The Relationship Between Aphid-Transmissibility of Potato Leafroll Virus and Surface Epitopes of the Viral Capsid

J. F. J. M. van den Heuvel, M. Verbeek, and D. Peters

First and second authors: DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands. Third author: Department of Virology, Wageningen Agricultural University, P.O. Box 8045, 6700 EM Wageningen, The Netherlands.

Accepted for publication 15 June 1993.

ABSTRACT

van den Heuvel, J. F. J. M., Verbeek, M., and Peters, D. 1993. The relationship between aphid-transmissibility of potato leafroll virus and surface epitopes of the viral capsid. Phytopathology 83:1125-1129.

A panel of nine monoclonal antibodies (MAbs) was used to investigate the relationship between aphid-transmissibility and surface epitopes of the capsid of potato leafroll virus (PLRV). In immunoblocking experiments, mixtures of purified PLRV and MAbs were fed to 1-day-old *Myzus persicae* nymphs, and their transmission efficiency was then studied. The MAbs were applied in triple-antibody sandwich enzyme-linked immunosorbent assay (TAS ELISA) to characterize antigenically two phenotypic variants of the Wageningen isolate of PLRV (PLRV-Wag) that differ in transmissibility and a highly aphid-transmissible isolate of PLRV. It was previously established that these MAbs reacted with epitopes exposed on the surface of the virion. However, the MAbs can be separated into two groups on the basis of their reactivity with intact and disrupted particles of PLRV. One group of six MAbs identified epitopes located on individual subunits of the viral capsid, since they reacted equally well with either conformational state of the virus. Four of these MAbs—WAU-A5, -A6, -A7, and -A13—affected the transmission of PLRV in immunoblocking experiments by significantly increasing the latency period of the virus in the aphid. The epitopes to which they are directed were previously found to be closely related topologically. WAU-A13 reacted significantly more strongly in TAS ELISA with the readily transmitted phenotypic variant of PLRV-Wag isolated from upper leaves of *Physalis floridana* than with the poorly transmissible variant from lower leaves. The other MAbs did not differentiate between these variants. Only WAU-A13 detected a highly aphid-transmissible isolate of PLRV (hat-PLRV) obtained after a selective transmission pressure was exerted on PLRV-Wag. Thus the epitope delineated by this MAb or topologically related capsid domains might be associated with the aphid transmission of PLRV. Western blot analysis of purified PLRV revealed the presence of two proteins with molecular masses of 23 and 56 kDa. Since WAU-A13 tagged both capsid-associated proteins, this MAb is specific for the major coat protein species of 23 kDa. The second group of MAbs—WAU-A12, -A24, and -B9—reacted exclusively with intact particles of PLRV, which suggests that they are directed to epitopes that depend on the quarternary protein structure. These MAbs did not interfere in PLRV transmission in the immunoblocking experiments, did not detect hat-PLRV, and were hardly reactive to the readily transmissible variant of PLRV-Wag compared to the poorly transmissible one. The epitopes delineated by these MAbs therefore could not be associated with aphid-transmissibility.

Additional keywords: luteovirus, persistent virus transmission.

Potato leafroll virus (PLRV), a member of the luteovirus group (27), is transmitted in a persistent manner by several aphid species, of which *Myzus persicae* (Sulzer) is the most efficient vector (18). Luteoviruses are transmitted in a circulative fashion by their vectors. To be transmitted by aphids, ingested virus particles must pass epithelial cell linings of the aphid's hindgut and salivary gland to be excreted with the saliva and hence inoculate plants.

Ultrastructural localization studies of virions of barley yellow dwarf virus strains in vector and nonvector aphids have led to the development of a model of receptor-mediated endocytosis-exocytosis to describe this transcellular virus transport (7,8). The model predicts that a receptor-mediated attachment of virus particles to membranes controls acquisition and vector specificity (1,7). Current evidence supports the hypothesis that the viral capsid plays a key role in this process (14) and determines both transmission and vector specificity of a luteovirus. It is, however, still a matter of speculation whether the major coat protein species or other capsid-associated proteins expressed through read-through of the coat protein gene termination codon (25) impart this transcellular transport (2,26).

Massalski and Harrison (12) showed that two PLRV isolates

poorly transmitted by *M. persicae* reacted weakly with two monoclonal antibodies (MAbs) to PLRV, compared to isolates readily transmitted by the aphid. They concluded that the poorly transmitted isolates lacked one or more antigenic determinants, presumably dependent on the quarternary protein structure, that may regulate the passage of PLRV particles from the hemolymph to salivary gland cells of *M. persicae.* The importance of the viral capsid in vector-dependent virus transmission has also been reported in other studies (e.g., 4,5,11).

