to an electronic thermometer (type 1605, Comark Electronics Ltd, Sussex).

DNA base composition analysis. Base composition of virus DNA was determined by the method described by Bendich (1957). Samples of purified DNA from MV-L3, Escherichia coli and calf thymus were hydrolysed in 88 % formic acid at 175 °C for 30 min, dried under nitrogen, dissolved in N-HCl and chromatographed on Whatman No. 1 paper with an isopropanol/HCl solvent system using thymine, adenine, guanine and cytosine as reference markers. The location of each base was determined by u.v. illumination, the Rf was calculated, the spot was cut out and the base was eluted in 0.1 N-HCl. The extinction spectrum was recorded and the quantity of each base was calculated as described (Bendich, 1957).

Protein analysis. The protein content of purified MV-L3 was measured by the Hartree
modification of the Lowry estimation and by the quantitative detection of amino acids with ninhydrin after acid hydrolysis of the virus (Moore & Stein, 1954). All analyses were calibrated using bovine serum albumin, porcine pancreatic trypsin and porcine gastric pepsin from Sigma London Chemical Co. Ltd.

The virus structural polypeptides were analysed by electrophoresis of disrupted virus through 5% polyacrylamide gel in the presence of 0.1% SDS and 0.1 M-sodium phosphate buffer, pH 7.2. Dissociation of the virus was brought about by heating samples of purified virions at 100°C for 2 min in 0.01 M-sodium phosphate, pH 7.2, containing 1% SDS and 1% β-mercaptoethanol. The protein bands were located by staining with Coomassie brilliant blue and their approximate mol. wt. were determined from their mobility relative to bromophenol blue, using bovine haemoglobin (17000), porcine pancreatic trypsin (24000), porcine gastric pepsin (34500) and bovine serum albumin, monomer (69000) and dimer (138000), as standards.

RESULTS

Virus purification

The data presented in Table 1 illustrate the recovery of virus during the purification procedure. The initial titre of the virus used in the experiments described in this paper ranged from $2 \times 10^9$ to $2 \times 10^9$ p.f.u./ml. Precipitation with 50% saturated ammonium sulphate was found to be adequate to recover more than 95% of the infectivity and to reduce the volume for subsequent sedimentation steps. A great deal of non-virus protein, derived from lysed mycoplasmas and serum in the medium, was co-precipitated with virus, but much of this was removed during the sedimentation through 10% sucrose. Nonidet-P40 was included at this stage to liberate virus that had adsorbed onto fragments of host membrane.

A profile of the gradient formed after equilibrium sedimentation of the pelleted virus in CsCl solution is shown in Fig. 1(a). It is clear that the virus bands at a location where it is free from contaminating proteins and Table 1 shows the titre of this band of virus after collection and dialysis to remove the CsCl.

At this stage the preparation is the 'purified virus' referred to during the remainder of this article. Fig. 1(b), however, presents a profile of a second isopycnic equilibrium run formed by mixing 0.5 ml samples of fractions 10, 11 and 12 from the gradient in Fig. 1(a), adding a further 4 ml of a CsCl solution of density 1.52 g/ml and centrifuging in the 3 x 6.5 ml Ti rotor at 15000 g, for 19 h. There is a single band of material, as measured by extinction, corresponding to infective virus. Since no further purification was achieved by a second isopycnic equilibrium sedimentation, this step was omitted from the routine preparative procedure.

The buoyant density at which MV-L3 bands in CsCl solution was found to be in the range 1.47 to 1.48 g/ml, as shown in Fig. 1. Since this value was unexpectedly high the buoyant density in CsCl was accurately determined for each virus preparation produced. The mean value for 10 such determinations was 1.477 g/ml with a range from 1.473 to 1.482 g/ml.

Buoyant density in potassium tartrate

Isopycnic sedimentation of unpurified and partly purified MV-L3 preparations in potassium tartrate resulted in two bands of virus as determined by extinction and infectivity. Most of the virus was located at a density of 1.32 g/ml with a smaller amount, somewhat variable in quantity, at 1.26 g/ml. From the appearance of the denser band it would seem that MV-L3 is rather insoluble in 42% potassium tartrate; the virus retained its infectivity,
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Table 1. Recovery of virus during purification

