Isolation and Properties of Potato Virus Y Ribonucleic Acid

K. M. Makkouk and D. J. Gumpf

Graduate Student and Assistant Professor, respectively, Department of Plant Pathology, University of California, Riverside 92502.

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ABSTRACT

Potato virus Y (PVY) ribonucleic acid (RNA) isolated from purified virus preparations was found to be infectious, susceptible to ribonuclease, and resistant to deoxyribonuclease. The sedimentation coefficient of PVY-RNA was 39S as determined in linear-log sucrose density gradients. Formaldehyde-treated PVY-RNA sedimented at 25S. The estimated molecular weight is 3.1 $\times 10^6$. Heat-treated PVY-RNA shows breakdown after heating at 40 C for 10 min, with complete destruction at 60 C for 10 min. Heat treatment of virus preparations prior to RNA isolation indicated that the coat protein provided some protection to heat. Breakdown of the RNA was not observed until 50C, and complete destruction did not occur even after heating at 60C for 10 min.

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Isolation of an infectious nucleic acid from potato virus Y (PVY) has thus far been unsuccessful. We were likewise unable to isolate infectious PVY-RNA using 1.0 N HCl or 6 M Guanidine (8) or by using phenol or bentonite (5). Brakke and Van Pelt (3) reported a method using an ammonium carbonate buffer pH 9.0 containing 2% sodium dodecyl sulfate (SDS) to isolate RNA from the long flexuous rod shaped wheat streak mosaic virus (WSMV). Pring and Langenberg (6) also successfully isolated maize dwarf mosaic virus (MDMV)-RNA using this same method. The structural similarities of WSMV, MDMV, and PVY suggested that an alkaline buffer containing detergent might also be useful for the isolation of PVY-RNA. This paper reports the successful isolation of an infectious single stranded RNA from PVY.

MATERIALS AND METHODS.–*Culture and purification of virus.*–The PVY cultures used in this work were a North Carolina isolate (NC-57) provided by G. V. Gooding and a California isolate (PVY-4) originally obtained from pepper in southern California. The PVY isolates were inoculated to *Nicotiana tabacum* 'Havana 425' and infected leaves were harvested 10-12 days after inoculation. Subsequent regrowth was harvested for a period of 4 wk. The virus was purified using a modification of the method described by Purcifull and Gooding (7). Systemically infected tobacco leaves were ground in a Waring Blendor with 0.5 M sodium citrate containing 1% mercaptoethanol (1.0 ml/g of tissue) and the fluid was expressed through two layers of cheesecloth, followed by clarification with 6.5-7% butanol and low-speed centrifugation (12,000*g* for 30 min). The supernatant fluid was kept overnight at 4 C and then centrifuged at 12,000*g* for 15 min. Virus was precipitated from the preceding supernatant fluid with 4% polyethyleneglycol and 3% sodium chloride, and collected by centrifuging at 12,000*g* for 15 min. Virus pellets were resuspended in 0.02 M ortho borate buffer pH 8.2 containing 1mM EDTA. The virus was concd and purified by two cycles of differential centrifugation followed by sucrose density gradient centrifugation. Sucrose for 100-400 mg/ml sucrose density gradients was dissolved in 0.02 M orthoborate buffer pH 8.2. Ultraviolet absorbing virus zones in the density gradient columns were collected using an ISCO density-gradient fractionator.

Preparation of nucleic acid.–About 1 mg of virus in 1 ml was added to an equal volume of buffer containing 0.2 M ammonium carbonate, pH 9.0, 2mM disodium-EDTA, 2% SDS, and 200 μg of EDTA-treated bentonite (5) per ml as described by

