Mutations in the potyvirus helper component protein: effects on interactions with virions and aphid styles

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Mutations of K → E in the highly conserved ‘KITC’ motif of the potyvirus helper component (HC) protein result in loss of HC function in aphid transmission, presumably because of inability to interact with virions, styles or both. In this study we show that HC of potato virus C (PVC), a naturally occurring variant of potato virus Y (PVY) that has the K → E mutation, lacks the ability to be retained in styles, whereas PVY HC is retained. The K → E mutation in either PVC or a site-directed mutant of tobacco etch virus (TEV) did not hinder binding to capsid protein, nor did deletion of the N-terminal 107 aa of TEV HC. An additional mutation, F → L at aa 10 of TEV HC, which renders HC non-functional but does not affect binding to capsid protein, is reported. Collectively, the results suggest that the N-terminal domain is required for interaction of HC with styles rather than for binding to virions.

Aphid transmission of members of the genus Potyvirus requires, in addition to virions, the acquisition of a non-structural protein, ‘helper component’ (HC), hypothesized to act as a ‘bridge’, binding to virions and to aphid mouthparts thus allowing retention of virions at a site from which they can subsequently be inoculated (Govier & Kassanis, 1974). Several studies support the bridge hypothesis (Berger & Pirone, 1986; Ammar et al., 1994; Wang et al., 1996; Blanc et al., 1997), but there is little information about the location of the virion-binding or stylet-binding functions on the HC molecule.

HC is encoded by the HC-Pro region of the potyvirus genome, and site-directed mutagenesis studies have mapped the HC domain to the N-terminal and central regions of the HC-Pro protein (Atreya et al., 1992; Atreya & Pirone, 1993; Huet et al., 1994). Those HCs that have been characterized have a subunit molecular mass of ~55 kDa and the biologically active, apparently dimeric, forms have a molecular mass of 100–150 kDa (Govier et al., 1977; Thornbury et al., 1985).

Mutations in two highly conserved motifs in the HC region of HC-Pro have been associated with loss of HC activity. One of these is a threonine to alanine mutation in the conserved PTK motif that begins at amino acid 308 in the HC-Pro region of zucchini yellow mosaic potyvirus (ZYMV) (Huet et al., 1994). This mutation has recently been shown to result in loss of virion-binding ability of ZYMV HC (Peng et al., 1998). This finding is compatible with the hypothesis that the capsid protein–HC portion of the bridge does not form and thus transmission cannot occur.

Loss of HC activity in tobacco vein mottling potyvirus (TVMV) occurs when glutamic acid (E) or other amino acids besides arginine (R) are substituted for lysine (K) in the highly conserved ‘KITC’ motif which begins at amino acid 51 of TVMV HC-Pro (Atreya et al., 1992; Atreya & Pirone, 1993). Naturally occurring mutants of potato virus Y (PVY) (Thornbury et al., 1990; Canto et al., 1995) and ZYMV (Granier et al., 1993) that produce transmission-defective HC also have mutations of K to E in this motif. The presence of a positively charged amino acid at this position thus seems essential for HC activity.

In the present study, PVC, the naturally occurring variant of PVY, which has the K to E mutation and produces non-functional HC, and a variant of tobacco etch virus (TEV-HCH XaEITC), created by site-directed mutagenesis of TEV-HCH Xa (Blanc et al., 1998), herein designated TEV-HCH XaKICT, were tested to determine whether the K to E mutation also affects the ability of HC to bind to virions. The HCs of PVY and PVC were purified as described (Thornbury et al., 1985) and those of TEV-HCH XaKICT and TEV-HCH XaEITC, which contain a histidine tag near the HC N terminus, were purified on nickel affinity columns (Blanc et al., 1998). The purified HCs were adjusted to equal concentrations based on quantitative Western blotting (Atreya & Pirone, 1993). When tested for their...
ability to effect the aphid transmission of purified PVY and TEV virions, as expected, the KITC-motif HCs were functional whereas those with the EITC motif were not (data not shown).

To test the virion-binding ability of PVY HC and PVC HC, a series of dilutions of the purified HCs was prepared in TSM buffer (100 mM Tris–HCl, pH 7.2, 500 mM NaCl, 0.05% Tween 20) and 10 µl was applied to nitrocellulose using a vacuum manifold. After blocking for 1 h with 5% dried milk in TSM the blot was incubated for 1 h with purified PVY virions at a concentration of 1 µg/ml. The blots were then washed for 10 min twice in TSM and once in TTBS (100 mM Tris–HCl, pH 7.5, 500 mM NaCl, 0.02% Tween 20) and incubated overnight with a polyclonal anti-PVY virion antibody diluted in TTBS–5% dried milk. After washing three times in TTBS the blots were incubated for 3 h with goat anti-rabbit alkaline phosphatase conjugate (GAR–AP) and chromogenic substrate. As shown in Fig. 1, there was no difference in the ability of the two HCs to bind virions.

For the TEV HCs, capsid proteins were electrophoresed on polyacrylamide gels under denaturing conditions, transferred to nitrocellulose membranes and processed as described (Blanc et al., 1997). Binding of HCs to the capsid protein was detected by probing with polyclonal TEV HC antibody followed by GAR–AP and chromogenic substrate. As shown in Fig. 2, the K to E mutation did not affect the ability of TEV HC to bind to capsid protein.