<table>
<thead>
<tr>
<th>Sample</th>
<th>Infectivity (p.f.u./ml)</th>
<th>Volume (ml)</th>
<th>Total virus (p.f.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>1.6 × 10^10</td>
<td>200</td>
<td>3.2 × 10^12</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitate</td>
<td>2.0 × 10^11</td>
<td>30</td>
<td>6.0 × 10^12</td>
</tr>
<tr>
<td>Pellet from Nonidet-P40</td>
<td>1.8 × 10^11</td>
<td>18</td>
<td>3.2 × 10^12</td>
</tr>
<tr>
<td>Band from gradient</td>
<td>3.5 × 10^13</td>
<td>2</td>
<td>7.0 × 10^12</td>
</tr>
</tbody>
</table>

Fig. 1. Isopycnic equilibrium sedimentation of MV-L3 in CsCl density gradients. Virus that had been treated with Nonidet-P40 and sedimented through 10% sucrose was resuspended in T-S and solid CsCl was added to a density of 1.5 g/ml. The solution was centrifuged for 18 h at 150000 g, and the resulting density profile is shown in (a). (b) Illustrates the profile of a second density gradient formed by sedimentation of the material from fractions 10, 11 and 12 of the gradient shown in (a).

Virus morphology

Electron microscopy of purified MV-L3 preparations negatively stained with uranyl acetate showed uniform-sized particles with a hexagonal outline and a single tail-like projection (Fig. 2). Measurements made on 100 negatively stained particles gave the dimensions as 57 nm between opposing planes and 61 nm between opposing vertices, both values ± 1 nm, with a tail approx. 25 nm long and 9 nm wide. It has not been possible to see any clear arrangements of subunits on the surface of the particle nor any distinct structure within the virus, but there is evidence that stain may penetrate through an outer wall, which is approx. 3 nm wide, to reveal a threadlike structure inside (see Fig. 2). There appears to be a ‘collar’ at the junction of the tail and the polyhedral head, more clearly visible in preparations negatively stained with potassium phosphotungstate (Fig. 2 b and c). Occasional electron micrographs showed particles that did not resemble MV-L3. These elongated forms, one of which is illustrated in Fig. 2(d), occurred so infrequently that it has not been
possible to obtain much information about them. The particle shown in Fig. 2(d), however, can be seen to be approx. 150 nm long and 40 nm wide having a wall thickness comparable to that of the typical virions, some internal structure and the suggestion of a tail at one end of the rod.

**Analytical ultracentrifugation**

Sedimentation velocity runs were carried out on fifteen virus samples ranging in concentration from 0.45 to 3.20 $E_{260}$/ml and these data were used to calculate the sedimentation coefficient. The results from a representative experiment are illustrated in Fig. 3. The virus sedimented as a single species with values of $s_{	ext{observed}} = 261.3$ and $s_{20,w} = 290 \pm 13$. There was no evidence of dependence of the sedimentation coefficient on the concentration of virus within the range used.

**Nucleic acid identification**

Fractions from an isopycnic equilibrium sedimentation of MV-L3 in CsCl were assayed for virus infectivity and for DNA by the diphenylamine reaction. These data, Table 2, indicate that the band of infectivity was diphenylamine-positive, indicating the presence of
DNA in the virions. When fraction 13 from the above gradient was assayed by the orcinol test for nucleic acid, using RNA and DNA solutions for calibration, the amount of colour that developed was equivalent to either 75 µg of DNA or 21 µg RNA. Since the diphenylamine test had indicated a DNA content of 60 µg/ml, it is most likely that the orcinol-positive material corresponds to DNA. Additional evidence was obtained from anthrone tests on purified virus, in which the only carbohydrate that could be detected was a deoxypentose as judged by the extinction maxima at 562 nm and 524 nm. Under the conditions of the test, a sample of DNA gave a spectrum similar to that produced with purified virus, whereas a sample of RNA showed no extinction maxima other than 626 nm, the wavelength at which most hexoses and pentoses absorb in this test.

The chemical tests that were applied to the virus indicated the presence of DNA but could not be used to exclude the presence of some RNA in the virions. In order to compare the relative proportions of DNA and RNA in the purified virus preparations, MV-L3 was grown in cultures containing either [3H]-thymidine or [3H]-uridine, as described in Methods. Samples were removed at stages during the purification procedure and assayed for infectivity, total radioactivity and radioactivity precipitable with 5% (w/v) trichloroacetic acid at 4 °C. The results are presented in Table 3. While the recovery of virus during purification of both preparations was comparable, only the virus grown in medium containing [3H]-thymidine retained a high proportion of the initial radioactivity, confirming that the viral genome is composed of DNA.