In this study, we used a panel of nine PLRV-specific MAbs to investigate further the involvement of surface epitopes of PLRV in transmission of the virus by *M. persicae.* The MAbs can be separated into two groups on the basis of their reactivity with intact and disrupted PLRV in triple-antibody sandwich (TAS) and antigen-coated plate enzyme-linked immunosorbent assay (ELISA), respectively (20). MAbs WAU-A2, -A5, -A6, -A7, -A13, and -A47 detect either form of the virus (intact or disrupted) equally well and therefore are most likely directed to surface epitopes located on individual subunits of the viral capsid. The other three MAbs—WAU-A12, -A24, and -B9—apparently detect quarternary epitopes, because they are reactive only to intact virus.

All of the MAbs were used in immunoblocking experiments designed to study the efficiency of virus transmission by *M. persicae* nymphs fed mixtures of purified PLRV and antibodies through artificial diets. The MAbs were also used to characterize a highly aphid-transmissible isolate of PLRV and to immunologically compare two phenotypic variants of the Wageningen isolate of PLRV (PLRV-Wag), isolated from lower and upper leaves of *Physalis floridana,* that differ in their transmissibility by *M. persicae* (19).

MATERIALS AND METHODS

Aphids. *M. persicae* biotype WMp2 was reared on *Brassica napus* L. subsp. *oleifera* (oilseed rape) in a greenhouse compartment at 20 ± 3 C with a photoperiod of 16 h/day. Cohorts of nymphs differing in age by less than 24 h were produced by daily transfer of mature apterae, confined to leaf cages, to fresh plants.

Virus. PLRV-Wag was maintained on *P. floridana* as previously described (22). The virus was purified from *P. floridana* plants by a modified enzyme-assisted procedure, the final step of which was a 20–50% sucrose gradient (20).

Two phenotypic variants of PLRV-Wag, purified from upper and lower leaves of *P. floridana,* were compared immunologically in TAS ELISA with MAbs. PLRV-Wag acquired by *M. persicae* from upper leaves was transmitted significantly more efficiently than virus acquired from lower leaves of the same plant (22). This difference in transmissibility was retained even when purified suspensions of the two variants were fed to *M. persicae* nymphs (19).

Antibodies. We used a panel of nine MAbs to PLRV, partially purified from ascitic fluids of mice and previously characterized (20), and a polyclonal antiserum specific to PLRV from mouse. Antibody stocks of 2 mg/ml were prepared. Rabbit anti-PLRV, mouse antithyroglobulin, and rabbit anti-blackeye cowpea mosaic virus (BlCMV) polyclonal antibodies were kindly provided by D. Z. Maat, A. Schots (Laboratory for Monoclonal Antibodies, Wageningen, The Netherlands), and H. Lohuis, respectively.

ELISA. In all the ELISA experiments, Nunc-Immunoplate Maxisorp F96 plates (Nunc, Roskilde, Denmark) were used, and the plates were washed between incubation steps with phosphate-buffered saline (PBS) (2 mM KH_2PO_4, 8 mM Na_2HPO_4, 0.14 M NaCl, and 2 mM KCl, pH 7.4) containing 0.05% Tween 20.

TAS ELISA was used to quantify the reactivity of the MAbs with the various purified PLRV isolates. Wells of the plates were sensitized with 150 µl of 2 µg/ml rabbit anti-PLRV antibodies in coating buffer (0.05 M sodium carbonate, pH 9.6) for 3 h at 37 C. Subsequently, 100 µl of sample buffer (21) containing 20 ng of purified virus was added to the wells and incubated overnight at 4 C. Viral antigen was probed by incubating MAbs to PLRV or mouse anti-PLRV polyclonal antibodies, diluted in sample buffer, for 3 h at 37 C. The wells were then filled with 100 µl of goat antimouse IgG:alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) diluted 2,000-fold and incubated for 3 h at 37 C. To monitor immobilized alkaline phosphatase, the conversion of 1 mg/ml *p*-nitrophenyl phosphate disodium salt in 10% diethanolamine, pH 9.8 (150 µl per well), after 2 h of incubation at room temperature was measured at 405 nm (A_{405nm}) with a Bio-Kinetics Reader EL312 (Bio-Tek Instruments, Inc., Winooski, VT).

Immunodiffusion assay. The reactivity of the MAbs with purified PLRV-Wag was tested in double-diffusion precipitin tests with 0.5% (w/v) agarose gels in PBS. The MAbs were applied at a concentration of 1 mg per milliliter of PBS, and purified PLRV was diluted in PBS to 250 µg/ml. Rabbit anti-PLRV polyclonal antibodies in PBS, at a concentration of 1 mg/ml, were included as a control.

