Two Protein and Two RNA Species in Particles of Strawberry Latent Ringspot Virus

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SUMMARY

Purified preparations of strawberry latent ringspot virus (SLRV) contain a major (126S) component which tends to aggregate, and sometimes a minor component of about 58S. The major component contains two RNA species, of mol. wt. $2.6 \times 10^6$ and $1.6 \times 10^6$; some particles contain one molecule of the larger species and others apparently contain two molecules of the smaller species. The virus particles migrated as a single component both in immunoelectrophoresis tests and when electrophoresed in polyacrylamide gels. In polyacrylamide they were retarded by an increase of gel strength slightly more than were particles of raspberry ringspot virus. Virus preparations yielded two polypeptides of mol. wt. 44000 and 29000 in a molar ratio of about 1 to 1.3. In size and number of polypeptide species, SLRV differs from other nepoviruses and resembles broad bean wilt virus and comoviruses, but it did not react with antisera to seven of these viruses. The cryptogram of SLRV is R/1:2.6/37+(2×1.6/42):S/S:S/Ne.

INTRODUCTION

Plant viruses in the nepovirus group are characterized by having nematode vectors and polyhedral particles, and they share several other properties in vitro and in vivo (Cadman, 1963; Harrison et al. 1971). More recent work further suggests that each nepovirus has a genome composed of two RNA molecules (Harrison, Murant & Mayo, 1972a, b; Jones & Mayo, 1972; Murant et al. 1973; Harrison et al. 1974) and particles containing a single polypeptide of mol. wt. 54000 to 60000 arranged as 60 sub-units in a $T = 1$ structure (Mayo, Murant & Harrison, 1971; Jones & Mayo, 1972). Strawberry latent ringspot virus (SLRV) resembles nepoviruses in its biological properties, and in the stability, shape and size of its particles (Lister, 1964); indeed, it was included in the nepovirus group by Cadman (1963). In this paper we describe studies which show that whereas the RNA of SLRV particles is like that of other nepoviruses, the protein is not.

METHODS

Virus propagation and assay. Two isolates of SLRV were used. (1) Isolate H, the ‘Hampshire’ strawberry isolate of Lister (1964). (2) Isolate J, from Malling Jewel raspberry growing in Fife, Scotland (Taylor & Thomas, 1968). The two isolates were serologically indistinguishable but differed somewhat in virulence. In most experiments SLRV was propagated in cucumber (Cucumis sativus cv. Lockie’s Perfection) grown at 15 to 25 °C in glasshouses.
with supplementary illumination in winter. The cotyledons were dusted with Corundum (Bausch & Lomb, Rochester, N.Y.) and inoculated by rubbing with a cheesecloth pad wet with inoculum. Nucleic acid preparations were diluted in freshly boiled and cooled phosphate buffer, pH 8, and plastic gloves were used during inoculation.

**Virus purification.** SLRV was purified from inoculated and systemically infected leaves of cucumber plants harvested about 10 days after inoculation or, in some experiments, from systemically infected leaves of *Chenopodium amaranticolor* or *Nicotiana clevelandii*. Raspberry ringspot virus was purified from systemically infected leaves of *N. clevelandii*. Extracts made in sodium and potassium phosphate buffer (0.06 M, pH 7, 1 ml/g tissue) containing 0.01 M-EDTA + 0.1 % thioglycollic acid were clarified by the butanol-chloroform procedure of Steere (1956), and concentrated by two cycles of differential sedimentation (10 min at 10000 g, then 4 h at 95000 g or 1 h at 300000 g). Preparations were used immediately or further purified by sedimentation in sucrose density gradients (Murant et al. 1972). Virus yields were 1 to 2 mg per 100 g leaf.

**Serology.** Antiserum to isolate H was prepared by injecting a rabbit once intravenously with about 0.5 mg virus, and after 3 weeks, once intramuscularly with about 2 mg virus emulsified with Freund’s complete adjuvant. Antiserum obtained 4 weeks after the intramuscular injection had a precipitin titre of 1/4096 in gel-diffusion tests. Procedures for gel-diffusion and immuneelectrophoresis were as described by Murant et al. (1972).

**Analytical ultracentrifugation.** Virus preparations were examined using an AnD rotor in a Beckman Model E ultracentrifuge using Schlieren optics. Sedimentation coefficients were calculated as described by Markham (1960).

