Nucleic Acids of Two Phloem-limited Viruses: Tobacco Necrotic Dwarf and Potato Leafroll

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SUMMARY

Particles of tobacco necrotic dwarf (TNDV) and potato leafroll (PLRV) viruses, both of which are phloem-limited and transmitted by aphids in the persistent manner, contain a single-stranded RNA of mol. wt. $2.0 \times 10^6$, estimated from their mobilities in polyacrylamide gel electrophoresis. The results suggest that both viruses belong to the group of luteoviruses. TNDV has two minor RNA components of mol. wt. approx. $1 \times 10^6$.

Little is known of the chemical and physical properties of most of the plant viruses that are phloem-limited, not mechanically transmissible but transmitted by aphids in the persistent manner. An exception is barley yellow dwarf virus (BYDV) which is the type virus of the luteovirus group (Fenner, 1976) and is known to have a single-stranded (ss) RNA of mol. wt. $2.0 \times 10^6$ (Brakke & Rochow, 1974). We have recently isolated a new virus, tobacco necrotic dwarf virus (TNDV), from tobacco plants in Japan (Kubo & Takanami, 1977) and have found that TNDV and potato leafroll virus (PLRV) are serologically related (Kubo & Takanami, 1978) and are morphologically indistinguishable (Takanami & Kubo, 1979a). Although, from its biological properties, PLRV seems to have a close affinity with luteoviruses, its nucleic acid has been reported to be double-stranded (ds) DNA (Sarkar & Blessing, 1973; Sarkar, 1976). This paper describes studies on the nature of the nucleic acids of TNDV and PLRV; a preliminary account has appeared (Takanami & Kubo, 1979b).

TNDV and PLRV were purified from Physalis floridana plants infected by the aphid Myzus persicae using the enzyme method previously reported by Takanami & Kubo (1979a). Purified preparations suspended in 0.01 M-phosphate (Na$_2$HPO$_4$-KH$_2$PO$_4$) buffer, pH 7.8, were stored frozen at $-80^\circ$C before use.

Electrophoresis of nucleic acids was carried out in 2.4 or 5% acrylamide gels under conditions described by Loening (1969). The electrophoresis buffer (36 mM-tris, 30 mM-NaH$_2$PO$_4$ and 1 mM-EDTA, pH 7.8) contained 0.1% SDS. Samples were layered on the gels and subjected to electrophoresis at a current of 5 mA/gel and 7.4 V/cm at ambient temperature. Then gels were washed in distilled water for 1 h and were scanned at 260 nm using a spectrophotometer equipped with a gel-scanning attachment.

Nucleic acids of TNDV and PLRV were prepared for electrophoresis using a direct dissociation method with SDS. Equal volumes of purified virus suspension (1 to 2 mg/ml) and the electrophoresis buffer containing 1% SDS and 10% sucrose were mixed and were directly electrophoresed. Under these conditions particles of both viruses were completely dissociated into nucleic acid and protein. The nucleic acid preparations of TNDV and PLRV always had a major slow migrating component and perfect co-electrophoresis of the major component of both viruses was observed. Small amounts of faster-moving, polydisperse components were also present (Fig. 1a, b, c). In addition, preparations from TNDV always contained low mol. wt. components, which were resolved into two distinct components in 5% gels (Fig. 1d). The nucleic acid preparation of PLRV also had small amounts of polydisperse components but no low mol. wt. components (Fig. 1b). The electrophoretic
Fig. 1. Polyacrylamide gel electrophoretic patterns of nucleic acid from TNDV and PLRV after direct dissociation of virions by 0.5 % SDS. Nucleic acids of (a) TNDV and (b) PLRV were run on 2.4 % gels (0.5 × 7 cm) at a current of 5 mA/gel for 105 min. Migration is from left to right. (c) Mixtures of nucleic acids of TNDV and PLRV were run for 120 min. Arrows indicate the positions of TMV RNA and *E. coli* 23S and 16S ribosomal RNAs in companion gels. (d) Nucleic acids of TNDV were run on a 5 % gel for 150 min. Arrow indicates the position of the CMV-associated RNA 5.

