Some Physicochemical Properties of Maize Rayado Fino Virus

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SUMMARY

Maize rayado fino virus (RFV) was purified from infected maize (Zea mays) leaves. The virus had two components: empty shells and complete virus particles with sedimentation coefficients of 54S and 120S respectively, and buoyant densities of 1.28 and 1.46 g/ml in CsCl and 1.24 and 1.37 g/ml in Cs2SO4. Virus particles were stable from pH 7 to pH 4 but not at pH 3. Isoelectric focusing of purified empty capsids revealed a single band in the pH range of 6 to 6.2. RFV contained 33 to 36% of single-stranded (ss)RNA of mol. wt. approx. $2 \times 10^6$. Dissociated coat protein migrated in acrylamide gel electrophoresis as a main band with an average mol. wt. of $2.1 \times 10^4$. RFV shares several biological and physicochemical properties with oat blue dwarf virus. These viruses appear to represent a new group of small RNA viruses, transmitted propagatively by their cicadellid vectors.

INTRODUCTION

Maize rayado fino virus (RFV) is a small isometric virus transmitted in a persistent manner by the cicadellid leafhopper Dalbulus maidis DeLong & Wolcott. It is widespread and economically important in maize growing areas of the American tropics. The existing information on RFV has been recently summarized (Gamez, 1980a, b). In the following study we describe several properties of the virion of RFV, its nucleic acid and coat protein, and demonstrate that these properties distinguish RFV from other known small RNA-containing plant viruses. Part of this information has been reported briefly (Gámez et al., 1978). We conclude that the particles of RFV and oat blue dwarf virus (OBDV) share several characteristics.

METHODS

Virus strain and maintenance. The Costa Rican strain of RFV (Gámez, 1969, 1973) used in this study was propagated in the Tico H-4 variety of maize (Zea mays L.). Young plants were inoculated in the greenhouse by means of viruliferous leafhoppers, as described previously (Gámez, 1973).

Purification. RFV was purified from 250 to 500 g batches of foliage of infected plants by procedures already established (Gámez, 1980b, Gámez et al., 1977). The relative virus concentration from each experiment was assessed by scanning the sucrose density gradient columns with an ISCO model UA-2 absorbance monitor at 254 nm, and/or by u.v. spectrophotometry in a Hitachi 200-20 spectrophotometer. Virus recovered from the sucrose gradient was precipitated with 8% (w/v) polyethylene glycol (PEG) 6000 (Sigma) in 0.3 M-NaCl, centrifuged at 4 °C for 15 min at 10000 g, and the pellets stored at −30 °C.

Electron microscopy. Negatively stained preparations were made by placing small drops of the virus suspensions on to grids with carbon-coated Collodion support films, which had been...
previously subjected to glow discharge in an Eico IB-3 (Ibaragi, Japan) ion coater. Excess fluid was removed with filter paper and the preparation stained with 2% neutral sodium phosphotungstate or 2% uranyl acetate pH 4. The specimens were then examined at 75 kV in a Hitachi HU-12A electron microscope calibrated with diffraction grating replicas.

Sedimentation coefficient. Sedimentation coefficients were determined by Dr R. F. Bozarth, Department of Life Sciences, Indiana State University, U.S.A. Virus samples were dialysed for 48 h against three changes of 0.1 M-potassium phosphate buffer pH 7, the viscosity of which had been determined as described previously (Bozarth, 1976). Sedimentation was carried out in a Beckman analytical ultracentrifuge, equipped with electronic speed control, multiplexer and scanner unit, at a virus concentration of about 0.01 mg/ml. Samples were centrifuged in duplicate in a ANF-Ti rotor at 20000 rev/min and $s_{20,w}$ values were calculated according to Chervenka (1969). The nucleic acid content in the bottom component was estimated from the sedimentation velocity of full and empty particles by the method of Reichmann (1965).