Northern blotting. Recombinant M13tg131 phage containing a plus-stranded *Eco*RI fragment congruent to position 5196–5495 on PLRV genomic RNA (23) was grown in *Escherichia coli* JM101. DNA was extracted, and a [^{32}P]labeled probe was made by extension of an M13 primer downstream of the insert, followed by a *Hin*dIII digestion, as described by Barker et al (3). Samples of supernatants obtained after PLRV and MAbs were mixed and spotted onto Hybond-N hybridization transfer membranes (Amersham Corp., Arlington Heights, IL), which were prewetted, soaked in 20× SSC, and air-dried. RNA was cross-linked to the membrane by ultraviolet radiation. Prehybridization was done at 42 C for 4 h in 50% formamide, 6× SSC, 5× Denhardt's solution, 200 µg of salmon sperm DNA, and 0.1% sodium dodecyl sulfate (SDS) per milliliter. After overnight hybridization at 42 C, membranes were washed in 2× SSC containing 0.1% SDS.

Immunoblocking experiments and infectivity testing. The effect of antibodies on PLRV-Wag transmission by *M. persicae* was studied in experiments in which the MAbs and the virus were simultaneously fed to 1-day-old nymphs. Mixtures of 6 µg of purified PLRV-Wag and 67 µg of the MAb to be tested were incorporated into 100 µl of the artificial diet MP148 (10) supplemented with 1% bovine serum albumin (BSA) (19) and offered between Parafilm membranes to nymphs during an acquisition access period (AAP) of 24 h. To determine the latency period (LP) of the virus in the nymphs, nymphs were transferred individually at six subsequent inoculation access periods (IAPs) of 24 h to new *P. floridana* seedlings, and the IAP in which a nymph infected a test plant for the first time was recorded. The LP is defined as the time interval from the start of the AAP to the end of the IAP in which the first transmission occurred. A log-probit transformation was used to estimate the median LP (LP_{50}), which represents the period of time after which 50% of the nymphs that transmitted the virus completed the LP (17). The LP_{50} and its 95% fiducial limits were calculated as described by Finney (6) with the SAS program (SAS Institute Inc., Cary, NC). Sixty nymphs were used per virus-antibody mixture. Acquisition and transmission experiments were performed in a climate chamber at 20±0.1 C with continuous illumination (10,000 lx).

Gel electrophoresis and western blotting. Antibodies were detected in aphids as follows: One-day-old nymphs were fed for 3 days on MP148 containing 100 µg of MAbs, then macerated. The extracts were electrophoresed under denaturing conditions on a 4% (w/v) polyacrylamide stacking gel and a 12% (w/v) separation gel. Proteins were transferred to nitrocellulose sheets, which were incubated with goat antimouse or goat antirabbit IgG:alkaline phosphatase conjugate in PBS-Tween containing 0.05% BSA, then with a mixture of 5-bromo-4-chloro-3-indolyl-phosphate *p*-toluidine salt and nitroblue tetrazolium chloride in 0.1 M Tris-HCl buffer, pH 9.5, containing 0.1 M NaCl and 5 mM $MgCl_2$, to visualize bands.

RESULTS

Effect of mixing MAbs and purified PLRV on the virus concentration. Before offering mixtures of MAbs and purified virus to *M. persicae,* we investigated whether the PLRV-specific MAbs

would affect the actual virus concentration in suspension by precipitating the virus. The outcome of these preliminary tests dictated the choice of which transmission parameter to use to measure the effect of MAbs on virus transmission: either a parameter that depends on the virus dose acquired (the percentage of viruliferous aphids) or one that is independent of the dose, such as the LP_{50} (19).

In immunodiffusion assays, none of the nine MAbs gave a precipitin line with purified PLRV-Wag, whereas clear lines were visible with the polyclonal antibodies (results not shown). Moreover, unlike the PLRV-specific polyclonal antibodies from mouse, none of the MAbs precipitated the virus when suspended in MP148 (Fig. 1). In this test, purified PLRV-Wag at a concentration of 60 μg per milliliter of MP148 was mixed with 67 μg of MAbs, incubated for 3 h at 20 C, and then centrifuged for 15 min at 10,000 rpm. Dilutions were made of the supernatants, and 1-μl aliquots were spotted onto membranes. The intensity of the spots on the autoradiograph indicated that some of the MAbs slightly affected the actual concentration of the virus in suspension compared to the controls in which either no antibody or an unrelated one was added (Fig. 1). We therefore decided to determine the LP_{50} as the transmission parameter in the infectivity tests.

