Electrophoresis of the potato spindle tuber viroid (PSTV) in 20% polyacrylamide gels revealed a single infectious component with an electrophoretic mobility corresponding to a molecular weight of ca. $5 \times 10^4$ daltons (1). In all our tests, PSTV was solely identified by its biological activity; and no clearly recognizable UV-absorbing component was correlated with infectivity distribution in the gels. Evidently, PSTV has a high specific infectivity and occurs in infected plants in exceedingly small amounts.

Detailed characterization of PSTV requires its isolation in amounts sufficient for conventional biophysical and biochemical analyses. As a first step toward this goal, the present communication reports the extraction of RNA from healthy and PSTV-infected tomato plants derived from large quantities of tissue, and some characteristics of the isolated RNA.

Five kilograms each of leaves from unoinoculated and PSTV-infected leaves were extracted in portions of 500 g by the previously described method (2), but with the following modifications: As before, each nucleic acid preparation (derived from 500 g of leaves) was incubated with deoxyribonuclease. However, instead of applying 2-3 ml portions of the preparation to Sephadex columns, nucleic acids were precipitated with ethanol immediately after incubation with deoxyribonuclease. After centrifugation, the resulting pellets were dissolved in 2 ml of 0.02 M glycine-NaOH buffer containing 3 mM MgCl$_2$, pH 9.0 (GM buffer). Undissolved material was removed by low-speed centrifugation (5000 g, 15 min); and the supernatant solution was added to a Sephadex G-100 column, as previously described (2).

Portions of 20 µl each (representing 500 g of leaf) of the preparation from healthy plants and of that from PSTV-infected plants were analyzed by electrophoresis for 7.5 hr in 20% polyacrylamide gels (1). The gel containing the preparation from infected leaves was sliced; and each slice was bioassayed as described previously (2).

Figure 1A shows the ultraviolet absorption profile of the RNA preparation from healthy tomato leaves after electrophoresis for 7.5 hr. Under the conditions used, 5S RNA moved almost to the bottom of the gel. At least three additional UV-absorbing components with relative electrophoretic mobilities
smaller than that of 5S RNA are discernible in the gel (I, III, and IV). Identity of these RNA species is unknown; evidently they are minor low molecular weight components of cellular RNA.

Figure 1B shows the ultraviolet absorption and infectivity distribution profiles of the RNA preparation from PSTV-infected leaves after electrophoresis in a 20% polyacrylamide gel under identical conditions as those used with the preparation from healthy leaves. In addition to 5S RNA, four UV-absorbing components are discernible in the gel. The positions in the gel of three of these (I, III, IV) coincide with components found in the preparation from healthy leaves. In addition, another prominent component is discernible (II). Bioassay of individual gel slices demonstrated that infectivity coincides with component II (Fig. 1B). This coincidence, the high level of infectivity, and the fact that component II does not occur in preparations from healthy leaves constitutes strong evidence that component II is PSTV.

To isolate the UV-absorbing component that correlates with infectivity distribution in the gels, the remainder of the preparation from infected leaves was layered in 20 μl portions onto 20% polyacrylamide gels and each gel was subjected to electrophoresis for 16 hr. Under these conditions, PSTV migrates close to the bottom of the gel and is more completely separated from component III. The gel portion containing PSTV was excised from each of 12 gels run for 16 hr. The combined gel portions were then crushed in a glass tissue grinder with 30 ml of 0.02 M glycine buffer, pH 9.0 (G buffer). The mixture was centrifuged (15 min at 6000g) and the supernatant solution was passed through a column of hydroxyapatite (1 cm diam X 1 cm bed height). The pellet was resuspended in 20 ml of G buffer; and the mixture was again centrifuged. The supernatant solution was then passed through the column of hydroxyapatite. The column was washed with G buffer until the effluent was essentially devoid of ultraviolet absorbance. The nucleic acid was then eluted from the column with 0.2 M K₂HPO₄-0.02 M glycine buffer, pH 9.0. The eluted RNA was precipitated with ethanol and the pellet dissolved in 2 ml of G buffer. Total yield of PSTV was about 80 μg.

