Coelectrophoresis of Double-Stranded RNA from Maize Rough Dwarf and Rice Black-Streaked Dwarf Viruses

D. V. R. REDDY

Provisional Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801

E. SHIKATA

Botanical Institute, Faculty of Agriculture, Hokkaido University, Sapporo, Japan

G. BOCCARDO

Laboratorio di Fitovirologia Applicata, via O. Vigliani 10135, Torino, Italy

AND

L. M. BLACK

Provisional Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801

Accepted April 29, 1975

Rice black-streaked dwarf virus was shown to contain ten dsRNA segments in a genome with an approximate molecular weight of \(18.96 \times 10^8\) or \(19.66 \times 10^8\), depending on the buffer employed for electrophoresis in polyacrylamide gels. In coelectrophoreses of genomes from rice black-streaked dwarf and maize rough dwarf virus, all segments coincided electrophoretically except segments 1, 3, and 10. In an earlier study of maize rough dwarf virus that had been maintained in a greenhouse, two types of genomes were distinguished by a slight difference in the electrophoresis of segment 10. In the present study, the virus samples were collected directly from the field, and they showed the same two kinds of segment 10.

Maize rough dwarf virus (MRDV) and rice black-streaked dwarf virus (RBSDV) were shown to be similar in particle morphology, host range, production of minute tumorous growths on the lower surface of leaves and transmission by the same plant-hopper, *Laodelphax striatellus* Fallen (1-3). Moreover, serological reactions were reported between MRDV and RBSDV (4). The dsRNA\(^2\) genome of MRDV was recently shown to possess ten segments (5, 6), and a close correspondence was demonstrated between the molecular weights for the genome segments of MRDV and those of Fiji disease virus (6).

The presence of dsRNA in RBSDV was indicated by the acridine orange test (7), but we know of no previous report on electrophoretic analysis of the RNA of RBSDV. Our electrophoretic analysis, reported below, demonstrated that RBSDV

\[\text{Tris, tris(hydroxymethyl)aminomethane; EDTA, disodium dihydrogen ethylenediamine tetracacetate dihydrate; 0.045 M PTE, 0.045 M sodium phosphate, 0.03 M Tris, and 0.002 M EDTA (final pH, 7.9-8.0).}\]
possesses ten dsRNA segments, seven of which occupied the same position in the gel as those of MRDV.

The purification procedure used for RBSDV was described by Kitagawa and Shikata (8). Infected corn leaves were macerated in 0.01 M phosphate buffer (pH 7.0) containing 0.01 M EDTA. The virus was concentrated by two cycles of differential centrifugation followed by treatments with 30% carbon tetrachloride and 20% fluorocarbon. Further purification was accomplished by subjecting the virus sample to one cycle of sucrose gradient centrifugation. MRDV was first purified by a method described by Redolfi and Boccardo (5) and was then subjected to one cycle of quasiequilibrium centrifugation. The sucrose density gradient columns were prepared in a solution of 0.1 M glycine and 0.01 M MgCl₂ of pH 7.0, as in the purification of wound tumor virus (WTV) described by Reddy and Black (9). Purified MRDV and RBSDV preparations were diluted in 0.1 M His-Mg buffer, pH 7.0, collected on a Millipore filter of 0.05-μm pore diameter by the technique of Kimura and Black (to be published), resuspended in autoclaved distilled water containing 10% glycerol and then treated with 2% sodium dodecyl sulfate to release the RNA. Some RNA samples were also extracted from both viruses by Kirby's method as described before (10).

Electrophoresis of the RNA was performed at room temperature (about 25°) in 5% acrylamide gels containing 0.1% Bis with a liter of buffer in each compartment of the apparatus. The buffers used were Loening's (11) and 0.045 M PTE (6). About 15–30 μg of RNA in 10–30 μl was loaded on each gel column and electrophoresis was performed at 3.5 V/cm for 35 hr. After completion of a run, gels were stained in 0.1% toluidine blue O and scanned at 540 nm in a Gilford spectrophotometer equipped with a linear transport. The electropherograms were used to compute approximate molecular weights of the segments. All molecular weight determinations were based on coelectrophoretic runs of MRDV and RBSDV in which MRDV genome segments were taken as standards.

The molecular weights of MRDV segments were those determined by Reddy et al. (6).

