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Interaction between components of pea early-browning virus

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Abstract

The purification of pea early-browning virus and the separation of a long and a short component are described. Experiments with the two separate components revealed that inoculation with long particles only, gives rise to formation of unstable virus. Inoculation with a mixture of long and short particles leads to the formation of stable virus. Short particles alone are not infectious. Thus, the interaction between components of this virus is the same as has been described for tobacco rattle virus by Lister (1966).

Introduction

Pea early-browning virus (PEBV) is related to tobacco rattle virus (TRV). Like TRV, PEBV has rod-shaped particles with a bimodal length distribution (Bos and van der Want, 1962; Gibbs and Harrison, 1964). PEBV is also serologically related to TRV (Maat, 1963). For TRV it is known that there is an interaction between its components during infection (Frost et al., 1967; Lister, 1966, 1968; Sänger, 1968; Semancik and Kajiyama, 1968). Inoculation with long particles gives rise to RNA formation of the long particles only. Inoculation with a mixture of long and short particles leads to formation of the RNA of long and short particles and to formation of the coat protein, which results in complete TRV particles. In this investigation I have searched for a similar interaction between the two types of PEBV particles.

Materials and methods

Virus. An isolate of PEBV designated Dik Trom 5 was used, originally obtained from pea by Dr H. A. van Hoof. This isolate had particles 87.5 and 225 mμ long. The diameter of both particles was 25 mμ. The virus was propagated in *Nicotiana rustica*. For virus purification the infected leaves were picked 10–14 days after inoculation.

Virus purification. A modification of a method described by Maat (1963) was used. The material was ground in a Waring blendor in a mixture of half the amount (w/v) of 0.18 McIlvaine's phosphate-citric acid buffer solution pH 7 (for convenience henceforth called the buffer), to which were added 0.1 % thioglycollic acid, and a quarter of the amount (w/v) diethyl ether plus a quarter of the amount carbon tetrachloride. The homogenate was centrifuged for 20 min at 4,000 *g*. The upper phase was decanted and centrifuged for 1½ h at 105,000 *g*. The pellets were resuspended in a quarter of the

original sap volume of the buffer. After another low and high speed centrifugation (20 min at 4,000 *g* and 1½ h at 105,000 *g*) the virus was resuspended in 1/100 of the original sap volume of the buffer.

Separation of the virus components. The PEBV components were separated by using a MSE B XIV zonal rotor. The rotor was filled at 2,500 rpm (460 *g*) with a gradient of 10–40% (w/v) sucrose in the buffer. The gradient was linear against volume as it was pumped in. The filling of the rotor with the gradient was done through the feedline leading to the edge of the rotor, pumping in first the 10% sucrose solution. After this the rotor was loaded with about 30 mg virus in 10 ml of the buffer containing 2% sucrose (w/v). (Virus concentrations were determined with a Zeiss spectrophotometer; $E_{260} = 2$ corresponding to 1 mg/ml). The virus was introduced via the feedline to the centre of the rotor. An overlayer of 100 ml of the buffer was then introduced to the centre of the rotor, just slightly to displace the virus from the centre. The greater centrifugal force thus made the virus start to move faster. When the rotor was finally filled with gradient, virus, and overlayer, it was accelerated to 30,000 rpm (67,000 *g*) and spun for 1½ h. It was then decelerated to 2,500 rpm and unloaded by pumping a solution of 45% sucrose (w/v) in the buffer through the feedline to the edge of the rotor. The rotor contents were led through an UV-absorption meter before being collected in fractions of 15 ml and recorded as illustrated in Fig. 1. Fractions containing predominantly long and short particles were pooled, diluted 1:1 with the buffer, and concentrated by ultracentrifugation (105,000 *g* for 3 h). Then, both components were subjected to another density-gradient centrifugation in the MSE B XIV zonal rotor. This time, also, the fractions of long and short particles were pooled, diluted 1:1 with the buffer and centrifuged for 3 h at 105,000 *g*. This was done to concentrate the preparations and to remove most of the sugar. The sugar was removed to reduce degradation of the virus during storage at 4 °C.

Just before inoculation small quantities of the preparations were subjected to a final density-gradient centrifugation in a Spinco SW 25.1 rotor, applying a linear gradient of 10–40% sucrose (w/v) in the buffer. I centrifuged for 1½ h at 83,000 *g*.

Inoculation experiments. The preparations were inoculated at a concentration of 0.01 mg virus/ml to *N. rustica*, which became systemically infected and to *Phaseolus vulgaris*

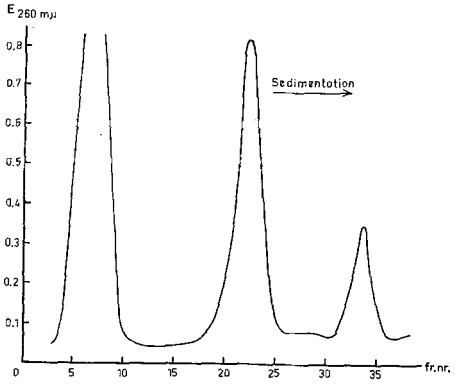

Fig. 1. UV-absorption of the fractionated rotor contents (from left to right: material of low molecular weight, short particles and long particles).

