Viroplasms of an Aphid-transmissible Isolate of Cauliflower Mosaic Virus Contain Helper Component Activity

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SUMMARY

Aphid transmission experiments with the transmissible isolate Cabbage B and the non-transmissible isolate Campbell of cauliflower mosaic virus (CaMV), using different combinations of purified viruses, viroplasms and cellular fractions, revealed that helper component (HC) activity for CaMV transmissibility was associated only with the viroplasm preparations of the transmissible isolate. In electrophoretic analyses of purified virions, viroplasms and cellular fractions from plants infected with both CaMV isolates as has already been described, a P18 polypeptide in the purified viroplasm preparations from the transmissible CaMV isolate was the only difference between the two isolates. These results suggest that the purified viroplasms of transmissible CaMV isolates contain the HC in a functional form, and that the P18 polypeptide is the active HC for CaMV aphid transmissibility or a part of it.

INTRODUCTION

Cauliflower mosaic virus (CaMV) has isometric particles about 50 nm in diameter which contain double-stranded circular DNA (Covey, 1985). Caulimoviruses induce in infected hosts the formation of cytoplasmic inclusion bodies called viroplasms, which are the major site of virus accumulation in the cell and contain a proteinaceous matrix (Rubio-Huertos, 1950; Shepherd et al., 1980).

Most natural CaMV isolates are aphid-transmitted in a non-persistent manner, but some isolates are not (Lung & Pirone, 1973). Aphid non-transmissible isolates can readily be transmitted if aphids first probe a plant infected with transmissible CaMV isolates or other transmissible caulimoviruses (Lung & Pirone, 1973; Markham & Hull, 1985). This, in addition to the fact that aphids cannot transmit either transmissible or non-transmissible CaMV isolates from purified preparations, although these are highly infective when assayed by mechanical inoculation, suggest that an aphid acquisition factor or helper component (HC) is required for the transmission of caulimoviruses, as has been shown for other viruses (Pirone & Thornbury, 1984).

Studies with aphid non-transmissible CaMV isolates constructed in vitro have shown that aphid transmissibility is related to the expression of the open reading frame (ORF) II of the CaMV genome (Woolston et al., 1983; Armour et al., 1983; Givord et al., 1984) and to the presence of a P18 polypeptide encoded by this gene (Woolston et al., 1983; Givord et al., 1984). It is noteworthy that there are some naturally occurring aphid non-transmissible isolates, lacking P18, which nevertheless show no deletion in ORF II (Markham & Hull, 1985). The ORF II product copurifies with viroplasms induced by aphid-transmissible CaMV isolates and seems to be involved not only in the aphid transmission process, but also in determining some of the viroplasm properties (Givord et al., 1984).

There is no direct evidence, however, that the ORF II gene product is the functional HC for CaMV aphid transmissibility. So far, neither P18 nor viroplasms have been directly assayed for
HC activity by determining their ability to promote the aphid transmission of purified viruses as has been done for potyvirus HC (Pirone & Thornbury, 1984).

The experiments described in this paper demonstrate that the HC activity copurifies with the viroplasms of an aphid-transmissible CaMV isolate.

**METHODS**

*Virus isolates and plants.* The aphid-transmissible Cabbage B and aphid non-transmissible Campbell isolates of CaMV were supplied by T. P. Pirone, University of Kentucky, Lexington, Ky., U.S.A., and were propagated in mustard plants (*Brassica perviridis* L, cv. Tendergreen), kept in a growth chamber with a 16 h photoperiod at a temperature of 20 ± 5 °C. Infected leaves were collected 4 to 5 weeks after inoculation for the purification of virus or viroplasms. In some cases tissue was kept frozen until use.

Mustard seedlings in the cotyledon stage were used as test plants in aphid transmission tests, and for infectivity assay by mechanical inoculation.

*Virus purification.* Virus particles were extracted and purified by differential and sucrose density gradient centrifugation as described by Hull et al. (1976). Virus concentration was estimated spectrophotometrically using \( E_{450}^{\text{mg/ml}} = 4.6 \). The preparations were kept at 4 °C for up to several months.

