Protein Coats of Two Strains of Cucumber Mosaic Virus Affect Transmission by *Aphis gossypii*

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ABSTRACT

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Two strains of cucumber mosaic virus (CMV) were studied to determine reasons for differences in transmission efficiency by *Aphis gossypii*. Strain CMV-T, which is highly transmissible from tobacco to cucumber (90%) and CMV-6 a poorly transmitted one (10%) were used. Difference in transmission was independent of host plant and not correlated with concentration of virus in the leaf. There were no indications of a helper factor associated with CMV-T or of an inhibitor that decreased transmission of CMV-6. Purified CMV-6 at high concentrations was acquired through a parafilm membrane almost as efficiently as was purified CMV-T and subsequently was transmitted by aphids to test plants. However, reducing virus concentration decreased transmission of CMV-6 markedly, whereas that of CMV-T was decreased considerably less. Differences in RNA profiles were observed after electrophoresis in acrylamide gels. Exchanging CMV-6 coat protein with CMV-T coat in vitro, increased aphid transmission rates markedly whereas replacing CMV-T coat by CMV-6 protein decreased transmission. These results were supported by enzyme-linked immunosorbent assay of single aphids. Apparently, the coat protein determines transmissibility of CMV strains by aphids.

Differences in the transmissibility of strains of a virus by an aphid vector have been reported by several workers (3,8,9,14,17). As reviewed by Pirone and Harris (12), the differences can be correlated in some, but not all, cases with variations in virus concentration. Differences in location of virus particles in cells also have been suggested to explain such specificities. In a number of cases, especially within the potato virus Y group, lack of aphid transmission of certain strains has been shown to be due to the absence of a helper factor (6). Intrinsic properties of the virus particles also could be instrumental in success of aphid transmission; studies with mixed infections of strains of barley yellow dwarf virus suggest that the viral capsid is associated with transmissibility (14).

Strains of cucumber mosaic virus (CMV) are differentially transmitted by apids (9), without indications of a helper factor (11). In this study, transcapsidation was used to show that differences in transmissibility of two CMV strains are associated with their protein coat, and not with differences in concentration of virus in the plant or the presence of a helper or inhibitory factor.

MATERIALS AND METHODS

Two CMV strains (CMV-T and CMV-Sq, originally isolated from squash) that are highly transmissible by aphids, and a poorly transmissible one, CMV Price's No. 6 (CMV-6) (13), were used. The strains were propagated through single lesions on cowpea, *Vigna sinensis* Endl. 'Blackeye' or *Chenopodium amaranticolor* Coste & Reyn. prior to use. The strains were propagated in *Nicotiana tabacum* L. 'Xanthi-nc'. Transmission rates from tobacco to cucumber with two aphids per plant after a 1.5-min acquistion access feeding period were 18/20 and 17/20 for CMV-T and CMV-Sq, respectively, and 2/20 for CMV-6 (1). Symptoms caused by CMV-T on tobaccos differed markedly from those caused by CMV-6; the latter showing distinct green and yellow areas while CMV-T gave a green-greenish mosaic. On cowpeas, CMV-T and CMV-6 produced local lesions, while CMV-Sq gave a systemic reaction.

A clone of melon aphids (*Aphis gossypii* [Glover]) that originated from a single female were reared on cucumber plants in a growth chamber. Before transmission tests, apterous aphids were starved for 1-2 hr. For transmission from plant to plant, two aphids per test plant (*Cucumis sativus* L. 'Bet Alpha') were used after 1.5 min of acquisition-access feeding. Transmission tests with purified virus were done with virus suspended in 0.005 M borate buffer, pH 9.0, containing 0.005 M EDTA and 5% sucrose. Aphids were allowed to probe the virus source through a parafilm membrane (10) for 30 sec. Probing was observed through a binocular microscope. After probing, a single aphid was placed on a test plant (cucumber). Aphids were allowed to remain on test plants for 2 hr (inoculation period), after which plants were sprayed with an insecticide and returned to the greenhouse at 22–25 C for symptom development.

