Double-stranded RNA of Rice Gall Dwarf Virus

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SUMMARY

Nucleic acid extracted from purified particles of rice gall dwarf virus (RGDV) was identified as double-stranded RNA because it (i) was susceptible to RNase A in 0.1 x standard saline citrate (SSC) but not in 1 x SSC, (ii) was resistant to nuclease S1, (iii) showed no hyperchromicity in u.v. absorption after treatment with formaldehyde at 37 °C, (iv) showed a sharp thermal transition in u.v. absorption in 0-01 x SSC and (v) had a buoyant density of 1.596 g/ml in Cs2SO4. Electrophoresis in polyacrylamide slab gel revealed that RGDV RNA was composed of 12 segments with a total mol. wt. of about 16.9 × 106. Co-electrophoresis of RNA from RGDV and rice dwarf virus demonstrated that the electrophoretic mobilities of the seven larger segments from the two viruses were similar but that the five smaller segments differed in this respect. These results confirm that RGDV is a new virus and a third member of the genus Phytoreovirus.

INTRODUCTION

Recently, a new virus disease of rice, named rice gall dwarf, was observed in Thailand. The causative agent, rice gall dwarf virus (RGDV), was found to have polyhedral particles about 65 nm in diameter. It is transmitted in a persistent manner by five species of leafhoppers, and causes characteristic symptoms including severe stunting and gall formation on rice plants (Omura et al., 1980; Inoue & Omura, 1982). No serological relationships were found between RGDV and eight other viruses from the genera Phytoreovirus and Fijivirus. The morphology and stability of the purified virus during some physical and chemical treatments suggested that RGDV was a new member of the genus Phytoreovirus (Omura et al., 1982). We now report some properties of the nucleic acids extracted from purified particles of RGDV and a comparison with those of rice dwarf virus (RDV) RNA.

METHODS

Extraction of nucleic acid. RGDV and RDV were purified after isolation from rice plants as described by Omura et al. (1982) and Kimura (1976), respectively. Nucleic acid from the purified viruses was extracted by the phenol–cresol–SDS method (Reddy & Black, 1973). In some electrophoresis experiments, nucleic acid was prepared by adding 1% SDS to purified virus particles suspended in distilled water containing 7.5% sucrose (Reddy & Black, 1973).

Effects of nuclease on nucleic acid. Denaturation of the viral nucleic acid and digestion of native and denatured nucleic acid with ribonuclease A were performed according to the method of Ikegami & Francki (1975), except that the concentrations of RNase A used were 10 μg/ml in 0.15 M NaCl, 0.015 M-sodium citrate (SSC) and 1 μg/ml in 0.1 x SSC, respectively. The RNase was heated for 10 min at 80 °C before use to inactivate any contaminating DNase. For digestion with nuclease S1, native or denatured nucleic acid was suspended at about 15 μg/ml in 30 mM-sodium acetate buffer pH 4.6, containing 0.2 M-NaCl and 1 mM-ZnSO4, and incubated at 45 °C in the presence of 200 units/ml nuclease S1.

The kinetics of digestion of the nucleic acid were estimated by the rate of increase in absorbance at 260 nm using
a Gilford Model 2400 S spectrophotometer equipped with a constant temperature circulator and an automatic cuvette programmer.

Reaction with formaldehyde. Nucleic acid was treated with formaldehyde as reported by Miura et al. (1966).

Thermal denaturation. Thermal denaturation kinetics of nucleic acid in 0.01 × SSC were estimated from the rate of increase in absorbance at 260 nm in a thermal block using a Gilford 2527 thermostimulator set to increase sample temperature by 0.50 °C/min from 30 °C to 100 °C.

Isopycnic density gradient centrifugation. Isopycnic ultracentrifugation was as described by Szybalski (1968). The nucleic acid (final concentration approx. 2.5 µg/ml) was mixed with Cs₂SO₄ (initial density 1.599 g/ml) in 0.01 M-Tris-HCl pH 8.0 and centrifuged for 96 h at 35000 r.p.m. (88000 g) at 25 °C in an MSE Centriscan 75 analytical ultracentrifuge equipped with u.v. optics.

Polyacrylamide gel electrophoresis. Electrophoresis was in 2.5% polyacrylamide slab gels (9.5 × 12.5 × 0.3 cm) prepared as described by Loening (1967). The electrophoresis buffer (36 mM-Tris, 30 mM-NaH₂PO₄, 1 mM-EDTA, pH 7.8; Loening, 1969) was circulated during electrophoresis. After completion of the electrophoresis at 4 V/cm for 10 h at room temperature, the gels were stained in 2 µg/ml ethidium bromide for 30 min, and both photographed under u.v. light (365 nm) and scanned in a Shimazu CS-910 chromatoscanner at 260 nm.

RESULTS

Effect of nucleases on RGDV nucleic acid

Preparations of nucleic acid isolated from purified RGDV showed u.v. absorbance spectra with a peak at 260 nm, a trough at 232 nm and an A₂₆₀/A₂₈₀ ratio of 1.89. When incubated with RNase A in 0.1 × SSC, both native and denatured nucleic acid of RGDV, and of RDV used as a control, increased rapidly in absorbance at 260 nm. When incubated in 1 × SSC, native nucleic acid was highly resistant to RNase digestion, whereas denatured nucleic acid was digested at a slower rate than in 0.1 × SSC (Fig. 1). Nuclease S1 also digested the denatured, but not the native, nucleic acid (Fig. 2). These results indicate that RGDV nucleic acid consists of double-stranded RNA.

