Role of Virion and Helper Component in Regulating Aphid Transmission of Tobacco Etch Virus

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ABSTRACT

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Three isolates of tobacco etch virus (TEV) were either highly, poorly, or nontransmissible by aphids (HAT, PAT, or NAT, respectively) from infected plants. Purified virus of these isolates acquired through membranes was transmitted with high, intermediate, and low efficiency, respectively, in the presence of helper component (HC). Thus, intrinsic differences in properties of the virions, most likely differences in the coat proteins, appear to be responsible for differences in transmissibility. Helper component could be extracted from plants infected with each of the three isolates, and HC activity was highest from PAT-infected plants, suggesting that absence or deficiency of HC was not responsibile for differences in transmissibility. However, since aphids given access first to HC and then to infected plants were able to transmit the NAT isolate, and transmitted the PAT isolate with increased frequency, a role for HC in regulating the efficiency of aphid transmission from plants cannot be ruled out.

There have been a number of reports of isolates or strains of potyviruses that either are not transmitted, or are transmitted poorly, from plants by aphids (2,13,16,18,19). When "typical" potyviruses, which are readily aphid-transmitted from plants, are purified, they cannot be transmitted unless a helper component (HC), which can be extracted from potyvirus-infected plants, but not from healthy plants, is present (6,14,17). One hypothesis to explain the poor- or nontransmissibility of certain potyviruses might thus be that a deficiency or lack of HC production in infected plants is responsible.

Another possible explanation is that differences in transmissibility are due to intrinsic differences in the properties of the virions. In the case of cucumber mosaic virus, which (like the potyviruses) is transmitted nonpersistently by aphids, as well as with pea enation mosaic virus and barley yellow dwarf virus, which have a circulative relationship with their aphid vector, the coat protein of the virion has been shown to regulate transmissibility (3,10,15).

The purpose of these experiments was to assess the roles of HC and the virus particle in determining the relative transmissibility of three isolates of tobacco etch virus.

MATERIALS AND METHODS

Virus isolates. Three isolates of tobacco etch virus (TEV) were chosen for study. The highly aphid-transmissible (HAT) isolate was obtained from field-infected tobacco in 1979 and had been maintained by single-aphid transfer. The poorly transmissible (PAT) isolate, which was described previously (4), had been maintained by periodic mechanical inoculation over a number of years. The nontransmissible (NAT) isolate was that described by Simons (18). The potato virus Y (PVY) isolate was previously described (7).

The HAT and NAT isolates produced symptoms on tobacco typical of those described by Bawden and Kassanis (1) for "severe etch virus," while the PAT isolate produced symptoms described for "mild etch virus" (1). All isolates produced wilt of Tabasco pepper and reacted, in SDS double-diffusion tests in agar, with an antiserum to the PAT isolate. The isolates all produced local lesions on *Chenopodium amaranticolor* (Coste & Reyn.). Lesions produced by the PAT isolate were about 1.0–1.5 mm in diameter and appeared ~5–6 days after inoculation, while those of the HAT and NAT isolates were smaller (<1 mm in diameter) and appeared in about 7–8 days. Concentrations of the three isolates in tobacco were similar, measured either by amount of purified virus recovered or by local lesion assay of crude leaf extracts (when corrected for the differences in specific infectivity described below).

Purification of virus and helper component (HC). Virus and HC were extracted from systemically infected leaves of tobacco (*Nicotiana tabacum* L. 'Burley 21') 2–4 wk after mechanical inoculation. The three TEV isolates were purified as described previously (14). Potato virus Y-HC was prepared by methods described previously (4,6). Midribs were removed from infected leaves and 100 g of the remaining tissue was cut into 4–5 cm rectangular pieces. The tissue was vacuum-infiltrated in cold extraction buffer (0.1 M ammonium acetate; 0.02 M Na_2-EDTA; 0.02 M Na-DIECA, pH 8.8), and then homogenized in a blender with an equal (w/v) volume of buffer. After filtration through cheesecloth the extract was centrifuged for 15 min at 8,000 *g*, and then the supernatant was centrifuged for 1 hr at 105,000 *g*. Concentrated HC was prepared from the high-speed supernatant by precipitating twice with 6% polyethylene glycol (PEG) as described by Govier et al (7). The final PEG pellet was resuspended in 1–2 ml of HC buffer (0.1 M tris; 0.02 M $MgCl_2$, pH 7.2).

