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The N-Terminal Region of the Luteovirus Readthrough Domain Determines Virus Binding to *Buchnera* GroEL and Is Essential for Virus Persistence in the Aphid

J. F. J. M. VAN DEN HEUVEL,[1]* A. BRUYÈRE,[2] S. A. HOGENHOUT,[1] V. ZIEGLER-GRAFF,[3] V. BRAULT,[3] M. VERBEEK,[1] F. VAN DER WILK,[1] AND K. RICHARDS[3]

Department of Virology, DLO Research Institute for Plant Protection (IPO-DLO), 6700 GW Wageningen, The Netherlands,[1] and Station de Recherche Grandes Cultures, INRA, Colmar 68021 Cédex,[2] and Institut de Biologie Moléculaire des Plantes du CNRS et de l'Université Louis Pasteur, Strasbourg,[3] France

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Luteoviruses and the luteovirus-like pea enation mosaic virus (PEMV; genus *Enamovirus*) are transmitted by aphids in a circulative, nonreplicative manner. Acquired virus particles persist for several weeks in the aphid hemolymph, in which a GroEL homolog, produced by the primary endosymbiont of the aphid, is abundantly present. Six subgroup II luteoviruses and PEMV displayed a specific but differential affinity for *Escherichia coli* GroEL and GroEL homologs isolated from the endosymbiotic bacteria of both vector and nonvector aphid species. These observations suggest that the basic virus-binding capacity resides in a conserved region of the GroEL molecule, although other GroEL domains may influence the efficiency of binding. Purified luteovirus and enamovirus particles contain a major 22-kDa coat protein (CP) and lesser amounts of an ~54-kDa readthrough protein, expressed by translational readthrough of the CP into the adjacent open reading frame. Beet western yellows luteovirus (BWYV) mutants devoid of the readthrough domain (RTD) did not bind to *Buchnera* GroEL, demonstrating that the RTD (and not the highly conserved CP) contains the determinants for GroEL binding. In vivo studies showed that virions of these BWYV mutants were significantly less persistent in the aphid hemolymph than were virions containing the readthrough protein. These data suggest that the *Buchnera* GroEL-RTD interaction protects the virus from rapid degradation in the aphid. Sequence comparison analysis of the RTDs of different luteoviruses and PEMV identified conserved residues potentially important in the interaction with *Buchnera* GroEL.

Species of the genus *Luteovirus* occur worldwide and infect a wide range of mono- and dicotyledonous plants, in which they replicate almost exclusively in the phloem tissue (45, 60). Two subgroups (I and II) are recognized within the genus based on genome organization and the type of RNA-dependent RNA polymerase (48). Luteoviruses are persistently transmitted by aphids in a circulative manner. Briefly, virions are ingested with phloem sap from infected plants and transcellularly transported through the gut into the hemocoel by receptor-mediated endocytosis-exocytosis (22). The hemolymph acts as a reservoir in which acquired virus particles are retained in an infective form for the aphid's lifespan, without replication (18). Upon contacting the basal lamina of the accessory salivary gland, virus particles may be transported through this gland, eventually arriving in the salivary duct from which they are excreted with the saliva when the aphid feeds (23). The high degree of vector specificity of luteoviruses among aphid species implies an intimate relationship between the surface domains of the viral capsid and aphid components (22, 61).

Symbionin (32), a protein released by the primary endosymbiotic bacteria (genus *Buchnera*) of aphids into the hemolymph, appears to be essential for luteovirus transmission (61). Symbionin-like molecules are immunologically closely related and share more than 80% sequence identity with the *Escherichia coli* heat shock protein GroEL, a member of the chaperonin 60 family (20, 50, 61). Chaperonins are essential for cell viability, since they bind and stabilize newly translated or translocated aggregation-prone polypeptides (8) and mediate their functional folding and assembly in an ATP-dependent manner (16, 24, 29). The structural characteristics of *Buchnera* GroEL are highly similar to those of GroEL of *E. coli* (27, 28, 50), and there is extensive amino acid sequence homology in functionally significant regions with *E. coli* GroEL (20, 50). Moreover, *Buchnera* GroEL from *Acyrthosiphon pisum* has been shown to be functional as a folding and assembly factor in a GroEL-deficient *E. coli* strain (50), to possess ATPase activity, and to be able to reconstitute dimeric ribulose 1,5-biphosphate carboxylase-oxygenase (RuBisCO) from its unfolded subunits in vitro (34). However, unlike *E. coli* GroEL, *Buchnera* GroEL is not restricted to the cytosol of the bacteria. It occurs at a high concentration extracellularly in the aphid hemolymph (20, 61).

Ligand binding assays have shown that potato leafroll virus (PLRV; subgroup II) and barley yellow dwarf virus (BYDV; subgroup I) have a high specific affinity for GroEL homologs of both vector and nonvector species (20, 61). Antibiotic treatment of *Myzus persicae* larvae dramatically decreased symbionin levels in the hemolymph, which was accompanied by inhibited transmissibility of PLRV and loss of capsid integrity in the hemolymph (61). These observations have led to the suggestion that luteoviruses associate with *Buchnera* GroEL in the hemolymph to retard proteolytic breakdown (20, 61).

The importance of the viral capsid in determining aphid transmissibility has been convincingly demonstrated (54). Two capsid-associated proteins have been detected: the major capsid protein (CP) with a molecular mass of ~22 kDa, which is encoded by open reading frame (ORF) 3; and a minor

* Corresponding author. Mailing address: Department of Virology, DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands. Phone: 31 317 476141. Fax: 31 317 410113. E-mail: J.F.J.M.vandenHeuvel@IPO.DLO.NL.

polypeptide, the readthrough domain (RTD), which is expressed as a result of translational readthrough of the ORF 3 termination codon into the neighboring ORF 5 (2, 14, 53, 66). In extracts of infected plants or protoplasts, the ORF 3-ORF 5 fusion protein of ~74 kDa is readily detected; however, in purified virus particles, the readthrough protein exists as a truncated ~54-kDa form which lacks the C-terminal region of the RTD (2, 6, 21, 45, 68). The truncated RTD is exposed on the surface of the virus particle and contains determinants necessary for virus transmission by aphids (6, 9, 20, 33).