The mol. wt. of TNDV and PLRV nucleic acids were estimated by comparing their electrophoretic mobilities in the acrylamide gels with those of TMV RNA (mol. wt. 2.05 × 10⁶; Caspar, 1963) and *E. coli* ribosomal RNAs (23S and 16S, mol. wt. 1.07 × 10⁶ and 0.55 × 10⁶; Stanley & Bock, 1965) used as internal markers. All samples were heated at 70 °C for 2 min, then chilled in an ice bath just before electrophoresis. The major component of the nucleic acids of TNDV and PLRV migrated slightly faster than TMV RNA (Fig. 1c) and their estimated mol. wt. were 2.0 × 10⁶. As we did not use any denaturing reagents of nucleic acids during electrophoresis, there might still be uncertainty about the values. When the low mol. wt. components contained in TNDV virions were separated into two components in 5 % gels, the slower moving one migrated slightly faster than the cucumber mosaic virus-associated RNA 5 which contains 335 nucleotide residues (Richards *et al.* 1978; Fig. 1d); thus the mol. wt. of TNDV small RNAs were considered to be around 1 × 10⁵.

To determine whether the nucleic acids of TNDV and PLRV are RNA or DNA, nucleic acid preparations were digested by RNase or DNase according to the procedures described
Fig. 2. Polyacrylamide gel electrophoretic patterns of nucleic acid from TNDV and PLRV after treatment with 0.1 μg/ml of RNase or 10 μg/ml of DNase at 28 °C for 20 min in 2 × SSC. Virus was treated with DNase in the presence of 0.05 M-MgCl₂. Samples were run on 2.4 % gels at a current of 5 mA/gel for 90 min. Migration is from left to right. (a) TNDV control; (b) TNDV treated with RNase; (c) TNDV treated with DNase; (d) PLRV control; (e) PLRV treated with RNase; (f) PLRV treated with DNase.

by Brakke & Rochow (1974) with slight modifications. For enzyme treatment, 40 μl of purified TNDV or PLRV (1 mg/ml) was mixed with 160 μl of 2 × SSC (0.3 M-NaCl, 0.03 M-sodium citrate, pH 7.0) containing 1 % SDS. After standing for 5 min at room temperature, SDS was precipitated by adding KCl to 0.05 M and incubated for 3 h at 0 °C. The precipitate was removed by centrifugation and RNase A (Worthington; final 0.1 μg/ml) or DNase I (Worthington, RNase free; final 10 μg/ml) was added to the supernatant; sufficient MgCl₂ to give 0.05 M was added before the DNase treatment. The RNase was heated for 10 min at 80 °C before use to avoid possible contamination of DNase. The samples were incubated for 20 min at 28 °C, then 2 vol. of cold ethanol were added to precipitate nucleic acid. After washing with 70 % ethanol, the precipitates were partially dried in vacuo, resuspended in 50 μl of the electrophoresis buffer containing 1 % SDS and 10 % sucrose and electrophoresed in 2.4 % gels (Fig. 2). The nucleic acids of TNDV and PLRV, including the low mol. wt. components of TNDV, were susceptible to digestion by RNase, but not by DNase. As these nucleic acids were digested by RNase even in 2 × SSC, they seemed to be ssRNAs. In a control experiment 2.5 μg/ml DNase digested only the peak of
DNA in total nucleic acids which were extracted from tobacco leaves and processed as described above for virions (data not shown).

The data in this report show that both TNDV and PLRV have a major component of ssRNA with a mol. wt. of about $2.0 \times 10^6$. The properties of the RNA of both viruses are similar to those of BYDV (Brakke & Rochow, 1974). It is strongly suggested that both TNDV and PLRV should be placed in the luteovirus group, because not only the biological and physical properties of the virus particles (Peters, 1970; Takanami & Kubo, 1979a) but also the nature of their nucleic acids are similar to those of BYDV.

Our results on the nucleic acid of PLRV differ from those of Sarkar (1976), who claimed that PLRV has a dsDNA of mol. wt. $0.56 \times 10^6$. The cause of this difference is at present unknown. The possibility that the Japanese PLRV is different from the European one can be ruled out, as Casper (1977) reported that antiserum against the Japanese PLRV reacted with German isolates of PLRV in the enzyme-linked immunosorbent assay.

The significance, if any, and the origin of the faster moving, polydisperse components contained in TNDV and PLRV particles and of the low mol. wt. components of TNDV RNA are unknown. Brakke & Rochow (1974) also showed the existence of the polydisperse components in preparations of BYDV RNA and they thought these RNAs might be degradation products of virus RNA. It is very interesting, however, that the low mol. wt. components are found in TNDV particles specifically. This is now under further investigation.

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REFERENCES


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