Characteristics of and Factors Affecting Helper-Component-Mediated Aphid Transmission of a Potyvirus

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ABSTRACT

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Purified tobacco etch virus, in the presence of helper component (HC), could be acquired and transmitted in the brief probes (<30 sec) characteristic of transmission of potyviruses from plants. The ability to transmit membrane-acquired virus following postacquisition fasting or feeding was also similar to that for plant-acquired virus, provided appropriate concentrations of HC and virus were used. Reduced retention times resulted when the concentration of either virus or HC was decreased, but loss of virus was the primary factor in determining the duration of retention. When HC and virus were acquired sequentially, HC efficacy was maintained in a number of buffers in the pH range 5–10, and buffer composition rather than its pH was more likely to adversely affect HC efficacy. The effect of "adverse" buffers was not to inactivate HC, because when HC was incubated in such buffers and virus was mixed with HC before acquisition, levels of transmission were much higher than when HC and virus were acquired sequentially. The implications of this finding relative to understanding the HC-virus interaction are discussed.

Additional key words: *Nicotiana tabacum*.

Viruses transmitted in a nonpersistent manner by aphids may be acquired from, and transmitted to, plants in a matter of seconds, and are retained by the vector for a relatively brief period of time, usually minutes to hours (8). The mechanism of transmission, the site(s) of virus retention in the aphid, and various other aspects of the transmission process are still poorly understood. For the potyviruses, the largest and most economically important group of viruses that are aphid-transmitted in a nonpersistent manner, the discovery that a "helper component" (HC) is required for successful transmission (2,5) has provided a key element to understanding the transmission process. Thus far, research on the helper component has focused on its characterization as a protein which is virus-coded (3,4,11), and on determining its specificity (7,10). Relatively little attention has been paid to the characteristics of the helper component-mediated transmission process.

One objective of the research described in this paper was to compare the characteristics of transmission of membrane-acquired virus with those of virus acquired from plants, with particular reference to the relative role of HC and virus in the retention of transmissibility. The second objective was to determine the effect of pH, buffer type, and salt regimes on the transmission process to improve understanding of how HC mediates the transmission process.

MATERIALS AND METHODS

The highly aphid transmissible (HAT) isolate of tobacco etch virus (TEV) (9) was used as source of virus in all experiments. For membrane acquisition the virus was purified from tobacco (*Nicotiana tabacum* L. 'Burley 21') by the method of Mohgal and Francki (6), resuspended in 0.05 M borate buffer, pH 8.0, and used at a concentration of 100 μg/ml unless otherwise noted.

Helper component (HC) was partially purified from potato virus Y (PVY)-infected tobacco leaves (9); final resuspension was in 0.1 M tris-H_2SO_4 buffer, pH 7.2, containing 0.02 M $MgSO_4$ (TSM buffer). The same HC preparation, frozen in 100 μl aliquots, was used in all experiments. The preparation was of high activity; a 1:100 dilution in TSM buffer mediated 100% transmission of TEV.

The concentrations of virus (100 μg/ml) and HC (1:20 dilution of the stock preparation), unless otherwise noted, were about five times those needed to give 100% transmission when acquired as a mixture under optimal buffer and pH conditions. These concentrations were used to assure that neither virus nor HC would be a limiting factor.

The diluent used for HC was TSM buffer except in the experiments in which other buffers or salts were being tested. Since the stock HC preparation was in TSM, low levels of tris-H_2SO_4 (5 mM) and $MgSO_4$ (1 mM) were also present in the experiments in which other buffers and salts were tested. While not an ideal protocol, preparation of HC in a single buffer (TSM), which maintained a high level of stability, was necessary to separate the effects of buffers and salts on HC stability from those on transmission. The composition of the acetate, citrate, citrate–phosphate, phosphate, tris-HCl, and borate (boric acid–borax for pH 8 and 9; borax-NaOH for pH 10) buffers was according to Gomori (1). Borate-KCl buffer was prepared according to Umbreit (12). Tris-H_2SO_4 buffer was prepared as described (11). In all cases, the indicated pH was achieved by titration and determined with a pH meter.

Myzus persicae (Sulz.), reared and handled as described previously (9), was used in all experiments. The aphids were kept in glass vials for 2 hr of preacquisition fasting. Procedures for acquisition of virus from plants or from mixtures of HC and virus contained in a Parafilm membrane were as described by Pirone and Thornbury (9). In sequential acquisition tests, acquisition was first through a Parafilm membrane containing HC and then through a second membrane containing virus suspension. In some experiments an intermediate access on a membrane containing only the salt and/or buffer under study was given. All preparations contained 20% sucrose. Unless otherwise stated, acquisition access was for 10 min and aphids were placed on test plants (Burley 21 seedlings) overnight for inoculation access. In experiments involving postacquisition fasting, aphids were kept in glass vials at room temperatures for the indicated length of time prior to inoculation access. Test plants were sprayed with insecticide and

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held for symptom development (9). Symptoms developed on infected plants in 5–7 days.