In this paper, we show that six subgroup II luteoviruses and the luteovirus-like pea enation mosaic virus (PEMV; genus *Enamovirus*) can bind to native GroEL homologs derived from *Buchnera* spp. of vector and nonvector aphids. In contrast to the situation reported for BYDV (20), we have found that the aforesaid viruses also readily bind to *E. coli* GroEL. Using beet western yellows luteovirus (BWYV) mutants with deletions in the RTD, we demonstrate that the presence of the RTD is indispensable for the interaction with GroEL. Finally, we have tested the fate of the BWYV RTD deletion mutants in the hemolymph of *M. persicae* and show that RTD-less virions are less persistent in the aphid.

MATERIALS AND METHODS

Aphids. A parthenogenic line of *M. persicae* biotype WMp2 was reared on *Brassica napus* subsp. *oleifera* at 20 ± 3°C under 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, which were confined to leaf cages, to fresh plants. Clones of *A. pisum* and *Rhopalosiphum padi* were maintained under similar conditions on *Pisum sativum* and *Avena sativa*, respectively.

Viruses and antibodies. PLRV and BWYV were maintained on *Physalis floridana*, and bean leafroll virus (BLRV) was maintained on *P. sativum* by repeated aphid transfers. The viruses were purified from frozen leaf material by a modified enzyme-assisted (Cellulase R-10 and Macerozyme R-10; Yakult Honsha Co., Tokyo, Japan) procedure (59). Purified virus was stored at −80°C in 0.1 M sodium citrate (pH 6.0) containing 25% sucrose. Purified beet mild yellowing virus (BMYV), cucurbit aphid-borne yellows virus (CABYV), and soybean dwarf virus (SDV) and their homologous antisera were kindly provided by O. Lemaire (INRA, Colmar, France), H. Lecoq (INRA, Avignon, France), and V. Damsteegt (USDA-ARS, Frederick, Md.), respectively. An aphid-transmissible isolate of PEMV was purified from *P. sativum* (12), and anti-PEMV immunoglobulin G (IgG) was obtained from S. Demler (Michigan State University, East Lansing, Mich.). Carnation ringspot dianthovirus (CRSV) was kindly provided by S. Lommel (North Carolina State University, Raleigh, N.C.) and was purified from *Nicotiana clevelandii* (42). Anti-BLRV was a gift of L. Katul (BBA, Braunschweig, Germany). The antiserum to native *Buchnera* GroEL from *M. persicae* was raised according to previously described procedures (61).

Purification of *Buchnera* and *E. coli* GroEL. Native tetradecameric *Buchnera* GroEL was purified from 5- to 6-day-old aphids as described before (34) with modifications. Aphids (0.25 g) were homogenized in 10 ml of phosphate-buffered saline (PBS; 2 mM KH_2PO_4, 8 mM Na_2HPO_4, 0.14 M NaCl, 2 mM KCl) containing 0.5% Triton X-100 and 0.1 mM phenylmethylsulfonyl fluoride. The homogenate was sonicated for 30 s with a Vibra Cell (Sonics & Materials, Inc., Danbury, Conn.) and centrifuged at 10,000 × *g* for 15 min to remove the debris. A 40% polyethylene glycol 8,000 solution was added to the supernatant, to a final concentration of 8%. The suspension was then incubated for 1.5 h on ice, followed by centrifugation at 18,000 × *g* for 20 min. The pellet was resuspended in 50 mM Tris-HCl (pH 7.6) containing 35 mM KCl, 25 mM NH_4Cl, 10 mM MgAc, and 1 mM dithiothreitol, and the suspension was incubated for 1 h on ice and centrifuged at 18,000 × *g*. The supernatant was sedimented through a 10 to 50% linear sucrose gradient in a Beckman SW41 rotor at 30,000 rpm for 16 h. The GroEL-containing bands were identified by Western blot analysis with an antiserum raised to *Buchnera* GroEL of *M. persicae*. *E. coli* GroEL was purified from DH5α cells grown at 37°C until an optical density at 600 nm of 0.6 was attained, when they were transferred to 45°C for 16 h (35). The cells were pelleted, resuspended in PBS, and sonicated three times for 1 min. Further purification was carried out as described for *Buchnera* GroEL. All steps were done at 4°C. Approximately 200 to 250 μg of the native protein was obtained from 100 mg (wet weight) of aphids or pelleted *E. coli* cells. GroEL suspensions were stored at −80°C.

BWYV mutants from agroinfected plants. The recombinant binary vectors containing wild-type BWYV full-length cDNA and BWYV RTD deletion mutants have been described previously (6, 7, 65) and are summarized in Fig. 3. BW_0 represents the wild-type construct (65). In mutant BW6.4, the entire RTD has been eliminated by deletion and frameshifting (53). The other constructs used, BW6.51, BW6.106, BW6.104, BW6.ΔTB, BW6.50, BW6.ΔE1, BW6.40, and BW6.41, contained short in-frame deletions at different locations in the RTD (7). *N. clevelandii* plants were agroinoculated according to previously described procedures (39). Infected plants were identified by enzyme-linked immunosorbent assaying (ELISA) (59) with BWYV-specific IgG, and virus was purified as described above.

