Transmission of Two Purified Carlaviruses by the Pea Aphid

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ABSTRACT

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Pea aphids (*Acyrthosiphon pisum* [Harris]) were able to transmit purified carlaviruses, pea streak virus (PSV) and red clover vein mosaic virus (RCVMV) after acquisition through membranes. Purity of virus preparations was monitored by ultraviolet spectrophotometry and sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Aphids also were able to transmit both viruses after feeding through an artificial membrane on crude sap extracts from infected plants. Purified PSV was more readily aphid transmissible than was purified RCVMV. There was no evidence that a second substance, acting as a helper agent to facilitate aphid transmission, was present. This is the first report of a purified filamentous virus being transmitted by aphids in the absence of accessory factors.

Only two plant viruses, alfalfa mosaic (5) and cucumber mosaic (7), have been demonstrated to retain stylet-borne aphid transmissibility after purification. Potyviruses (3) and caulimoviruses (4) lose aphid transmissibility upon purification due to separation from their respective helper agents. Others have speculated that the helper agent, an accessory factor produced as a result of infection, is involved in the aphid transmission of other plant virus groups (6). Our investigation deals with the aphid transmissibility of two carlaviruses, pea streak virus (PSV) and red clover vein mosaic virus (RCVMV), in the purified state. Evidence is presented that, after purification, these filamentous viruses were transmissible in the characteristic nonpersistent manner by the pea aphid, *Acyrthosiphon pisum* (Harris), and that no helper agent or accessory factor is involved.

MATERIALS AND METHODS

Pea aphids used in this study were reared on bell bean (*Vicia faba* var. *minor* Peterm. Beck) at a constant temperature (20 C) and photoperiod (18 hr/day). An Oregon colony collected from alfalfa in 1978 was used in all but three trials in which a Michigan clone (10) was used. In all cases, transmission trials were conducted with 5- to 7-day-old 4th instar nymphs.

To eliminate the possibility of confounding seedborne or other extraneous viruses, aphids routinely were starved for 12 hr after removal from the bell bean aphid culture plants (no viruses are known to be both seedborne in bell bean and aphid-transmitted in a persistent manner). Aphids cultured, starved, and tested before feeding on virus-source plants have not transmitted extraneous viruses.

Isolates of PSV and RCVMV were identified by host range, symptomology on pea (*Pisum sativum* 'Cascade'), particle morphology, and SDS-gel immunological reactions (8). For comparative purposes, we obtained Wisconsin pea streak virus (PSV-W) from D. J. Hagedorn and RCVMV-ATC from the American Type Culture Collection. All isolates were propagated in pea cultivar Cascade. PSV isolate ID-3-2 was obtained from an alfalfa stand in Idaho and RCVMV isolate WA-7641 originated in infected peas from Washington.

Virus extracts were prepared by vacuum infiltrating infected pea tissue with 0.018 M trisodium citrate, 0.165 M disodium phosphate, and 0.15% diethyldithiocarbamate at pH 9.0. Tissue was ground in a mortar with sterile sand in extraction buffer at a ratio of 1:2 (w/v). Buffered extract was clarified by centrifugation for 10 min at 12,000 *g* and virus was precipitated by the addition of polyethylene glycol, MW = 6000 (PEG 6000), to 6% (w/v). The virus was pelleted by centrifugation at 12,000 *g* for 10 min and resuspended in extraction buffer to one-fifth the original volume. Both the crude and concentrated extracts were highly infectious, as determined by mechanical inoculation onto pea.

Virus purification. Viruses were purified from frozen pea tissue, essentially as described by Veerisetty and Brakke (12). Tissue was blended in phosphate-citrate buffer (1:4, w/v), clarified by low-speed centrifugation, and precipitated virus was centrifuged at low speed and pellets were resuspended in buffer containing 1% Triton X-100, at one-tenth the molarity of the extraction buffer. Following a low speed centrifugation the virus in the supernatant was concentrated by centrifugation at 160,000 *g* through a layer of 30% sucrose containing buffer and 1% Triton X-100 in a Spinco 65 rotor (Beckman Instruments Inc., Palo Alto, CA 94304). Virus bands were collected and concentrated by a second differential centrifugation. Final virus pellets were resuspended in buffer without Triton X-100.

Relative concentrations of purified virus were estimated by UV absorption analysis on a Beckman Model 25 spectrophotometer (Beckman Instruments Inc., Palo Alto, CA 94304). The absorbance at 260 nm (A_{260}) was not corrected for the negligible amounts of light scattering that ocurred. Viral protein was analyzed electrophoretically on SDS polyacrylamide gels (5.6%) by using the methods of Fairbanks (2). Marker proteins used to estimate molecular weights of viral coat protein were bovine serum albumin, ovalbumin, human gamma-globulin, and alchohol dehydrogenase.