Preparation of active TEV-HC required an additional step involving treatment with DEAE cellulose. Precycled DEAE cellulose, equilibrated with 0.1 M ammonium acetate, 0.02 M Na_2-EDTA (pH 8.8) was filtered through Whatman 41 filter paper on a Büchner funnel. The moist cake was weighed and added to the HC-containing high-speed supernatant (prepared as described above) at the rate of 17 g per 100 g of starting leaf material. After being stirred for 30 min, the suspension was filtered through Whatman 41 paper. The filtrate was then concentrated by PEG precipitation and resuspended as described above.

In the experiments comparing the activity of HC from the three TEV isolates, equal amounts of starting leaf material were used and final resuspension was in equal amounts of buffer so that valid comparisons among the isolates could be made. For the experiments involving PVY-HC, and for those with TEV-PAT-HC, which did not involve comparison with HAT- or NAT-HC, the HC preparations were preassayed so that appropriate dilutions (ie, containing sufficient HC to promote transmission) could be chosen

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for the actual experiments (14). Frozen preparations of HC could be stored without loss of activity (7).

Transmission tests. *Myzus persicae* (Sulz.), reared and handled as described previously (14), were used in all tests. When virus acquisition was from infected plants, leaves similar to those used for virus and HC preparation were used. Aphids were allowed to probe the leaves for 30–90 sec and then were placed on healthy tobacco seedlings overnight (14–18 hr). Membrane acquisition tests were done as described by Govier et al (7). After inoculation access, test plants were sprayed with an insecticide and placed in a growth room or greenhouse for symptom development. With a single virus, symptom development was used as an indication of transmission. In the competition experiments, which involved acquisition of both TEV and PVY, specific antisera were used for identification of the virus(es) that was transmitted (5).

Electrophoresis. Coat proteins of the TEV isolates were analyzed by electrophoresis in 10% polyacrylamide gels containing sodium dodecyl sulfate (SDS) in the Laemmli buffer system (11). Virus (1 mg/ml) was mixed with an equal volume of 2× stacking gel buffer, 2% SDS, 2% mercaptoethanol, and 40% sucrose and heated to 100 C for 5 min. Gels were stained with 0.1% Coomassie brilliant blue R-250 in 50% methanol, 10% acetic acid, and destained in 10% methanol, 7.5% acetic acid. Intact virus was analyzed by cellulose acetate electrophoresis according to the procedures described by Morales (12), which are based on the methods used for SDS-dissociated proteins by Hiebert and McDonald (9). Purified virus (1 mg/ml) was applied with a serum applicator to cellulose acetate strips equilibrated with either 75 mM tris-H_2SO_4 (pH 9.0), 20 mM sodium phosphate (pH 7.0) or 24 mM potassium acetate (pH 4.0) and electrophoresed for 1 hr at 300, 300, and 160 V, respectively. Virus zones were stained with Coomassie brilliant blue R-250 and migration distances from the origins were measured.

RESULTS

Aphid transmission from plants. In seven experiments (10 plants per treatment in each experiment) transmission of the HAT isolate averaged 91% (range 80–100%), while that of the PAT isolate was 22% (0–50%), when 10 aphids per plant were used. Using one aphid per plant, transmission of the HAT isolate averaged 54% (10–80%) and that of the PAT isolate 3% (0–10%). No transmission of the NAT isolate occurred regardless of the number of aphids tested.

Effect of prior acquisition of PVY-HC. To test the hypothesis that either poor- or nontransmissibility of the PAT and NAT isolates was due to a lack of HC production in infected plants, aphids were given a 10-min acquisition access to HC prepared from PVY-infected plants and were then allowed to acquire virus from TEV-infected plants. Prior access to PVY:HC had no effect on transmission of the HAT isolate, while transmission of PAT increased and NAT became transmissible (Table 1). Transmission remained highest for the HAT isolate.

Extraction of HC from TEV-infected plants. The results of the previous experiments suggested that a deficiency or lack of HC was at least partially responsible for transmission differences. Numerous unsuccessful attempts were made to obtain HC from TEV-infected plants, using methods described for PVY-HC extraction (7,14). The modification described in the methods section consistently produced HC of reasonably high activity (about 10–20% that of a typical PVY-HC preparation) from plants infected with the PAT isolate, while the activity of HC from plants infected with the HAT or NAT isolates was much lower. Using purified TEV (HAT isolate) in assays for HC activity, eight preparations of HC from PAT-infected plants effected 60–100% transmission, while HC from six preparations from HAT-infected plants effected 0–30% transmission and three preparations from NAT-infected plants effected 10–30% transmission. In all of the above experiments each preparation was tested using 10 plants per test, 10 aphids per plant.