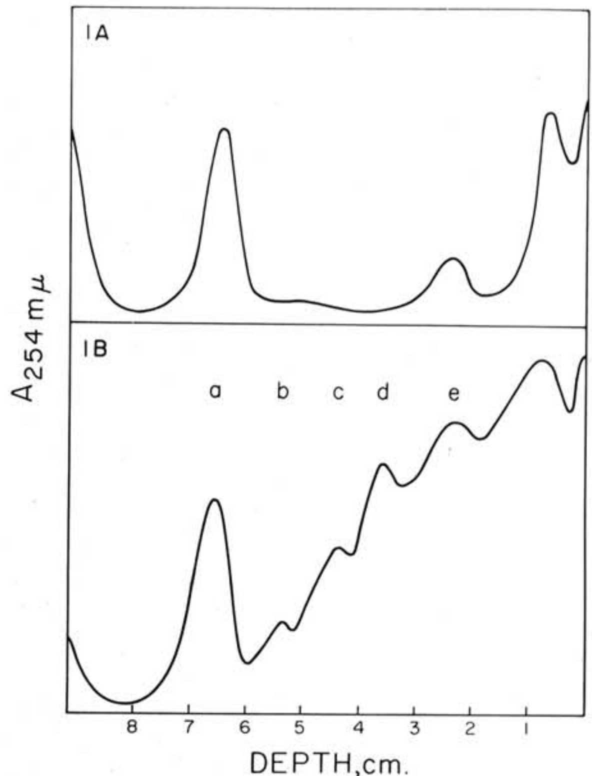

Fig. 1-(A,B). Ultraviolet light absorbancy scanning profiles of different RNA species centrifuged in linear-log sucrose density gradients at 6 C at 40,000 rpm in a Beckman SW41 rotor for 5.5 h. **A)** PVY-RNA. **B)** (a) PVY-RNA; (b) tobacco mosaic virus-RNA (31.1S); (c,d, and e) brome grass mosaic virus species of 25.3S, 20.5S, and 13.8S, respectively.

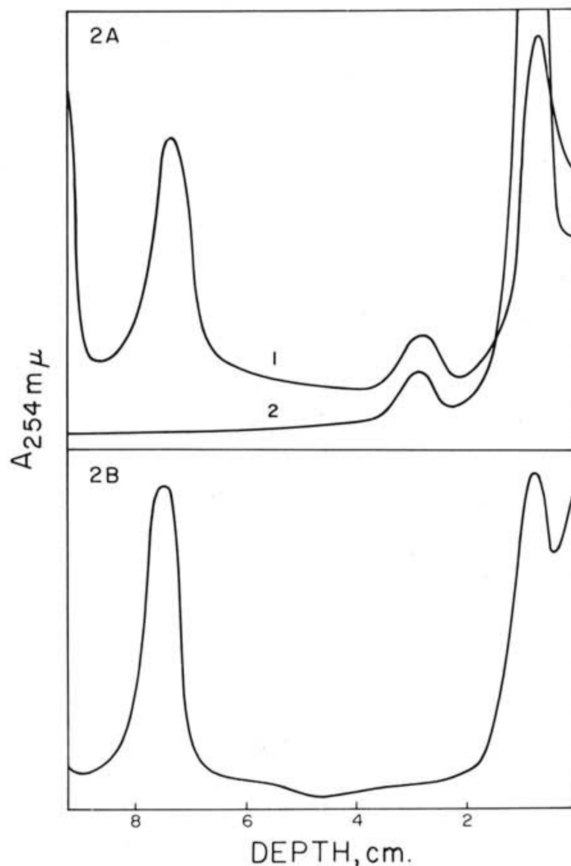

Fig. 2-(A,B). Ultraviolet light absorbancy scanning profiles of RNA preparations centrifuged in linear-log sucrose density gradient at 6 C at 40,000 rpm in Beckman SW41 rotor for 5.5 h. Preparations were treated with: **A-1)** Pronase, 10 μg/ml for 30 min; **A-2)** RNase, 3 μg/ml for 30 min; and **B)** DNase, 50 μg/ml for 1.0 h.

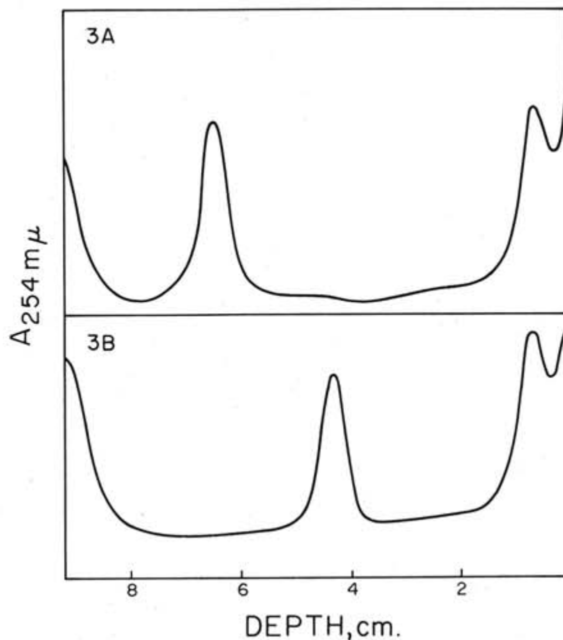

Fig. 3. Ultraviolet light absorbancy scanning profiles of RNA preparations centrifuged in linear-log sucrose density gradient at 6 C at 40,000 rpm in Beckman SW41 rotor for 5.5 h. **A)** Native PVY-RNA. **B)** Formaldehyde-treated PVY-RNA.