GroEL ligand assay. Immunoplates (Maxisorp F96; Nunc, Roskilde, Denmark) were sensitized with 100 μl of 10 μg of purified GroEL per ml of 0.05 M sodium carbonate (pH 9.6) (coating buffer) for 16 h at 4°C and incubated with 100 μl of purified virus at a concentration of 10-μg/ml SEB (PBS containing 0.05% Tween 20, 1% polyvinyl pyrrolidone, and 0.1% ovalbumin) for 16 h at 4°C. Then, the homologous IgGs at 1 μg/ml in SEB were incubated for 3 h at 37°C. The antigen-bound primary antibodies were detected by goat-anti-rabbit IgG alkaline phosphatase conjugate (Sigma, St. Louis, Mo.) in SEB for 3 h at 37°C. The amount of immobilized alkaline phosphatase was revealed by adding 1 mM *p*-nitrophenyl phosphate (disodium salt) in 10% diethanolamine (pH 9.8). Color development at 405 nm was measured with a Bio-Kinetics Reader EL312 (Bio-Tek Instruments, Inc., Winooski, Vt.).

Mg-ATP dissociation of GroEL. *Buchnera* GroEL was dissociated by procedures previously described for *E. coli* GroEL (41). Briefly, 2 μg of purified GroEL was incubated in 50 μl of 50 mM Tris-HCl (pH 7.5) containing 5 mM $MgCl_2$, 5 mM ATP, 5 mM creatinine phosphate (sodium salt), and 2 U of creatine phosphokinase for 30 min at 20°C. Subsequently, 25 μl of 40% sucrose was added and 10-μl samples were loaded onto an sodium dodecyl sulfate (SDS)-free 4% polyacrylamide gel. Following polyacrylamide gel electrophoresis (PAGE), proteins were transferred to nitrocellulose (57), after which immunodetection with anti-*Buchnera* GroEL IgG and alkaline phosphatase-conjugated anti-rabbit IgG was carried out (61).

Aphid microinjection. Seven-day-old *M. persicae* nymphs were microinjected (49) with 60 nl of purified virus at 80 μg/ml by using calibrated glass capillaries (Gabay Instruments, Geneva, Switzerland). The microinjected aphids were transferred to healthy potato plants which were maintained at 20 ± 0.1°C and with 16 h of light per day. After 2, 6, 24, 72, and 120 h, batches of three aphids were collected and stored at −80°C until further processing by triple-antibody-sandwich (TAS)-ELISA.

Detection of virus by TAS-ELISA. Prior to sample incubation, the immunoplates were coated with 150 μl of 1-μg/ml anti-BWYV IgG in coating buffer for 16 h at 4°C. The samples consisted of three aphids triturated in 150 μl of SEB and were incubated for 16 h at 4°C. Viral antigen was detected with monoclonal antibody WAU-A12 (59) and goat-anti-mouse IgG linked to alkaline phosphatase.

RESULTS

Purification of GroEL. Native tetradecameric GroEL was isolated from the endosymbiotic bacteria of *M. persicae*, *A. pisum*, *R. padi*, and from *E. coli* cells. SDS-PAGE of GroEL revealed a single ~60-kDa band corresponding to GroEL subunits (Fig. 1A, lane 1). *Buchnera* GroEL, like *E. coli* GroEL, is an oligomer of 14 identical subunits arranged into two stacked heptameric rings (5, 20, 28). PAGE of purified GroEL under nondenaturing conditions, followed by Western blot analysis with an antiserum to *Buchnera* GroEL, revealed a single band of 14-meric GroEL (Fig. 1B, lane 1). Electron microscopy of the purified *M. persicae* GroEL suspension clearly showed the multimeric nature of the protein (Fig. 1C). As observed for GroEL of *E. coli* (43) and *Buchnera* GroEL of *A. pisum* (34), *Buchnera* GroEL of *M. persicae* underwent partial dissociation into lower-molecular-mass species upon incubation with Mg-ATP (Fig. 1B, lane 2). However, no dissociation was observed when the GroEL 14-mer was incubated overnight in ELISA coating buffer (Fig. 1B, lane 3), indicating that the protein retains it oligomeric state under the conditions used during its immobilization onto immunoplates for tests of luteovirus binding (see below). The purified GroEL proteins from *A. pisum*, *R. padi*, and *E. coli* behaved similarly in the aforesaid tests (data not shown). Finally, nondenaturing PAGE of hemolymph samples taken directly from *M. persicae* (61) established that the GroEL 14-mer prevails in the aphids' body fluid (Fig. 1B, lanes 1, 2, and 4).

GroEL binding of luteoviruses and PEMV. Purified GroEL proteins from *M. persicae*, *A. pisum*, *R. padi*, and *E. coli* were immobilized onto immunoplates, and their affinities for six subgroup II luteoviruses (BWYV, BMYV, PLRV, CABYV, BLRV, and SDV) and PEMV were tested in the GroEL ligand