Buoyant densities in CsCl and Cs$_2$SO$_4$. The buoyant densities of RFV were determined by equilibrium banding of full and empty particles in a 65P Hitachi ultracentrifuge using the RP65T rotor. CsCl or Cs$_2$SO$_4$ (Merck, Darmstadt, F.R.G.) was dissolved in 0.05 M-tris-HCl pH 7 and viral pellets were resuspended in this solution. Refractive indices of the initial solutions of CsCl and Cs$_2$SO$_4$ were set at 1.370 and 1.363 respectively, using a Bausch & Lomb Abbé-3L refractometer. Centrifugation was performed at 38 000 rev/min for 40 to 60 h at 18 °C. Gradient tubes were scanned at 254 nm in an ISCO UA-2 analyser; fractions were collected and the refractive indices determined using the relationships given in Brakke (1967).

Nucleic acid. Based on the buoyant densities of RFV, the nucleic acid content in the bottom component was calculated according to Sehgal et al. (1970). Nucleic acids were prepared by resuspending virus pellets in 0.02 M-tris pH 8, 0.125 M-NaCl, 2 mM-EDTA and 1% SDS. Extraction was done with buffer-saturated redistilled phenol or with a mixture of chloroform and isoamyl alcohol (25 : 1) until no denatured protein remained at the interphase. The nucleic acid in the aqueous supernatant was precipitated with 2.5 vol. cold ethanol, after adding magnesium acetate to 1 mM. The precipitated material was resuspended in 3% potassium acetate pH 5.2 and reprecipitated with ethanol (Wen et al., 1974). U.v. spectra were obtained in a Hitachi model 200-20 spectrophotometer. Hyperchromicity of the RNA was estimated after adding 5 µl pancreatic RNase A (Sigma) heat-treated for 5 min or T1 RNase (Sigma) to blank and sample. The effect of DNase I (Sigma) was also tested. Hydroxyapatite columns (Bio-Rad) used as described by Bernardi (1969) were eluted with 0.01 to 0.4 M-potassium phosphate gradients. The purified nucleic acid molecules were examined by electron microscopy after surface spreading in 50% formamide, following the procedure of Davis et al. (1971); MS-2 and Qβ RNA (Miles Laboratories, Elkhart, Ind., U.S.A.) were used to standardize length measurements of RFV RNA. Carbon-coated Collodion-covered grids were used; preparations were rotary-shadowed at a 7° angle with platinum/palladium 80 : 20. Acrylamide gel electrophoresis of the nucleic acids was according to procedures already described (Wen et al., 1974), except that 0.8% agarose was used to stabilize the gels and staining was done with 0.04% methylene blue (Peacock & Dingman, 1968). Escherichia coli ribosomal RNA (Sigma) mol. wt. 1.05 × 10$^6$, 0.54 × 10$^6$, 2.8 × 10$^4$ and 2.5 × 10$^4$; Qβ RNA (Miles Laboratories) mol. wt. 1.2 × 10$^6$ and TMV RNA mol. wt. 2 × 10$^6$, isolated from a local strain (P. León & R. Gámez, unpublished results) were used as mol. wt. markers. A preliminary estimate of the base composition was made by descending paper chromatography following acid hydrolysis of purified RNA, according to the procedures of Lin & Maes (1967). Cytosine, adenosine, guanine and uracil (Sigma) were included as standards.
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Coat protein. The mol. wt. of the viral protein was estimated by SDS-disc gel electrophoresis in 12% acrylamide, according to the procedure of Laemmli (1970). Isoelectric focusing in 7% acrylamide gels with 9 M-urea and Nonidet P40 (NP40) was done as described by O’Farrell (1975). After each run gels were stained with Coomassie Brilliant Blue R250 (Sigma) and scanned with a Helena scanner. For mol. wt. determinations the standards used were bovine serum albumin, ovalbumin, ribonuclease A, cytochrome c and chymotrypsin (all from Sigma); mol. wt. of standards were taken from Weber & Osborn (1969). Markers used for isoelectric point determinations were serum albumin, ribonuclease A and cytochrome c (the corresponding isoelectric pHs are 4.9, 9.6 and 10.6). The pH gradient was estimated as described by O’Farrell (1975).

