Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: effects on aphid transmission and binding to purified virions


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Transmission of zucchini yellow mosaic virus (ZYMV) by aphids was examined by introducing mutations within the highly conserved proline-threonine-lysine (PTK) motif of the helper component proteinase (HC-Pro) using a cDNA full-length clone. Replacement of proline by alanine (ATK) in the PTK motif abolished transmission almost completely both from plants and from membranes. Substitution of the basic lysine by glutamic acid (PTE) did not reduce the rate of transmission compared with the wild-type. Replacement of threonine by valine (PVK) or serine (PSK) resulted in a rate of transmission that was lower than that of the wild-type. The rate was lower for PSK than for PVK. Western blot comparison did not permit attribution of HC-Pro functionality in transmission to its level in the host. The HC-Pro of strains that effected transmission (with the wild-type PTK motif, and with the mutated PTE and PVK motifs) could also bind in vitro to virions of ZYMV. HC-Pro with a PSK motif, which was less effective in assisting transmission, could bind only weakly to virions, while HC-Pro of the almost non-transmissible strains (with PAK and ATK motifs) did not bind at all. Interestingly, positive binding was recorded for transmission-defective ZYMV-Ct, which has a PTK motif but has glutamic acid instead of lysine in the lysine-leucine-serine-cysteine (KLSC) motif. These findings support the ‘bridge hypothesis’, and confirm the binding of the HC-Pro to the virus. The possible role of the PTK and KLSC motifs in binding to the virus and to the mouthparts of the aphid is discussed.

Introduction

Zucchini yellow mosaic virus (ZYMV), a member of the potyvirus group, is a major pathogen of cucurbit crops worldwide (Lisa et al., 1981). Successful transmission of potyviruses by aphids requires the presence of two virus-encoded proteins, the coat protein (CP) and the helper component proteinase (HC-Pro) (Murant et al., 1988; Pirone & Blanc, 1996). A number of ZYMV strains have been found to be non-transmissible by aphids, either due to a deficient CP (Antignus et al., 1989; Lee et al., 1993) or a deficient HC-Pro (Lecoq et al., 1991; Granier et al., 1993; Grumet et al., 1992). Other cases of non-transmissible strains of potyviruses have been reported and are included in a recent review by Pirone & Blanc (1996). The role of the helper in assisting aphid transmission has been known since the 1970s (Gover & Kassanis, 1974a,b). Since then, the following additional functions have been attributed to the HC-Pro protein: affecting symptom severity (Atreya & Pirone, 1993), serving as a protease (Carrington et al., 1989), being involved in long-distance movement (Cronin et al., 1995; Rojas et al., 1997), and playing a role both in genome amplification (Kasschau et al., 1997) and in nucleic acid binding (Maia & Bernardi, 1996).

Major progress in recognizing the domains that control aphid transmission was made in the last decade when the sequences of a number of potyviruses became available. Comparison of the sequence of the HC-Pro gene in aphid-transmissible and aphid non-transmissible strains revealed mutations in two highly conserved motifs, the lysine-isoleucine-threonine-cysteine (KITC) (Thornbury et al., 1990) and the proline-threonine-lysine (PTK) (Granier et al., 1993; Huet et al., 1994). Direct proof for the role of these motifs in HC-Pro activity was obtained by introducing mutations into full-length cDNA clones and testing the progeny viruses for transmission by aphids. A change from K to E in the KITC motif of the HC-Pro of tobacco vein mottling virus (TVMV) resulted in loss of HC-Pro activity (Atreya et al., 1992). A thorough mutational analysis of the KITC motif and its adjacent sequences in TVMV HC-Pro was done by Atreya &
Pirone (1993). They reported that replacement of K by other amino acids resulted, in most cases, in loss of activity in transmission and in attenuation of symptoms.

Similarly, the role of the PTK motif in aphid transmission was proved by introducing a change from T to A in this motif of ZYMV HC-Pro. This substitution resulted in almost total loss of HC-Pro activity (Huet et al., 1994).