Table 2. Detection of DNA in purified virus

<table>
<thead>
<tr>
<th>Fraction no.</th>
<th>Infectivity (p.f.u./ml)</th>
<th>DNA concentration (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>8.5 x 10^8</td>
<td>24</td>
</tr>
<tr>
<td>13</td>
<td>5.0 x 10^9</td>
<td>60</td>
</tr>
<tr>
<td>14</td>
<td>2.0 x 10^10</td>
<td>72</td>
</tr>
<tr>
<td>15</td>
<td>1.5 x 10^11</td>
<td>46</td>
</tr>
<tr>
<td>16</td>
<td>4.5 x 10^10</td>
<td>20</td>
</tr>
<tr>
<td>17</td>
<td>2.8 x 10^10</td>
<td>8</td>
</tr>
</tbody>
</table>

* DNA concentration as determined by the diphenylamine reaction (Burton, 1956).
Table 3. Recovery of infectivity and radioactivity during the purification of MV-L3 grown in medium containing [\( ^{3}\text{H} \)]-thymidine or [\( ^{3}\text{H} \)]-uridine

<table>
<thead>
<tr>
<th>Sample</th>
<th>Virus type*</th>
<th>Radioactivity (ct/min)</th>
<th>Infectivity (total p.f.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total†</td>
<td>Acid-insoluble‡</td>
</tr>
<tr>
<td>Starting material T</td>
<td>T</td>
<td>3.2 × 10⁶§</td>
<td>4.2 × 10⁶§</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>2.2 × 10⁵</td>
<td>5.5 × 10⁵</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitate</td>
<td>T</td>
<td>1.5 × 10⁶</td>
<td>5.2 × 10⁶</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>4.9 × 10⁵</td>
<td>2.5 × 10⁵</td>
</tr>
<tr>
<td>Pelleted material T</td>
<td>T</td>
<td>2.7 × 10⁶</td>
<td>2.6 × 10⁶</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>1.0 × 10⁵</td>
<td>1.0 × 10⁴</td>
</tr>
<tr>
<td>Virus band from CsCl§</td>
<td>T</td>
<td>1.5 × 10⁶</td>
<td>1.5 × 10⁶</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>&lt; 10³</td>
<td>&lt; 10³</td>
</tr>
</tbody>
</table>

* Virus type: virus grown in medium containing either [\( ^{3}\text{H} \)]-thymidine (type T) or [\( ^{3}\text{H} \)]-uridine (type U).
† Total radioactivity was determined in 10 μl of sample added to NE250 scintillant.
‡ Acid-insoluble radioactivity was detected by precipitating 25 μl of sample with 5% trichloroacetic acid (TCA), filtration through a Whatman GF/A disc, successive washings with 5% TCA and ethanol and counting in NE250 scintillant.
§ The values quoted refer to the total infectivity and radioactivity recovered at each stage of the purification procedure.

Thermal denaturation of viral DNA

DNA was isolated from a purified virus preparation using method 1 (pronase/SDS/phenol) as described in Methods. The sample was divided into two parts; one was dialysed against SSC and the other against 0.1 × SSC (0.015 M-NaCl: 0.0015 M-citrate, pH 7.0) and both samples were heated in a dual beam spectrophotometer. The temperature of the sample in SSC was measured with a thermocouple and the extinction at 260 nm was measured for each sample against an appropriate reference of SSC or 0.1 × SSC. Fig. 4 presents these data plotted as relative extinction against temperature. The nucleic acid showed a sharp thermal transition when heated at either salt concentration and a hyperchromicity of 42 to 50%. The mean melting temperature, \( T_m \), from several experiments was 85.5 °C, which would correspond to a GC content of approx. 39.5% (Marmur & Doty, 1962).

Buoyant density of viral DNA

When viral DNA solutions, prepared by method 1, were subjected to isopycnic equilibrium sedimentation in CsCl a single band of DNA was found at a buoyant density of 1.674 g/ml (Fig. 5). The sharpness of the peak suggested that there was very little contamination of the DNA by protein and yet double-stranded DNA containing 39.5% GC would be expected to band at a density of 1.699 g/ml (Schildkraut, Marmur & Doty, 1962). Viral DNA, prepared by method 1, was further treated with 1% sodium lauroyl sarcosinate to remove any residual protein, a technique that had been shown to be useful with the DNA from cauliflower mosaic virus (Shepherd et al. 1970). Such treatment, however, did not change the buoyant density of MV-L3 DNA. It was found that treatment of purified virus with 1% sodium lauroyl sarcosinate followed by sedimentation in CsCl density gradients containing 0.1% of the detergent (method 2) produced a band of DNA of the same density as with preparations from method 1. Since this abnormally low buoyant density of the virus DNA might have resulted from the adhesion of a mycoplasma constituent which is not removed by pronase, detergent or phenol, virus DNA was extracted using a technique that was developed for the extraction of mycoplasma DNA, method 3 (Bak & Black, 1968). MV-L3
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DNA prepared by this procedure, using SDS, high salt and chloroform to deproteinize the nucleic acid, was not detectably different from the samples prepared by either methods 1 or 2 as detailed above.