Aphid transmission of the HAT and PAT isolates of TEV, in the presence of HC prepared from plants infected with these isolates, was compared in a series of reciprocal experiments. Each TEV isolate was at a concentration of 40 μg/ml and the HC preparations, prepared as described in Materials and Methods, were used undiluted. The HAT isolate was transmitted at a higher frequency than the PAT isolate, regardless of the source of HC (Table 2). As suggested in the preliminary experiments, HC from plants infected with the PAT isolate effected better transmission of either the HAT or PAT isolate than did HC from plants infected with the HAT isolate.

Aphid transmissibility of purified TEV isolates. The previous experiments suggested that the virus isolates differed in intrinsic transmissibility. Since the activity of HC from plants infected with the HAT or NAT isolates was too low to allow meaningful comparisons, HC from PAT-infected or PVY-infected plants was used in tests with the three TEV isolates, at a series of virus concentrations. Transmission of the HAT isolate was highest, that

TABLE 1. Effect of prior access to partially purified potato virus Y (PVY) helper component (HC) on the ability of aphids to transmit tobacco etch virus isolates from infected tobacco leaves

Exp. no.	Transmission (%)					
	HAT isolate		PAT isolate		NAT isolate	
	Direct[a]	HC first[b]	Direct	HC first	Direct	HC first
1	80[c]	80	20	70	0	50
2	80	80	0	40	0	50
3	100	90	30	50	0	30
Avg.	87	83	17	53	0	43

[a] Control aphids were given 10-min acquisition access to buffered sucrose prior to 30- to 90-sec acquisition probes on virus source.
[b] Aphids given 10-min acquisition access to PVY-HC, prior to 30- to 90-sec acquisition probes on virus source plant.
[c] Percent infected test plants; 10 plants per treatment in each experiment; 10 aphids per plant.

TABLE 2. Aphid transmission of the highly aphid transmissible (HAT) and poorly aphid transmissible (PAT) isolates of tobacco etch virus mediated by helper component prepared from PAT- or HAT-infected plants[a]

HC Source	Exp. no.	Transmission (%) of:	
		HAT	PAT
HAT	1	10[b]	0
	2	0	0
	3	30	0
PAT	1	100	0
	2	100	30
	3	90	20

[a] Concentration of each virus was 40 μg/ml.
[b] Percent infected test plants; 10 plants per treatment per experiment, 10 aphids per plant.

TABLE 3. Aphid transmission of three purified isolates of tobacco etch virus (TEV) in the presence of helper component (HC)[a]

HC source	TEV isolate	Transmission (%) at a concentration (μg/ml)[b] of:		
		200	20	2
TEV-PAT	HAT	93	97	37
	PAT	45	37	17
	NAT	13	3	0
PVY	HAT	90	80	45
	PAT	70	24	20
	NAT	45	5	0

[a] No transmission of any isolate was obtained in the absence of HC.
[b] Averages of three to 10 experiments; 10 aphids per treatment per experiment, 10 aphids per plant. Differences among the levels of transmission of three isolates were highly significant ($P = >0.01$) at each concentration (analysis of covariance).

of NAT lowest, and that of PAT intermediate, at all concentrations tested, regardless of the HC source (Table 3).

Specific infectivity of TEV-isolates. To determine whether the preceding results could be explained on the basis of lower specific infectivity of the PAT and NAT isolates, infectivity was assayed by manual inoculation of *C. amaranticolor*. This was not the case and, in fact, the specific infectivity of the PAT isolate was about 10 times higher than that of the HAT or NAT isolates (Table 4). Similar results were obtained when quantitative assays were made by mechanical inoculation of tobacco and the data expressed in terms of the number of plants systemically infected at each virus concentration. When inoculated with virus at 2 μg/ml, 100% of the assay plants were infected with all three isolates; at 0.2 μg/ml, 50–60% infection was obtained with HAT and NAT, and 90–100% with PAT; at 0.02 μg/ml, 0–10% infection occurred with HAT and NAT, and 40–50% with PAT.

The infectivity of the RNA from the HAT and PAT isolates, prepared as described (8), was also compared. The results were similar to those with intact virus: in two experiments, RNA of the PAT isolate produced an average of 29 lesions per half-leaf of *C. amaranticolor* at a concentration of 7.5 μg/ml and six lesions at a concentration of 1.5 μg/ml. The RNA of the HAT isolate produced an average of three and less than one lesion per half leaf, respectively, at these concentrations.