Recently, Blanc et al. (1997) made an important contribution to the understanding of potyviral transmission by aphids by showing a direct in vitro association between a potyviral HC-Pro and the CP of transmissible strains.

The present study centres on the effects of mutations made within the PTK motif of ZYMV HC-Pro on transmission by aphids and their effect on in vitro binding to ZYMV virions.

**Methods**

- **Virus strains.** Four strains were used in the present study. (1) A wild-type ZYMV that has a biologically active helper component, (with an unchanged PTK motif) (Fig. 1b); this is the original strain used for the preparation of the ZYMV full-length cDNA clone and is here designated ZY-PTK. (2) The laboratory-made strain ZYMV(DRA), which has an almost inactive HC-Pro, with A instead of T in the PTK motif (Huet et al., 1994). This strain is designated ZY-PAK in this study (Fig. 1b). (3) The wild-type ZYMV-Ct, which has a biologically inactive helper with E instead of K in the lysine-leucine-serine-cysteine (KLSC) motif (Grumet et al., 1992) (in ZYMV the KITC motif appears as KLSC). (4) An aphid-transmissible strain (ZYMV-HAT) that was used for virus purification; the virions were used in membrane feeding and in binding experiments.

- **Virus purification and host plants.** ZYMV was raised on squash (Cucurbita pepo cv. Maayan) plants and purified by the method of Moghal & Franci (1976) with modifications. Squash plants were grown in the greenhouse and served as the principal virus host. Squash and cucumbers (Cucumis sativus cv. Dilla) were used for testing the infectivity of the clones, for HC-Pro extraction and for aphid transmission.

- **HC-Pro purification.** Squash seedlings were inoculated with ZY-PTK, ZY-PAK or with each of the ZYMV HC-Pro mutants (Fig. 1b). Two weeks after inoculation, HC-Pro was extracted from the systemically infected leaves using the procedure described previously (Antignus et al., 1989; Lecoq et al., 1991): HC-Pro were then used in transmission studies. Recently, we found that the HC-Pro of wild-type and mutant ZYMV strains can be purified on a Ni²⁺-resin affinity column without adding histidine for tagging (D. Kadoury, B. Raccah & A. Gal-On, unpublished results). The quantity and purity of HC-Pro obtained by this method was greater than that obtained by the ultracentrifugation method. Ni²⁺-resin-purified HC-Pro preparations were also used for estimation of HC-Pro titre in plants that were infected with ZYMV mutants and for testing HC-Pro binding to dot-blotted ZYMV virions on membranes. The same preparation that was tested in binding experiments was also used in transmission tests from membranes.

- **Aphid transmission.** Squash seedlings were mechanically inoculated with either ZY-PTK or HC-Pro mutated virus strains (Fig. 1b). These seedlings were then used as source plants for acquisition access feeding. Green peach aphids (Myzus persicae) were fasted for 1 h, then allowed a 5 min acquisition access feeding; thereafter, they were transferred in groups of three aphids for an 18 h inoculation access feeding. Green peach aphids were allowed 10 min acquisition access feeding and an 18 h inoculation access feeding as described elsewhere (Antignus et al., 1989; Raccah & Pirone, 1984). Transmission tests were also carried out with the preparations of Ni²⁺-resin purified HC-Pro used in binding tests. The transmission protocol was identical to that used for HC-Pro purified by ultracentrifugation.

- **Insertion of mutations within the PTK motif in the full-length infectious clone.** Five mutations were generated within the region encoding the PTK motif in HC-Pro. The mutations that changed T to S or V in the PTK motif were prepared by site-directed mutagenesis according to Huet et al. (1994) using primers No. 1 and 2 (Fig. 1b).