A plausible explanation for the loss of HC function is that the K to E mutation renders the HC incapable of interacting with the stylets. To test this, aphids (Myzus persicae) were allowed to acquire a mixture of TEV virions and either purified PVY HC or PVC HC through Parafilm membranes. The aphids were processed for sectioning, immunogold labelling with HC-specific antibodies, and examination by electron microscopy as described before (Ammar et al., 1994; Wang et al., 1996). Thin sections of the stylet bundle were incubated with a 1/20 dilution of monoclonal antibodies to PVY HC (kindly provided by Ramon Jordan; USDA, Beltsville, MD, USA) and then with goat anti-mouse IgG conjugated to 10 nm gold under the conditions described by Ammar et al. (1994). These PVY HC antibodies had been found to react equally well with PVY HC and PVC HC on thin sections of infected plants as well as in dot-blot tests with purified HCs. Fifty sections of the stylets of each of 25 aphids fed on the respective HCs were examined. HC was found in the food canal of 15 aphids that acquired the EITC-motif PVC HC whereas HC was not detected in the food canal of any of the aphids that acquired the KITC-motif PVC HC (Table 1). Taken together, the evidence indicates that a K to E mutation does not affect HC–capsid protein binding but rather results in loss of HC function by affecting the HC–stylet interaction.

In further experiments, two additional TEV mutants that produce non-functional HC were analysed for their ability to bind to capsid protein. The TEV-DD1 mutant described by Dolja et al. (1997) has a deletion of 107 amino acids at the N terminus of the HC (which includes the KITC motif). Purification of the truncated HC from TEV-DD1 on a nickel affinity column provided a large amount of a polypeptide with
an $M_r$ of 39000 which was specifically labelled with an antibody against TEV HC. Aphids that acquired a mixture of the truncated HC and purified wild-type TEV virions were unable to transmit the virus. Despite the lack of helper component activity, the HC from TEV-DD1 was still able to interact with the virus capsid protein (Fig. 2), thus indicating that the domain involved in virion-binding is not included in the N-terminal 107 amino acids of the TEV HC.

Production of TEV-HCH XaKITC involved the construction of an intermediate, TEV-HCH (Blanc et al., 1998). Although it contains the KITC motif, TEV-HCH was not aphid transmissible despite extensive tests; evidently changes had inadvertently occurred during the cloning process. The sequence of TEV-HCH was compared to that of wild-type TEV and two non-conservative changes, from phenylalanine (F) to leucine (L) at amino acid 10 and from glutamic acid to glycine at amino acid 74, were found in the HC coding region. (These changes were not present in TEV-HCH XaKITC due to the strategy used in its construction.) To determine which of these mutations was associated with the loss of aphid transmissibility, we engineered two revertant viruses designated TEV-HCH10 and TEV-HCH74, depending on the amino acid position that was reverted to match the TEV wild-type sequence. TEV-HCH10 was transmitted from plant to plant by aphids at a level comparable to wild-type TEV whereas TEV-HCH74 was not transmitted. Thus the lack of aphid transmissibility of TEV-HCH, and consequently of TEV-HCH74, was due to the mutation (from F to L) at amino acid 10 of the HC. Capsid protein-binding assays such as those described above demonstrated that the loss of transmissibility was not due to inability of the HC from TEV-HCH and/or TEV-HCH74 to interact with virus particles (data not shown).

Other mutations that result in loss of HC activity but do not involve a K to E change in the KITC motif have been described. These include a glycine to aspartic acid change at position 35 in PVY HC (Canto et al., 1995) and a glycine to glutamic acid change in the position immediately preceding the KITC sequence in the HC of turnip mosaic virus (Nakashima et al., 1993). These mutations involve charge changes and are located either within or near the cysteine-rich region surrounding the KITC motif. Each of these changes may thus have an effect on HC activity comparable to that of the K to E mutation in the motif itself, i.e. inability to be retained in the stylets. The F to L mutation at amino acid 10 of the HC of TEV-HCH is particularly interesting because it falls in a region that is distinct from the cysteine-rich domain, and in which aromatic residues are somewhat conserved among potyviruses. Sequences near the N terminus (including position 10 in TEV) and the cysteine-rich domain (including the KITC motif) may have different roles in the molecular mechanisms of HC activity. Alternative hypotheses for the effect of the F to L mutation such as effects on folding or dimer formation, or directly on stylet interaction, remain to be tested. It should be noted that PVY has an L at position 10 and yet is fully functional for transmission. The context within which the L lies in PVY HC is, however, different than that of the L in TEV HC. This indicates that, as is the case for the DAG motif in the potyvirus capsid protein (I. J. López-Moya & T. P. Pirone, unpublished), the surrounding amino acids can affect the functioning of motifs involved in aphid transmission.

The analysis of the several forms of potyviral HC presented in this paper, together with previous data (Thornbury et al., 1990; Atreya et al., 1992; Atreya & Pirone, 1993; Canto et al., 1995; Granier et al., 1993) suggest that the N-terminal domain of HC is involved in interaction with the aphid mouthparts. It appears that the region of HC responsible for virion binding is located outside the N-terminal domain, presumably in the central region (Peng et al., 1998).

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