**Chemical composition of the virus**

A preparation of purified virus was used for the chemical analysis of DNA (10 determinations) with deoxyribose, calf thymus DNA, *Escherichia coli* DNA and *Micrococcus lysodeikticus* DNA as standards, of protein (12 determinations) and with serum albumin, trypsin and pepsin as standards and of the carbohydrates fucose, sialic acid, pentoses, hexoses and hexosamines, each in duplicate. The results are shown in Table 4 as the concentration of each component in the virus sample and expressed as percentages (w/w) of the total detectable material.

**DNA base composition analysis**

The data presented in Table 5 show the relative proportions of guanine, cytosine, adenine and thymine determined in duplicate assays of DNA from calf thymus and *Escherichia coli*, with their standard deviations, and the values for three separate analyses of virus DNA, with their means and standard deviations. It is apparent that the variation in these values for the virus DNA is considerably greater than the corresponding figures for the reference DNA species and prevents any definitive values for the virus DNA composition being given.
Fig. 5. Isopycnic equilibrium sedimentation of virus DNA. MV-L3 DNA, labelled with [\textsuperscript{3}H]-methyl-thymidine, was suspended in a solution of CsCl at a density of 1.70 g/ml. After 16 h sedimentation at 150000 \textit{g}_{av} the density gradient was fractionated by siphon. Solution density (---) was determined by refractometry and radioactivity (bullet) was measured in NE250 scintillant with a Packard 2425 spectrometer.

Table 4. Chemical composition of MV-L3

<table>
<thead>
<tr>
<th></th>
<th>(\mu g/ml)</th>
<th>(% \text{w/w})*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>92.2</td>
<td>35.2</td>
</tr>
<tr>
<td>Protein</td>
<td>165.0</td>
<td>63.0</td>
</tr>
<tr>
<td>Fucose</td>
<td>4.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>nd†</td>
<td>--</td>
</tr>
<tr>
<td>Pentoses</td>
<td>nd</td>
<td>--</td>
</tr>
<tr>
<td>Hexoses</td>
<td>nd</td>
<td>--</td>
</tr>
<tr>
<td>Hexosamines</td>
<td>nd</td>
<td>--</td>
</tr>
</tbody>
</table>

* Expressed as the amount of each component as a percentage of the total material detected.
† None detectable.

It would appear, however, that the amounts of the purines and the pyrimidines may be approximately similar in accordance with the double-stranded nature of the DNA, and that the GC content is in the order of 30 %.

Chromatography of the hydrolysed virus DNA, as described, resulted in the separation of four bases whose Rf values and absorption spectrum characteristics were identical with those isolated from DNA of \textit{Escherichia coli} and calf thymus and with the four bases guanine, cytosine, adenine and thymine which were included as chromatographic markers.
Table 5. Base composition analysis of MV-L3 DNA

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>C</th>
<th>A</th>
<th>T</th>
<th>% GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf thymus DNA</td>
<td>Mean*</td>
<td>22.6†</td>
<td>21.9</td>
<td>29.3</td>
<td>26.2</td>
</tr>
<tr>
<td></td>
<td>s.d.</td>
<td>0.21</td>
<td>1.56</td>
<td>0.99</td>
<td>0.28</td>
</tr>
<tr>
<td>Escherichia coli DNA</td>
<td>Mean*</td>
<td>25.3</td>
<td>25.6</td>
<td>25.1</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>s.d.</td>
<td>1.56</td>
<td>2.19</td>
<td>0.78</td>
<td>0.07</td>
</tr>
<tr>
<td>MV-L3</td>
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* The values for the mean and standard deviation (s.d.) were calculated from two experiments.
† The data are presented as molar percentage composition of the four bases guanine, cytosine, adenine and thymine.
‡ Calculated from the three experiments quoted.

Fig. 6. Estimation of mol. wt. of MV-L3 structural polypeptides from their relative mobility in SDS-polyacrylamide gel.

