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Properties of viruses of the potyvirus group. 1. A simple method to purify bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus and potato virus Y^N

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Abstract

Bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus, and potato virus Y^N were purified by homogenizing and clarifying infected leaves in a mixture of 0.1 M tris-thioglycollic acid buffer pH 9, carbon tetrachloride and chloroform, followed by differential centrifuging applying moderate centrifugal forces.

Introduction

Viruses of the potyvirus group (Harrison et al., 1971) are difficult to purify because of their tendency to aggregate, both end to end and side by side (Shepherd and Pound, 1960). Nevertheless, there have been several reports on the purification of various viruses of this group. Damirdagh and Shepherd (1970) used clarification by n-butanol, and concentration by polyethylene glycol (PEG) precipitation and differential centrifuging, to purify, among others, tobacco etch virus. Stace-Smith and Tremaine (1970) applied clarification by heat and ethanol and concentration by centrifuging for potato virus Y. Van Oosten (1972) successfully added a treatment with Triton X-100 to the method of Wetter (1960) for isolating plum pox virus. The general conclusion from these reports is that with rather complicated procedures only small amounts of homogeneous virus preparations were obtained and that the methods successfully used with a certain virus can not generally be applied for the purification of other elongate viruses. As a result, there is a striking lack of information on the chemical and physical properties of the viruses of the potyvirus group. The study of their relationships has been based primarily on morphological features, transmissibility, and in some cases serological properties (Brandes and Wetter, 1959; Bercks, 1961; Bartels, 1964; Purcifull and Shepherd, 1964). However, it has become evident, that the degree of relationship between viruses within the potyvirus group can not be assessed properly on the basis of those properties alone (Taylor and Smith, 1968; Bos, 1970). I have now tried to purify some viruses of this group in substantial amounts, thus enabling a more detailed study of their chemical and physical properties on which I will publish later.

Materials and methods

Virus isolates and host plants. Bean yellow mosaic virus (BYMV) B25 (*/*: */*:E/E: S/Ap) and pea mosaic virus (PMV) E198 (*/*:*/*:E/E:S/Ap) were obtained from Dr L. Bos. Lettuce mosaic virus (LMV) (*/*:*/*:E/E:S/Ap) was obtained from Mr N. Huyberts, and Dr J. H. Venekamp provided us with potato virus Y^N (*/*:*/*:E/E: S/Ap). BYMV B25, PMV E198 and the LMV isolate were described earlier by Bos (1970), PVY^N by de Bokx (1964). BYMV B25, PMV E198, and LMV were propagated on *Pisum sativum* 'Koroza'. Seedlings were inoculated twice on successive days when they were about 2 cm high. Systemically infected leaves were harvested when they showed symptoms all over. LMV could well be propagated on *Lactuca sativa* 'Portato' too. Lettuce was inoculated when the seedlings had 3 leaves. A few days after the leaves showed symptoms they were harvested. PVY^N was propagated on *Nicotiana tabacum* 'Samsun NN'. The non-inoculated systemically infected leaves were harvested when they showed symptoms.

Homogenization and clarification. Infected leaves were homogenized in a Waring blendor in a mixture of 150 ml buffer, 40 ml of carbon tetrachloride, and 40 ml of chloroform. The buffer was either 0.18 M phosphate-citric acid pH 7 (PCA) containing 0.1 % thioglycollic acid or 0.01 or 0.1 M tris-thioglycollic acid pH 9. The homogenate was centrifuged for 10 min at 10,000 *g* to separate the water phase containing the virus from the remainder.

Concentration procedures. Virus was precipitated by different amounts of PEG or sedimented by centrifuging at different centrifugal forces, and resuspended in PCA or tris-HCl pH 9 buffers.

Analytical ultracentrifuging. Centrifugal analyses were done in a Spinco Model E ultracentrifuge using Schlieren optics. Sedimentation coefficients were determined by the graphical method of Markham (1960).

Electron microscopy. Samples were put on to 200 mesh grids and negatively stained with 1% potassium phosphotungstate pH 6.5. Preparations were examined in a Philips EM 300.

Results

Homogenization and clarification. Homogenizing the material was convenient when 50 g of pea or tobacco leaves or 100 g of lettuce leaves were ground in the mixture mentioned under 'Materials and methods'. After centrifuging the homogenate a clear yellow-brown supernatant fluid was obtained. The highest yields of unaggregated virus particles were obtained when the 0.1 M tris-thioglycollic acid pH 9 buffer was used in the homogenization mixture.

