Studies on a Virus Isolated from *Gonometa podocarpi* (Lepidoptera: Lasiocampidae)

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SUMMARY

Some properties of a small RNA virus isolated from larvae of *Gonometa podocarpi* (Lepidoptera: Lasiocampidae) are described. The virus develops in the cytoplasm of gut and fat body and is 32 nm in diameter. The sedimentation coefficient of virus was 180 S and the buoyant density was 1.35 g/cm³. The RNA:protein ratio was 37:63 and the RNA was single-stranded. Four principal polypeptides were found with mol. wt. in the range 12 100 to 36 500. It is proposed that this virus be included in the enterovirus group.

INTRODUCTION

A small RNA virus of *Gonometa podocarpi* was described by Harrap et al. (1966); applications of this virus exerted a dramatic control of larval populations of *G. podocarpi* on *Pinus patula* in Uganda. The virus was isolated in high yield from larvae and pupae and the mean diameter of the particles was then reported to be 38 nm. The present paper describes additional properties of the virus and it is suggested that it should be included in the enterovirus group. The *G. podocarpi* virus is compared with a similar one from a sympatric lasiocampid, *Pachymetana* sp.; this insect also feeds on *P. patula* and possibly may have been exposed to the *G. podocarpi* virus.

METHODS

**Virus purification.** The virus was isolated from dead larvae which had been stored at −20 °C; the larvae were triturated in the frozen state in a pestle and mortar and the thawed slurry was diluted with 0.1 M-phosphate buffer, pH 7.2 at 4 °C (1 ml of buffer/larva). This suspension was clarified at 10000 g for 30 min and the virus was sedimented from the supernatant fluid at 100000 g for 4 h. The pellets were resuspended in 10 ml buffer and were clarified at 10000 g for 30 min. The virus was then purified by two cycles of sucrose gradient centrifugation, first on a 20 to 50% (w/w) linear sucrose gradient in a BXIV zonal rotor at 40000 rev/min for 3 h, followed by centrifugation on 20 to 50% linear sucrose gradients in a MSE three-place 23 ml swinging bucket rotor at 100000 g for 4 h. Purified virus samples were stored at −20 °C in 0.1 M-phosphate buffer, pH 7.2.

**Serology.** An antiserum was prepared in rabbits against purified *G. podocarpi* virus.
One mg of virus in 1 ml of 0.1 M-phosphate buffer, pH 7.2, emulsified with an equal vol. of Freund's complete adjuvant was injected subcutaneously at 2 weekly intervals. A final injection of 1 mg of virus in 1 ml of buffer was administered intravenously on the third week. The rabbits were bled from the ear vein and the separated sera were stored at -20 °C.

**Determination of sedimentation coefficients.** Sedimentation coefficients were determined in a Spinco Model E analytical ultracentrifuge fitted with both Schlieren and u.v. absorption optics. One mg of the virus in 0.1 M-NaCl was centrifuged at 20,410 rev/min in an AND rotor with temperature controlled at 20 °C.

**Determination of the chemical composition of the virus.** The presence of RNA was determined by the orcinol reaction of Mejbaum (1939) and that of DNA was tested by the diphenylamine reaction as modified by Burton (1956). Protein concentration was determined by the Folin method of Lowry et al. (1951) as modified by Eggstein & Kreutz (1955).

RNA was extracted either by the phenol-cresol method of Kirby (1965) or by the method used by Nair & Lonberg-Holm (1971) for extraction of rhinovirus RNA. The nucleic acid was dispersed in 0.1 M-NaCl and sedimentation velocity was measured at 44,770 rev/min in the analytical ultracentrifuge.

The buoyant density of the virus particle was determined in caesium chloride by a method similar to that described by Rowlands, Sangar & Brown (1971) for picornaviruses. Caesium chloride solutions were prepared in 0.1 M-phosphate buffer, pH 7.6. Linear gradients containing 15.0 ml were made with the aid of a gradient-making machine from 45 and 30% (w/w) caesium chloride solutions and 0.5 ml samples of the virus were then layered on top. The tubes were centrifuged at 20 °C in the three-place swinging bucket rotor in an MSE 65 ultracentrifuge for 6 h at 28,000 rev/min. The gradients were sampled by puncturing a hole in the bottom of the tube and collecting 0.5 ml fractions.

**Electron microscopy.** Ultrathin sections of gut and fat body of fourth-instar larvae were prepared and examined as described by Harrap & Robertson (1968). Purified virus preparations were negatively stained with 1% uranyl acetate and specimens were examined on an AEI EM6B at an accelerating voltage of 60 kV.

**Polyacrylamide gel electrophoresis of virus particles.** Large pore gels with a tris–glycine discontinuous buffer system were those used by Strauss & Kaesberg (1970) for Qβ virus. Electrophoresis was carried out on 2.5% gels at a constant current of 2 mA/tube, for 45 to 90 min.