Incorporation of two additional mutations and deletion of the PTK motif were done by the PCR method. Primer Nos 3–5 (Fig. 1b) harbouring the mutations and the AvaI restriction site were used from the 5′ ends and a specific primer representing a conserved region, which coincides with the putative 3′ end of the HC-Pro gene starting at position 1367 from its putative 5′ end (5′ CCAACTCTGTAATTTTTCAT), was used from the 3′ ends in the PCR reaction. The PCR products (457 bp) were cloned into T-cloning vector pUC-57 (MBI-Fermentas). The resulting clones were double digested by AvaI and BamHI and the excised section from each mutated fragment was ligated into the 5′ clone of ZYMV pKS16B (Gal-On et al., 1991). The mutations within the HC-Pro gene were verified in pSK16B by sequencing and restriction analysis. Finally, the mutated pSK16B clone was transferred into the full-length clone pKS35SZYPTKNOS (Gal-On et al., 1995) by exchanging BseFII–BamHI fragments as shown in the schematic presentation (Fig. 1a).

- **Infection of plants with clones.** The full-length clones harbouring the mutations of PVK and PSK were transcribed and mechanically inoculated as described before (Huet et al., 1994). The resulting viruses were named ZY-PVK and ZY-PSK accordingly. Three additional full-length clones, pKS35SZYPKNOS, pKS35SZYATKNOS and pKS35SZYPTKNOS (with a deletion of the PTK motif), were inoculated by particle bombardment as described by Gal-On et al. (1996). pKS35SZYPTKNOS was not infectious; therefore, work with this clone was discontinued. Infection with the other two clones resulted in viruses that were named ZY-PTE and ZY-ATK.

- **Verification of the mutations in the progeny virions.** The presence of each mutation in progeny viruses was verified by RT–PCR as described by Huet et al. (1994). The primers used were 5′ CTAGCAAGGCCTGATATTG and the specific primer (5′ CCAACTCTGTAATTTTTCAT) described above. Each mutant was engineered to incorporate a unique restriction site within the new mutation (Fig. 1b). The presence of each mutation was ascertained after digestion with the appropriate restriction enzyme.

- **Estimation of HC-Pro titre in infected plants by Western blot analysis.** The HC-Pro titre of each mutant in the plants was estimated 8–10 days after inoculation. Samples of leaves (2 g) were extracted and then passed through an Ni²⁺-resin affinity column. The resulting eluent containing HC-Pro was separated on discontinuous 12% SDS–PAGE. The HC-Pro titre was visualized by Western blot analysis (Towbin et al., 1979) using antibodies to HC-Pro (the antibody used to detect ZYMV HC-Pro was kindly given by D. E. Purcell and E. Hiebert from the University of Florida, USA, who prepared the antibody against papaya ringspot virus amorphous inclusion protein) at 1:1500 and goat anti-rabbit alkaline phosphatase conjugate (Promega) at 1:4000.

- **In vitro binding of HC-Pro to dot-blotted virions of ZYMV.** The binding reaction between HC-Pro and ZYMV virions was carried
Fig. 1. (a) Schematic representation of the clones used for introducing mutations within the PTK motif of the full-length cDNA clone pKS35SZYPTKNOS [this ZYMV clone was constructed and designated pKS35SZYMVNOS by Gal-On et al. (1996)]. (b) Virus strains and the primers used to generate mutations within the PTK motif of the HC-Pro of ZYMV and designation of names for progeny viruses.
out according to Blanc et al. (1997) with some modifications. Purified virions (1–1.5 μg) were blotted onto nitrocellulose membrane. The membranes were blocked for 24 h at 4 °C in TSM (100 mM Tris–HCl buffer, pH 7.2, 20 mM MgCl$_2$) containing 5% dry milk powder (DMP). Then, ca. 40 μg of Ni$^{2+}$-resin-purified HC-Pro was added to micro dishes containing 0.5 ml TSM + DMP for 24 h at 4 °C. After allowing time for binding, the membrane was washed three times with PBS–TWEEN buffer for 10 min at room temperature. Positive binding was ascertained by immunoblot assay; membranes were placed for 4 h at 4 °C in 0.5 ml TSM containing DMP with antibodies to HC-Pro at 1:3000 dilution. After an additional session of washings with PBS–TWEEN, the membranes were probed with goat anti-rabbit alkaline phosphatase conjugate (1:5000) (Promega) for 45 min at room temperature. The binding of HC-Pro to nitrocellulose membrane (without virus), which was then processed as described above.