The experimental units consisted, in most experiments, of 10 test plants on each of which were placed 10 aphids. Statistical analysis was by analysis of variance after arcsin transformation for proportion of transmission. Means of transformed data were compared by least significant difference.

RESULTS

Acquisition and inoculation times for membrane-acquired virus. Aphids were allowed to probe a virus-HC mixture for 15–30 sec; probing behavior and duration were determined by microscopic observation. Individual aphids transmitted the virus at an average rate of 12.7% in five experiments. When groups of 10 aphids were placed on each test plant, the transmission rate was similar—12%. During a 5- to 10-min acquisition access period (actual probing duration not determined), transmission by individual aphids was 16–20% in three experiments while transmission by groups of 10 aphids was 90–100%.

Aphids given a 10-min acquisition access period were placed on test plants, allowed a 15–30 sec inoculation probing period, and then removed from the test plant. Individual aphids transmitted at a rate of 10% while transmission by groups of 10 aphids was 40%. In parallel experiments, transmission by aphids given an overnight inoculation access period was 16–25% for individual aphids and 100% for groups of 10 aphids.

TABLE 1. Retention of transmissible tobacco etch virus (TEV) acquired by aphids from infected plants or from a mixture of TEV and helper component (HC)[w]

Retention time (hr)[x]	Percent transmission from[y]	
	Plants	TEV + HC
0	90 a[z]	100 a
2	50 d	83 b
4	17 e	67 c
16	0 f	0 f

[w] Aphids allowed a 10-min acquisition access on plants inoculated 2–3 wk previously or on a Parafilm membrane containing a mixture of TEV at 100 μg/ml and HC at a 1:20 dilution, in TSM buffer and 20% sucrose.

[x] Aphids fasted in glass vials at room temperature for indicated time between acquisition access and inoculation access.

[y] Means of three experiments; 10 test plants per treatment; 10 aphids per test plant.

[z] Means followed by the same letter are not significantly different, $P = <0.01$, according to Duncan's multiple range test.

Comparative retention of transmissible virus acquired from plants or from a virus-HC mixture. A series of experiments was conducted to compare retention of transmissible virus acquired from plants with that acquired through a Parafilm membrane and to assess the roles of virus and HC in retention. In the first type of experiment, retention during postacquisition *fasting* was compared for virus acquired from plants or from the standard virus-HC mixture. As shown in Table 1, transmission declined more rapidly for plant-acquired than for membrane-acquired virus, but no transmission occurred after 16 hr in either case.

To determine whether retention was dependent on virus or HC concentration, a series of experiments was done in which the relative concentrations of virus and HC were varied; transmission was compared with that from infected plants. As shown in Fig. 1, reduction of the virus concentration from the 100 μg/ml concentration used for the experiments in Table 1 to 5 μg/ml resulted in a pattern of retention similar to that for plant-acquired virus, while reduction of either the virus concentration (to 1 μg/ml) or the HC concentration (to 1:100) resulted in a more rapid decline in transmission with postacquisition fasting.

Retention during postacquisition *feeding* was also similar for plant and membrane-acquired virus, provided that appropriate HC and virus concentrations were used. In these experiments aphids