Concentration. Precipitation by 2–4% PEG induced severe aggregation, which made it impossible to resuspend the virus in small amounts of buffer. When concentrating by centrifuging at different centrifugal forces was applied, it was found that two cycles

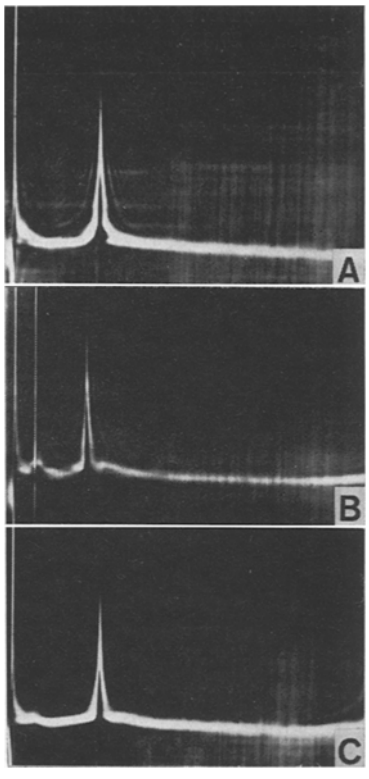

Fig. 1. Analytical u tracentrifuge patterns of A. BYMV B25; B. PMV E198 and C. LMV. Sedimentation was from left to right. The pictures were made about 10 min after the selected rotor speed, 21,740 rpm, was reached. Bar angle 50°, rotor temperature 20°C.

Fig. 1. Analytische-ultracentrifugebeelden van A. BYMV B25; B. PMV E198 en C. LMV. De sedimentatie verliep van links naar rechts. De foto's werden ongeveer 10 min. na het bereiken van de gekozen draaisnelheid van 21.740 rpm opgenomen. De hoek bedoeg 50°. De rotortemperatuur was 20°C.

of differential centrifuging (1.5 h at 26,500 *g* and 10 min at 8,000 *g*) yielded 10–25 mg of unaggregated virus/kg leaves and caused negligible breakage of the virus particles. Higher centrifugal forces produced considerable amounts of broken particles. The virus was resuspended in 1/100 to 1/50 of the original amount (v/w) of 0.1 M tris-HCl ph 9.

Analytical ultracentrifugation. The purified preparations of BYMV B25, PMV E198 and LMV each sedimented as a single peak (Fig. 1). The S-values at infinite dilution in 0.1 M tris-HCl pH 9 and 20°C did not differ significantly and varied between 140 and 143. PVY^N also sedimented as a single peak, but due to lack of material we were not able to determine the S value properly.

Electron microscopy. The electron micrographs of the four viruses, presented in Fig. 2, also show that the purified preparations are highly homogeneous.

Discussion

In purifying elongate viruses there are two main difficulties to overcome: aggregation and fragmentation. It was demonstrated here that for BYMV B25, PMV E198, LMV, and PVY^N the aggregation could be avoided by using a high molar buffer solution

Fig. 2. Electron micrographs of A. BYMV B 25, B. PMV E198; C. LMV and D. PVY^N. Preparations were stained with 1 % potassium phosphotungstate pH 6.5. Magnification × 29,000.

