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Separation of long and short particles of tobacco rattle virus with polyethylene glycol

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Abstract

Two methods to separate long and short particles of tobacco rattle virus with polyethylene glycol 6,000 (PEG) are described. The first is based on specific precipitation, the second on specific solubilization of particles with different lengths at different PEG concentrations. The results of the separations are comparable to, or better than those obtained by sucrose-gradient centrifuging. The advantage of the methods using PEG is that no expensive equipment is required.

Introduction

Tobacco rattle virus (TRV) is a multiparticle virus, consisting of long and short rod-shaped nucleoprotein particles. Both long and short particle types contain about 5% RNA (Harrison and Nixon, 1959). No antigenic differences have been detected between the different particle types of a particular strain of TRV (Harrison and Nixon, 1959; Lister and Bracker, 1969), though long and short particles may possibly differ in charge (Harrison and Roberts, 1968). However, they differ in genetic information (Sänger, 1968) and for genetic research separation and purification of the two particle types is of great importance.

In 1963 Hebert reported that plant viruses of different shape could be precipitated with various concentrations of polyethylene glycol 6,000 (PEG). Precipitation of the rod-shaped viruses occurred at relatively low (4%) PEG concentrations. The spherical ones were found to precipitate at higher concentrations (8%) of PEG. Leberman (1966) also found that a rod-shaped virus precipitated at a much lower concentration than a spherical one. These data indicate that for precipitation of small viruses higher PEG concentrations are required than for larger ones.

Another way of sorting virus particles according to length with PEG is that of the solubility-concentration gradients (Clark, 1970).

I have now separated the long and short particle types of TRV with PEG.

Materials and Methods

Virus. A strain of TRV, originally isolated from a soil at Lisse by Sol and Seinhorst (1961), was used. Its long particles are 185 nm and the short ones 67.5 nm long (Huttinga, 1972). TRV-Lisse was purified from infected *Nicotiana rustica* plants

according to the ether-tetra method (Huttinga, 1969). An analytical ultracentrifuge pattern of a purified virus preparation is presented in Fig. 1A.

Specific precipitation with PEG. To a TRV-Lisse suspension, containing 2 mg virus/ml, PEG and NaCl were added ranging from 3 to 5% and 0.1 to 0.5 M, respectively, to find a combination of PEG and NaCl at which only the long particles would precipitate. In each case the precipitate was collected by centrifuging for 15 min at 12,000 *g*. The supernatant was thoroughly removed and the pellet resuspended in 0.18 M phosphate-citric acid buffer pH 7 (PCA buffer) of a volume equal to the initial suspension. Subsequently, the concentration of PEG and NaCl was increased to 10% and at least 0.25 M, respectively, to precipitate all the virus left in the suspension. The resulting precipitate was also collected by centrifuging, and resuspended in PCA buffer. All fractions were tested in the analytical ultracentrifuge.

PEG solubility-concentration gradients. The virus was precipitated with 8% PEG and 0.08 M NaCl. The precipitate was spun for 30 min at 10,000 *g* on a 10–30% sucrose gradient in a Spinco SW 25.1 tube containing also a gradient of 1–8% PEG in 0.08 M NaCl opposite to the sucrose gradient. The virus bands were visualized by light scattering.

Results and discussion

With 3% PEG and 0.1 M NaCl the long particles precipitated and the short ones remained in the solution. When the PEG or the NaCl concentration was increased, the short particles also started to precipitate. Figs 1B and C show that in the analytical ultracentrifuge the preparations appeared homogeneous. The separation is very good and fully comparable to separations obtained with sucrose-gradient centrifuging in a zonal rotor (Huttinga, 1972). These results confirm those of Clark (1968), who precipitated alfalfa mosaic virus (AMV), packed it in a column and eluted this with a continuously decreasing concentration gradient of PEG. By this procedure a partial sorting of the virus into three groups according to length was achieved.

With the PEG solubility-concentration gradients the virus aggregates move through the gradient towards smaller PEG concentrations until they reach a concentration at which they dissolve again. At that site the virus is immobilized because the applied centrifugal force is too low to move single virus particles through the sucrose gradient. (Clark, 1970). With TRV-Lisse three bands were obtained, which proved to consist, from top to bottom, of extra short, short and long particles, respectively. Separation of long and short particles was comparable to that obtained by normal sucrose-gradient centrifuging, but the separation between short and extra short particles was improved as compared to that obtained in normal sucrose-gradient centrifuging. With the same method Clark and Lister (1971) were able to sort particles of AMV and TRV according to length.

The experiments described in this paper demonstrate that it is possible to separate virus particles according to length by specific precipitation or solubilization in PEG media. The results are comparable to, or better than those obtained by density-gradient centrifuging. However, the methods with PEG have the great advantage that they can be performed with relatively simple equipment.