Reactivity with formaldehyde

The u.v. absorbance spectrum of native RGDV RNA was little affected by incubation with 1.8% formaldehyde at 37 °C for 22 h. On the other hand, similar treatment of denatured RNA caused a hyperchromic shift of 24-3% at the peak absorbance and a 3 to 4 nm shift of maximum absorbance wavelength (Fig. 3).

![Graph](image-url)
Double-stranded RNA of RGDV

Fig. 2. Comparison of the kinetics of digestion of RGDV RNA and RDV RNA by nuclease S1 as measured by the increase in absorbance at 260 nm. About 15 μg/ml of native (nat.) or heat-denatured (den.) nucleic acids were incubated at 45 °C in 30 mM-sodium acetate containing 0-2 M-NaCl and 1 mM-ZnSO₄, pH 4-6, with 200 units/ml nuclease S1.

Thermal denaturation

RGDV RNA, unlike tobacco mosaic virus (TMV) RNA used as a control, showed a sharp thermal transition with a melting temperature of 76-2 °C and a hyperchromicity of 30-6% when heated in 0-01 × SSC (Fig. 4), which indicates its double-stranded nature. Under the same conditions, the Tₘ of RDV RNA was 78-6 °C (data not shown).
Fig. 4. Comparison of the thermal denaturation kinetics of native RGDV RNA and TMV RNA. RNA (about 20 μg/ml) was suspended in 0.01 x SSC and its absorbance at 260 nm was measured as the temperature was increased at 0.5 °C/min.

Fig. 5. Absorbance profile of native RGDV RNA after isopycnic ultracentrifugation in Cs₂SO₄ as described in the text.

Buoyant density

The buoyant density of native RGDV RNA in Cs₂SO₄ was 1.596 g/ml (Fig. 5) and that of native RDV RNA was 1.599 g/ml. These values are similar to those of 1.599 g/ml for wound tumour virus (WTV) RNA (Szybalski, 1968) and 1.60 g/ml for Fiji disease virus (FDV) RNA (Ikegami & Francki, 1975), and therefore further suggest that both RNAs are double-stranded. In contrast, the buoyant densities of single-stranded TMV RNA and of RNA from cowpea mild mottle virus were found to be 1.632 g/ml and 1.636 g/ml, respectively, under the same conditions (Iwaki et al., 1982). The buoyant densities of heat-denatured RGDV and RDV RNA in Cs₂SO₄ were 1.614 and 1.611 g/ml, respectively, and were thus lower than those of single-stranded viral RNA. These data, as well as the slower digestion rates of the denatured RGDV and RDV RNAs by RNase in 1 x SSC, suggest either that the denaturation by heating at 100 °C for 10 min in 0.1 x SSC followed by rapid cooling in an ice–ethanol bath was incomplete, or that partial renaturation of the denatured RNA occurred rapidly in the buffers used.

Molecular weight and segmentation of RGDV RNA

Electrophoresis of RGDV RNA for 10 h in 2.5% acrylamide gel resolved 11 distinct bands (Fig. 6b). Electropherograms also suggested that the 6th band from the origin contained two
Double-stranded RNA of RGDV

Fig. 6. Electropherograms of double-stranded RNA segments from RDV (a) and RGDV (b) in 2.5% polyacrylamide gel. About 20 μg of each RNA preparation was electrophoresed for 10 h. Each lane of the gel was scanned at 260 nm.

Table 1. **Approximate molecular weights of double-stranded RNAs of RGDV**

<table>
<thead>
<tr>
<th>Segment no.</th>
<th>RGDV*</th>
<th>RDV†</th>
<th>WTV†</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>12</td>
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<td>0.52</td>
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<tr>
<td>Total</td>
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<td>17.26</td>
<td>16.67</td>
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* Calculated by using double-stranded RNA segments of RDV as standards.
† Data from Reddy et al. (1974).

different co-migrating RNA segments, because the peak area of the band was larger than that expected from the assumption that all virus particles contain a set of 12 distinct RNA segments. Recently, the 6th band has been found to separate into two when electrophoresis was performed in a 7.5% polyacrylamide gel using the method described by Omura et al. (1983) (data not
shown). Thus, it was concluded that the RNA genome of RGDV consists of 12 segments. The molecular weights of these were calculated by comparing their electrophoretic mobilities to those of RDV RNA segments separated in the same slab gel. The approximate molecular weights of the RGDV RNA segments ranged from $0.32 \times 10^6$ to $3.10 \times 10^6$ and the sum of molecular weights for the total RGDV genome was about $16.92 \times 10^6$ (Table 1).

**DISCUSSION**

The properties of the nucleic acid of RGDV described establish that its particles contain double-stranded RNA in 12 segments, with a total mol. wt. of approximately $16.92 \times 10^6$. The approximate mol. wt. of the genome segments of RGDV, RDV and WTV (Table 1) suggest that the molecular weights of segments 1 to 7 of RGDV are similar to those of the corresponding segments of RDV, but not to those of WTV, and that the molecular weights of segments 8 to 12 of RGDV are different from those of the corresponding segments of RDV. Since RGDV, like WTV but unlike RDV, can induce tumours in infected plants, it is possible that the genetic information for tumorigenesis is carried in one or more of segments 8 to 12, although more detailed analysis of the structure and function of each segment is required to test this. The lower $T_m$ and lower buoyant density of RGDV RNA than those of RDV RNA also suggest that the G + C contents of the RNAs from the two viruses are different. These results, in addition to the biological and morphological properties reported previously (Omura et al., 1980, 1982), confirm that RGDV is a new virus and a third member of the genus *Phytoreovirus* (Matthews, 1982).

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**REFERENCES**


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