Virus purification. Virus was purified from Xanthi-nc plants, grown at 22–25 C, following the method of Scott (16). However, instead of the second final high-speed centrifugation the redissolved pellet (semipurified virus) was further purified by sucrose density gradient centrifugation (10–40% sucrose in 0.005 M borate buffer, pH 9.0; 70,000 *g* for 2.5 hr in a Spinco SW 27 rotor) to remove the G_2 fraction (host protein). The gradients were fractioned with an ISCO Model 640 gradient fractionator. The virus fractions were pelleted by ultracentrifugation for 2.5 hr at 78,000 *g*, after dilution (1:3) with 0.005 M borate buffer, pH 9. Then the pellets were dissolved in 0.005 M borate buffer, pH 9.0, containing 0.005 M EDTA. Virus infectivity was assayed on primary leaves of 8- to 11-day-old cowpea plants.

Dissociation and reassembly of virus. Purified virus was dissociated in 0.02 M Tris-HCl buffer pH 7.2, containing 2 M LiCl and 10^{-3} M Cleland's reagent (5). Viral RNA was precipitated and recovered by centrifugation for 10 min at 12,000 *g*; the supernatant contained the viral coat protein. Infectivity of the protein solution was tested on cowpea plants and found to be negative, indicating that no nondegraded virus remained. Purity of RNA and protein preparations was tested spectrophotometrically at 260 and 280 nm. Values of $A_{260\ nm}/A_{280\ nm}$ for RNA from CMV-6 and CMV-T were 0.8/0.36 = 2.22 and 1.0/0.48 = 2.08, respectively. Values of $A_{280\ nm}/A_{260\ nm}$ for protein from CMV-6 and CMV-T were 0.7/0.28 = 2.5 and 0.75/0.34 = 2.2, respectively; indicating a high degree of

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purity for both RNA and protein preparations.

For reassembly of virus from CMV-protein and CMV-RNA the dilution method (4) was used. RNA and protein were mixed stoichiometrically (four protein for one RNA, w/w) in 0.02 M Tris-HCl buffer pH 7.2, containing 0.5 M LiCl and 10^{-3} M Cleland's reagent. The suspension was immediately diluted to low ionic strength (0.2 M LiCl in 0.02 M Tris-HCl and 10^{-3} M Cleland's reagent) in the cold. The virus then was pelleted by ultracentrifuging the mixture for 2.5 hr at 78,000 *g*. The pellet was resuspended in 0.005 M borate-EDTA buffer, pH 9.0, containing 5% sucrose.

Profiles of RNA components. RNA was obtained according to Lot et al (7) by phenol extraction of purified virus suspended in 0.1 M phosphate buffer pH 7.0, containing 0.15% sodium dodecyl sulphate and 0.02 mg/ml bentonite. Electrophoresis was done in 2.4% acrylamide gels as described by Wood and Coutts (18). Gels were scanned at 260 nm with a Gilford Model 2400 spectrophometer.

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed as described before (1).

RESULTS

Effect of host plant and vector species on transmission. Host plant did not affect transmission rates. Thus, CMV-6 was poorly transmissible (10–15%) from cucumbers (6/40), *N. glutinosa* (3/30), and *N. tabacum* 'White Burley' (3/20), but CMV-T was efficiently transmitted (60–90%) from cucumber (34/40), *N. glutinosa* (30/36), and *N. tabacum* 'White Burley' (17/20). With *Myzus persicae* (Sulz.) transmission rates from tobacco were 4/40 and 34/40 for CMV-6 and CMV-T, respectively. Young 9- to 10-day-old cucumber plants were used as test plants in all experiments.

Virus concentration in the infected plant. To investigate the possibility that low concentrations of virus in the leaf tissue are responsible for the poor transmission of CMV-6, aphids were allowed to probe infected apical leaves at different times after mechanical inoculation. At the completion of each aphid transmission test, virus concentration in the same apical leaves was assayed by mechanical inoculation. The concentration of CMV-6 was similar to, if not higher than, that of CMV-T, but CMV-T consistently was transmitted more efficiently than CMV-6 (Table 1).