### Results

#### Infectivity and effects on symptoms

Attempts to infect plants with clone pKS355ZYA PTK NOS (with a deletion of the PTK motif) failed. Therefore, work with this clone was discontinued. cDNA full-length clones harbouring other mutations within the PTK motif (Fig. 1 b) were infectious. The resulting viruses (ZY-PVK, ZY-PSK, ZY-PTE and ZY-ATK) produced symptoms as severe as the wild-type ZY-PTK. Symptom attenuation was only seen in plants infected with ZY-PAK (data not shown).

### Effects of mutations in the PTK motif on HC-Pro activity as tested by aphid transmission

The effect on aphid transmission was tested from plant-to-plant (Table 1a) and from membranes using partially purified HC-Pros (Table 1 b). As shown, substitution of K with E in the PTK motif resulted in HC-Pro with the ability to assist transmission (more than 60% transmission both for the wild-type and for ZY-PTE when acquired from plants and more than 70% when acquired from membranes). A loss of HC-Pro activity was seen when P was substituted by A in the PTK motif (both in plants and from membranes). On the other hand, substitution of T for the hydrophobic V resulted in a reduction in HC-Pro activity that was more pronounced when acquired from plants than from membranes (11.2 and 20.0%, respectively). It was however surprising to note that the change of T to S (both polar amino acids) resulted in loss of HC-Pro activity effecting only 2-1% transmission from plants and 5-7% from membranes.

In order to rule out the possibility that poor transmission was a result of a low titre of HC-Pro in the plant, the HC-Pro concentration was determined in plants. The following data were obtained when the intensity of the HC-Pro bands seen in Fig. 2 was analysed using the BioMax 1D Image Analysis Software (Kodak). The relative intensities of each HC-Pro (assuming the intensity of the wild-type ZY-PTK HC-Pro is 1.0) are: ZY-PTK, 1.82; ZY-ATK, 1.96; ZY-PAK, 1.02; ZY-PVK, 2.03; and ZY-PSK, 0.83. Thus, an intensity of a similar order of magnitude is seen for the HC-Pros of the non-transmissible ZY-ATK and the transmissible ZY-PTE and ZY-PVK. On the other hand, lower but similar band intensities were seen for the HC-Pro of the transmissible ZY-PTK and the almost non-transmissible ZY-PAK. These data lead us to conclude that lack of HC-Pro activity is not a result of a low HC-Pro concentration in the host plant.

### Table 1. Transmission of wild-type and mutated ZYMV strains by aphids

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Infected/tested</th>
<th>Percentage (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY-PTK (wild-type)</td>
<td>65/86</td>
<td>76.1 ± 10.3*a</td>
</tr>
<tr>
<td>ZY-PSK</td>
<td>2/92</td>
<td>2.05 ± 2.8*b</td>
</tr>
<tr>
<td>ZY-PVK</td>
<td>9/83</td>
<td>11.2 ± 8.8*b</td>
</tr>
<tr>
<td>ZY-PAK</td>
<td>0/78</td>
<td>0*</td>
</tr>
<tr>
<td>ZY-PTE</td>
<td>51/82</td>
<td>64.9 ± 7.7*b</td>
</tr>
<tr>
<td>ZY-ATK</td>
<td>0/76</td>
<td>0*</td>
</tr>
</tbody>
</table>

*a Acquisition access feeding of 5 min; inoculation access feeding of 18 h.
+b Mean of five replicates, three aphids per test/plant. Means followed by a common letter are not significantly different (at P < 0.05; χ² test).