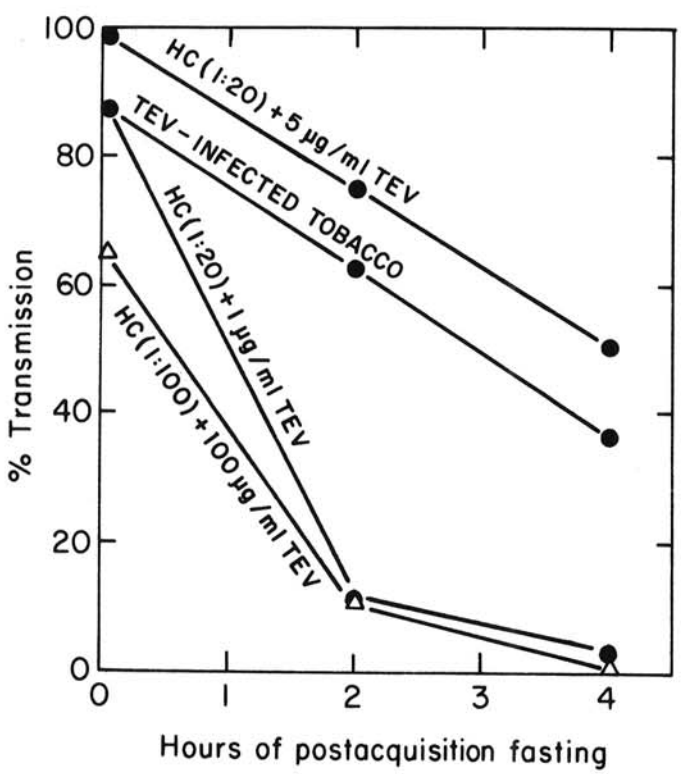

Fig. 1. Retention of transmissible tobacco etch virus (TEV) acquired by aphids from infected plants or from mixtures of TEV and helper component (HC) at the indicated concentrations. Aphids fasted, for the indicated time, after a 10-min acquisition access and prior to the inoculation access. Each point is the average of three experiments, 10 test plants per experiment, 10 aphids per test plant. (LSD = 14%; $P = <0.05$).

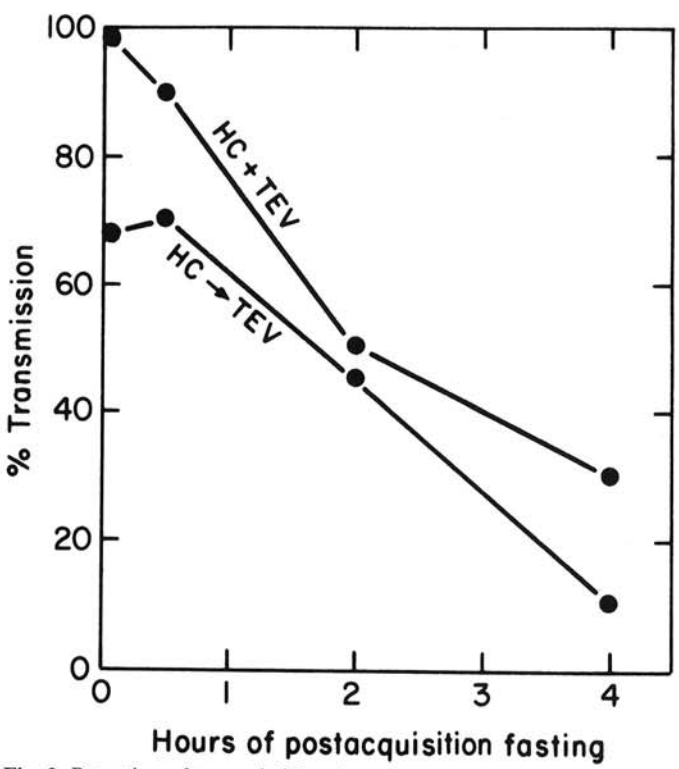

Fig. 2. Retention of transmissible tobacco etch virus (TEV) acquired by aphids from a mixture of TEV and helper component (HC), or in the sequence HC then TEV. Virus was at 5 μg/ml and HC was at a 1:20 dilution in both treatments. Aphids fasted for indicated time, between a 10-min acquisition access to TEV and the inoculation access. Each point is the average of three experiments, 10 test plants per experiment, 10 aphids per test plant. (LSD = 22%; $P = <0.01$).

were allowed a 10-min acquisition access to infected leaves or to a solution containing 5 μg/ml TEV and a 1:20 dilution of HC. These concentrations were used since retention was similar to that from plants in the postacquisition fasting experiments (Fig. 1). After acquisition, aphids were maintained on uninfected tobacco leaves, on which they were allowed to probe and feed for the indicated period of time, following which they were placed on test plants. In three experiments, transmission from plants averaged 97 and 22% after 0 and 10 min of postacquisition feeding, respectively. Transmission from membranes was 90 and 28%, for those two time periods. No transmission occurred after 60-min postacquisition feeding in either case.

Retention after sequential acquisition. The above experiments were done with mixtures in which HC and virus were acquired simultaneously. Sequential acquisition experiments were thus designed in an attempt to independently assess the role of HC and virus in retention.

In preliminary experiments, the rate of transmission as a function of postacquisition fasting was compared for virus acquired in a mixture and sequentially. The rate of loss was similar for the mixed and sequential treatments, although the percentage of transmission was higher from the mixture (Fig. 2).

The effect of postacquisition fasting on retention of transmissibility was then tested in sequential acquisition experiments. Aphids that were fasted after acquiring HC, but before acquiring virus, transmitted at a higher rate than aphids fasted after acquisition of HC and then the virus (Table 2).