*Fractionation of infected tissue.* Preparations of viroplasms were made by the method of Al Ani et al. (1980). The final pellet, consisting of viroplasms and starch grains, was resuspended in 1 ml of MES buffer (5 mM-MES, 1 mM-CaCl₂, 5 mM-2-mercaptoethanol, pH 6.5) per 25 g of tissue, and either stored frozen or used on the following day for aphid transmission tests. The supernatant fraction that remained over the sucrose cushion after the first centrifugation of the leaf homogenate, called the viroplasm supernatant (VS) fraction, was pelleted by centrifugation for 2.5 h at 40000 r.p.m. in a Beckman Ti50 rotor, resuspended in 1 ml of MES buffer per 25 g of tissue and kept at -20 °C.

The purity of viroplasm preparations was ascertained by light and electron microscopy as described by Shockey et al. (1980). The preparations contained mainly intact vacuolar viroplasms and starch grains with little cellular debris.

*Infectivity tests.* The infectivity of all virus and viroplasm preparations used for aphid transmission was tested by mechanical inoculation in *B. perviridis*, and it was always positive. Viroplasm preparations of the transmissible CaMV isolate stored at -20 °C retained their aphid transmission activity for up to 2 months.

*Aphid transmission tests.* Aphids (*Myzus persicae* Sulz.) were allowed to probe through Parafilm M membranes into solutions which contained either (i) purified viroplasms from either CaMV isolate, (ii) mixtures of purified viroplasms from both CaMV isolates, (iii) mixtures of purified viroplasms plus purified viruses or (iv) mixtures of purified viroplasms plus VS fractions. To standardize the amounts of viroplasms and virus in each test, the mixtures were always made with viroplasms at the equivalent of 25 g tissue/ml and purified viruses at a concentration of 2 mg/ml. All solutions contained 20% sucrose. After 5 to 10 min of access, aphids were placed on seedlings (10 to 15 per plant) and allowed to feed overnight. After 4 weeks, plants that showed symptoms of CaMV infection were assayed by mechanical inoculation to *Datura stramonium*, in which the Campbell isolate induces local lesions, but the Cabbage B isolate is symptomless (Lung & Pirone, 1972).

*Electrophoretic analysis.* Samples from virus or viroplasm preparations and from VS fractions were denatured by heating for 2 min at 100 °C in 0.0625 M-Tris-HCl pH 6.8, 2% SDS, 2% 2-mercaptoethanol and 0.05% bromophenol blue, and subjected to electrophoresis in 12.5% polyacrylamide–SDS slab gels with a 4.5% stacking gel. The buffer system used was that of Laemmli (1970). The polypeptides were stained with Coomassie Brilliant Blue G250.

**RESULTS**

*Aphid transmission of virus from viroplasm preparations*

When aphids were allowed to probe through Parafilm membranes into each viroplasm preparation from each CaMV isolate, they transmitted CaMV from the viroplasm preparations only of the Cabbage B isolate (Table 1).

To test whether viroplasm preparations from the transmissible isolate could act as a helper source for the non-transmissible CaMV isolate, as happens with infected tissue (Lung & Pirone, 1973, 1974), aphids were allowed to feed from a solution containing a mixture of viroplasms from both isolates. This was so, because the aphids transmitted both CaMV isolates (Table 1). Table 1 also shows that aphids could not transmit the Campbell isolate from a mixture of the VS fraction obtained from tissue infected with the transmissible isolate and purified viroplasm preparations of the non-transmissible isolate. This suggests that the helper component for aphid transmission was associated only with the viroplasms of the transmissible isolate and not with smaller cellular components.
CaMV viroplasms contain helper activity

Fig. 1. SDS-PAGE analysis of proteins of Cabbage B viroplasm preparations and of VS fractions. Lane 1, mol. wt. standards (mol. wt. are indicated on the left); lane 2, VS fraction; lane 3, viroplasms.