Brakke and Van Pelt (3). The solution was incubated for 24 h at 4-5 C before it was layered on linear-log sucrose density gradients designed for the Spinco SW41 Rotor (2), using 0.15 M sodium chloride, 15mM sodium citrate (SSC), pH 7.0, as the sucrose solvent. The gradients were preformed and placed at 4 C for 16-18 h before use, and were centrifuged at 40,000 rpm for 5.5 h at 6 C. Gradients were fractionated with an ISCO density gradient fractionator. Bromegrass mosaic virus (BMV)-RNA and tobacco mosaic virus (TMV)-RNA standards for S value estimates, were prepared by the method described by Brakke and Van Pelt (3).

RESULTS.–*Isolation and infectivity of nucleic acid components.*–Linear-log density gradient centrifugation of the alkaline buffer treated purified virus revealed two ultraviolet (UV) light absorbing zones (Fig. 1-A). An average sedimentation coefficient of 39S for the faster sedimenting material (Fig. 1-B) was determined from 11 experimental values ranging from 37 to 41S. The slower sedimenting component had sedimentation coefficients in four separate experiments ranging from 13-14S. No differences in sedimentation behavior between the nucleic acid preparations from the two PVY isolates could be determined.

The nucleic acid components from 6 mg of purified virus were collected separately and inoculated to tobacco plants. Six days following inoculation, two of 10 plants inoculated with material from the faster-sedimenting 39S zone showed vein-clearing symptoms typical of PVY. Sap from these plants reacted positively with PVY antiserum. The 10 plants inoculated with the slower-sedimenting material failed to show symptoms and sap from these plants failed to react with PVY antiserum. In another infectivity experiment with nucleic acid prepared from 6 mg of purified virus, three of 15 plants inoculated with the faster 39S component developed symptoms and extracted juice from them reacted with PVY antiserum.

Nuclease and pronase treatment.–No nucleic acid zones were evident in the 39S region of density-gradient columns after centrifugation of a nucleic acid preparation which had been incubated for 30 min at 37 C with 3 μg/ml of RNase (Fig. 2). Similar treatment of a preparation with DNase at a concn of 50 μg/ml had no effect (Fig. 2) on the faster-sedimenting material, but it did hydrolyze the slower-sedimenting material. Pronase treatments (10 μg/ml) had no effect on any of the material present in the nucleic acid preparations. The faster-sedimenting zone thus appears to contain single-stranded RNA, while the slow-moving zone contains DNA.The ratio