Influence of buffers and pH on the transmission process. The pH and buffer composition have been shown to affect the in vitro stability of HC (3). We examined their effect on transmission to gain some insight into factors regulating the HC-virus interaction and the transmission process. A series of buffers, at pH values of 4–10, was tested. Aphids were first allowed to acquire HC in the indicated buffer and then to acquire virus in 0.05 M borate buffer, pH 8.0. Reasonable levels of transmission over the pH range 5–10 occurred, provided an appropriate buffer was used (Table 3). In this pH range the buffer constituents, rather than the pH, seemed to be the more important factor.

Effect of magnesium ions. Magnesium ions have been shown to stabilize the activity of HC (3), and their effect on transmission was thus tested in sequential acquisition experiments done in the same manner as those described in Table 3. The presence of 0.02 M $MgCl_2$ in the HC buffer resulted in increased transmission in all buffers at pH ≥7.2, when compared with the same buffers without $MgCl_2$. The results were most pronounced with "adverse" buffers. For example, with HC in borate buffer, pH 8.0, transmission averaged 10 and 70%, in the absence and presence of Mg^{++}, respectively. With HC in borate buffer, pH 9.0, transmission averaged 7 and 53%, in the absence and presence of Mg^{++}, respectively. Addition of $CaCl_2$, KCl, and NaCl at 0.02 M did not have a similar, beneficial effect (*unpublished*).

Effect of KCl. One hypothesis for the mode of action of HC is that it may act by binding to virus and to putative receptor sites within aphid mouthparts (5). If this binding is electrostatic, high salt concentrations might be expected to reduce the interaction. Since 0.02 M KCl did not adversely affect transmission, a series of increased concentrations of KCl was tested. Aphids were allowed to acquire HC in TSM buffer pH 7.2 containing KCl. Acquisition was either simultaneous, from an HC-virus mixture, or sequential. Transmission rates after simultaneous acquisition were 100% for 0.02 M, 97% for 2 M, and 63% for 4 M KCl. On the other hand, transmission rates after sequential acquisition were 65, 45, and 10% for the respective KCl concentrations. (Means of four experiments of each type.) The difference between the modes of acquisition could not be attributed to the probability of acquisition of both HC and virus by the same aphids, as the reduction at 4 M for sequentially acquired virus was greater than the reduction at 0.02 M. The possibility that prior feeding on 4 M KCl affected aphid behavior and consequently virus uptake in the subsequent acquisition access was ruled out; for aphids fed first on 4 M KCl and then on a virus-HC mixture the transmission was 100%.

Effect of virus on the efficacy of HC. One explanation for the above results is that the presence of virus in a mixture with HC reduced the sensitivity of HC to adverse conditions. The following experiment was conducted to determine whether the adverse effects were due to exposure of HC in vitro or in the aphid. Aphids were allowed to acquire HC from two test solutions known to be unfavorable for transmission: 0.1 M borate, pH 8.0, and 4 M KCl in TSM buffer. These were offered in three combinations: HC and virus were acquired from a mixture without prior incubation, HC was allowed to incubate in vitro in the test solution for 30 min and then virus was added to the solution prior to acquisition by aphids, or HC was first acquired from the test solution then followed by acquisition of the virus. From Table 4, it is apparent that these test solutions adversely affected the HC-mediated transmission process only if HC was acquired in the absence of virus. That HC was still active in these solutions is demonstrated by the efficient transmission that occurred even after 30 min of incubation, provided virus was added before acquisition.

TABLE 2. Effect of postacquisition fasting (PAF) of aphids, after acquisition of helper component (HC) or after acquisition of HC and then tobacco etch virus (TEV), on the retention of transmissible TEV[w]

Postacquisition fasting (hr)[x]	Percent transmission[y] following the sequence:	
	HC→PAF→TEV	HC→TEV→PAF
0	73 ab[z]	67 ab
½	77 a	60 b
2	60 b	30 c
4	43 c	10 d

[w] Aphids allowed a 10-min acquisition access period for HC and for TEV, with postacquisition fasting in the indicated sequence for the designated periods of time. Virus at 100 μg/ml and HC at a 1:20 dilution in TSM buffer and 20% sucrose.
[x] Aphids fasted in glass vials for indicated time.
[y] Means of three experiments, 10 test plants per treatment; 10 aphids per test plant.
[z] Means followed by the same letter are not significantly different at $P = <0.01$, according to Duncan's multiple range test.