**Polyacrylamide gel electrophoresis of virus polypeptides.** Acrylamide gel electrophoresis in SDS was carried out according to the method of Shapiro, Vinuela & Maizel (1967). The mol. wt. of the structural proteins of the *G. podocarpi* virus were determined by a modification of the procedure of Weber & Osborne (1969). Polyacrylamide gels (10%) were used, containing 0.1% SDS and 8 M-urea in 0.1 M-phosphate buffer, pH 7.8. Virus samples of 1 mg/ml were disrupted in a water bath by incubating with 1% SDS and 8 M-urea in 0.01 M-phosphate, pH 7.0, at 100 °C for 1 min. Samples of 20 to 50 μl were loaded on to each gel, and electrophoresed for 30 min at 2 mA/tube, followed by electrophoresis for 4 h at 6 mA/tube. The gels were stained overnight in a 1% solution of Coomassie Brilliant Blue in methanol:acetic acid:water (5:1:5) and destained in methanol:acetic acid:water. The destained gels were stored in 7% acetic acid. Estimation of the mol. wt. of the virus proteins were made with reference to the mobility of ovalbumin, pepsin, bovine serum albumin, myoglobin, and tobacco mosaic virus protein. The gels were scanned in a Joyce LoebI microdensitometer.
RESULTS

Electron microscopy

All larvae received from Uganda had advanced infections and the internal organs were fragile. Nevertheless, sections of both tissues showed extensive virus development. The cytoplasm of columnar cells was completely filled with virus particles (Fig. 1) and mitochondria and cell membranes showed pronounced degeneration. The nuclei were unaffected. Commonly, groups of virus particles were present in the cytoplasm, bounded by a membrane. Virus development in these membrane-bound areas was associated with the presence of amorphous electron dense material. Extensive virus development was also observed in the goblet cells of the midgut. Frequently the fat body had degenerated to the extent that none was available for examination, but such sections as were possible indicated that virus also developed in the cytoplasm of fat body cells; microcrystalline arrays of virus particles, bounded by a membrane being observed there.

Negatively stained preparations of virus particles (Fig. 2) showed a regular hexagonal outline with a mean diameter from apex to apex of 32 nm. Tobacco mosaic virus particles were used as an internal standard.
Fig. 3. Absorption spectra of Gonometa podocarpi virus (△—△) and virus RNA (○—○).

Chemical composition of the virus

The virus gave a positive orcinol reaction and was estimated to contain 37% RNA. The diphenylamine reaction of the virus was negative. Using the Folin test an equivalent virus sample contained 63% protein. The $E_{260}$ (uncorrected) for the virus was 9.75 and after correction for light scattering was 8.72. The absorption spectra of the virus and the isolated RNA are shown in Fig. 3.

When the RNA was hydrolysed with pancreatic ribonuclease at 25°C using 25 μg RNA/ml and 0.2 μg ribonuclease/ml in 1 × SSC (SSC = 0.15 M-sodium chloride, 0.15 M-sodium citrate), 24.0% hyperchromicity was observed. The melting profile of the RNA in 1 × SSC showed a gradual increase in absorption at 260 nm with no marked $T_m$. The increase in absorption was at a maximum at 80°C and was 21.3%. When the RNA was reacted with 2% formaldehyde in 1 × SSC there was a shift of $E_{max}$ from 258 to 260 nm and an 18.3% increase in absorption at 260 nm. These results indicated that the RNA of G. podocarpi virus was single-stranded.

The G. podocarpi cadavers used in these experiments had been stored for many months, and the virus was not infective for five European lasiocampids, or for several other lepidopterous larvae, when these were infected per os, or by injection of virus particles into the haemocoel. The RNA was extracted by the methods described by Kirby (1965) and by Nair et al. (1971) and neither method gave uniform high mol. wt. nucleic acid. It was therefore likely that the RNA had become degraded.

Sedimentation experiments

The virus had a marked tendency to aggregate on further purification on linear sucrose gradients in a swinging bucket rotor and in these rate zonal gradients, in addition to the main virus band, other peaks were obtained which were presumed to contain dimers and trimers of the virus. The main peak corresponding to the monomer was isolated and the
buoyant density of the virus in caesium chloride was determined as 1.35 g/cm³ at pH 7.6, similar to that described for the acid-stable mammalian picornaviruses. The acid-stability of the *G. podocarpi* virus was examined; virus preparations of 0.3 mg/ml were dialysed against buffers of pH 7.6, 6.4 and 5.0 overnight and then were centrifuged on preformed caesium chloride gradients at pH 7.6 for 6 h. There was no evidence of breakdown of the virus within this pH range and in each case the buoyant density was 1.35 g/cm³.

The sedimentation coefficient ($s_{20,w}$) of purified virus was calculated to be 180S, there being no dependence of sedimentation coefficient on concentration in the range 0.4 to 1.2 mg/ml.