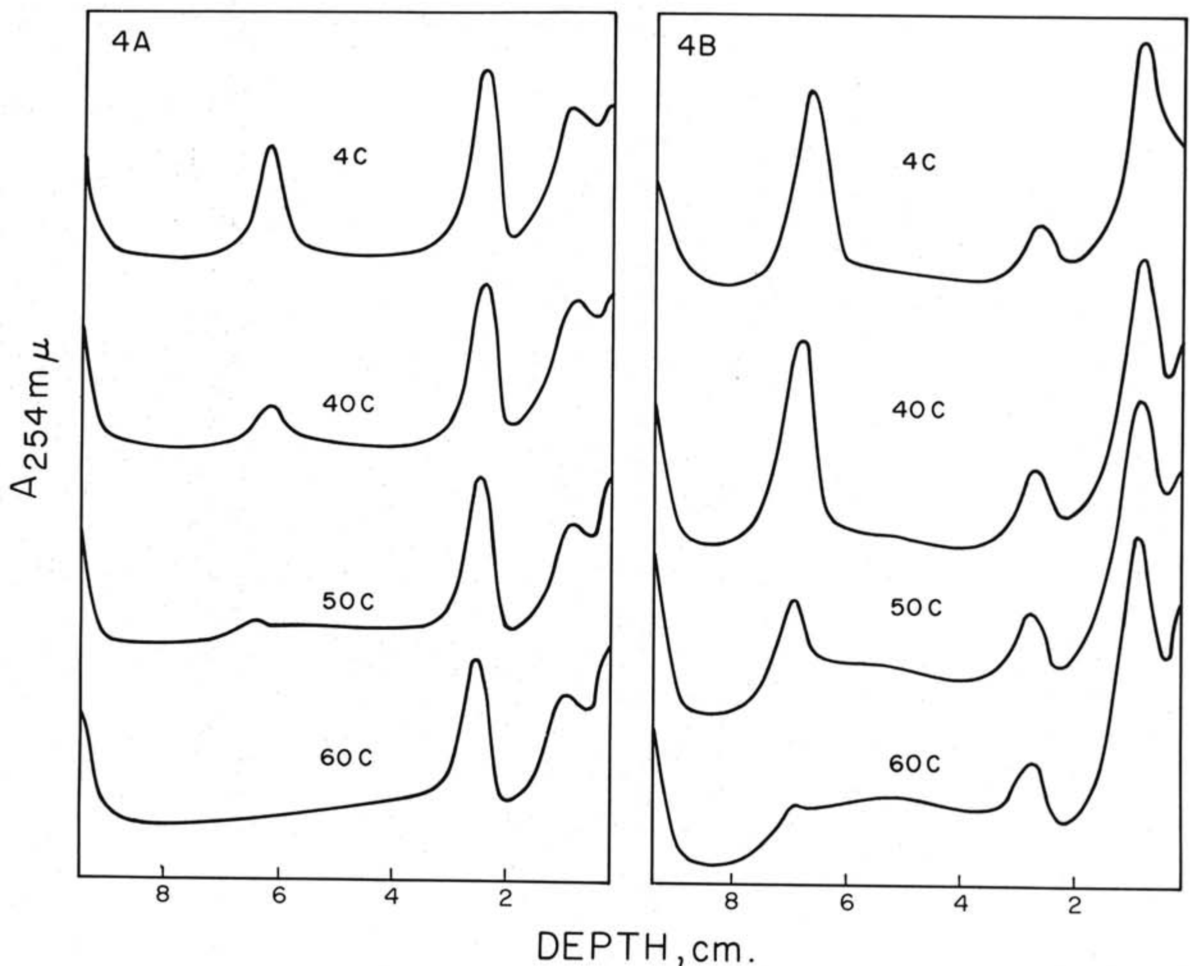

Fig. 4-(A,B). Ultraviolet light absorbancy scanning profiles of RNA preparations centrifuged in linear-log sucrose density gradients at 6 C at 40,000 rpm in Beckman SW41 rotor for 5.5 h. **A)** Virus preparation was heat treated after incubation with the ammonium carbonate buffer, pH 9.0, while in **B)** the heat treatment was before the addition of the buffer.

of the RNA fraction to the DNA fraction was not consistent in our experiments.

Formaldehyde treatment.–An average value of 25S was obtained for the sedimentation of PVY-RNA after dialysis for 12 h at 40 C against 1.1 M formaldehyde in 1mM EDTA at pH 7.0 (Fig. 3). This value is an average of 11 separate determinations with coefficients ranging from 24 to 27S. Also, when formaldehyde-treated PVY-RNA was cocentrifuged with BMV-RNA, the 25.3S component of BMV and the formylated PVY-RNA appeared as one distinct band in the sucrose gradients.

Heat treatment.–Samples of PVY-RNA were heat treated at 40, 50, and 60 C for 10 min, rapidly cooled, and then centrifuged through density gradient columns. A sample maintained at 4 C served as the control.

The RNA was observed to be susceptible to the heat treatment as absorbancy profiles (Fig. 4) showed a decrease in UV absorption after treatment at 40 C for 10 min. After 60 C for 10 min there was no UV absorption in the area of the column where PVY-RNA normally sediments. When intact purified virus was heat-treated and then RNA isolated, the RNA zone was unaffected by pretreatment at 40 C for 10 min as compared to the control. Even at 60 C for 10 min there was some UV (254 nm) absorption in the 35-40S region of the gradient column.

DISCUSSION.–Ammonium carbonate buffer was adequate to release PVY-RNA. Procedures (5, 8) using bentonite, phenol, or guanidine were not applicable for the release of infectious PVY-RNA. The basis for our conclusion that nucleic acid from PVY is an infectious single-stranded RNA is its susceptibility to RNase, resistance to DNase, infectivity tests, and its reaction with formaldehyde. The slow-moving band was identified as DNA, based on susceptibility to DNase, and resistance to RNase and Pronase. Also, when this zone was dialyzed against SSC buffer, ethanol precipitated, and resuspended in SSC, it gave a 260:280 ratio of 1.95. It is most probable that this DNA fraction is of host

origin, possibly becoming attached to the virus during the extraction procedure, and carried along through the purification steps. When the purified virus preparation was treated with ammonium carbonate buffer, host DNA was released along with the PVY-RNA. The amount of the DNA fraction varied with different PVY-RNA preparations and in a few instances was absent completely, lending further support to this idea.

According to Boedtker (1), when the secondary structure of RNA is destroyed by formaldehyde treatment, the sedimentation coefficient should depend only on molecular weight. Brakke and Van Pelt (3) obtained a relationship between the sedimentation coefficient of formaldehyde-treated RNA (S), and its molecular weight before formaldehyde treatment (M) which is $S = 0.83\ M^{0.38}$. Duplicating the experimental conditions of Brakke and Van Pelt, the molecular weight, using the above formula, was calculated to be 3.1×10^6.

Heat inactivation experiments of PVY-RNA showed it to be heat sensitive, with the RNA starting to break down at 40 C after 10 min. Data also suggest that the protein coat affords the nucleic acid some protection against heat. The reported thermal inactivation of 55-60 C for PVY (4) is in agreement with the results of our experiments.

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