Competition experiments. Previous studies have suggested a bias toward preferential transmission of the homologous virus, when transmission was from a mixture of two viruses and helper component homologous and heterologous to the respective viruses (14). To determine whether the intrinsic transmissibility of the heterologous virus would have an effect on this bias, aphid transmission of the HAT and PAT isolates, respectively, and of PVY, in the presence of PVY-HC was tested. From a mixture of HAT and PVY, preferential transmission of HAT occurred in three of four experiments, while PVY was preferentially transmitted from a mixture of PAT and PVY (Table 5).

Electrophoretic comparison of isolates. Comparison of the coat proteins of the three isolates by gel electrophoresis revealed no consistent difference that could be associated with transmissibility.

TABLE 4. Specific infectivity of tobacco etch virus isolates as measured by local lesion production on *Chenopodium amaranticolor*[a]

	Avg. no. of local lesions per half leaf at three virus concentrations (μg/ml)		
Isolate	20	2	0.2
HAT	75	6	<1
PAT	>200	78	8
NAT	83	5	<1

[a] Average of three experiments.

TABLE 5. Aphid transmission of purified potato virus Y (PVY) and the HAT and PAT isolates of tobacco etch virus, alone and from the indicated mixtures[a,b]

	Transmission (%)						
	Single virus			Mixture of viruses[c]			
Exp. no.	HAT	PAT	PVY	HAT + PVY		PAT + PVY	
1	100	10	70	80	0	0	80
2	90	50	90	100	20	20	60
3	50	20	50	40	0	30	30
4	90	20	60	40	75	5	75
Avg.	85	25	68	65	24	14	61

[a] Potato virus Y helper component was present, at a standard concentration, in all virus preparations.
[b] Concentration of each virus was 40 μg/ml in all cases.
[c] Transmission of each virus from the indicated combination is listed in the column under that virus.
[d] Percent infected test plants; 10 plants per treatment per experiment, 10 aphids per plant.

Most preparations of HAT and NAT produced a single band of M.W. ~33,000, while the PAT isolate produced a similar band as well as a faster moving band of M.W. ~32,000. However, occasional preparations of HAT and NAT, with transmission characteristics typical of these isolates, produced two bands similar to those of PAT. Furthermore, in recent studies with a fourth isolate, the aphid transmissibility of which, either from plants or purified preparations, is virtually identical to PAT, the coat protein produced a single band with a molecular weight virtually identical to that of HAT coat protein.

When samples of intact viruses were electrophoresed on cellulose acetate at pH 4, 7, and 9, resolution was poor, but there were no apparent differences in mobility. Other methods used to detect possible charge differences with intact virions included electrophoresis in agarose gels and isoelectric focusing in granulated Sephadex gel beds. In these systems, the virus either failed to migrate or was not detectable by standard methods.

Selection experiments. Poorly- or nontransmissible isolates of potyviruses have been reported to occur as a result of repeated transfer by mechanical inoculation (16–18). Although no data are available on the aphid transmissibility of the PAT isolate when first obtained, the fact that it had been periodically transferred by mechanical inoculation for over 15 yr suggests that it may have become poorly transmissible as a result of this. An attempt was made to select a revertant, highly transmissible, isolate by transmitting the PAT isolate, using individual aphids, to a series of 10 tobacco plants. Transmission was no greater from the 10th transfer than from the initial transfer.

DISCUSSION

The aphid transmissibilities of the three TEV isolates used in this study are intrinsically different. Differences in aphid transmissibility of two strains of cucumber mosaic virus, a nonpersistent virus, have been shown to be associated with differences in protein coats (3), as have differences in transmissibility of isolates of pea enation mosaic virus (10) and barley yellow dwarf virus (15), both of which have a circulative relationship with their aphid vector. Differences in the coat proteins of the TEV isolates would also seem to be the most likely reason for differences in transmissibility, although as yet we have not obtained direct evidence of this.

The role of the HC is less unequivocal. The fact that HC can be extracted from plants infected with the PAT and NAT isolates obviously demonstrates that HC is produced by infection with these isolates. However, prior access to PVY-HC increases aphid transmission of PAT, and makes NAT transmissible from infected plants. Since the activity of HC from PAT-infected plants was consistently higher than that of HAT-HC this would seem to rule out a deficiency of HC in PAT-infected plants as an explanation of poor transmissibility. However, no single procedure is suitable for extracting HC of all potyviruses (this report and *unpublished*); possibly the procedure used for preparation of TEV-HC was less effective for HAT- and NAT-HC. Possible explanations for the apparent ineffectiveness of HC in NAT- and PAT-infected plants include the possibility that aphids are unable, or less able, to acquire active HC due to its being bound or inhibited. However, such explanations are purely speculative at this point.

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