Sequential feeding. To determine whether transmission of CMV-6 by aphids requires a helper that might be present in CMV-T- or CMV-Sq-infected plants, consecutive acquisition probe tests were done. Aphids were allowed to probe first on CMV-Sq- or CMV-T-infected cucumber seedlings and then on CMV-6-infected cucumber plants, before being placed on cucumber test seedlings. When test plants developed symptoms they were assayed on cowpea plants, where CMV-6 produces local lesions and CMV-Sq a systemic reaction; or on Xanthi-nc plants, which react with different mosaic symptoms when infected with CMV-6 or CMV-T. No marked increase in transmission of CMV-6 was obtained after probing on plants infected with a highly transmissible strain (Table 2).

To see whether the poor transmission of CMV-6 is due to an inhibitory substance present in the infected tissue, and assuming that this substance is also inhibitory to CMV-T, consecutive probing tests were done, where aphids were allowed to probe first into CMV-6 infected plants or extracts from such plants, and then into a purified CMV-T preparation. Preprobing did not affect transmission of CMV-T (Table 3). Addition of a leaf extract from CMV-6-infected leaves to a purified CMV-T solution also did not affect transmission rates, when compared with those obtained from a mixture containing purified CMV-T and extracts from CMV-T-infected plants (Table 3).

Transmission of purified virus. Normand and Pirone (9) reported that their MCJ CMV-strain, which rarely was transmitted from plant to plant, was transmitted efficiently from purified concentrated preparations to a degree similar to that of a highly transmissible strain; therefore, we were interested to see whether our strains behaved similarly, and if transmission of the two strains remained the same at various dilutions of purified preparations.

The two strains, CMV-T and CMV-6, were purified and adjusted to an equal concentration on the basis on infectivity and absorbance at 260 nm before being fed to the aphids through parafilm membranes. Results, averages from two experiments (Table 4), show that purified CMV-6 at high concentrations (undiluted ~40 $A_{260\,nm}$ units) is transmitted at a high rate (55%). Decreasing virus concentration reduced transmission of CMV-6 markedly–with no transmissions at a dilution of 1:10, but those of CMV-T were reduced considerably less, with transmission rates of

TABLE 1. Virus concentration and transmission rates from CMV-6- and CMV-T-infected cucumber plants by *Aphis gossypii*, at different times after inoculation

Days after inoculation	Virus concentration[a]		Transmission[b]	
	CMV-6	CMV-T	CMV-6	CMV-T
4	33 ± 11	16 ± 6	4/40	32/40
5	203 ± 77	135 ± 79	6/40	38/40
6	163 ± 78	129 ± 58	8/40	36/40
7	95 ± 32	85 ± 38	6/40	34/40
8	68 ± 13	84 ± 19	6/40	36/40

[a] Number and standard deviation of numbers of local lesions per cowpea leaf (average from 12–18 leaves) that had been inoculated with inoculum prepared from four 5-mm disks from infected plants homogenized in 3 ml 0.05 M phosphate buffer, pH 7.5.

[b] Denominator, number of plants used; numerator, number of plants infected, with two aphids per plant. Acquisition access feeding time was 2 min, and inoculation access feeding time was 2 hr. Transmission rates were analyzed statistically by the χ^2 test. Transmissions of CMV-6 were significantly lower ($P = 0.01$) from those of CMV-T at every day after inoculation.

TABLE 2. Rates of transmission cucumber mosaic virus strain CMV-6 by *Aphis gossypii* after probing on healthy and virus infected cucumber plants

Probing sequence	Transmissions[a]	
	(no./total)	(%)
CMV-6 only	12/120	10
Healthy, then CMV-6	0/60	0
CMV-Sq, then CMV-6	18/120	15
CMV-T, then CMV-6	5/60	8.3

[a] Denominator = number of plants used; numerator = number of plants infected. Two aphids were used per cucumber test plant. Acquisition access feeding time on intermediate plant and on a CMV-6 infected cucumber plant for 2 and 1.5 min, respectively. Inoculation access feeding time was 2 hr.