Effect of MAbs on virus transmission. To study the effect of MAbs on transmission of PLRV by *M. persicae,* we fed 1-day-old nymphs for 24 h on mixtures of 6 μg of purified PLRV-Wag and 67 μg of MAbs in 100 μl of MP148. PLRV was purified from *P. floridana* plants 5 wk after inoculation. The ratio of antibody molecules to virus particles in the mixtures was about 200:1, which we calculated assuming that on average 50% of the protein in the MAb suspensions consisted of antibodies (10–90% has been reported [9]) and that the particle masses of antibodies and PLRV are 1.5×10^5 Da (9) and about 6.3×10^6 Da (15), respectively. Rabbit anti-BlCMV antibodies (67 μg per 100 μl of MP148) served as a control in this experiment, since BlCMV is not related to PLRV. Preliminary experiments had shown that the addition of these antibodies to a virus suspension in MP148 did not have a significant effect on the LP_{50}. Compared to the control, MAbs WAU-A5, -A6, -A7, and -A13 significantly increased the LP_{50} of PLRV in the aphid (Fig. 2) and thus affected the transmissibility of PLRV. The other MAbs did not have a significant effect on the LP_{50}.

Exposing aphids first to MAbs suspended in MP148 before they acquired PLRV from infected *P. floridana* did not consistently influence virus transmission (data not shown), most likely because the antibodies failed to accumulate in the gut or hemocoel of the aphid, as could be demonstrated by western blotting. No

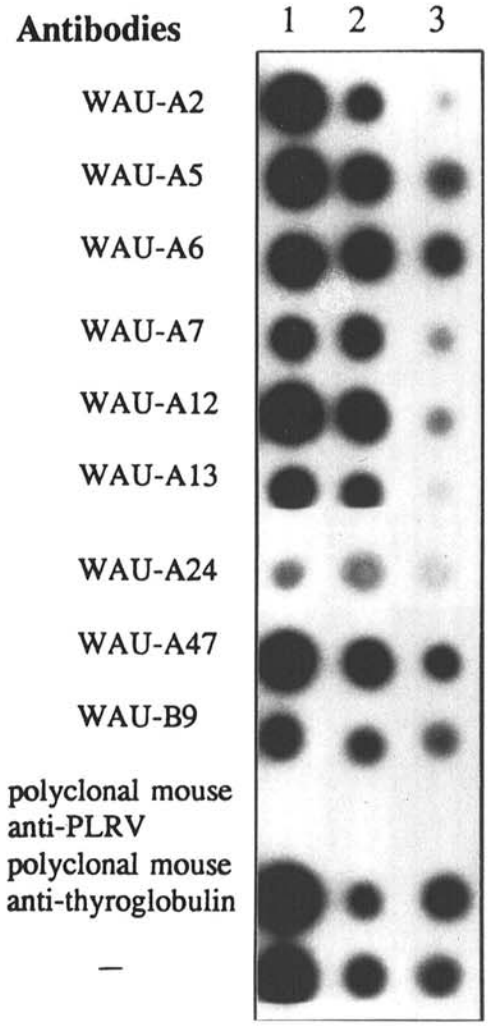

Fig. 1. Autoradiograph showing the effect of mixing 67 μg of monoclonal antibodies or unrelated antibodies with 6 μg of purified potato leafroll virus (PLRV) on the amount of virus in suspension in 100 μl of the artificial aphid diet MP148. After incubation for 3 h at room temperature, the mixture was centrifuged at a low speed, and 1-μl aliquots of the supernatant diluted fourfold (lane 1), eightfold (lane 2), and 16-fold (lane 3) were spotted onto nitrocellulose for molecular hybridization. The dash indicates that no antibody was added to the virus suspension.

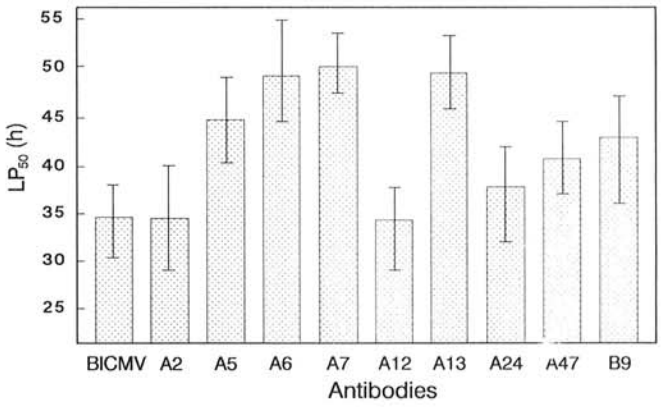

Fig. 2. The median latency period (LP_{50}) of potato leafroll virus (PLRV) in *Myzus persicae* aphids fed mixtures of purified PLRV-Wag isolate (6 μg) and virus-specific or nonspecific antibodies (67 μg) in 100 μl of the artificial diet MP148. The mixture was offered between Parafilm membranes to 1-day-old nymphs during an acquisition access period of 24 h. The nymphs were then transferred every 24 h for 1 wk to fresh *Physalis floridana* seedlings, and the LP_{50} was determined. Sixty nymphs were used for each mixture tested.