#### (b) Transmission of purified ZYMV-HAT virions mixed with partially purified HC-Pro molecules derived from wild-type and mutated ZYMV

<table>
<thead>
<tr>
<th>Source of HC-Pro*</th>
<th>Infected/tested†</th>
<th>Percentage (Mean ± SD)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY-PTK (wild-type)</td>
<td>49/70</td>
<td>70.0 ± 12.9*a</td>
</tr>
<tr>
<td>ZY-PSK</td>
<td>4/70</td>
<td>5.7 ± 7.9*b</td>
</tr>
<tr>
<td>ZY-PVK</td>
<td>14/70</td>
<td>20.0 ± 17.3*b</td>
</tr>
<tr>
<td>ZY-PAK</td>
<td>0/70</td>
<td>0*</td>
</tr>
<tr>
<td>ZY-PTE</td>
<td>63/80</td>
<td>77.2 ± 21.5*a</td>
</tr>
<tr>
<td>ZY-ATK</td>
<td>0/80</td>
<td>0*</td>
</tr>
</tbody>
</table>

*a Acquisition from a mixture of 40 μl partially purified helper in 0.2 M Tris–H$_2$SO$_4$ buffer pH 7.2 containing 0.02 M MgCl$_2$, 20% sucrose and 100 μg of ZYMV-HAT in 10 μl 0.1 M borate buffer, pH 8.† Acquisition access feeding for 10 min; inoculation access feeding of 18 h. Ten aphids which completed access feeding were placed on each test plant.† Means of five replicates; means followed by a common letter are not significantly different (at P < 0.05; χ² test).
Transmission of ZYMV and binding to HC-Pro

**Fig. 2.** Comparison of the HC-Pro titre in plants infected with wild-type and mutant ZYMV strains determined by Western blot analysis.

**Fig. 3.** In vitro binding assay between different ZYMV HC-Pro and dot-blotted virions of ZYMV-HAT: (1) virions alone; (2) virions reacting with virus-free plant extract; (3) reaction of dot-blotted virions with antibodies to ZYMV CP; (4) Western dot blot of equivalent amounts of the different HC-Pro; (5) reaction of virions with HC-Pro. Strips 1, 2, 4 and 5 were probed with antibodies to HC-Pro. All treatments were then probed with goat anti-rabbit conjugated alkaline phosphatase and stained with NBT/BCIP.

**Table 2.** Transmission of purified ZYMV-HAT virions mixed with two distinct non-functional HC-Pros derived from ZY-PAK and ZYMV-Ct

<table>
<thead>
<tr>
<th>Source of HC-Pro*</th>
<th>Infected/tested†</th>
<th>Percentage (Mean ± SD)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY-PTK</td>
<td>67/75</td>
<td>89.33 ± 5.67*</td>
</tr>
<tr>
<td>ZY-PAK</td>
<td>0/74</td>
<td>0—a</td>
</tr>
<tr>
<td>ZY-Ct</td>
<td>0/70</td>
<td>0—a</td>
</tr>
<tr>
<td>ZY-PAK + ZY-Ct</td>
<td>0/63</td>
<td>0—a</td>
</tr>
</tbody>
</table>

* Acquisition from a mixture of 40 µl partially purified helper in 0·2 M Tris–H₂SO₄ buffer pH 7·2 containing 0·02 M MgCl₂, 20% sucrose and 100 µg of ZYMV-HAT in 10 µl 0·1 M borate buffer, pH 8. 1, the ZY-PTK (wild type) HC-Pro, has unaltered KLSC and PTK motifs (Huet et al., 1994); 2, ZY-PAK HC-Pro has a change from T to A in the PTK motif (Huet et al., 1994); 3, ZYMV-Ct HC-Pro has a change from K to E in the KLSC motif (Grumet et al., 1992).
† Acquisition access feeding for 10 min; inoculation access feeding of 18 h. Ten aphids which completed access feeding were placed on each test plant.
‡ Means of five replicates; means followed by a common letter are not significantly different (at P < 0.01; χ² test).