RESULTS

Properties of purified virus preparations

In sucrose density gradients RFV sedimented as two components (Gámez, 1980b), visible as distinct light-scattering zones, coincident with peaks of u.v. absorbance at 254 nm (Fig. 1). When examined in the electron microscope, both fractions appeared homogeneous and contained empty shells (33 nm in diam.) and complete particles (31.5 nm in diam.) in the top and bottom component respectively (Fig. 2). The two components were serologically identical in Ouchterlony gel double-diffusion tests. The u.v. absorption spectra of empty shells and complete particles were typical of proteins and nucleoproteins, with maxima at 278 and 262 nm and minima at 252 and 244 nm respectively. Mean values of $A_{260}/A_{280}$ were 0.87 for the shells and 1.58 for the complete particles, corrected for light scattering (Noordam, 1973) which was negligible at low virus concentrations. The sedimentation coefficients ($s_{20,w}$) for top and bottom components and their standard deviations were $54S \pm 4$ and $120S \pm 1$. The
Fig. 2. Electron microscopy of top and bottom components from sucrose gradients. (a) Empty shells; (b) complete virions.

Fig. 3. Absorption spectra of viral nucleic acids after purification through hydroxyapatite. Ten μl RNase A (final concn. 5 μg/ml) were added to 2 ml cuvettes with 10 μg/ml RNA in 0.05 M-tris pH 7.4, 0.1 M-NaCl. Absorption scans from 320 to 220 nm were performed at 1 min time intervals. DNase I treatment (final concn. 900 units/ml) was done in a similar fashion except that MgCl₂ was first added to a concentration of 1 mM. Trace A represents the spectrum before treatment, trace B after treatment with DNase I and trace C after treatment with RNase A.

nucleic acid content in the bottom component, estimated from the sedimentation velocity of full and empty particles, is approx. 33%.

In isopycnic gradients of caesium salts both types of particles were stable (Fig. 1 b, c), empty capsids and full virions banding at 1.28 and 1.46 g/ml in CsCl and at 1.24 and 1.37 g/ml in Cs₂SO₄ respectively. On the basis of density the nucleic acid content of RFV was estimated as 36%.

The stabilities of empty virus capsids and whole particles were tested in sucrose gradients buffered with citric acid–sodium citrate 0.1 M solutions, at pH values of 6, 5, 4 and 3. Both
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Fig. 4. (a) Purified RNA was dissolved in 0.05 M-Tris pH 8, 0.1 M-NaCl, 50% formamide and 5 mM-EDTA. Cytochrome c was added to a final concentration of 100 μg/ml, and spreading was done over a water hypophase. The grids were rotary shadowed with platinum/palladium at a 7° angle before visualization in the electron microscope. (b, c) Histogram of RNA molecules from (b) RFV (n = 83) and (c) MS-2 (n = 53; 1.1 × 10⁶ mol. wt.) spread over a distilled water hypophase, and measured from enlarged photographs with a map measuring wheel.

empty capsids and whole particles were unstable at pH 3 but sedimented as discrete bands at higher pH values.

Properties of the nucleic acid

Virus nucleic acid obtained from purified bottom component rapidly developed a marked hyperchromicity at 260 nm after treatment with T1 or pancreatic RNase but not with DNase I (Fig. 3). Elution from hydroxyapatite columns using phosphate gradients occurred at an approximate molarity of 0.17, corresponding with the behaviour of an RNA of limited double helicity (Bernardi, 1969). Surface spreading of the purified nucleic acid revealed the presence of linear molecules of 0.9 ± 0.2 μm in length (Fig. 4), which under our conditions of spreading corresponds with a mol. wt. slightly under 2 × 10⁶. The appearance of this
molecule is suggestive of a single-stranded nucleic acid. The mobility of the isolated undenatured nucleic acid in 2.6% acrylamide/agarose gels corresponded to a mol. wt. of $2 \times 10^6$, when compared with *E. coli* ribosomal RNA, Qβ RNA and TMV RNA. As the value of denatured RNA might be different, we conclude that the genome of RFV is a linear ssRNA molecule of approx. $2 \times 10^6$ mol. wt.

RNA purified through hydroxyapatite was subjected to acid hydrolysis and the bases were separated by descending paper chromatography. Molar concentrations were estimated by dissolving the u.v.-fluorescent spots in 0.1 M-HCl and using the appropriate extinction coefficients. Purified bases were also included as standards in each run and the relative mobilities were determined. The average percentage of four independent runs with different samples and the corresponding standard deviations were as follows: adenine 24 $\pm$ 2; guanine, 30 $\pm$ 2; cytosine, 26 $\pm$ 2; uracil, 21 $\pm$ 2, thus confirming the single-stranded nature of the nucleic acid.