TABLE 1. The reactivity[a] of monoclonal antibodies specific to potato leafroll virus (PLRV) to two phenotypic variants[b] of the Wageningen isolate of PLRV (PLRV-Wag) in triple-antibody sandwich enzyme-linked immunosorbent assay

Antibody	Antibody dilution	PLRV-Wag variant[b]	
		Upper leaves	Lower leaves
WAU-A2	10,000	0.454 ± 0.037	0.423 ± 0.043
WAU-A5	100	0.282 ± 0.018	0.257 ± 0.012
WAU-A6	80,000	0.448 ± 0.025	0.398 ± 0.018
WAU-A7	80,000	0.454 ± 0.080	0.423 ± 0.074
WAU-A12	20,000	0.117 ± 0.025	0.325 ± 0.104
WAU-A13	1,000	0.313 ± 0.061	0.245 ± 0.043
WAU-A24	10,000	0.025 ± 0.006	0.190 ± 0.017
WAU-A47	40,000	0.565 ± 0.043	0.546 ± 0.018
WAU-B9	20,000	0.006 ± 0.006	0.153 ± 0.031
Mouse anti-PLRV	10,000	0.614	0.613
$LSD_{0.05}$[c]		0.055	

[a] Mean absorbance values at 405 nm (A_{405}) ± standard error after 1 h of substrate incubation.

[b] A readily transmissible variant isolated from upper leaves of *Physalis floridana* and a poorly transmissible variant purified from lower leaves of *P. floridana*. Two different batches of each phenotypic variant were tested three times; purified virus was applied at 20 ng per 100 μl of sample buffer.

[c] Least significant difference.

bands representing either the light or the heavy chain of the antibodies could be observed in macerates of five *M. persicae* nymphs fed for 3 days on MP148 containing 100 μg of MAbs or polyclonal antibodies per 100 μl (data not shown). Mixing homogenized aphids with known quantities of MAbs showed that as little as 1 ng of the MAbs could be identified on western blots.

Immunological differentiation of PLRV variants. To associate surface epitopes of the virus with transmissibility by *M. persicae,* we compared a readily and a poorly transmissible phenotypic variant of PLRV-Wag in TAS ELISA with the panel of PLRV-specific MAbs. The readily transmissible variant was isolated from asymptomatic upper leaves of *P. floridana* plants infected with PLRV-Wag at the seedling stage 5 wk before. The poorly transmissible one was obtained from lower leaves with pronounced interveinal chlorosis that were infected 8 wk before purification. Two different batches of each variant were purified and tested three times in TAS ELISA at a concentration of 20 ng per well. The MAbs and the PLRV-specific polyclonal antibodies from mouse were applied at different dilutions to compensate for differences in affinity (Table 1).

The polyclonal antibodies detected the two variants equally well (Table 1). The reactions obtained with the MAbs, however, differed markedly. WAU-A12, -A24, and -B9, which had previously been shown to detect quarternary epitopes, were significantly ($P < 0.001$) less reactive to the virus isolate from upper leaves than to the isolate from the bottom leaves. The subunit-specific MAbs, except for WAU-A13, all reacted equally well with both variants ($P < 0.05$). The epitope recognized by WAU-A13 was detected significantly more strongly on the readily transmitted PLRV variant from upper leaves than on the poorly transmissible one from the bottom leaves (Table 1).

Highly aphid-transmissible PLRV. A highly aphid-transmissible PLRV isolate (hat-PLRV) was obtained via biweekly transfers of PLRV-Wag under a selective transmission pressure. In each transmission series, 1-day-old nymphs were given a 24-h AAP on infected *P. floridana* plants, followed by a 6-h IAP on *P. floridana* seedlings. Infected plants from each transmission series served as a virus source in the next series. Thirty seedlings (three nymphs per plant) were inoculated in each transfer.

During the first two transfers, 3% of the plants became infected (Fig. 3), which is typical for PLRV-Wag with a 24-h AAP and a 6-h IAP. Beginning with the third transfer, the transmission efficiency of the isolate gradually increased until the sixth transfer, when about 70% of the test plants became infected (Fig. 3). The high aphid-transmissibility of this PLRV isolate could then be maintained in consecutive transfers to *P. floridana* even without applying the selection pressure. Homogenized leaf material infected with hat-PLRV was tested in TAS ELISA from the sixth transmission onward. It reacted strongly only with WAU-A13; no positive reaction was observed with any other MAb or with the PLRV-specific polyclonal antibodies from mouse.