Aphid transmission tests. Aphids were starved for 12 hr before transmission tests. For plant-to-plant transmission studies, aphids were allowed 2–5 min acquisition access periods (AAP) on source plants after which 3–10 aphids were transferred by microaspirator to each test plant. For solution-to-plant transmission studies, aphids were allowed 10-min AAP to either solutions of purified

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virus or comparable noninfectious solutions sandwiched between two layers of stretched Parafilm 'M' (American Can Co., Greenwich, CT 06830). For determinations of aphid transmissibility, 5–10 aphids that had assumed characteristic feeding postures were transferred to each test plant. Feeding solutions were made 20% sucrose (w/v). Aphids were allowed inoculation access periods (IAP) on test plants of 8–12 hr. Determinations of transmission were based on test plant symptomatology. Virus content in plants with questionable symptoms was verified by electron microscopy or serology.

After completion of aphid AAP, virus solutions were removed from membranes and mechanically inoculated onto pea plants which then were maintained as experimental controls.

RESULTS

In preliminary plant-to-plant aphid transmission experiments, we determined that the aphid clones (*A. pisum*) from either Oregon or Michigan readily transmitted RCVMV and PSV under the conditions of our study. In these separate tests, rates of transmission from infected to healthy Cascade pea plants by three to 10 aphids per test plant were 5/10, 7/16, and 5/8 for RCVMV and 5/8, 3/18, and 3/16 for PSV. Aphids also were able to transmit both viruses when given access through membranes to nonconcentrated or PEG-concentrated aqueous extracts from infected plants (Table 1). In four separate control tests, aphids which had fed upon noninfectious buffer-sucrose solutions failed to transmit RCVMV or PSV to test plants. Each test involved 40 aphids and 16 test plants.

TABLE 1. Aphid transmission of red clover vein mosaic (RCVMV) and pea streak (PSV) viruses, acquired by the pea aphid, *Acyrthosiphon pisum*, via membrane feedings[a]

Virus	Treatment	Exp No.	Aphids per test plant	Remarks	Transmission[b]
RCVMV					
WA-7641	Buffered sap[c]	1	10		2/10
		2	10		1/8
	PEG precip.[d]	1	10[e]	Conc. ×5	4/10
		2	10	Conc. ×5	1/8
	Purified	1	10[e]	A_{260}: 0.1	0/7
		2	10[e]	0.5	1/9
		3	8	1.0	1/10
		4	5	1.0	0/10
		5	5	3.8	1/6
		6	5	5.3	1/8
PSV					
ID-3-2	Buffered sap	1	10		1/8
	PEG precip.[d]	1	10	Conc. ×5	1/8
		2	10	Conc. ×1.6	4/8
	Purified	1	5	A_{260}: 0.2	0/8
		2	5	0.5	3/8
		3	5	1.0	1/8
		4	5	1.5	2/8
		5	5	1.5	3/8
		6	5	2.0	4/8
		7	5	2.5	3/8
		8	5	2.5	3/8
		9	5	3.0	3/8
		10	5	3.0	5/8

[a] Aphids were routinely control-tested for vectoring ability and freedom from contaminating plant viruses in each experiment. Typically, 20–50 aphids were included in such test controls.

[b] Numerator: number of infected plants. Denominator: number of test plants.

[c] Pea tissue homogenized in 0.018 M Na_3-citrate, 0.165 M Na phosphate, and 0.15% Na diethyldithiocarbamate pH 9.0 1:2 (w/v).

[d] Sap concentrated by precipitation with polyethylene glycol (PEG 6000) and resuspension in extraction buffer.

[e] Aphids used in these trials from East Lansing, MI, clones; Oregon colony used in other trials.

Aphid transmission and characteristics of purified RCVMV. Aphid transmissibility of purified RCVMV was demonstrated at low levels over an O.D. A_{260} range of 0.1 to 5.7 (Table 1). Varying the number of aphids per test plant and the concentration of virus failed to increase transmission rates. Although only four of 50 plants fed upon by aphids after access to purified RCVMV became infected, each infection occurred in a separate trial, which demonstrated reproducibility. The pathogenicity of purified RCVMV fed to aphids was indicated by typical, severe, streak-like symptoms that rapidly developed in plants inoculated with preparations from feeding membranes at the conclusion of each test.