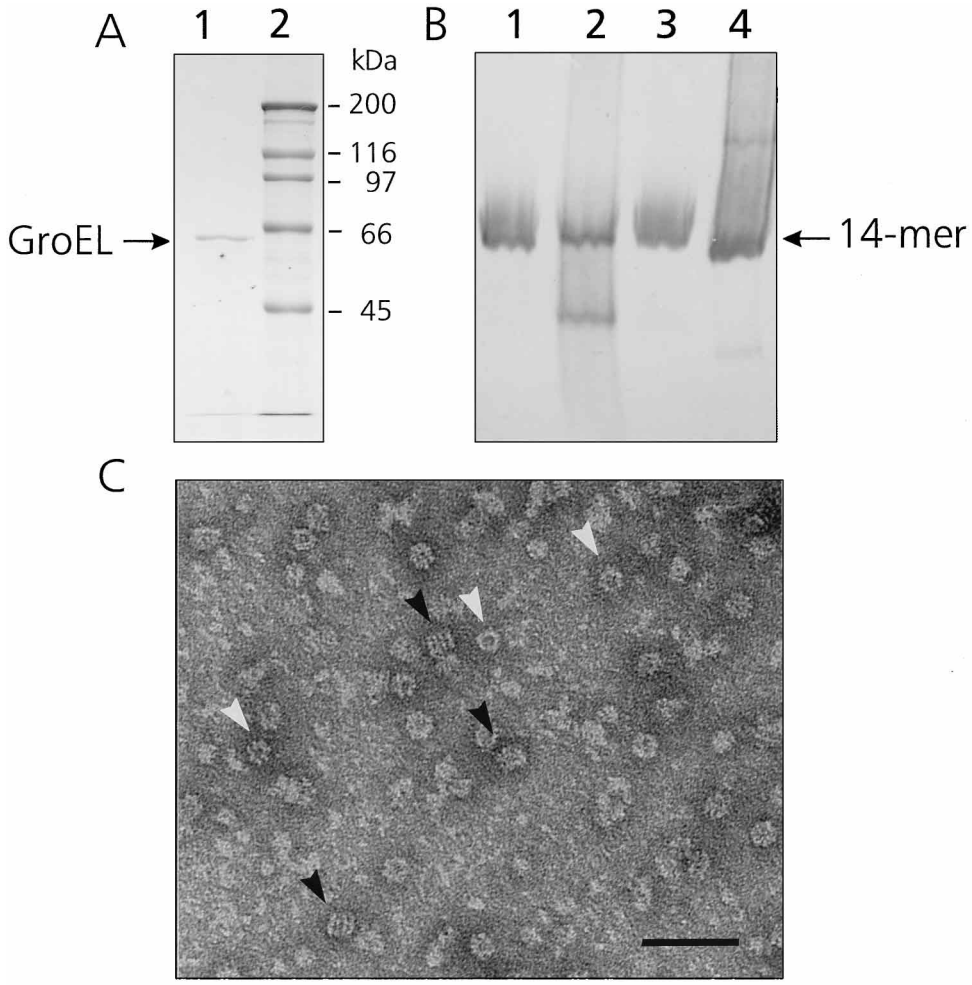


FIG. 1. Characterization of purified *Buchnera* GroEL from *M. persicae*. (A) A 1-μg amount of sucrose density gradient-isolated GroEL on an SDS-8.5% PAGE stained with Coomassie brilliant blue (lane 1). Lane 2, molecular markers. (B) Nondenaturing PAGE of native and dissociated GroEL followed by Western blot analysis with anti-*Buchnera* GroEL IgG. Lanes: 1, native GroEL; 2, Mg-ATP-incubated GroEL; 3, GroEL incubated overnight in ELISA coating buffer; 4, hemolymph sample from *M. persicae*. (C) Electron micrograph of GroEL oligomeric complexes stained with 2% (wt/vol) uranyl acetate. Black and white arrows indicate side and top views of GroEL, respectively. Bar, 50 nm.

assay (Fig. 2). PEMV was included because of the striking similarities with luteoviruses concerning aphid transmissibility, genomic organization of PEMV RNA 1, and incorporation of a 55-kDa coat protein-RTD polypeptide in the viral capsid (13).

The results show that PLRV bound to *Buchnera* GroEL from the nonvector aphids *A. pisum* and *R. padi* and to *E. coli* GroEL with an avidity similar to that of *M. persicae*, its primary vector (Fig. 2A). All luteoviruses tested and PEMV also bound to the four GroEL homologs (Fig. 2), but with different affinities, which were not related to whether GroEL was derived from a vector or a nonvector aphid. Thus, BWYV and BMYV bound more efficiently to *Buchnera* GroEL of *A. pisum*, a rather poor vector (31), than to the GroEL of the efficient vector *M. persicae* (Fig. 2C). Likewise, *A. pisum* is a very efficient vector of BLRV, SDV, and PEMV; however, these viruses did not bind to *A. pisum* GroEL with an affinity notably higher than that to the GroEL proteins from other sources (Fig. 2B, E, and F). The finding that these viruses have affinity for GroEL homologs derived from aphid species which do not transmit or inefficiently transmit them is entirely consistent with the observations that aphids can acquire and retain luteoviruses that they do not transmit (46, 55). Plant viruses which are not aphid transmitted in a circulative fashion did not show affinity for any of the GroELs in the ligand-binding assay (data not shown). The viruses tested were from the genera *Potyvirus* (blackeye cowpea mosaic virus), *Tospovirus* (tomato spotted wilt virus), *Comovirus* (cowpea mosaic virus), *Furovirus* (beet necrotic yellow vein virus), and *Dianthovirus* (CRSV). Because CRSV is a spherical virus with dimensions similar to those of the luteoviruses, it was used as a negative control in the remaining experiments.

Identifying the region on the BWYV capsid implicated in GroEL binding. The fact that different luteoviruses all have affinities for *Buchnera* GroEL suggests that a conserved feature(s) of the luteovirus capsid is involved. Comparing the derived amino acid sequences of the luteovirus major CPs (12, 25, 26, 53, 58, 62, 66) revealed that 25% of the residues are identical. The N-terminal half of the RTD, which is also present in purified luteovirus particles (see introduction), contains 16% of globally identical residues. Global sequence identity in the C-terminal region of the RTDs, which are not present in purified particles, was negligible.