Western blot analysis. As indicated in a previous report (20), none of the MAbs used in this study reacted with purified virus on western blots when hybridoma culture supernatants were tested. Viral capsid-associated proteins were detected, however, when the MAbs derived from ascitic fluids were applied at a high concentration (100-fold dilution of the antibody stocks). In this assay, the virus (500 ng per lane) was electrophoresed under denaturing conditions on a 4% stacking and a 12% separating polyacrylamide gel. Blots on nitrocellulose were incubated overnight in the MAb suspensions and in a PLRV-specific polyclonal antiserum from mouse (diluted 1,000-fold). Alkaline phosphatase-conjugated goat antimouse antibodies were applied as a secondary antibody. The MAbs previously shown to affect virus transmission were applied, and only WAU-A7 and -A13 revealed two bands of 23 and 56 kDa (Fig. 4).

DISCUSSION

Our results demonstrate that four PLRV-specific MAbs (WAU-A5, -A6, -A7, and -A13) significantly increase the LP_{50} of the virus in *M. persicae* when they are mixed with purified PLRV-Wag and fed to the aphids (Fig. 2). The other five MAbs tested did not affect the transmissibility of the virus in these immunoblocking experiments.

Competitive binding assays based on TAS ELISA indicated that the epitopes assigned by these four MAbs are topologically closely related and are located on individual subunits of the viral capsid (20). WAU-A5 and -A13 react with different epitopes, whereas WAU-A6 and -A7 are most likely directed against the same epitope (20).

Two virion-associated proteins of 23 and 56 kDa were identified by western blot analysis of purified PLRV-Wag (Fig. 4). The major coat protein species of 23 kDa is encoded by ORF 4, and the 56-kDa polypeptide is believed to be expressed by a read-through of the ORF 4 termination codon. Since both protein species were detected by both WAU-A7 and -A13, these MAbs

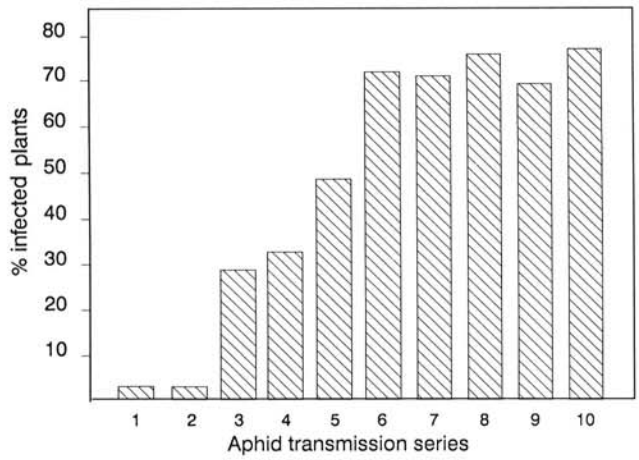

Fig. 3. Percentage of infected plants in a series of aphid transmissions designed to select a highly aphid-transmissible potato leafroll virus (PLRV) isolate (hat-PLRV) from the Wageningen isolate PLRV-Wag. After an acquisition access period of 24 h, three *Myzus persicae* nymphs were placed on a fresh *Physalis floridana* seedling for an inoculation access period of 6 h. Transfers were done at biweekly intervals. Infected plants from the previous transfer series served as virus source in the next series.

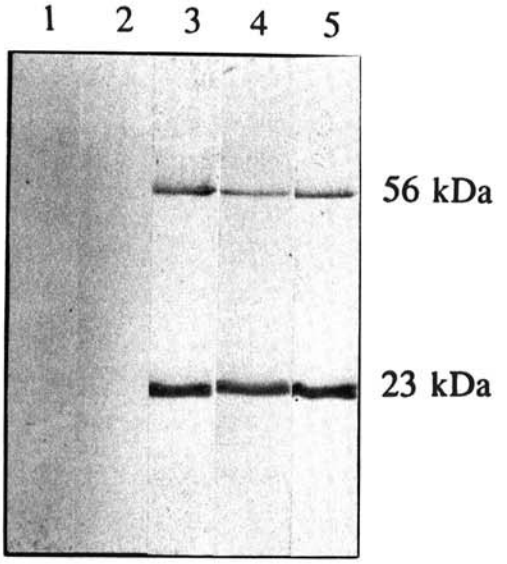

Fig. 4. Western blot analysis of virion-associated proteins from the Wageningen isolate of potato leafroll virus (PLRV). Purified virus (500 ng per lane) was separated under denaturing conditions on a 12% polyacrylamide gel and blotted onto nitrocellulose. Monoclonal antibodies WAU-A5 (lane 1), WAU-A6 (lane 2), WAU-A7 (lane 3), WAU-A13 (lane 4), and PLRV-specific polyclonal antibodies from mouse (lane 5) were applied as detecting antibodies.