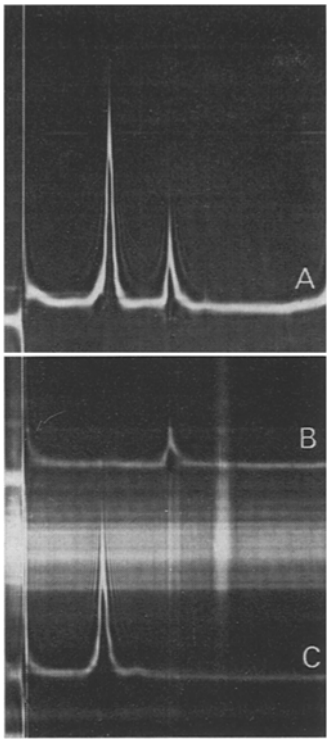

Fig. 1. Analytical ultracentrifuge patterns of: A. TRV-Lisse; B. long particles of TRV-Lisse precipitated at 3% PEG and 0.1 M NaCl from a normal TRV-suspension; C. short particles of TRV-Lisse precipitated at 10% PEG and 0.25 M NaCl from a solution out of which the long particles had been removed by precipitation at 3% PEG and 0.1 M NaCl. Sedimentation is from left to right. The pictures were made 10 min after the selected rotor speed, 21,740 rpm, was reached. The rotor temperature was 20°C.

Fig. 1. Analytische-ultracentrifugebeelden van: A. TRV-Lisse; B. lange deeltjes van TRV-Lisse, neergeslagen met 3% PEG en 0,1 M NaCl uit een normale TRV-suspensie; C. korte deeltjes van TRV-Lisse, neergeslagen met 10% PEG en 0,25 M NaCl uit een oplossing waaruit de lange deeltjes waren verwijderd door neerslaan met 3% PEG en 0,1 M NaCl. De sedimentatie verliep van links naar rechts. De foto's werden 10 min na het bereiken van de gekozen draaisnelheid, 21.740 rpm, opgenomen. De rotortemperatuur bedroeg 20°C.

Samenvatting

Scheiding van lange en korte deeltjes van het tabaksratelvirus met polyethyleenglycol

Twee methoden om lange en korte deeltjes van het tabaksratelvirus met behulp van polyethyleenglycol 6000 (PEG) te scheiden worden beschreven. De ene is gebaseerd op specifieke precipitatie, de andere op specifieke oplosbaarheid van deeltjes met verschillende lengten bij verschillende PEG-concentraties. De resultaten van de scheidingen zijn even goed als of beter dan die van scheidingen verkregen door centrifugeringen op een suikergradiënt. Het voordeel van de PEG-methoden is gelegen in het feit dat er geen kostbare apparatuur voor nodig is.

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Book review

J. A. de Bokx (Ed.): Viruses of potatoes and seed-potato production. 232 pp., cloth bound with dust jacket, 8 colour plates, 66 figs., subject index. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands, Price Dfl. 36.

In this book a number of experts have collected information on the growing of (seed) potatoes and their virus diseases.

Most of the data presented have been obtained from experience in the Netherlands. The book contains the following chapters: Introduction to plant virology; Graft and mechanical transmission; Aphids: their life cycles and their role as virus vectors; Soilborne viruses; Virus purification; Electron microscopy; Serology; Test plants; Histological, cytological and biochemical methods; Potato viruses: properties and symptoms; Virus translocation in potato plants and mature-plant resistance; Incidence of infection in commercial crops and consequent losses; Therapy; Control of aphid vectors in the Netherlands; Breeding for resistance; Dutch techniques of growing seed potatoes; Inspection and quality grading of seed potatoes. Descriptions of potato varieties mentioned in the chapters are given in a separate list before the subject index.

This handbook provides the reader with a wealth of information on potato virus diseases. One only wonders whether here and there the scope is too broad. The chapters on 'Introduction to plant virology', 'Electron microscopy' and 'Serology' would be more appropiate in a handbook on general plant virology than in one on potato virus diseases. A rather detailed description of the principle of the electron microscope and the different methods used in electron microscopy, for instance, seem out of place here (particularly as, in the chapter on purification, the principles of ultracentrifugation and density gradient centrifugation were not elaborated on). In these chapters and also in that on purification the number of references pertaining to potato viruses is very small so that it will not be easy for the reader to obtain additional information on the subject he is particularly interested in.

Very good contributions which exactly fit in the scope of this book are those on 'Aphids: their life cycles and their role as virus vectors', on 'Therapy', on 'Dutch techniques of growing seed potatoes' and on 'Inspection and quality grading of seed potatoes'.

As this is a publication from the Netherlands and also meant for the staff of inspection and plant protection services in this country, a list of Dutch synonyms of potato viruses would have been a welcome addition. In general the quality of the photographs is good. The text is carefully printed.

This book will certainly make its mark among those interested in both practical and fundamental aspects of potato virus diseases in relevance to potato production.

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