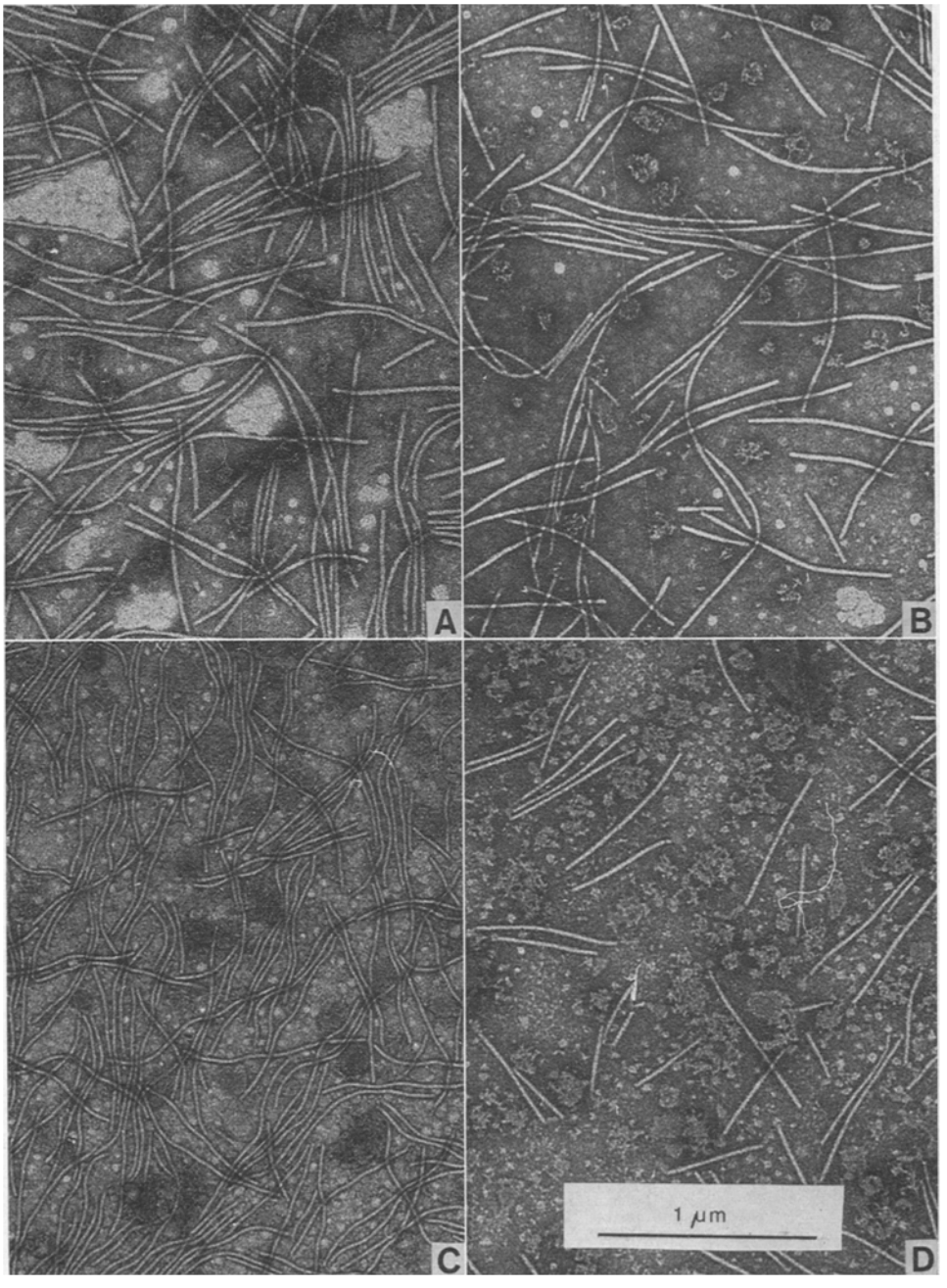

Fig. 2. Elektronenmicroscopische foto's van A. BYMV B25; B. PMV E198; C. LMV en *D. PVY^N. De preparaten werden negatief gekleurd met 1 % kaliumwolframaat pH 6,5. Vergroting 29.000 ×.*

with a high pH: 0.1 M tris-HCl pH 9. The fragmentation was kept at a minimum by centrifuging at moderate centrifugal forces. These data suggest that in the past a lot of purifications have suffered from the use of too high centrifugal forces. They also imply that viruses of the potyvirus group can be purified by differential centrifuging using a so-called low-speed centrifuge only.

Samenvatting

Eigenschappen van virussen van de potyvirusgroep. 1. Een eenvoudige methode om het bonescherpmozaïekvirus, het erwtemozaïekvirus, het slamozaïekvirus en het aardappelvirus Y^N te zuiveren

Het bonescherpmozaïekvirus B25, het erwtemozaïekvirus E198, het slamozaïekvirus en het aardappelvirus Y^N konden worden gezuiverd door geïnfecteerde bladeren te vermalen in een mengsel van 0,1 M tris-thioglycolzuur pH 9, tetrachloorkoolstof en chloroform, en de geklaarde suspensie vervolgens differentiëel te centrifugeren bij matige centrifugaalkrachten.

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