To ascertain which of the two capsid-associated proteins is

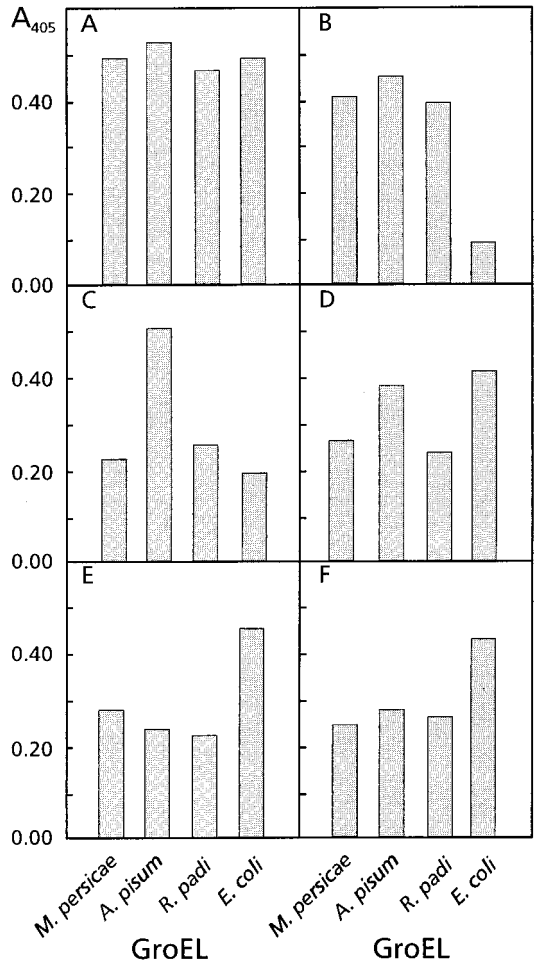


FIG. 2. Affinity binding of PLRV (A), BLRV (B), BWYV (C), CABYV (D), SDV (E), and PEMV (F) to GroEL homologs purified from the endosymbiotic bacteria of *M. persicae*, *A. pisum*, and *R. padi* and from *E. coli*. All samples were tested in duplicate, and the mean absorbency values at 405 nm (A_{405} [ELISA value]) are given. Binding data for BMYV are comparable to those for BWYV (not shown). Virus binding to ovalbumin and CRSV binding to the GroEL homologs (negative controls) gave ELISA values of less than 0.02.

responsible for the interaction with *Buchnera* GroEL, experiments with particles of BWYV mutants engineered to contain deletions of different portions in the RTD were carried out. In mutant BW6.4 (53), the entire RTD had been eliminated by deletion and frameshifting (Fig. 3). In mutants BW6.ΔE1, BW6.40, and BW6.41, in-frame deletions eliminated 85, 128, and 79 amino acid residues from the C-terminal half of the RTD (see reference 7 for descriptions of these and the following mutants). In mutant BW6.50, 33 residues spanning the junction between the conserved and the nonconserved portions of the RTD were eliminated, and, in mutants BW6.51, BW6.106, BW6.104, and BW6ΔTB, 21, 7, 15, and 39 residues, respectively, of the conserved N-terminal portion of the RTD were deleted. We have shown elsewhere (7) that no readthrough protein can be detected in virions of any of the conserved domain mutants and that trace amounts were present in only some preparations of BW6.50 virions (7). By contrast, particles of BW6.ΔE1, BW6.40, and BW6.41 contain C-terminally truncated readthrough protein in amounts similar to those observed in wild-type virions (7).

When used in the GroEL ligand assay, the mutants with deletions in the C-terminal half of the RTD, which produce particles which are similar in capsid composition to wild-type BWYV, bound as efficiently to *Buchnera* GroEL of *M. persicae* as did wild-type virus (Fig. 3). The mutants which produced particles that were deficient in the RTD, on the other hand, did not bind (Fig. 3). The fact that BW6.4, whose capsid contains only CP subunits, did not show affinity for *Buchnera* GroEL indicates clearly that this protein is not directly involved in binding. Therefore, we conclude that it is the RTD and, more particularly, the conserved N-terminal half of the RTD which is implicated in the interaction with *Buchnera* GroEL.

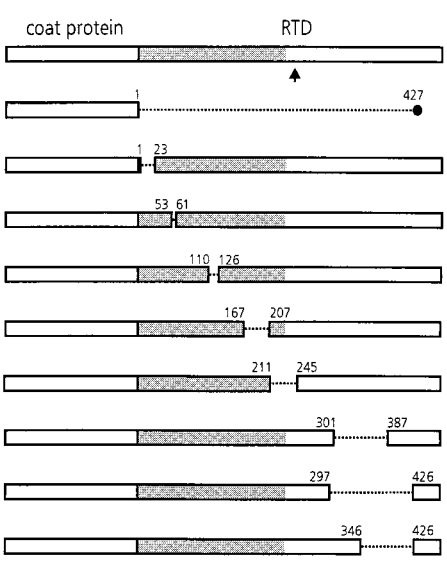
Fate of microinjected BWYV mutants. To investigate the role of the RTD-*Buchnera* GroEL interaction in vivo, purified wild-type virus (BW_0) and BW6.ΔE1, which both bind to *Buchnera* GroEL, and two nonbinding mutants, BW6.4 and BW6.50, were microinjected directly into the aphid's hemocoel. Although virus particles devoid of the RTD are stable in the intestine of the aphid (6) and are able to cross the gut epithelium (9), the gut-hemocoel interface was bypassed in these experiments, since it is not clear whether the RTD modulates the efficiency of the passage. The fate of the viruses in the microinjected aphids was monitored by TAS-ELISA with a monoclonal antibody that reacts with quaternary surface epitopes of the virus (59) and thus specifically recognizes intact virus particles. The ELISA readings (Fig. 4) directly reflect the amount of virus present in the aphid.