**Sedimentation in CsCl.** This was done using a step gradient technique for attaining rapid equilibrium (Brunk & Leick, 1969). CsCl solutions were made to the required refractive index (n) in 0.006 M-sodium and potassium phosphate buffer, pH 7.3. Purified virus (B component) in 2.5 ml CsCl solution of n = 1.376 was layered over 2.5 ml CsCl solution of n = 1.390 in 2 × 0.5 in. Beckman SW 50.1 centrifuge tubes which were then centrifuged at 40000 rev/min for 15½ h. All solutions and the centrifuge bowl were kept at 2 to 4 °C. After collection from gradients by upward displacement, the refractive index and extinction of alternate four-drop fractions were determined. The density of the fraction with greatest $E_{265}$ was calculated from the interpolated refractive index.

**Electrophoresis of protein in polyacrylamide gels.** Protein was prepared by heating virus (1 mg/ml) in 0.01 M-sodium phosphate, pH 7, containing 1 % SDS and 0.1 % dithiothreitol, in boiling water for 90 s. Electrophoresis was in 7.5 % acrylamide gels containing 0.1 % SDS (Mayo et al. 1971), using a similar buffer system, 0.1 M-sodium phosphate + 0.1 % SDS + 0.1 % thioglycerol, in a 4-cell slab-type electrophoresis apparatus (Universal Scientific Ltd). Protein was alkylated with iodoacetamide as described by Mayo & Jones (1973). Protein bands were detected by staining with 0.25 % Coomassie blue in methanol: water: acetic acid (5:5:1, v/v/v) and stained gels were scanned using a microdensitometer (Joyce–Loebl, Mark IIIc).

**Preparation and electrophoresis of RNA in polyacrylamide gels.** Nucleic acid was extracted from purified virus preparations using the pronase/SDS method (Murant et al. 1972). After overnight extraction, RNA preparations were shaken with an equal vol. 0.1 % 8-hydroxyquinoline in water-saturated phenol + m-cresol (9:1, v/v), the aqueous phase was washed with diethyl ether and the RNA then precipitated by adding 2 vol. ethanol. One such RNA preparation, dissolved in 0.03 M-phosphate buffer, pH 8.0, infected *Chenopodium quinoa* at $E_{260} = 0.004$ but not at a fifth of this concentration. Before electrophoresis in 2.2 to 2.4 % acrylamide gels, RNA was heated at 60 °C for 15 min in buffer containing 8 M-
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Fig. 1. U.v. absorptiometer trace of a preparation of strawberry latent ringspot virus after sedimentation in a sucrose gradient for 75 min at 26,000 rev/min.

urea (Murant et al. 1972). For irradiation with u.v. light (Mayo et al. 1973) virus preparations were diluted to $E_{260} = 2$ to 3 and placed in flat dishes to give a layer 1 to 2 mm deep. Preparations irradiated for 4 min were concentrated by sedimentation and RNA was extracted from the pellets.

Electrophoresis of virus preparations. Gels of 3 to 3.4% acrylamide were prepared as described by Loening (1967), omitting SDS from the buffers. Electrophoresis was in 0.02 M-tris-phosphate + 0.002 M-EDTA pH 6.7, at 10 V/cm, 8 mA/gel of 6 mm cross-sectional diam., at 4 °C. Buffer was recirculated between electrode reservoirs. Virus bands were located by a u.v. densitometer (UV Scanner, Joyce–Loebl) and also by staining gels with amido black.

RESULTS

Sedimentation in sucrose density gradients

When preparations (1 ml) of either isolate were floated on sucrose gradients and centrifuged for 75 min at 26,000 rev/min in 3.5 × 1 inch tubes in a Beckman SW 27 rotor, a light-scattering zone formed 23 to 26 mm from the meniscus corresponding in position to the B component (130S) zone of tobacco ringspot and raspberry ringspot viruses. With some preparations (Fig. 1) additional zones formed at about 8 to 10 mm, corresponding in position to the T component (52S) zone of raspberry ringspot virus, and at 30 to 32, 36 to 37 and 39 to 41 mm, presumably representing various aggregation forms of B component. Electron microscope observations showed that the 8 to 10 mm zone contained 'empty' particles most of which were penetrated by potassium phosphotungstate, whereas the other zones contained 'full' particles most of which were not penetrated. These results resemble those reported by Richter & Proll (1970) with a German isolate of SLRV from peach.