**Polyacrylamide gel electrophoresis of virus particles**

The *G. podocarpi* virus migrated readily in the 2.5% large pore gels. Samples of 50 to 100 μg were loaded on the gels and the progress of electrophoresis was observed by light scattering. Electrophoresis was stopped when the refractile bands had migrated 1 to 2 cm into the gels as after this point resolution was impaired. Three such gels are shown in Fig. 4. In gels 1 and 2, two bands were observed, which had migrated quite close together; two different virus preparations are represented, in gel 2 the virus was freshly isolated from larvae and in gel 1 the virus has been stored at 4 °C for several months. Both preparations gave a single peak in caesium chloride gradients in which their buoyant density was 1.35 g/cm³. Gel 3 contains virus from the monomer and dimer peaks from the sucrose gradients described above.

**Polyacrylamide gel electrophoresis of virus polypeptides**

The mol. wt. of the virus proteins were estimated in 10% SDS-urea polyacrylamide gels following the method of Weber & Osborne (1969). Five polypeptides were present; their mol. wts. and molar proportions are shown in Table 1, and the acrylamide gel profile in Fig. 5. Polypeptides V2 and V3 ran as one band in SDS gels without urea and in SDS-urea...
Table 1. The structural polypeptides of Gonometa podocarpi virus

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Mol. wt.</th>
<th>Peak area (%)</th>
<th>Molar proportions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPo</td>
<td>47,500</td>
<td>0.81</td>
<td>—</td>
</tr>
<tr>
<td>VP1</td>
<td>36,500</td>
<td>4.07</td>
<td>1.23</td>
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<td>VP2</td>
<td>32,000</td>
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</tr>
<tr>
<td>VP3</td>
<td>29,000</td>
<td>2.19</td>
<td>0.83</td>
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<tr>
<td>VP4</td>
<td>12,000</td>
<td>4.4</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* Mol of component/109,500 of protein, calculated by dividing the product of column 2 times the subunit weight (109,500) by the mol. wt. of each polypeptide.

Fig. 6. Immunodiffusion test showing identity between viruses from Gonometa podocarpi (G) and Pachymetana sp., (P) when reacted with antiserum to the former (GA).

gels in which electrophoresis was continued for less than 3.5 h. There was no change in this profile when 2-mercaptoethanol was used in dissociation of the virus and incorporated in the system. Furthermore, when virus was purified by electrophoresis through large pore gels, the SDS-urea profile was unchanged. The profile obtained from a morphologically similar isolate from a sympatric lasiocampid, Pachymetana sp., was identical with that of the G. podocarpi isolate.

Serology

The isolates from G. podocarpi and Pachymetana sp. were serologically identical; in immunodiffusion tests using an antiserum to the former isolate, a pattern of identity was obtained (Fig. 6). Furthermore, the G. podocarpi virus did not react in immunodiffusion tests with antisera to the following insect RNA viruses: Nudaurelia cytherea capensis, Antheraea eucalypti (I. R. M. Juckes, J. F. Longworth & C. Reinganum, in preparation), or to the ‘P’ virus of Drosophila melanogaster.

DISCUSSION

The G. podocarpi isolate replicates primarily in the cytoplasm of cells of the midgut. The virus is 32 nm in diameter, has a sedimentation coefficient of 180S and contains approximately 37% single-stranded RNA. The buoyant density of the virus is 1.35 g/cm³, and is not affected within the pH range of 5.0 to 7.6. The virus particle is stable at pH 5.0. Thus, the isolate has many properties in common with the enterovirus group, and the cryptogram for this virus is as follows: R/1; */37; S/S; I/O. The size and s₂₀,₅₀ value reported here are slightly high for the picornavirus group, however the accepted diameter (15 to 30 nm) of picornaviruses does not include the reported size of a significant number of animal entero-
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viruses (McFerran, Clarke & Connor, 1971). If the G. podocarpi virus RNA is liable to breakdown then further studies including the mol. wt. of the RNA and its base composition must await fresh supplies of infected material.

In the large pore gels (Fig. 4), two distinct light-scattering bands were formed in the position of the monomer of the virus particle. This preparation gave one band in caesium chloride gradients and one precipitin line in immunodiffusion tests. Similar electrophoretic heterogeneity has been reported for turnip yellow mosaic virus in polyacrylamide-agarose gels by Wolf & Casper (1971), and slight degradation of the virus particle was suggested.

The polypeptide composition of several members of the animal picornavirus group is remarkably similar (Talbot & Brown, 1972). Typically, there are three larger polypeptides in equimolar amounts, with mol. wt. ranging from 37 to $24 \times 10^3$ and a smaller polypeptide in a half molar amount relative to the others, with a mol. wt. in the range of $7$ to $13.5 \times 10^3$.

The polypeptide composition of the G. podocarpi virus conforms to this general pattern (Table 1), though the molar ratios which in this case were derived from a densitometer trace of a stained gel, are not in close agreement. A further polypeptide of mol. wt. $47.5 \times 10^3$ was present in the G. podocarpi virus preparations. This may be analogous to the epsilon polypeptide in ME virus (Medappa, McLean & Rueckert, 1971), the presence of which in mature virus has been attributed to the failure of a few of the sixty epsilon chains to cleave during the final maturation step.

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REFERENCES


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