Table 1. Aphid transmission of CaMV from viroplasms or mixtures containing viroplasms

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Aphid transmission*</th>
<th>No. plants infected with Campbell†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1. Viroplasms (Cabbage B)</td>
<td>13/38</td>
<td>–</td>
</tr>
<tr>
<td>Viroplasms (Campbell)</td>
<td>0/38</td>
<td>–</td>
</tr>
<tr>
<td>Expt. 2. Viroplasms (Campbell) + Viroplasms (Cabbage B)</td>
<td>7/48</td>
<td>4</td>
</tr>
<tr>
<td>Viroplasms (Cabbage B) + VS fraction (Cabbage B)</td>
<td>3/30</td>
<td>–</td>
</tr>
<tr>
<td>Viroplasms (Campbell) + VS fraction (Cabbage B)</td>
<td>0/30</td>
<td>–</td>
</tr>
<tr>
<td>Expt. 3. Viroplasms (Campbell) + CaMV (Cabbage B)</td>
<td>0/38</td>
<td>–</td>
</tr>
<tr>
<td>Viroplasms (Cabbage B) + CaMV (Campbell)</td>
<td>7/38</td>
<td>6</td>
</tr>
</tbody>
</table>

* Total number of plants infected in three (Expt. 2) or two (Expt. 1 and 3) experiments: number of plants showing symptoms/number of test plants; 10 to 15 aphids were placed on each test plant.
† Determined by local lesion assay on D. stramonium.

In further tests aphids were allowed to probe into mixtures containing combinations of purified virus particles and viroplasms. In these experiments (Table 1), aphids transmitted the purified Campbell isolate in the presence of Cabbage B viroplasms but they did not transmit the Cabbage B isolate in the presence of Campbell viroplasms. These results confirm that the HC activity was present only in the Cabbage B viroplasm preparations.

Electrophoretic analysis

When the proteins of purified virions and viroplasms of the two CaMV isolates were analysed by SDS-PAGE no differences were found between the virion coat proteins of the two CaMV isolates (data not shown) and viroplasm proteins gave similar patterns except that an 18 000 mol. wt. polypeptide (P18), as described by Modjtahedi et al. (1985), was associated only with viroplasm preparations extracted from plants infected with the transmissible Cabbage B isolate (Fig. 1). P18 was not detected in the VS fraction obtained from plants infected with this CaMV isolate (Fig. 1).
DISCUSSION

Caulimoviruses require a helper component for transmission by aphids, and the polypeptide product (P18) of CaMV ORF II is involved in this process (Pirone & Thornbury, 1984). The assignment of this role to the CaMV ORF II gene product came from deletion experiments in this region of the CaMV genome and the concomitant loss of P18 and aphid transmissibility (Woolston et al., 1983; Armour et al., 1983; Givord et al., 1984).

The functional form and the mode of action of the caulimovirus HC are unknown. We have shown in this paper that the HC activity copurifies with viroplasms extracted only from plants infected with the transmissible Cabbage B isolate and that a P18 polypeptide is associated with these preparations. No other differences were found between the proteins of viroplasms from the Cabbage B and Campbell isolates nor between the VS fractions. We have prepared a polyclonal antiserum against P18. Preliminary results using this serum labelled with gold clearly localize the P18 within the viroplasm of the transmissible Cabbage B isolate by electron microscopy (D. Rodriguez, D. López-Abella & J. R. Díaz-Ruíz, unpublished results). Similar conclusions have been drawn by Givord et al., 1984), who have reported that the P18 protein is not only involved in aphid transmissibility, but also in maintaining the compact structure of the viroplasms.

All these lines of evidence strongly suggest that the viroplasms of the CaMV transmissible isolates contain the HC activity in a functional form. Whether P18 alone is the functional moiety or part of a multipolypeptide complex, remains to be resolved. We are attempting to use the P18-specific antisera to isolate native P18 to test these possibilities.

Since the transmission-defective Campbell isolate used does not have deletions in the ORF II region of the genome (Woolston et al., 1983), the reason for the absence of P18 is unknown. Regulatory sequences for the expression of the P18 gene, located in other positions of the genome and affecting either the transcription or the processing of the mRNA which encodes the P18 polypeptide, may be necessary (Modjtahedi et al., 1985).

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