Virus particles seemed more prone to aggregation following sedimentation through sucrose gradients, and this step was omitted when purifying some preparations.
Fig. 2. Analytical sedimentation of a preparation containing about 0.7 mg/ml B component of strawberry latent ringspot virus. Schlieren angle 50°, photograph taken after 8 min at 30000 rev/min.

Fig. 3. Immunoelectrophoresis of strawberry latent ringspot virus in 1% Oxoid I.D. agar containing 0.025 ionic strength barbitone-acetate buffer, pH 8.6. Electrophoresis was for 2 h at a constant current of 40 mA. Starting voltage was 130, final voltage was 120. Troughs contained serum at a dilution of 1/10 (upper) and 1/100 (lower). Cathode is to the left.

Moving boundary analytical sedimentation

When examined by analytical sedimentation a preparation of B component of isolate J formed four peaks, three of which were small (Fig. 2). Estimated sedimentation coefficients ($s_{20,w}$) were 58S, 123S, 175S and 218S. The main peak (126S at infinite dilution) corresponded to the band collected from the sucrose density gradient; the faster-sedimenting components probably represent dimers and trimers of the 126S particles. The slower peak may be T component material possibly resulting from a breakdown of the 126S particles. Other estimates for the sedimentation coefficient of the main component of SLRV are 134S (Allen, Davidson & Briscoe, 1970), 128 to 130S (Brunt, 1964) and 130S (Richter & Proll, 1970).

Preparations of isolate H contained two minor components with sedimentation coefficients of 108 and 116S, and a major component of 128S. Interpretation of this observation is complicated by the presence of an additional RNA species in this isolate (see below).

Sedimentation in CsCl

Preparations of both isolates formed a single band of highly aggregated material when centrifuged to equilibrium in CsCl in the preparative ultracentrifuge. When the virus preparation illustrated in Fig. 2 was centrifuged to equilibrium, the band of aggregated virus particles corresponded to a density of 1.46 g/ml and, when diluted to $E_{260} = 0.00012$, the material from the band infected Chenopodium amaranticolor and Chenopodium quinoa.
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Table 1. The effect of acrylamide gel strength on the electrophoretic mobility of particles of strawberry latent ringspot (SLRV) and raspberry ringspot (RRV) viruses

<table>
<thead>
<tr>
<th>Expt.*</th>
<th>Virus</th>
<th>Mobilities† at gel strength of</th>
<th>Retardation ratio‡</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>3 %</td>
<td>3·2 %</td>
</tr>
<tr>
<td>1</td>
<td>SLRV</td>
<td>56·38</td>
<td>35·8</td>
</tr>
<tr>
<td></td>
<td>RRV-E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>SLRV</td>
<td>46</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>RRV-S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>SLRV</td>
<td>52</td>
<td>36§</td>
</tr>
<tr>
<td></td>
<td>RRV-S</td>
<td></td>
<td></td>
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</tbody>
</table>

* Expt. 1, electrophoresis for 16 h at approx. 20 °C; Expt. 2, electrophoresis for 22 h at 4 °C; Expt. 3 electrophoresis for 20½ h at 4 °C.
† Mobilities are distances in mm on densitometer traces between the gel surfaces and the peaks of virus.
‡ The retardation ratio for each virus is the mobility in the stronger gel divided by the mobility in the 3 % gel.
§ Figures are the means of results from 2 to 4 gels, each gel containing both viruses.
|| Strains E and S of raspberry ringspot virus were those described by Murant et al. (1972).
¶ 3·25 % gels.

Fig. 4. U.v. densitometer traces of 2·2 % acrylamide gels following electrophoresis of RNA from B component of strawberry latent ringspot virus: (a) electrophoresis of RNA from isolate J for 180 min, (b) electrophoresis of RNA from isolate H for 190 min. Electrophoresis was in 0·02 M-tris-phosphate + 0·002 M-EDTA + 0·2 % SDS, pH 7·8, and migration is from left to right.

