The Application of Polyethylene Glycol Precipitation for Purification of Potato Leaf-Roll Virus

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小島 誠*・村山大記*: ポリエチレングリコールによる
ジャガイモ葉巻病ウィルスの沈澱

A large scale purification is required for biochemical and biophysical research of aphid-borne circulative plant viruses, because of the low virus content in the host plants. The current procedure for purification of potato leaf-roll virus (PLRV) from plant materials is fairly laborious¹⁾. Since Hebert²⁾ reported polyethylene glycol (PEG) precipitation of plant viruses, many workers have applied this PEG precipitation for purifications of bacteriophages and animal viruses^{3,4)}. We have applied PEG precipitation to PLRV, as a modification of the purification procedure described previously¹⁾.

In preliminary experiments, assays by aphid-injection showed that precipitates obtained by the addition of 4 or 8% PEG to clarified plant juice retained infectivity. However, the amount of virus in the precipitates could not be accurately measured by the bioassay. Accordingly, comparative assays using precipitates obtained by the addition of PEG at various concentrations were made by an analytical sucrose density-gradient centrifugation⁵⁾.

Frozen tissue of *Physalis floridana* plants infected with PLRV was ground by an electric meat grinder. The macerate was then homogenized with a Waring Blender in 0.5 M phosphate buffer (pH 7.4). The homogenate was emulsified with a chloroform and *n*-butanol mixture (1:1) in a fruit mixer. The preparation was clarified by centrifuging at 6,000 rpm for 15 minutes in 9B-2 rotor of a Marusan high-speed centrifuge. PEG 6,000 was added at various concentrations to the resultant aqueous phase, which was stirred for 60 minutes and then kept in a centrifuge tube for 60 minutes at room temperature. Precipitates obtained after centrifugation in the same rotor at 6,000 rpm for 15 minutes were suspended in 0.01 M phosphate buffer (pH 7.4) with a chilled Teflon-glass homogenizer. The resuspended preparation was again clarified with fluorocarbon (Daifron S-3) in a cold room, and further purified by two cycles of differential centrifugation (at 40,000 rpm for 120 minutes; at 10,000 rpm for 15 minutes) in RP40 rotor of a refrigerated Hitachi 40P ultracentrifuge. Two milliliters each of the concentrated preparation (from 400 g of tissue) was layered on a sucrose gradient column, and was centrifuged at 23,000 rpm for 180 minutes in RPS25 rotor of the same ultracentrifuge. Density-gradient columns, made by layering of 4, 7, 7 and 7 ml of 100, 200, 300 and 400 mg of sucrose per milliliter of phosphate buffer (0.01 M, pH 7.4), respectively, in 1×3 inch cellulose nitrate centrifuge tubes, were used after standing overnight at 4°C. For an analytical density-gradient centrifugation, centrifuged columns were scanned with an ISCO gradient column fractionator and flow densitometer attached with an external recorder.

A 10 mm light path cell and sensitivity range 0-0.5 at 253.6 nm wavelength were used. Scanning patterns of the centrifuged gradient columns on which partially purified preparations using PEG at

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various concentrations were layered are shown in Fig. 1. The scanning pattern did not show an extra component at 25–27 mm from the meniscus, when partially purified preparation obtained by using 4% PEG was layered. However, scanning patterns with a peak were obtained when preparations obtained from 6, 8 and 10% PEG precipitates were layered. In these gradient columns, a zone visible by scattered light was observed at 25–27 mm from the meniscus. These patterns suggested that PLRV was partially precipitated by the addition of 6% PEG, and completely by 8% PEG. On the other hand, the scanning pattern did not have a peak at 25–27 mm from the meniscus when preparation obtained from healthy *P. floridana* plants with 8% PEG was layered (Fig. 1).

Fig. 2 shows scanning pattern obtained from an experiment with the same purification procedure as described above, using 8% PEG to precipitate virus from 1,000 g of starting material. This pattern indicates that the sedimentation rate of PLRV would be greater than that of barley yellow dwarf

Fig. 1. Scanning patterns of gradient columns centrifuged after layering 2 ml each of partially purified preparations of potato leaf-roll virus concentrated by PEG precipitation at various concentrations, followed by two cycles of differential centrifugation. The columns, prepared from 4, 7, 7 and 7 ml of 100, 200, 300 and 400 mg sucrose per milliliter, respectively, were centrifuged at 23,000 rpm for 180 minutes. These tubes were scanned with a 10 mm light path and sensitive range (0–0.5) of densitometer.

Fig. 2. Scanning pattern of gradient column centrifuged after layering of 2 ml of partially purified preparation of potato leaf-roll virus concentrated 500 $\times$ (i.e., 2 ml from 1,000 g infected plant tissues) by 8% PEG precipitation followed by two cycles of differential centrifugation. Columns were centrifuged for 180 minutes at 23,000 rpm.

Fig. 3. Electron micrograph of phosphotungstate-stained PLRV ($\times 150,000$).

virus (115-118 S), based on comparison of the present result with those of Rochow and Brakke⁶⁾. The yield of purified virus from 1,000 g of infected plants was about 10 μ g, assuming an A_{260} of 5.0 for a 1 mg/ml concentration.

It was confirmed that this peak region was associated with infectivity and contained many uniform virus particles (Fig. 3).

The results obtained here suggest that the PEG precipitation method may be useful as the first step to facilitate purification of aphid-borne plant viruses.

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