TABLE 3. Transmission rates of purified CMV-T by *Aphis gossypii*: 1) after probing on healthy or CMV-6 infected cucumbers or extract; and 2) from mixtures[a] containing plant extract

Probing sequence	Transmissions[b]	
	(no./total)	(%)
1. purified CMV-T only	40/50	80
healthy, then CMV-T	18/20	90
CMV-6-infected plant, then CMV-T	21/30	70
healthy extract [c], then CMV-T	24/30	80
CMV-6 extract, then CMV-T	35/40	88
2. healthy extract + CMV-T	16/20	80
CMV-6 extract + CMV-T	24/40	60
CMV-T extract + CMV-T	21/40	53

[a] Mixtures at 1:1 (virus solution to plant extract).

[b] Denominator, number of plants used; numerator, number of plants infected, with one aphid per plant. Acquisition access feeding time on purified virus or mixtures was 30 sec, preprobing on plants was 2 min, and inoculation access feeding time was 2 hr.

[c] Extracts were prepared by homogenizing 0.5 g of leaves in 3 ml of 0.005 M borate EDTA buffer, pH 9.0, containing 5% sucrose.

50% even at a dilution of 1:20.

RNA profiles and aphid transmission of reassembled virus. Results of the foregoing experiments gave no indications that differential transmission of the two strains is caused by a helper or inhibitory factory, or to differences in virus concentration. Therefore the possibility that intrinsic properties of the virus determine its transmissibility was investigated. Representative RNA profiles (from five separate experiments), as determined by gel-electrophoresis, showing significant differences between CMV-T and CMV-6, are given in Fig. 1. When aphids were allowed to probe on reassembled virus in sucrose solution, where CMV-6 coat protein was exchanged by CMV-T coat, transmission rates increased markedly and were comparable to those obtained with CMV-T. Conversely, exchanging CMV-T coat protein by CMV-6 coat decreased aphid transmission rates, similar to those obtained by CMV-6 (Table 5). These results were supported by ELISA of single aphids allowed to probe on suspensions of reassembled virus (Table 5). Thus, $E_{405\ nm}$ values of 0.07–0.08 were obtained when CMV-T protein was the coat, compared with 0.03–0.04 when CMV-6 protein was the coat. In all tests symptoms that developed on *N. tabacum* 'Xanthi-nc' after mechanical inoculation with reassembled virus were those characteristic of the RNA component.

DISCUSSION

The difference in transmissibility by aphids between two CMV strains is independent of host plant and not correlated with the concentration of virus in the leaf tissue; this is similar to the results of Normand and Pirone (9). No indications of a helper factor associated with the transmissible strain or an inhibitor present with the poorly transmissible one could be obtained. Transmissibility is an intrinsic property of the virus, ie, of its protein coat. By

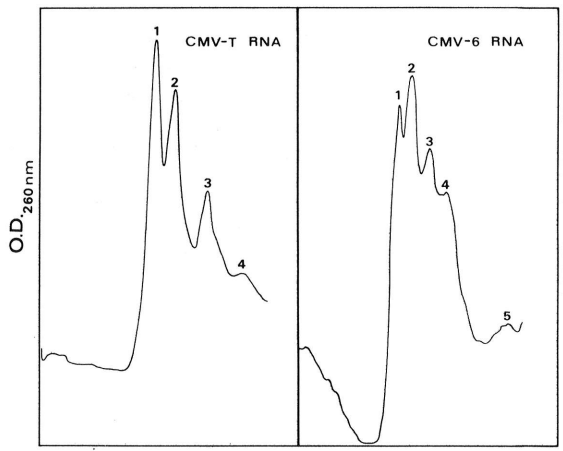

Fig. 1. Polyacrylamide electrophoresis diagrams showing the RNA component composition of cucumber mosaic virus strain CMV-T (highly transmissible by *Aphis gossypii*) and strains CMV-6 (poorly transmissible by *A. gossypii*). Electrophoresis was conducted at 5 mA/gel for 75 min. Migration was from left to right.