As in the case of MRDV, all RNA components of RBSDV migrated as sharp zones in 5% acrylamide gels in accord with their double-stranded nature. The RNA of RBSDV was incubated for 30 min at 37° in a solution containing 1 μg/ml of RNase A (Worthington Biochemicals) as described by Reddy and Black (12). Preparations treated with RNase and control untreated RNA samples yielded the same electrophoretic pattern for genome segments.

All ten genome segments of RBSDV were resolved when 0.045 M PTE was used (Figs. 1 and 2). In Loening's buffer, segments 3 and 4 were not clearly resolved (Figs. 3 and 4). The observed and theoretical values for areas under the peaks of segments 1–10 indicated that these segments are present in all virions in equimolar amounts. The molecular weight of the RBSDV genome was estimated as ~19.66

![Fig. 1. Electrophoresis of dsRNA genome segments from MRDV (a), MRDV + RBSDV (b) and RBSDV (c) in 0.045 M PTE buffer. Approximately 25 μg of RNA was run in a 5% acrylamide gel at ~3.5 V/cm for 30 hr, stained in 0.1% toluidine blue O and destained in distilled water. Movement was from top to bottom. Each corresponding dsRNA segment of the two viruses, excepting segments 1 and 10, occupied the same position when coelectrophoresed.](image)
FIG. 2. Electropherogram of dsRNA segments of RBSDV in 0.045 M PTE buffer after ~3.5 V/cm for 30 hr, staining in 0.1% toluidine blue O, destaining in distilled water and scanning at 540 nm. Movement was from left to right.

FIG. 3. Electrophoresis of dsRNA segments from RBSDV (a), MRDV + RBSDV (b) and MRDV (c) in Loening’s buffer. Other experimental details as in Fig. 1. Each corresponding dsRNA segment of the two viruses, excepting segments 1, 3 and 10, occupied the same position in the gel after coelectrophoresis.

MRDV segment 10 appears in two forms, 10a and 10b.

Fig. 4. Electropherogram of dsRNA segments of RBSDV in Loening’s buffer after ~3.5 V/cm for 30 hr, staining in 0.1% toluidine blue O, destaining in distilled water and scanning at 540 nm. Movement was from left to right.

TABLE 1
APPROXIMATE MOLECULAR WEIGHTS OF DSrna SEgments of RBSDV and MRDV DETERMINED IN Loening’s and 0.045 M PTE BURFERS

<table>
<thead>
<tr>
<th>Segment No.</th>
<th>Loening’s buffer</th>
<th>0.045 M PTE buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBSDV</td>
<td>MRDV</td>
<td>RBSDV</td>
</tr>
<tr>
<td>1</td>
<td>2.88</td>
<td>2.85</td>
</tr>
<tr>
<td>2</td>
<td>2.52</td>
<td>2.52</td>
</tr>
<tr>
<td>3</td>
<td>2.48</td>
<td>2.50</td>
</tr>
<tr>
<td>4</td>
<td>2.48</td>
<td>2.48</td>
</tr>
<tr>
<td>5</td>
<td>2.25</td>
<td>2.25</td>
</tr>
<tr>
<td>6</td>
<td>1.82</td>
<td>1.82</td>
</tr>
<tr>
<td>7</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>8</td>
<td>1.32</td>
<td>1.32</td>
</tr>
<tr>
<td>9</td>
<td>1.24</td>
<td>1.24</td>
</tr>
<tr>
<td>10</td>
<td>1.17</td>
<td>1.13</td>
</tr>
<tr>
<td>Total</td>
<td>19.66</td>
<td>19.61</td>
</tr>
</tbody>
</table>

* Molecular weights for the dsRNA of RBSDV were calculated by using dsRNA segments of MRDV.

* Molecular weights were the same as those calculated by Reddy et al. (6).
population of MRDV is maintained by transovarial passage in the vector, without alternate passage through plant and vector, the history of this part of the virion population would resemble that of the above wound tumor virus strain maintained only in vector-cell culture. Therefore, MRDV segments 10a and 10b may have arisen from long continued transovarial passage, and it is interesting to note that in RBSDV, which is not known to be transmitted through the egg of the vector (14), genome segment 10 appeared only in one form.

ACKNOWLEDGMENTS

We thank Dr. Y. Kitagawa and Mr. T. Senboku for their kind help in the purification of rice black-streaked dwarf virus and Dr. C. M. Wilson for the use of his Gilford spectrophotometer.

REFERENCES