Purified RCMVM isolate WA-7641 exhibited a UV absorption spectrum typical of that for filamentous viruses. Within the 270–240 nm wavelength range, absorption maxima and minima occurred at 260.0 nm and 246.5 nm, respectively. The $A_{260/280}$ ratio of purified RCVMV was 1.24 ± 0.02. Purified WA-7641 was determined by electron microscopy to consist of particles with a modal length of 660 nm and to be free from discernible host material. Viral protein, prepared by sodium dodecyl sulfate (SDS) disruption of pure virus, migrated as a single band in SDS polyacrylamide gel electrophoresis. The molecular weight of RCVMV protein subunits was estimated to be 31,000 daltons based on measured electrophoretic mobilities of five protein standards. Protein from isolate WA-7641, mixed with equal amounts of disrupted protein from RCVMV-ATC, also migrated as a single band in gel electrophoresis. Purified RCVMV was highly antigenic. Antiserum prepared against WA-7641 produced a conterminous precipitin band between homologous antigen and RCVMV-ATC in SDS agar gels.

Aphid transmission and characteristics of purified PSV. Purified PSV isolate ID-3-2 was more readily aphid transmissible than was purified RCVMV (Table 1). There was a positive relationship between transmission frequency and concentration of purified PSV. Aphids fed on purified virus suspensions with A_{260} values ranging from 2.0 to 3.0 transmitted PSV to 18 of 40 test plants, whereas those fed on suspensions with A_{260} values of 0.2 to 1.5 transmitted PSV to nine of 40 test plants. Probability analysis (9) of these proportions indicated ($Z = -2.123$) that they differed statistically, $P = 0.05$.

Purified PSV fed to aphids was highly infectious, as evidenced by the typical, severe streaking symptoms that rapidly developed in plants inoculated with preparations removed from feeding membranes.

Purified PSV isolate ID-3-2 exhibited a UV absorption spectrum similar to that of RCVMV. Within the 270- to 240-nm wavelength range, absorption maxima and minima occurred at 260.0 nm and 247.0 nm, respectively. The $A_{260/280}$ ratio of purified virus was $1.15 \pm .02$. Purified ID-3-2 consisted of particles with a modal length of 630 nm and were free from discernible host material. The protein of isolate ID-3-2 migrated in SDS polyacrylamide gels as a major band (29,000 daltons, based on electrophoretic standards) and two to three minor bands (26,000 to 20,000 daltons), in agreement with recently reported results (11) for a Wisconsin isolate of PSV. Minor bands that occurred inconsistently in repeated tests, were inducible by incubation at 60 C, and were concluded to represent breakdown products of PSV protein. Purified PSV was highly antigenic and antiserum against isolate ID-3-2 produced a conterminous precipitin band between homologous antigen and PSV-W in SDS agar gels.

DISCUSSION

The data reported here demonstrate that PSV and RCVMV are both aphid transmissible after purification. Although no new or novel procedures were developed to exhaustively test for minute quantities of possible helper agent which could have remained attached to virus particles despite treatment with nonionic detergent and isopycnic centrifugation, our procedures were

essentially equivalent to those of previous workers who succeeded in separating helper agents from potyviruses (3) and caulimoviruses (4).

Purified cucumber mosaic virus, which is aphid transmissible either from infected plants or in the purified state, is not transmissible in the presence of sap from infected plants (7). Our results demonstrate that both RCMVM and PSV are readily aphid transmitted when sap is present.

Aphids feeding on either infected pea tissue or on sap extracts through artificial membranes readily transmitted both PSV and RCVMV. After purification, however, RCVMV was less easily transmitted than was PSV at equivalent optical densities. We considered the possibility that the multiple banding pattern of PSV coat protein represented factors that could influence aphid transmissibility, in light of recently published correlations between aphid transmissibility of pea enation mosaic virus with a second coat protein (1). However, since coat protein from all Northwest isolates and the Wisconsin isolate of PSV exhibited this multiple banding pattern, it was not possible to test aphid transmissibility in the absence of these smaller-molecule proteins. Furthermore, these smaller proteins associated with PSV coat protein differed significantly from the helper agents reported in crude sap extracts from plants infected with potyviruses (3) and caulimoviruses (4) in that they persisted throughout the purification procedure which included treatment with nonionic detergent and isopycnic centrifugation.

Based on an extinction coefficient of 2.0 (13) for both carlaviruses studied, the concentration of purified virus required for aphid transmission was estimated to be 0.1 mg/ml. Increases in aphid transmission rates were less than proportional to increases in virus concentrations. Transmission efficiency achieved by workers with potyviruses (3) was much greater than ours. Because of the observed tendency for plant viruses to precipitate upon ageing, it is possible that low virus transmission rates obtained in this study were related to the limited virus particle stability provided by the extraction buffer. Use of different extraction media possibly could have provided more efficient transmission of purified virus. Transmission rates of purified virus, relatively lower than obtained with potato virus Y (3) also may reflect inherent transmissibility of PSV and RCVMV; ie, properties intrinsic to virus particles that are specifically interactive with those of aphid tissues.

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