Determination of the UV absorption spectrum of PSTV (Cary Model 14 recording spectrophotometer) disclosed a maximum at 260 nm and a 260/280 nm ratio of 2.2. The maximum/minimum ratio, however, was only 1.25 and the minimum was at 238 nm and not at 230 nm. Control experiments with

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1 Mention of specific equipment or a commercial company does not constitute its endorsement by the U. S. Government over similar equipment or companies not mentioned.
nucleic acid-free gel portions (treated as described) revealed that a nondialyzable impurity elutes from the gel. Elution of an impurity from polyacrylamide gels has previously been observed (3).

In an attempt to remove this impurity, the preparation was subjected to gel filtration on a column of G-100 Sephadex (1 cm diam × 200 cm). Fractions containing PSTV were reprecipitated by chromatography on hydroxyapatite, followed by ethanol precipitation of the RNA and resuspension in 2 ml of 0.01 × SSC (SSC = 0.15 M sodium chloride-0.015 M sodium citrate, pH 7.0). The preparation was then dialyzed for 24 hr against three changes of 1000 vol each of 0.01 × SSC. The maximum/minimum ratio of the reconcentrated RNA was now 1.41 and the minimum was at 234 nm. The small amount of RNA available precluded attempts at further purification.

Comparison of the UV absorption spectrum of the impurity eluted from the gel with that of PSTV still containing some of the impurity revealed that at 260 nm only 2.4% of the absorbance was due to the impurity, the remainder being due to PSTV. Furthermore, solutions of the impurity disclosed only negligible hyperchromicity at 260 nm when heated to 100 C; thus, thermal denaturation of PSTV could be accurately determined in the presence of the impurity. The RNA was heated in 3-ml glass-stoppered cuvettes in a Gilford model 2400 spectrophotometer equipped with reference compensator and thermosensor. Heating was carried out at 0.9 C per min.

Thermal denaturation properties of PSTV were determined in 0.01 × SSC, i.e., at low ionic strength, because (1) denaturation of double-stranded RNAs, irrespective of their base composition, occurs at lower temperatures than in high ionic strength buffer and is complete below 100 C (4-8), (2) thermal denaturation of double-stranded RNA occurs over a narrow temperature range (4-8), and (3) thermal denaturation curves of various double-stranded RNAs (4-8), of transfer RNA (6), and of ribosomal RNA (6) in 0.01 × SSC have been published.

As shown in Fig. 2, the total hyperchromic shift of PSTV in 0.01 × SSC is about 24% and the Tm about 50 C. The thermal denaturation curve indicates that PSTV is not a regularly base-paired structure such as double-stranded RNA, since in this case, denaturation would be expected to occur over a narrower temperature range and at higher temperatures (4-8). The thermal denaturation curve does not, however, rule out an irregularly base-paired single-stranded RNA molecule, similar to transfer RNA, in which single-stranded regions alternate with base-paired regions (see 6 for thermal denaturation curves of transfer and ribosomal RNA).

![Fig. 2. Thermal denaturation curve of PSTV in 0.01 × SSC (—) and absorbance change during slow cooling (---).](image-url)
RNAs in 0.01 \times SSC). If double-helical segments occur in PSTV, these must be relatively short or imperfect, because only in this case is \( T_m \) appreciably less than that of high molecular weight double-stranded RNA (9).

Cooling of previously heated PSTV did not lead to full recovery of the originally present hypochromicity; only about 70% was recovered after slow cooling to 20 C (Fig. 2). Evidently, heated and subsequently cooled PSTV has a less ordered secondary structure than unheated PSTV. Since PSTV can be heated to 90 C and quick-cooled without appreciable loss of infectivity (10), it appears that the secondary structure of the RNA is not essential for infectivity.

The present results confirm—by physical means—earlier conclusions that were based entirely on infectivity assays (1, 2). Isolation of PSTV as a ribonuclease-sensitive, UV-absorbing component with an absorption maximum at 260 nm and with thermal denaturation properties typical of nucleic acids demonstrates that the infectious agent is, indeed, an RNA. The observation that this RNA penetrates into 20% polyacrylamide gels and moves through such gels as a monodisperse, UV-absorbing band confirms that the RNA is of very low molecular weight. Finally, the thermal denaturation curve of PSTV confirms earlier conclusions based on chromatographic properties of PSTV which had indicated that the RNA is not a regularly base-paired duplex molecule (11).

ACKNOWLEDGMENT

The author thanks Mr. D. R. Smith for excellent technical assistance.

REFERENCES


T. O. DIENER

Plant Virology Laboratory
Plant Protection Institute
Agricultural Research Service
Beltsville, Maryland 20701
Accepted August 3, 1972