Fig. 1. UV-absorptie van de gefractioneerde rotorinhoud (van links naar rechts: laag-moleculair materiaal, korte deeltjes en lange deeltjes).

'Bataaf', which reacted with local lesions only. If a mixture of long and short particles was inoculated, the concentration of both components was 0.01 mg/ml. In that case the number of short particles was about twice that of the long particles, since the former are about half as long as the latter.

After about 10 days discs (diam. 1 cm) were punched from those parts of the leaves which showed symptoms. The discs were divided into two equal portions. One was ground in the buffer, the other in the buffer containing 25 mg bentonite per ml. Both inocula were tested for infectivity on primary 'Bataaf' leaves.

Results

The method of virus purification described yielded lower quantities of PEBV than I experienced with TRV. With TRV the yields were 150–200 mg virus/kg leaf material and with PEBV 50–80 mg virus/kg leaf material in summer (detailed results will be published later). In winter the yields were lower and the long particles in particular were present in very small quantities.

With the zonal rotor a good separation of the components was obtained at quantities up to 55 mg virus per run (Fig. 1) using the procedure previously described. If more virus was used per run the bands tended to overlap, which resulted in a relatively smaller recovery of separate components. It might be possible to separate more virus per run if other gradients or gradient materials were used. The recovery from our separation procedure was low. In each run we found a lot of material of low molecular weight at the meniscus. In the first runs this could be explained by assuming that the virus was not yet sufficiently pure. In the second series of zonal rotor runs the peak of low molecular weight material was assumed to be formed by particles broken during ultracentrifugation. To remove such broken particles the final density-gradient centrifugation in the Spinco SW 25.1 rotor was introduced. Thus inoculations were always made with preparations containing intact particles only. Inocula consisting of long particles or of a mixture of long and short particles were found to induce symptoms on bean and *N. rustica*. Inoculations with short particles exclusively did not lead to infection. There was a great difference in the infectivity of virus from plants inoculated with long particles only and those inoculated with a mixture of long and short particles. In the former case the virus was unstable, that is to say, it could not be transmitted

Table 1. Results of the inoculation experiments with components of pea early-browning virus.

Inoculum source:	Transmission in		Kind of virus in
N. rustica plants inoculated with	buffer	buffer + bentonite	inoculum source
long particles	−	+	unstable
long and short particles	+	+	stable
short particles	−	−	no virus

− = unsuccessful transmission
\+ = successful transmission

Table 1. Resultaten van de inoculatieproeven met componenten van het vroege-verbruiningsvirus van erwt.

from *N. rustica* to bean in buffer extracts. However, with extracts from infected *N. rustica* leaves made with the buffer containing 25 mg bentonite per ml, the beans could easily be infected. Inocula consisting of a mixture of long and short particles gave rise to infected leaves that contained stable virus: it could readily be transmitted from *N. rustica* to bean in buffer extracts. The results of the inoculation experiments are shown in Table 1.

Discussion

It may be concluded from this work that PEBV is quite unstable compared with TRV. The long particles in particular are easily lost in purification and separation procedures.

Our finding that short particles of PEBV are not infectious confirms the results of Harrison (1966). In 1964 Gibbs and Harrison stated that PEBV isolates could be distinguished in two types, irrespective of their source. With buffer extracts one type was readily transmitted, the other only with difficulty, although extracts of the latter type made with water-saturated phenol gave very infective preparations. The preparations I produced can be differentiated into two types, in a manner similar to that described by Gibbs and Harrison (1964). When plants were inoculated with long particles only, unstable virus, probably a free RNA form, was obtained. Such free RNA was described by Sänger and Brandenburg (1961) and Cadman (1962) for TRV. After inoculation with a mixture of long and short particles I obtained stable virus. Thus, the present results show that there is a similarity between PEBV and TRV with regard to the interaction between components during infection. In analogy with Lister's hypothesis, concerning TRV (Lister, 1966), the following interaction for PEBV components is proposed. Long particles of PEBV give rise to RNA formation of the long particles only. Inoculation of a mixture of long and short particles leads to the formation of the RNA of both types of particles and the formation of the coat protein, which results in the formation of complete PEBV. As with TRV, the most obvious explanation of this phenomenon is that the genetic information in the RNA of the long particle lacks the genetic code for coat protein production. The latter code is most probably located in the RNA of the short particle which, however, lacks the genetic code for its own multiplication. Thus both types of particles would be functionally defective and mutually complementing.

Further work on this and related subjects is in progress.

Samenvatting

Wisselwerking tussen twee componenten van het vroege-verbruiningsvirus van erwt

Er worden methoden beschreven om het vroege-verbruiningsvirus van erwt te zuiveren en te scheiden in een lange en een korte component. De resultaten van inoculatieproeven (Tabel 1) wijzen uit dat tussen de twee componenten van het vroege-verbruiningsvirus van erwt eenzelfde wisselwerking bestaat als door Lister (1966) werd gevonden voor de twee componenten van het ratelvirus van tabak. Inoculatie met alleen lange deeltjes van vroege-verbruiningsvirus van erwt leidt tot de vorming van onstabiel virus, waarschijnlijk vrij RNA van het lange deeltje. Inoculatie met een mengsel van lange en korte deeltjes leidt tot de vorming van stabiel virus. Korte deeltjes alleen zijn niet infectieus.

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