Wild-type BWYV and the GroEL-binding mutant BW6.ΔE1 were readily detected in the microinjected aphids, and, although a slight decline in virus titer was visible, the total amount of virus present at 120 h postinjection was still ~67% of the amount injected. The virus titer declined slowly and gradually over the entire experimental period: about 16% during the first 2 h and 21% from 2 to 120 h. In contrast, the level of the nonbinding BWYV mutants BW6.4 and BW6.50 declined rapidly immediately after injection and thereafter. During the first 2 h, a greater-than-60% decline in virus content was observed, and from 2 to 120 h the level fell by another 70%. In total, only 10% of the injected virus was detected at 120 h postinjection.

DISCUSSION

The RTD plays an important role in the infection cycle of luteoviruses; it harbors determinants implicated in the accumulation of virus in plants after agroinfection (6, 9, 71) and in virus transmission by aphids (6, 7, 20, 33, 68). With respect to aphid transmission, PEMV resembles a luteovirus, since it requires the RTD to be incorporated in the viral capsid (12). Here, we have shown by mutational analysis of a full-length infectious clone of BWYV that the RTD is also important for the interaction with *Buchnera* GroEL in vitro (Fig. 3) and that this interaction might determine virus retention by the aphid in vivo (Fig. 4). BWYV mutants deficient in the RTD were quickly degraded in the aphid hemolymph after microinjection. These data corroborate earlier findings on the loss of PLRV capsid integrity in *Buchnera* GroEL-deficient aphids (61).

In vitro binding to *Buchnera* GroEL is a phenomenon common to all plant viruses transmitted by aphids in a circulative nonreplicative manner. Six subgroup II luteoviruses (Fig. 2), BYDV-PAV (subgroup I [20]), and the type species of the genus *Enamovirus*, PEMV (Fig. 2), all displayed a strong affinity for native GroEL homologs from endosymbiotic bacteria of aphids. Thus, it is most likely that highly conserved regions on the RTDs of luteoviruses and PEMV are involved. *E. coli* GroEL binds substrate polypeptides by an apparent hydrophobic interaction (40). Structural features recognized by GroEL



	RTD incorporation	Transmissibility	GroEL-binding (A_{405})
BW_0	+ + +	+	0.501 ± 0.014
BW 6.4	− − −	−	0.017 ± 0.011
BW 6.51	− − −	−	0.012 ± 0.015
BW 6.106	− − −	−	0.018 ± 0.002
BW 6.104	− − −	−	0.009 ± 0.001
BW 6.ΔTB	− − −	−	0.008 ± 0.010
BW 6.50	− − +	−	0.020 ± 0.017
BW 6.ΔE1	+ + +	+	0.460 ± 0.100
BW 6.40	+ + +	+	0.462 ± 0.117
BW 6.41	+ + +	+	0.493 ± 0.080

FIG. 3. Affinity binding of BWYV RTD mutants to *Buchnera* GroEL of *M. persicae*. The structures of the readthrough proteins of wild-type BWYV (BW_0) and the various deletion mutants (7) are shown to the left. The conserved portion of the RTD (see text) is shaded; vertical arrow, the approximate site of cleavage to yield the C-terminally truncated form of readthrough protein associated with purified wild-type virus. The deletion in each mutant is indicated by a dotted line, and the numbering refers to the amino acid coordinates of the deletion boundaries relative to the beginning of the RTD (see Fig. 5). The deletion in mutant BW6.4 provoked a frameshift, and the resulting missense sequence is indicated by a small circle. The GroEL-binding data to the right give the amount of virus (as measured by ELISA) which bound to immobilized *Buchnera* GroEL from *M. persicae* in the GroEL ligand assay. The values are the mean ELISA readings (A_{405} ± standard errors) for three samples from different batches of purified virus. CRSV was used as a negative control and yielded a mean A_{405} value of 0.023. Data on the ability of aphids to transmit the mutants and on the incorporation of the RT protein into purified virions are taken from reference 7.

are predominantly hydrophobic surfaces typically exposed by partially folded polyproteins (30) but also certain amino acid sequence patterns (36) and specific secondary structures (56). Comparing the deduced amino acid sequences of the luteovirus and PEMV RTDs revealed that only the N-terminal half of the readthrough proteins are conserved and contain hydrophobic regions (Fig. 5). The C-terminal halves of the luteovirus RTDs, from residue 241 onward (Fig. 5), have no significant sequence identity, nor do they contain regions of a hydrophobic nature. Furthermore, most of the C-terminal region is missing from the PEMV RTD. Therefore, it is concluded that the GroEL binding capacity resides in the N-terminal conserved region of the RTD. This coincides well with the size of the RTD present in purified virus particles. Based on mutational analysis and mass spectroscopy, the calculated C terminus of the truncated RTD of BYDV-PAV was mapped to amino acid residue 242 (Fig. 5) (21). The highest overall level of sequence similarity in the RTD extends from positions 184 to 223 (Fig. 5), where about 23% of the residues are identical. Moreover, this region is relatively hydrophobic compared to the rest of the RTD. Amino acid replacement studies are required to verify whether the determinants for the interaction with *Buchnera* GroEL reside in this region of the RTD.

In addition to *Buchnera* GroEL binding, all luteoviruses and PEMV showed affinity for GroEL of *E. coli* (Fig. 2), which indicates that the capacity of GroEL to interact with these viruses resides in a conserved part on the GroEL molecule rather than in a variable domain as was previously suggested (20). Three domains are distinguished on the GroEL subunit: the apical and the equatorial domains, in which polypeptide and nucleotide binding sites are located, and the intermediate domain, which harbors ATPase activity and potential hinge functions, allowing allosteric movement of the other domains relative to each other (5, 10). In *E. coli*, GroEL facilitates productive folding through cycles of protein binding and release, which is a process which may require ATP hydrolysis. The ATPase activity of the GroEL subunit is regulated by GroES (44, 67), a single heptameric ring of 10-kDa subunits also encoded by the *GroE* operon. Functional chaperonins may involve symmetrical or asymmetrical GroEL-GroES complexes (17), and different binding sites on the GroEL molecule are involved. In the asymmetrical complex, with GroES blocking one end of the GroEL cylinder, the monomeric substrate binds within the cylinder at the level of the apical domains (37) which expose a putative binding surface toward the cavity (4, 10, 19, 47). Although it was previously suggested that luteoviruses may bind to the apical domain of *Buchnera* GroEL or to its flexible C termini which are projected into the cylinder (20), this is highly unlikely to occur in the aforesaid manner, since the dimensions of the luteovirus particle (diameter, 23 nm) should prohibit virions from entering the central cavity, which is approximately 50 to 80 Å wide (10). Typically, the GroEL cylinder accommodates substrates ranging from 15 to 60 kDa (reviewed in reference 29).