Comparison of the in vitro binding of HC-Pros to ZYMV-HAT virions

The HC-Pro of the wild-type ZY-PTK, the defective ZY-PAK (Huet et al., 1994) and the four engineered mutants (Fig. 1b) were compared for their ability to bind to ZYMV-HAT virions that were dot blotted onto nitrocellulose membranes. Our attempts to detect binding of ZYMV HC-Pro to electroblotted ZYMV CP following the protocol of Blanc et al. (1997) did not succeed (data not shown). Instead, when intact ZYMV virions were dot blotted onto membranes, binding became consistent and evident (Fig. 3). Positive binding was obtained for the strains that have active HC-Pro (namely, ZY-PTK, ZY-PTE and ZY-PVK). Weak binding was recorded for the HC-Pro of ZY-PSK while the HC-Pros of the almost non-transmissible strains (ZY-ATK and ZY-PAK) did not bind to dot-blotted virions (Fig. 3).

A sample of each of the Ni²⁺-resin-purified HC-Pro preparations that was used in binding tests was taken to test their functionality in transmission tests. Transmission rates obtained for HC-Pro purified by the Ni²⁺-resin method were consistent but higher than those obtained with HC-Pro purified by the ultracentrifugation method (data not shown).

In order to examine the binding capacity of the KLSC motif of the HC-Pro to ZYMV virions, we made use of the ZYMV-Ct strain (kindly provided by Rebecca Grumet from the University of Michigan, USA). The ZYMV-Ct strain is aphid non-transmissible, has a non-functional HC-Pro, with E instead of A in the PTK motif.
of K in the KLSC, but harbours an unchanged PTK motif (Grumet et al., 1992). As seen in Fig. 4, the HC-Pros of the wild-type ZY-PTK and the ZYMV-Ct bound to dot-blotted virions, while that of ZY-PAK did not.

**Testing complementation between two non-functional HC-Pros differing in their mutated motif by aphid transmission from membranes**

An attempt to complement two non-functional HC-Pros that differ in the mutations that they harbour in their motifs is shown in Table 2. The HC-Pro of ZYMV-Ct (E instead of K in the KLSC motif) and that of ZY-PAK (A instead of T in the PTK motif) did not assist ZYMV transmission from membranes, either when they were mixed together (ZYMV-Ct + ZY-APK) or when presented alone.

**Discussion**

The present study provides additional information about the involvement of the PTK motif of ZYMV HC-Pro in assisting aphid transmission and the first direct evidence for the role of this motif in binding to ZYMV virions. Virus strains with a mutation in the PTK motif that rendered their HC-Pro non-functional in transmission failed to bind to dot-blotted virions, while those with a functional HC-Pro did bind. Furthermore, a positive binding to virions was seen for the ZYMV-Ct strain that has a non-functional HC-Pro that is mutated in the KLSC motif (E instead of K) but not in the PTK motif. These findings suggest that the KITC motif is not functional in direct binding of the HC-Pro to virions.

In a previous study (Huet et al., 1994), we raised the hypothesis that two motifs are involved in binding, one (either KLSC or PTK) binds to the virion and another (either KLSC or PTK) binds to the aphid mouthparts (Huet et al., 1994). In view of the findings in this study, we propose that the PTK motif that binds to the virion and speculate that KITC is involved in binding of HC-Pro to the aphid’s mouthparts.

In addition to a role in aphid transmission, the PTK motif may be the site where the HC-Pro is associated with virus particles in the host cells. An interaction between the HC-Pro and the capsid protein and the role of these proteins in cell-to-cell movement was recently reported for two potyviruses (Rojas et al., 1997).