Structural polypeptide analysis

Electrophoresis of dissociated MV-L3 through 5% polyacrylamide gels in the presence of 0.1% SDS revealed five polypeptides associated with the virion (Fig. 6). The most abundant structural polypeptide had an estimated mol. wt. of 43000 and accounted for approx. 80% of the total virus protein. The remainder consisted of four polypeptides, two
of which had estimated mol. wt. of 68000 and 81000 comprising approx. 10.5% and 7.5% of the total protein respectively, and two minor components of 73000 and 172000 each of which was present at about 1% of the total. None of the polypeptide bands stained with a periodic acid/Schiff's reagent, suggesting that there is no appreciable amount of glycoprotein in the virion.

**DISCUSSION**

Purified preparations of MV-L3 contain virus particles that consist of a head 57 nm x 61 nm, a tail approx. 25 nm long and at the junction of head and tail, a collar. The virions are composed of 35.2% double stranded DNA, 63% protein consisting of three major and two minor polypeptides and 1.8% fucose.

These characteristics obviously distinguish MV-L3 from the two other viruses of Acholeplasma laidlawii that have been described and it is of interest whether they relate MV-L3 to any other group of viruses. The presence of 30 to 40% double stranded DNA in a naked polyhedral particle having a tail shows some resemblance to the T-even bacteriophages of Escherichia coli, which are considerably larger than MV-L3, and to λ-phage which is more similar in size to MV-L3 but has a larger DNA content. MV-L3 bears a closer resemblance to the blue-green algal virus LPP-1 (Safferman & Morris, 1963), which has an icosahedral head of 56 nm average diam. and a tail approx. 14 nm long (Schneider, Diener & Safferman, 1964). There is no obvious collar in this virus but a tail plate at the junction of head and tail has been described (Smith et al. 1966).

It is clear that MV-L3 cannot be classified into any of the well defined groups of viruses, and even though it shows some similarity to certain viruses, more information is required before any useful classification can be made.

The buoyant density of MV-L3 in CsCl solution, 1.477 g/ml, is rather high for a virus of this type. The density at which a virus bands in CsCl is related to its chemical composition, the degree of hydration of the particle and the extent of interaction between the nucleic acid and caesium ions. A virion consisting of 35% DNA would be expected to have a buoyant density of no higher than 1.40 to 1.42 g/ml; these values are derived from the combined densities of protein (approx. 1.3 g/ml) and DNA (approx. 1.68 g/ml) in CsCl solution.

It is possible that the high particle density is caused by the presence of structural glycoproteins having densities higher than 1.3 g/ml but only deoxyribose and a small quantity of fucose were found in the virion and none of the structural polypeptides reacted when polyacrylamide gels were stained for carbohydrate. This does not exclude the possibility that one or more polypeptides are fucosylated, however, as the amount present in each band may be below the limit of detection or the carbohydrate may be linked in a way that resists periodate oxidation. Alternatively, the particles may bind additional caesium ions due to the presence of a structural component as yet undetected.

The DNA extracted from MV-L3 exhibits some unusual characteristics. Following a variety of deproteinizing steps, the nucleic acid banded in CsCl at a density of 1.674 g/ml which is consistent with a GC content of approx. 14%. Upon heating, however, the DNA melts with a very large hyperchromicity at a temperature corresponding to a GC content of 39.5%. The results of base composition analysis were subject to an unusually high level of variation for which there is no obvious explanation, but the GC content is clearly in the order of 30%, intermediate between the values obtained from melting temperature and buoyancy studies. These discrepancies may be due to some virus material bound to the DNA, but it is not likely to be protein since the variety of methods used to extract the DNA should effect total deproteinization. The presence of unusual bases in the DNA may inter-
Characterization of MV-L3

ference with the determination of GC content by the methods used. There was no evidence of unusual bases, however, as the DNA hydrolysate chromatographed into only four spots which corresponded to guanine, cytosine, adenine and thymine in their Rf values and absorption spectra.

The fucose present in the virion may be in the form of glycopeptides as discussed above. The possibility exists, however, that fucose is associated with the DNA bases in a similar way to the glycosylated hydroxymethyl-cytosine of the T-even bacteriophages of *Escherichia coli*. We have found no evidence of hydroxymethyl-cytosine in MV-L3 DNA; the quantity of fucose present would represent approximate molar proportions of 1 fucose:2 cytosine, or 1 fucose:3 thymidine and it might be expected that this amount of a base that could accept fucose would have been detected. Moreover, it is not known whether fucosylation would confer unusual characteristics upon the DNA even if it were to occur. The explanation of these abnormal properties will have to await a more detailed investigation of the nature of MV-L3 DNA.

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REFERENCES


D. J. Garwes AND OTHERS


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