TABLE 4. Transmission of purified cucumber mosaic virus strains CMV-T and CMV-6 at different concentrations by *Aphis gossypii*

CMV strain	Dilution ratio	Infectivity[y]	A_{260}	Transmissions[z] (no./total)
T	undiluted	94.5 ± 10.8	40	17/20 a
	1:5			15/20 ab
	1:10			11/20 ab
	1:20			10/20 b
6	undiluted	100 ± 21.6	40	11/20 ab
	1:5			2/20 c
	1:10			0/20 c
	1:20			0/20 c

[y] Number of local lesions per cowpea leaf, average from 11 leaves, and standard deviation, after 1:100 dilution of purified virus.
[z] Denominator, number of plants used; numerator, number of plants infected, with one aphid per plant. Acquisition access feeding time was 30 sec, inoculation access feeding time was 2 hr. Ratios followed by the same letter are not significantly different ($P = 0.01$).

TABLE 5. Transmission rates and ELISA absorbance values (E_{405}) of single *A. gossypii* after probing on reassembled virus

Treatment	Virus concentration (mg/ml)	Infectivity[x]	Transmissions[y] (no./total)	E_{405} in aphids[z]
Intact CMV-T	7.1	66 ± 9.8	17/20 a	0.085 a
	5.0	45 ± 8.6	17/20 a	0.073 a
Intact CMV-6	7.1	65 ± 12	6/20 b	0.030 b
	5.0	43 ± 7.1	3/20 b	0.034 b
Protein-T, RNA-T	7.1	51 ± 18	16/20 a	0.073 a
	5.0	38 ± 7.4	15/20 a	0.062 a
Protein-6, RNA-T	7.1	47 ± 12.8	3/20 b	0.032 b
	5.0	39 ± 6.7	4/20 b	0.033 b
Protein-6, RNA-6	7.1	46 ± 7.7	7/20 b	0.035 b
	5.0	36 ± 6.8	2/20 b	0.038 b
Protein-T, RNA-6	7.1	45 ± 12	15/20 a	0.078 a
	5.0	33 ± 6.8	15/20 a	0.063 a

[x] Number of local lesions per cowpea leaf, average from 10–11 leaves, and standard deviation, after 1:100 dilution of intact or reassembled virus.
[y] Denominator, number of plants used; numerator, number of plants infected, with one aphid per plant. Acquisition access feeding time was 30 sec and inoculation access feeding time was 2 hr. Ratios followed by the same letter are not significantly different ($P < 0.01$).
[z] Average from eight replicates; absorbance of control (0.005 M borate buffer, pH 9.0, in 5% sucrose) = 0.006. Means followed by the same letter are not significantly different ($P < 0.01$).

exchanging the coat protein of the poorly transmissible strain with that of the transmissible one in vitro, transmission rates increased markedly. Conversely, they decreased if coat protein of the transmissible strain was exchanged with that of the poorly transmissible one. This was correlated with ELISA absorbance values of aphids allowed to probe on intact or reassembled virus. Significantly more virus was detected serologically if aphids probed on virus with coat protein originating from CMV-T. Apparently the coat protein is responsible for transmissibility, perhaps by facilitating adherence of the virus particles to the aphid's foregut. Preliminary experiments, showed that ELISA detection of CMV almost entirely was associated with the foregut (authors, *unpublished*).

From studies of mixed infections Rochow (15) concluded that vector specificity of barley yellow dwarf isolates also appears to be a function of the virus capsid; and Harris (2) suggested reciprocity between recognition sites on the coat protein of this virus and aphid salivary gland membranes for effective transmission. Since CMV is a nonpersistent virus and its transmission is associated with the aphid foregut (12), it could well be that recognition sites for coat protein also are present at that site.

Analysis of our data showed that purified CMV-6 at high concentration (8 mg/ml) was transmitted almost as efficiently as purified CMV-T; these results are similar to those of Normand and Pirone (9). However, reducing virus concentration to one-fifth (1.6 mg/ml) reduced transmission of CMV-6 markedly, but that of CMV-T was almost unaffected. The relatively high transmission rate from concentrated CMV-6 preparations does not seem to be due to contamination with CMV-T or other highly transmissible CMV particles, as strains were passed through single lesions before use. Furthermore, after three serial transfers of CMV-6 from cucumber to cucumber by aphids, no significant increases in transmission rates were observed (authors, *unpublished*). It seems that when concentration of virus particles in the suspension is high, all aphid attachment sites are saturated with virus despite the incomplete compatibility of CMV-6 strain with the *A. gossypii* vector.

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