On the other hand, symmetrical GroEL-GroES complexes (with the central cavity capped on both sides by GroES) stably bind and assist the folding and assembly of large multimeric macromolecules such as RuBisCO and malate dehydrogenase on their external envelope (1). The equatorial domain seems to be responsible for binding of these large multimeric macromolecules (69). It may well be that luteoviruses and PEMV employ sites on the GroEL subunit similar to those of RuBisCO and malate dehydrogenase, thus overcoming the size limitations imposed by the central cavity. Interestingly, it was shown that in the absence of GroES, GroEL also binds polypeptides with unstable secondary structure and transiently maintains them in a soluble, folding-competent conformation (3, 4, 10, 19, 37, 38). This observation may be of importance in understanding the luteovirus-*Buchnera* GroEL interaction. Although the *Buchnera GroE* operon accommodates a gene for a

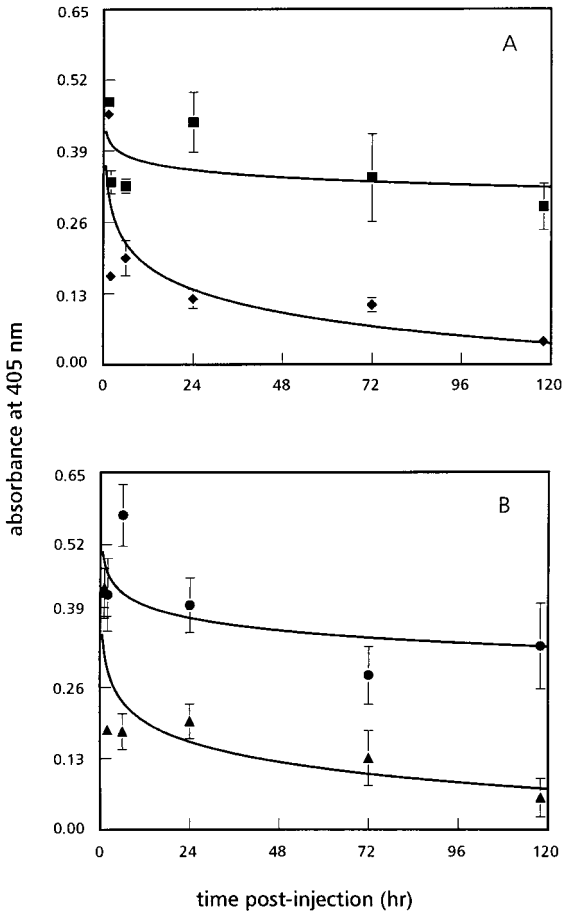


FIG. 4. Retention of BWYV wild-type and readthrough deletion mutants in *M. persicae* tested by ELISA (A_{405}). (A) Wild-type BW_0 (■) and BW6.4 (◆). Each point represents the mean (± standard error) of three samples from three aphids. (B) BW6.ΔE1 (●) and BW6.50 (▲). Seven samples from three aphids were tested. The aphids were microinjected with ~5 ng of virus. Due to rapid degradation of the BWYV mutants BW6.4 and BW6.50, time zero of the four viruses was established by adding purified virus directly to aphid homogenates. Mock-microinjected aphids gave ELISA values of less than 0.01 (not shown).

```
      1                                                  36
BWYV  VDEE..... .PGPSPGPSP SPQ......P TPQK.KYRFI VYTGVPVTRI
BMYV  VDKE..... .PGPSPGPSP SPQ......P TPSK.KYRFI VYTGVPVTRI
PLRV  VDSG..... .PEPGPSPQP TPT......P TPQK.HERFI AYVGIPMLTI
 SDV  VDGE..... .PGPKPGPDP APQPTPTPKP TPAK.HERFI AYTGTLSTLI
CABYV VDGS..... .SPPPPSPSP TPPPPPPPQP QPQPCAQRFW GYEGNPQNKI
BYDV  VDSSTSEPQP APEPTPTPQP TPAPQPAPEP TPAPVPKRFF EYIGTPTGTI
PEMV  GDD..... .APPSPGPDP GPQPPPPPPP SPTPVGARFW GYEGVPESRM
       *                 * * * * *       *  *     **   * *

      37                                                  86
BWYV  MAQSTDDAIS LYDMPSQRFR YIEDENMNWT NLDSRWYSQN SLKAIPMIIV
BMYV  MAQSTDDAIS LYDMPSQRFR YIEDENMNWT NLDSRWYSQN SLKAIPMIIV
PLRV  QARESDDQII LGSLGSQRMK YIEDENQNYT NVSSEYYSQS SMQAVPMYYF
 SDV  SARQSSDSIS LYSIRNQRIR YIEDENSSWT NIDAKWYSQN SVEAIPMFVY
CABYV LTAENSRNID SRPLNFVQMY KWEDEKWDKV NLQAGYSRND RRCMETYLTI
BYDV  STRENSDSIS VSKLGGQSMQ YIENEKCETK VIDSFWSTNN NVSAQAAFVY
PEMV  ISERNDHDID VKPLSFITMY KWEDESWTSV KLSASYLQND QVEATPYFLI
            *                 * *

      87                                                 136
BWYV  PVPQGEWTVE ISMEGYQPTS STTDPNKDKQ DGLIAYNDDL SEGWNVGIYN
BMYV  PVPQGEWTVE ISMEGYQPTS STTDPNKDKQ DGLIAYNDDL KEGWNVGVYN
PLRV  NVPKGQWSVD ISCEGYQPTS STSDPNRGRS DGMIAYSNAD SDYWNVGEAD
 SDV  PVPEGTWSIE ISCEGYQAAS STSDPHRGKC DGMIAYDDDS SKVWNVGQQN
CABYV PADKGKFHVY LEADGEFVVK HIGDELDGSW LGNIAY.DVS QRGWNVGNYK
BYDV  PVPEGSYSVN ISCEGFQSVD HIGGNEDGYW IGLIAYSNSS GDNWGVGNYK
PEMV  PSSKGKFSVY IECEGFQAVK SIGGKSDGCW GGLIAY.NRK KDGWQARAYT
        *         *                      ***         *

      137                                                183
BWYV  NVEITNNKAD NTLKYGHP.. .DMELNGCHF NQGQCLERDG DLTCHIKTTG
BMYV  NVEITNNKAD NTLKYGHP.. .DMELNSCHF NQGQCLERDG DLTCHVKTTG
PLRV  GVKISKLRND NTYRQGHP.. .ELEINSCHF REGQLLERDA TISFHVEAPT