are specific to the 23-kDa product. None of the MAbs in our panel specifically detected the 56-kDa polypeptide, which means that we found no direct evidence for the suggested involvement of this product in virus transmission (2,26). Also, the analysis of the readily and poorly transmissible variants of PLRV-Wag by polyacrylamide gel electrophoresis did not reveal any difference in the relative amounts of the readthrough protein present in those preparations (data not shown). Moreover, repeated freezing and thawing of a purified PLRV suspension did not abolish the readthrough product, as was shown for the NY-RPV isolate of barley yellow dwarf virus (25). The use of in vitro-expressed readthrough protein and readthrough product-specific antibodies in virus acquisition by aphids and mutational analysis of full-length cDNA clones of luteoviruses might provide more insight into the role of this protein in virus transmission.

Immunological studies of the two phenotypic variants of PLRV-Wag that differ in transmissibility by *M. persicae* revealed that the variants differed markedly in capsid integrity (Table 1). WAU-A13 was the only MAb tested that detected the readily transmissible variant significantly better than it detected the poorly transmissible one (Table 1). Moreover, this MAb also detected the highly aphid-transmissible isolate obtained by exerting selective pressure on PLRV-Wag, whereas the other MAbs did not. We conclude from these observations that the epitope detected by WAU-A13, or a surface domain closely related to it, is associated with the transmission of the virus.

Conformational alterations of these epitopes, caused by changing physiological conditions in aging leaves, may have changed the aphid transmission phenotype of PLRV-Wag in bottom leaves. With respect to the MAbs that presumably detect epitopes relying on the quarternary protein structure, it is remarkable that they all reacted better with the poorly transmissible variant from the lower leaves than with the readily transmissible one from the upper leaves (Table 1). Two of these MAbs, WAU-A24 and -B9, detected virus from the upper leaves only weakly. Since these MAbs are directed to epitopes that consist of amino acid residues of two joining protein subunits, it appears that the virus particles in the upper leaves occur in an expanded state. Specific sites, exclusively exposed by virions in this conformational state, may therefore play an essential role in intrinsic properties of the particle in processes such as virus transmission.

Massalski and Harrison (12) identified epitopes that depended on the quarternary protein conformation being involved in the passage of PLRV particles from the hemolymph to the salivary gland cells of *M. persicae*. The absence of these epitopes on a poorly transmissible PLRV isolate apparently did not influence virus acquisition by or retention in *M. persicae* (12). This apparent discrepancy in results with MAbs recognizing epitopes formed by the quarternary protein structure might indicate that the transcellular transport of luteovirus particles across cell linings of the aphid hindgut and the salivary glands is mediated by different protein domains on the viral capsid-associated proteins.

Transport of immunoglobulins across the gut epithelial lining and accumulation in the hemocoel have been reported for a number of insects (13,16,24). However, we were unable to show the presence of these proteins in extracts of *M. persicae* nymphs that fed for 3 days on antibody-containing diets. Apparently, the MAbs did not accumulate either in the gut lumen or in the hemocoel of *M. persicae*. The failure to detect the antibodies suggests that they degrade rapidly in the gut lumen of *M. persicae*. The increase in the LP_{50} measured after ingestion of MAb-treated PLRV-Wag by *M. persicae* could then be explained by a delay in the entry of the virus into the hemocoel of the aphid as a result of temporary blocking of a specific domain on the 23-kDa coat protein either directly or indirectly through steric hindrance.