TABLE 3. The effect of pH and buffer on the efficacy of helper component in mediating the transmission of tobacco etch virus by aphids[a]

pH	Buffer	No. of expts.	Virus transmission (%)[b] (mean ± SD)	Buffer	No. of expts.	Virus transmission (%)[b] (mean ± SD)
4.0	Citrate	4	0			
4.0	Acetate	3	0			
5.0	Acetate	3	20 ± 0	Citrate-PO_4	4	60 ± 22
6.1	Acetate	3	40 ± 17	Phosphate	3	73 ± 21
7.2	Tris-HCl	3	33 ± 6	Tris-H_2SO_4	5	78 ± 23
8.0	Borate	6	3 ± 8	Tris-H_2SO_4	5	41 ± 36
9.0	Borate	16	8.4 ± 11	Tris-H_2SO_4	5	39 ± 27
10.0	Carbonate	3	0	Borate-KCl	3	60 ± 10

[a] All buffers were at 0.1 M. Aphids were given a 10-min acquisition access to HC (1:20 dilution) in the indicated buffer followed by a 10-min acquisition access to TEV (100 μg/ml) in 0.05 M borate buffer, pH 8.0. All solutions contained 20% sucrose.
[b] Ten to 20 test plants per treatment; 10–15 aphids per test plant.

TABLE 4. Effect of the presence of tobacco etch virus (TEV) on the efficacy of helper component (HC) in adverse buffer or salt conditions[x]

Buffer	Transmission[y] (%)		
	HC + TEV	HC (30 min) + TEV	HC→TEV
0.1 M borate, pH 8.0	73.3 a	60 a	0 b
0.1 M TSM[z]; 4 M KCl	56 b	83 a	13.3 c

[x]Aphids allowed 10-min acquisition access to a mixture of HC + TEV in the designated buffer; to a mixture of HC + TEV to which the TEV was added after HC had been incubated in the buffer for 30 min; or allowed sequential 10-min acquisition accesses to HC in the indicated buffer and then to TEV in 0.05 M borate buffer, pH 8.0. An additional 2.5 mM borate was present in the mixture as the result of addition of TEV from the stock solution (2 mg/ml in 0.05M borate, pH 8.0). Virus concentration was 100 μg/ml and HC was at a 1:20 dilution in all cases.

[y]Means of three experiments for each buffer, means in the same row followed by the same letter are not significantly different ($P = <0.05$).

[z]TSM is 0.1 M tris-H_2SO_4 buffer, pH 7.2, containing 0.02 M $MgSO_4$.

DISCUSSION

Purified virus, in the presence of HC, can be acquired and transmitted in the brief probes (~30 sec) characteristic of transmission of potyviruses from plants (8). Thus brief acquisition and inoculation times are characteristic of the virus and virus-aphid interaction regardless of the source of virus acquisition.

Potyviruses and other nonpersistent viruses are retained in their vectors for minutes to hours. In the present study, we made use of the purified virus-HC system to establish the dependence of retention on the concentration of either HC or the virus in the test solution. When an appropriate dilution of HC (1:20) and concentration of TEV (5 μg/ml) is used, the rate of virus retention for aphids acquiring the suspension by feeding through artificial membranes is similar to that for aphids acquiring virus from infected tobacco plants. However, reduction in the concentrations of either TEV or HC in the mixture resulted in a corresponding reduction in the retention rate. It thus seems evident that either HC concentration or virus concentration can affect retention. However, the data in Table 2, in which identical fasting periods were given either after acquisition of HC or after acquisition of virus, suggest that when the concentration of neither moiety is limiting, loss of virus is the primary factor in determining retention of transmissibility.

Helper component was shown to be effectively acquired over a wide range of pH and buffer conditions. In general, however, pH and buffer conditions found to be most suitable for maintaining the stability of HC in vitro (3,11) were also those most suitable for acquisition. Furthermore, even buffers from which transmission was poor could serve for acquisition of HC provided Mg^{++} ions were added. The role of Mg^{++} is unknown, but it appears to be specific, as Ca^{++} does not have a similar effect.

From the standpoint of increasing understanding of how HC mediates the transmission process, the most interesting results were those obtained with acquisition from adverse buffer or salt solutions (Table 4). Simultaneous acquisition of HC and virus resulted in relatively high transmission rates even if HC was incubated for up to 30 min in the adverse solution before the addition of virus. However, when HC was acquired alone, under adverse conditions, transmission of subsequently acquired virus was either abolished or drastically reduced. One possible explanation is that conditions within the aphid's mouthparts are normally inappropriate for the HC-virus interaction to take place. In the presence of "favorable" buffers these conditions are ameliorated, but if HC is acquired in "adverse" buffers they are not, and transmission is either poor or does not occur.

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