 SDV  NVTITNNKAD NDWKYGHPDP LDLMINGDRF DQNQVVEKDG IISFHLVTTG
CABYV GCKITNYQSN TVFVAGHP.. .DATMNGKSF DTARAVEVDW FASFELECDD
BYDV  GCSFKNFLAT NTWRPGHK.. .DLKLNDCQF TDGQIVERDA VMSFHVEATG
PEMV  GTVLSNYRST TTVINGHP.. .DCEVNDCKF KPDRGVESDL ICSFHLEA.E
                   **          *   *       * *

      184                                                230
BWYV  DNASFFVVGP AVQKQSKYNY AVSYGAWTDR MMEIGMIAIA LDE...QGSS
BMYV  DNASFFVVGP AVQKQSKYNY AVSYGAWTDR MMEIGMIAIA LDE...QGSS
PLRV  D.GRFFLVGP AIQKTAKYNY TISYGDWTDR DMELGLITVV LDE.HLEGTG
 SDV  PNASFFLVAP AVKKTAKYNF CVSYGDWTDR DMEFGMVSVV LDE.HLEGAR
CABYV EEGSWAIYPP PIQKDSSYNY TVSYGNYTEK YCEWGAISVS IDE.DNNGNE
BYDV  TDACFYLMAP KTMKTDKYNY VVSYGGYTNK RMEFGTISVT CDESDVEAER
PEMV  EDSYWALQAP PIQKSSDYNY VVSYGGYTEK SIEWGSVSIS IDEVNQTASA
            *    *   **    ***       *         **

      231                                                278
BWYV  GSVKTERPKR VGH.SMAVST WETIKL.PEK GNSEGYETSQ RQDSKTPPTA
BMYV  GSAKIERPKR VGH.SMAVST WETINL.PEK ENSGEFKTDQ RQDLKTPPTS
PLRV  SANRVRRPPR EGH.TYMASP REPEGK.PVG NKPRDETPIQ TQERQPDQTP
 SDV  SSQYVRKSPR PGHFGVNRSH RLQDSFTPVE YVSDDDSSSS SSIVSNRPST
CABYV .......PRR IPRGVMAWST PEPSFSGDDS QRQDFNTPSL EERGSDALES
BYDV  ITRHAETPIR FKHILVSEQY EQPLPTIIDQ CLCDVQTPEQ EQTLVDE...
PEMV  SP....WRGR ARKLAILQET VVPLPFPPGG AMDYRLGDRE GDQTGTSEKG
            *
```

FIG. 5. Comparison of the N-terminal RTD-deduced amino acid sequences from subgroup I (56) and II (25, 26, 52, 62, 66) luteoviruses and PEMV (12). Asterisks, identical amino acid residues; boldface, hydrophobic regions. There is no significant level of sequence homology beyond residue 240. Based on mass spectroscopy and mutational analysis, the C terminus of the truncated full-length CP-RTD of BYDV was suggested to map to residue 242 (21), which is located in the middle of the RTD. The full-length RTD of PEMV is only 20 amino acids longer than the presented sequence. The numbering refers to the position of the amino acid residues on the BWYV RTD. Hydrophobicity is based on the output of the PeptideStructure program (Genetics Computer Group, Inc., Madison, Wis.).

10-kDa protein that is highly homologous to *E. coli* GroES (50), it seems to be repressed at the translational level, since *Buchnera* GroES was not detected in the aphid (34).

In conclusion, our data may provide insight into how luteoviruses escape destruction in the hemolymph of the aphid vector. Indeed, the hemolymph of invertebrates constitutes a potentially hostile environment (51). In several hematophagous insects and ticks, host serum components including antibodies are readily detectable in the hemolymph after engorgement, although they disappear quickly immediately after cessation of feeding (11, 63, 64). Even isolated *Buchnera* cells directly injected into aposymbiotic aphids lyse rapidly (15). Several hypotheses have been put forward concerning possible mechanisms involved in the survival of pathogens and parasites within compatible invertebrate hosts (70). Among the possibilities evoked are evasion of host recognition either by molecular mimicry or active acquisition of host molecules and interference with the host defense mechanism. Although specific data on aphid immunology are rare, it may well be that association with *Buchnera* GroEL provides the virus with a means of escaping the host's immune response.

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