Gel electrophoresis of virus preparations

Preparations of B component of isolate J migrated as a single component when examined either by immunoelectrophoresis in 1 % agar (Fig. 3) or by electrophoresis in 3 to 3·4 % acrylamide gels. The electrophoretic mobilities of preparations of SLRV-J in polyacrylamide gels of differing strengths were compared with the mobilities of preparations of raspberry ringspot virus in the same gels. The mobilities (Table 1) of the two viruses are different because their proteins differ in charge; however, the increased friction caused by
increasing gel strength retarded SLRV particles proportionately more than raspberry ringspot virus particles, suggesting that the particles of SLRV were slightly larger.

**Electrophoresis of RNA in polyacrylamide gels**

RNA was extracted from virus preparations purified with or without sucrose density-gradient sedimentation. When subjected to electrophoresis in polyacrylamide gels, RNA from either type of preparation was resolved into two major components with estimated mol. wt. of $2.6 \times 10^6$ (RNA-1) and $1.6 \times 10^6$ (RNA-2) (Fig. 4a, b). Both RNA species migrated slightly more slowly than those from raspberry ringspot virus and, by analogy with other nepoviruses, both are probably functional parts of the genome. RNA from isolate H but not that from isolate J contained a third component of mol. wt. $0.4 \times 10^6$ (Fig. 4b). This component formed a broader band than is usual for RNA species and is possibly somewhat heterogeneous in size. This RNA is not essential for multiplication of SLRV because it is absent from isolate J, and it may be similar in nature to the ‘satellite’ RNA reported in some isolates of tobacco ringspot (Schneider, 1969) and tomato black ring (Murant et al. 1973) viruses. RNA extracted from a preparation of SLRV which had been irradiated with u.v. light for 4 min contained less RNA-2 and more material of similar size to RNA-1 than RNA extracted from an untreated preparation. Also RNA-1-like material from irradiated virus formed a broader band in gels than did either RNA from untreated virus. These effects are similar to those of u.v.-irradiation on RNA in particles of raspberry ringspot virus (Mayo et al. 1973) and, as with this virus, suggest that some particles of SLRV contain two molecules of RNA-2 which become covalently joined when u.v.-irradiated.

**Electrophoresis of protein in polyacrylamide gels**

Protein preparations examined by electrophoresis in polyacrylamide gels consistently produced two prominent bands of mol. wt. 44000 and 29000 (24 determinations) (Fig. 5). In some preparations a less prominent third band was detected with mol. wt. 79000. The estimates of mol. wt. and the relative staining intensities of these bands were unaffected when the proteins were alkylated with iodoacetamide before electrophoresis. Estimates of the mol. wt. of the two major components were similar when electrophoresis was in 6 %, 8 % or 10 % acrylamide gel. Similar results were obtained when protein was prepared from
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virus purified from infected Chenopodium amaranthicolor, Cucumis sativus or Nicotiana clevelandii, and proteins from particles of isolates H and J were indistinguishable. The same two bands were found in protein prepared from a virus preparation centrifuged to equilibrium in CsCl, and in protein extracted from the band formed after electrophoresis of virus particles in 3% polyacrylamide gel. In all gels the band of higher mol. wt. protein was slightly more intensely stained than the band of lower mol. wt. protein. When 50% trichloroacetic acid was used in fixing and staining procedures to minimize any possible loss of protein (Butler, 1970), microdensitometer traces of the gels indicated that the areas under the peaks of the higher and lower mol. wt. proteins were in the ratio of 1:1.5:1.

Serology

In protein composition SLRV resembles cowpea mosaic virus (Wu & Bruening, 1971; Geelen, van Kammen & Verduin, 1972) and broad bean wilt virus (Doel, 1973), and is different from other nepoviruses studied. Tests were therefore made to see whether SLRV is serologically related to comoviruses or to broad bean wilt virus. A purified preparation of cowpea mosaic virus (yellow strain) did not react even with undiluted antiserum to SLRV, nor was any reaction obtained between purified preparations of SLRV isolate J and any of the following antisera, used either undiluted or at a dilution of 1/10: (1) cowpea mosaic virus (yellow strain), titre 1/1000, obtained from A. van Kammen; (2) cowpea mosaic virus (severe strain), titre unknown, obtained from A. van Kammen; (3) bean pod mottle virus, titre unknown, obtained from H. A. Scott; (4) radish mosaic virus, titre 1/512, obtained from R. N. Campbell; (5) broad bean stain virus, titre 1/1024, obtained from A. J. Gibbs; (6) echtes Ackerbohnemosaik Virus, titre 1/512, obtained from A. J. Gibbs; (7) squash mosaic virus, titre 1/1024, obtained from A. J. Gibbs; (8) broad bean wilt virus (PV3 isolate), titre 1/2048, obtained from J. A. Frowd.