**Coat protein**

The virus protein obtained from purified capsids or full particles migrated in SDS-polyacrylamide gels as a single band of approx. $2.1 \times 10^4$ mol. wt. When the gels were overloaded, bands of lower mobility appeared which may have corresponded to dimers, tetramers or hexamers of the native protein. In addition we frequently encountered a second low mobility band, which stained faintly with Coomassie Brilliant Blue, of approx. $2.4 \times 10^4$ mol. wt. The origin of this second band is presently under investigation. Isoelectric focusing of purified virus revealed a band in the pH range of 6 to 6.2 (Fig. 5) in urea–acrylamide gels with a pH gradient from 4 to 10 generated with ampholites and a sodium hydroxide/phosphoric acid system. Caution should be exercised in interpreting this result, since anomalous isoelectric points are sometimes obtained in the presence of urea (Ui, 1971).

**DISCUSSION**

This report provides new information on the composition of RFV (Gámez, 1980a, b) which contains an ssRNA of $2 \times 10^6$ and a protein of mol. wt. $2.1 \times 10^4$. The presence of empty shells in all purified preparations suggests that, as in turnip yellow mosaic virus (TYMV) (Matthews, 1977), protein–protein interactions are predominantly responsible for capsid stability in RFV. Preliminary observations on the morphology suggested an icosahedral symmetry and 180 subunits in the virus capsid (Gámez et al., 1978). This corresponds to a total mol. wt. for the complete particle of approx. $5.7 \times 10^6$, which is in
<table>
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<tr>
<th>Virus</th>
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<th>S value (nm)</th>
<th>Size (nm)</th>
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<th>Density in CsCl</th>
<th>No. of subunits</th>
<th>Particles (x10^-6)</th>
<th>RNA (x10^-6)</th>
<th>Protein (x10^-3)</th>
<th>RNA (%)</th>
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<th>Reference†</th>
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<td>LH</td>
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* Vector: B, beetle; A, aphid; LH, leafhopper.
† 1, Matthews (1977); 2, Rowhani & Stace-Smith (1979); 3, Rochow & Israel (1977); 4, Gingery et al. (1978); 5, Banttari & Zeyen (1973); 6, Gámez (1980b) and information from this paper.
good agreement with values obtained from the sedimentation coefficient of the full particles and the spectral data reported in the results.

There are now sufficient data on RFV for a useful comparison with other similar viruses, either grouped (Matthews, 1979) or ungrouped. RFV differs from most well-characterized viruses in the combination of properties of its virion, RNA, coat protein and biological characteristics. Some properties of the viruses which apparently most resemble RFV are shown in Table 1. TYMV and RFV share most of their properties, but these viruses differ significantly in the appearance of the virus capsid, base composition, type of host plants, symptoms and vector and the type of biological relation with these vectors. The differences in properties between RFV and potato leafroll virus (PLRV) and barley yellow dwarf virus (BYDV) in the luteovirus group and maize chlorotic dwarf virus (MCDV) are more evident. Other additional differences also exist in their virus–host and virus–vector relationships. BYDV, PLRV, TYMV and MCDV, and 49 additional small isometric plant viruses, which include representatives of six recognized groups, are not serologically related to RFV (Gámez, 1980a; R. Koenig, personal communication).

OBDV and RFV share a number of properties which include size, shape and sedimentation coefficient of the virion and type, strandedness and mol. wt. of the nucleic acid. Both viruses are transmitted by cicadellid leafhoppers in a persistent manner. OBDV multiplies in its leafhopper vector; RFV is transmitted in a manner typical of propagative viruses. No serological relationship, however, exists between them (Gámez, 1980b).

Based on this information, we consider that, as stated by Black (1979), RFV and OBDV probably represent a new group of plant viruses, with virions much smaller than those of viruses previously known to multiply in their leafhopper vectors. Sequence data may help establish whether these two viruses represent ‘natural groupings’ in the evolutionary sense, or convergent forms that do not share a common origin.

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