LITERATURE CITED

1. Adam, G., Sander, E., and Shepherd, R. J. 1979. Structural differences between pea enation mosaic virus strains affecting transmissibility by *Acyrthosiphon pisum* (Harris). Virology 92:1-14.
2. Bahner, I., Lamb, J., Mayo, M. A., and Hay, R. T. 1990. Expression of the genome of potato leafroll virus: Readthrough of the coat protein termination codon in vivo. J. Gen. Virol. 71:2251-2256.
3. Barker, J. M., McInnes, J. L., Murphy, P. J., and Symons, R. H. 1985. Dot-blot procedure with [^{32}P]DNA probes for the sensitive detection of avocado sunblotch and other viroids in plants. J. Virol. Methods 10:87-98.
4. Briddon, R. W., Pinner, M. S., Stanley, J., and Markham, P. G. 1990. Geminivirus coat protein gene replacement alters insect specificity. Virology 177:85-94.
5. Chen, B., and Francki, R. I. B. 1990. Cucumovirus transmission by the aphid *Myzus persicae* is determined solely by the viral coat protein. J. Gen. Virol. 71:939-944.
6. Finney, D. J. 1962. Probit Analysis, a Statistical Treatment of the Sigmoid Response Curve. Cambridge University Press, Cambridge.
7. Gildow, F. E. 1987. Virus-membrane interactions involved in circulative transmission of luteoviruses by aphids. Pages 93-120 in: Current Topics in Vector Research, Vol. 4. K. F. Harris, ed. Springer-Verlag, New York.
8. Gildow, F. E. 1993. Evidence for receptor-mediated endocytosis regulating luteovirus acquisition by aphids. Phytopathology 83:270-277.
9. Harlow, E., and Lane, D. 1988. Antibodies; A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
10. Harrewijn, P. 1983. The effect of cultural measures on behaviour and population development of potato aphids and transmission of viruses. Meded. Fac. Landbouwwet. Rijksuniv. Gent 48:791-798.
11. Harrison, B. D., and Robinson, D. J. 1988. Molecular variation in vector borne plant viruses: Epidemiological significance. Pages 121-136 in: The Epidemiology and Ecology of Infectious Disease Agents. R. M. Anderson and J. M. Thresh, eds. The Royal Society, London.
12. Massalski, P. R., and Harrison, B. D. 1987. Properties of monoclonal antibodies to potato leafroll luteovirus and their use to distinguish virus isolates differing in aphid transmissibility. J. Gen. Virol. 68:1813-1821.
13. Nogge, G., and Giannetti, M. 1980. Specific antibodies: A potential insecticide. Science 209:1028-1029.
14. Rochow, W. F. 1970. Barley yellow dwarf virus: Phenotypic mixing and vector specificity. Science 167:875-878.
15. Rowhani, A., and Stace-Smith, R. 1979. Purification and characterization of potato leafroll virus. Virology 98:45-54.
16. Schlein, Y., Spira, D. T., and Jacobson, R. L. 1976. The passage of serum immunoglobulins through the gut of *Sarcophaga falculata* Pand. Ann. Trop. Med. Parasitol. 70:227-230.
17. Sylvester, E. S. 1965. The latent period of pea-enation mosaic virus in the pea aphid *Acyrthosiphon pisum* (Harris)—An approach to its estimation. Virology 25:62-67.
18. Sylvester, E. S. 1980. Circulative and propagative virus transmission by aphids. Annu. Rev. Entomol. 25:257-286.
19. van den Heuvel, J. F. J. M., Boerma, T. M., and Peters, D. 1991. Transmission of potato leafroll virus from plants and artificial diets by *Myzus persicae*. Phytopathology 81:150-154.
20. van den Heuvel, J. F. J. M., De Blank, C. M., Goldbach, R. W., and Peters, D. 1990. A characterization of epitopes on potato leafroll virus coat protein. Arch. Virol. 115:185-197.
21. van den Heuvel, J. F. J. M., and Peters, D. 1989. Improved detection of potato leafroll virus in plant material and in aphids. Phytopathology 79:963-967.
22. van den Heuvel, J. F. J. M., and Peters, D. 1990. Transmission of potato leafroll virus in relation to the honeydew excretion of *Myzus persicae*. Ann. Appl. Biol. 116:493-502.
23. van der Wilk, F., Huisman, M. J., Cornelissen, B. J. C., Huttinga, H., and Goldbach, R. W. 1989. Nucleotide sequence and organization of potato leafroll virus genomic RNA. FEBS Lett. 245:51-56.
24. Vaughan, J. A., and Azad, A. F. 1988. Passage of host immunoglobulin G from blood meal into hemolymph of selected mosquito species (Diptera: Culicidae). J. Med. Entomol. 25:472-474.
25. Vincent, J. R., Lister, R. M., and Larkins, B. A. 1991. Nucleotide sequence analysis and genomic organization of the NY-RPV isolate of barley yellow dwarf virus. J. Gen. Virol. 72:2347-2355.
26. Vincent, J. R., Ueng, P. P., Lister, R. M., and Larkins, B. A. 1990. Nucleotide sequences of coat protein genes for three isolates of barley yellow dwarf virus and their relationships to other luteovirus coat protein sequences. J. Gen. Virol. 71:2791-2799.
27. Waterhouse, P. M., Gildow, F. E., and Johnstone, G. R. 1988. Luteovirus group. No. 339 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, England.