Discussion

Our results show that SLRV particles contain two polypeptides of mol. wt. 44000 and 29000. Virus isolates obtained from different hosts and localities, and preparations purified from different species or using additional steps, such as rate zonal sedimentation in sucrose gradients, equilibrium sedimentation in CsCl or electrophoresis of virus particles in polyacrylamide gels, gave the same result. We found no evidence that the preparations contained particles of two different viruses, or two types of particle with different proteins.

The larger and smaller SLRV polypeptides apparently occurred in relative molar amounts of about 1 to 1:3. Although we cannot be certain, it is possible that the polypeptides were in an equimolar ratio. Assuming this were so, the simplest icosahedral shell that could be constructed from them would contain 60 molecules of each polypeptide and have a mol. wt. of 4.4 × 10^6. In particles consisting of such a shell plus one RNA molecule of 2.6 × 10^6 mol. wt. the RNA would comprise 37% by weight; in particles containing two RNA molecules of 1.6 × 10^6 mol. wt. the RNA would comprise 42%. The estimated density of aggregated SLRV B component particles in CsCl was 1.46 g/ml, which gives, by calculation using the empirical relation derived by Sehgal et al. (1970), a value of 38% RNA. Particles with the suggested structure, and a density of 1.46 g/ml, would have a diam. about 10% larger than that of raspberry ringspot virus particles (mol. wt. 5.7 × 10^6, density in CsCl 1.51 g/ml; Murant et al. 1972). Hence both the density of SLRV particles, and the greater retardation of SLRV particles than of raspberry ringspot virus particles in acrylamide gels of increasing strength, are compatible with the structure we suggest for SLRV. However, further work
is needed to show whether our suggestion is correct, and whether, as we would doubt, the smaller polypeptide is derived from the larger.

SLRV has several properties typical of nepoviruses; a nematode vector, transmissibility through seed, moderate thermal inactivation point and concentration in sap, wide host range and two RNA species of appropriate size. However, its protein differs from that of several nepoviruses, whose particles contain 60 molecules of a single polypeptide of mol. wt. 54,000 (raspberry ringspot, arabis mosaic, cherry leaf roll), 57,000 (tobacco ringspot), 59,000 (tomato black ring) or 60,000 (cocoa necrosis) (Mayo et al. 1971; Jones & Mayo, 1972; Kenten, 1972; Walkey, Stace-Smith & Tremaine, 1973; M. A. Mayo, unpublished results). The two SLRV polypeptides are, however, similar in size to those in particles of two comoviruses, cowpea mosaic (Wu & Bruening, 1971) and radish mosaic (Kassanis, White & Woods, 1973), and of broad bean wilt virus (Doel, 1973). These four viruses also each have two RNA species, whose sizes are similar in the different viruses. However, the comoviruses are beetle-transmitted, have higher thermal inactivation points, occur in greater concentration in sap and have narrower host ranges than SLRV, and broad bean wilt virus occurs in greater concentration and is aphid-transmitted but not seed-borne. Also, no serological relationship was detected between SLRV and either broad bean wilt virus or any of six comoviruses.

In a general classification of viruses, the possession of a single atypical character should not be enough to exclude an anomalous virus from a virus group, and for the present we feel that, despite its different protein, SLRV should continue to be considered a nepovirus. However, no other group of plant viruses is known to contain members differing in the number and size of their coat proteins and, if a grouping of viruses is considered indicative of phylogenetic relatedness, it becomes necessary to explain how the different proteins of SLRV and other nepoviruses could have had a common origin. One possibility is that nepovirus coat proteins are derived from a larger precursor molecule, and that both products of a 'maturation' cleavage occur in SLRV particles, whereas the precursor protein of other nepoviruses is cleaved in a different place and the smaller product is not found in the virus particles. If such an explanation were correct the dissimilarity between the proteins of SLRV and of other nepoviruses would indeed be of little significance. But whatever its origin, the two-polypeptide structure of SLRV suggests that this virus, and possibly other nepoviruses, may have closer affinities with broad bean wilt virus and comoviruses than with other plant viruses.

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REFERENCES


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