The first report of the presence of helper activity in plants infected with a potyvirus was of a strain of potato virus Y (PVY) (Kassanis & Govier, 1971). Using membrane feeding, evidence was then provided that the so-called ‘helper’ is a component other than the virion (Govier & Kassanis, 1974b). Later, membrane feeding became instrumental in distinguishing between virus mutants that were deficient in the CP or the HC-Pro (Pirone & Thornbury, 1983; Antignus et al., 1989; Lecoq et al., 1991). These studies led in due time to sequence comparisons that resulted in the identification of two highly conserved mutated motifs: the KITC in potato virus C (Thornbury et al., 1990; Atreya et al., 1992) and the PTK in ZYMV (Huet et al., 1994).

Our research is a direct follow-up of two important recent studies. The first is the report of Schmidt et al. (1994), showing an association between the cauliflower mosaic virus (CaMV) aphid transmission factor and CaMV virions. The second is that of Blanc et al. (1997), which provides convincing evidence for direct binding between HC-Pro and potyviral CP, and that non-functional CPs (with a mutation in or close to the DAG motif) fail to bind to the HC-Pro.

Our attempts to complement two HC-Pros that are deficient in these motifs (one with PAK instead of PTK and another with ELSC instead of KLSC) by presenting them in a mixture with virions for membrane feeding resulted in no transmission. This suggests that both motifs must be present on the same HC-Pro molecule to ensure biological activity.

Several models have been proposed to explain the mode of action of the HC-Pro. The first was the suggestion that the HC-Pro connects between the virus and the aphid stylet (Govier & Kassanis, 1974b). Other models have speculated on an effect of the HC-Pro on the virion that allows direct binding of the virion to the stylet or of providing protection to the virion in the aphid’s stylet (see review by Pirone & Blanc, 1996). In view of the work of Blanc et al. (1997) and the findings presented here, it seems that the original ‘bridge model’ of Govier & Kassanis (1974b) is the most probable explanation for the HC-Pro mode of action.

The importance of HC-Pro in the retention of virus particles in the aphid’s mouthparts was demonstrated by a combination of electron microscopy and 125I-labelling of virions (Berger & Pirone, 1986). Later, both 125I- and gold-labelling allowed visualization of virions attached to the epicuticle of the aphid mouthparts, confirming retention only when an active HC-Pro is used (Wang et al., 1996).

The fact that deletion of the PTK motif results in a non-infectious clone may imply that this motif is essential for virus movement or replication. This issue could have been resolved by inoculation of protoplasts, but this line of research was beyond the scope of the present study.

The replacement of each of the three amino acids in the PTK triplet resulted in viable viruses. Surprisingly, the most drastic change in charge, occurring when K was replaced by E, did not alter the HC-Pro activity. On the other hand, replacing P by A and T by A resulted in almost total loss of HC-Pro activity. This suggests the possibility that the change from polar to hydrophobic amino acids is the cause for the loss in HC-Pro activity. However, when the polar amino acid T was replaced with the polar S, HC-Pro activity was not retained and transmission was greatly reduced. From these data, it is not possible to attribute the activity of the PTK motif to changes in charge or polarity. Thus, a structural interaction between the HC-Pro and the virion may exist.

The PTK motif of the HC-Pro is highly conserved among the potyviruses that have been reported until now. However,
in order to generalize about the role of this motif in binding to potyviral virions, there is a need to repeat the experiments reported with additional potyviral systems.

A different situation was described for the KITC motif of TVMv where the K or the similarly charged R is crucial for HC-Pro activity; changes to Q or H resulted in loss of functionality (Atreya & Pirone, 1993). The position of the KITC motif within the ‘zinc finger’ region of the HC-Pro (Robaglia et al., 1989) may lead to speculation that this region is involved in an association with charged ‘receptors’ that are present in the lining of the aphid epicuticle mouthparts.

In the process of inoculation, the virus must be discharged by the viruliferous aphid to the plant. In this case, two models may exist: either (1) the virus is discharged while still attached to the HC-Pro, or (2) the virus is discharged alone, and the HC-Pro remains bound to the aphid’s mouthparts. The elucidation of this issue will become possible when we know more about the role of the KITC motif in the transmission process.

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