Research Note

Comparison of Tobamovirus Coat Protein Structural Features That Affect Elicitor Activity in Pepper, Eggplant, and Tobacco

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Specific mutations designed to affect the tertiary, quaternary, and surface structures of the tobacco mosaic tobamovirus (TMV) coat protein (CP) were compared for their effect on CP elicitor activity in pepper (*L¹* gene), eggplant, and tobacco (*N'* gene). Recognition by all three hosts was dependent upon the maintenance of the CP's α -helical bundle. However, variations in recognition resulted from mutations that affected different CP surface features and/or quaternary configurations. The relationship between CP structure and recognition in these hosts is discussed.

Additional keywords: hypersensitive response.

Plants have evolved sophisticated resistance mechanisms that function in the recognition and control of specific pathogens. One such mechanism, the hypersensitive response (HR), is often characterized as a gene-for-gene relationship involving the interaction of pathogen avirulence gene products, termed elicitors, with the products of host-encoded resistance (*R*) genes. Interestingly, the molecular analysis of several plant *R* genes indicates they share remarkably similar structural motifs. In particular, the majority of *R* genes encode leucine-rich repeats that have been implicated in ligand binding and signal transduction (Parker and Coleman 1997; Kobe and Deisenhofer 1995). In contrast, pathogen elicitors include a variety of molecules that share no common motifs (Keen and Dawson 1992). These findings suggest that plants share a common mechanism that is capable of recognizing a diverse array of pathogen components. Understanding this mechanism will require detailed investigations into the structural properties involved in *R* gene-elicitor interactions.

The structural characteristics of the tobacco mosaic tobamovirus (TMV) coat protein (CP) responsible for the elicitation of the *N'* gene HR from *Nicotiana sylvestris* have been

investigated. Within this system, site-directed mutations were created based on the known structure of the TMV CP (Fig. 1) and analyzed for their effects on *N'* gene recognition. Muta-

Fig. 1. Alpha carbon tracing showing the three-dimensional structure of the 17.5-kDa CP (coat protein) of TMV (tobacco mosaic tobamovirus; Namba et al. 1989). The central portion of CP consists of four right-handed, anti-parallel alpha-helices designated left and right slewed (LS and RS) and left and right radial (LR and RR). A short hairpin loop connects the LS and RS helices. The long loop connects the LR and RR helices. A short beta-sheet of four strands braces the four helix bundle. The N and C termini are located on the outer surface of the virus.

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tions that modified structural features on the right face of the CP's α -helical bundle were found to disrupt the ability of the TMV elicitor P20L CP (this mutation confers elicitor activity to the CP of TMV strain U1) to induce the N' gene HR, while similar mutations that disrupted structural features outside of this region had no effect on the elicitor activity of P20L CP (Taraporewala and Culver 1996). The structural properties of this region, termed the elicitor active site, were characteristic of a recognition surface (Janin and Chothia 1990), suggesting that this region may serve as a receptor binding site. The elicitor active site is also structurally and functionally conserved among different tobamovirus CPs that vary widely in sequence similarity but share a common three-dimensional fold (Taraporewala and Culver 1997), indicating that the overall structural configuration of CP plays a significantly greater role in N' gene recognition than the position of any specific amino acid.

In addition to eliciting the N' gene HR, the CP of TMV has also been shown to function as an elicitor of the HR in *Cap-sicum* spp. (pepper) carrying the L^1 (*C. annum*), L^2 (*C. frutescence*), or L^3 (*C. chinense*) genes as well as a genetically uncharacterized HR in eggplant (C. D. Dardick and J. N. Culver, unpublished data; de la Cruz et al. 1997; Berzal-Herranz et al. 1995; Dardick and Culver 1997). The ability of the TMV CP to elicit these HRs provides a unique opportunity to investigate how different R genes recognize the same pathogen elicitor. In this study, a set of previously constructed TMV CP mutants were examined for their ability to elicit the HRs in pepper (L^1 gene) and eggplant. Results were then compared with those reported for these CPs in tobacco (N' gene). In vitro transcripts, generated from mutagenized, full-length cDNA clones of TMV, were used to mechanically inoculate each host (Taraporewala and Culver 1996). Plants were maintained in environmental growth chambers at 25 or 29°C under a 12-h photoperiod and monitored for symptom development for a period of at least 3 weeks. The expression of each mutant CP in each of the different hosts was monitored by Western (immunoblot) analysis of inoculated leaf tissue, as in Taraporewala and Culver (1996; and data not shown).

The ability of divergent tobamovirus CPs to elicit the HR in pepper was determined with chimeric TMV constructs in which the CP open reading frame (ORF) was replaced by the ORFs of either TMV U2, ondtoglossum ringspot virus (ORSV), or cucumber green mottle mosaic virus (CGMMV) (Hilf and Dawson 1993; Taraporewala and Culver 1997). These CPs share 71, 73, and 37% identity to the U1 CP, respectively, but have nearly identical three-dimensional structures (Namba et al. 1989; Pattanayek and Stubbs 1992; Wang and Stubbs 1994). Results demonstrated that U1, U2, ORSV, and CGMMV CPs elicited phenotypically similar HRs in pepper. In comparison, only the U1 CP failed to elicit an HR in tobacco, while only the CP of CGMMV failed to elicit the HR in eggplant (Taraporewala and Culver 1997; Dardick and Culver 1997). Thus, recognition in pepper appears to be more tolerant of the structural variations between these tobamovirus CPs than either the tobacco or eggplant HRs, suggesting that these R genes may target different CP structural features for detection.

The overall three-dimensional fold of the TMV CP has been shown to be an essential feature necessary for elicitor activity in tobacco (Saito et al. 1989; Taraporewala and Culver 1996).

To determine the importance of the CP's three-dimensional fold in eliciting the pepper (L^1 gene) and eggplant HRs, a set of P20L CP mutants containing either deletions or single amino acid substitutions was tested for elicitor activity in these hosts (Fig. 2). Results showed that deletions of amino acids 1 through 14 (ΔN) or 148 through 158 (ΔC) from either the N or C termini did not interfere with the elicitor activity of CP in either pepper or eggplant. However, deletion of amino acids 102 through 119 (mutant CP25; Dawson et al. 1988), which make up a portion of the RNA-binding loop as well as the left radial α -helix, resulted in the loss of elicitor activity in both pepper and eggplant. In comparison, the same N- and C-terminal deletions disrupted P20L CP's ability to elicit the tobacco HR (Taraporewala and Culver 1996), while the CP25 deletion did not affect recognition. Both the ΔN and the ΔC deletions would be expected to significantly alter the structural conformation at the outer radius of CP, while the CP25 deletion should affect CP conformation at its innermost radius. These deletions may also indirectly affect the orientation and/or configuration of the mid-radius α -helical bundle. Differential recognition of these deletion mutants suggests that CP structural features affected by residues at the outer radius of CP are important for recognition in tobacco, while structural features affected by residues at the inner radius of CP are more important for recognition in pepper and eggplant.

CP residues W17 and F48 are both located within the hydrophobic interior of the molecule and contribute significantly to the stability of the α -helical bundle (Fig. 2). CP substitutions W17R and F48S were designed to disrupt the hydrophobic interior of the molecule and, thus, interfere with the ability

A

Mutant	N' gene HR	L^1 gene HR	Eggplant HR
ΔN	-	+	+
ΔC	-	+	+
CP25	+	-	-
W17R	-	-	-
F48S	-	-	-

B

Fig. 2. Differential responses to TMV (tobacco mosaic tobamovirus) CP (coat protein) mutants designed to alter tertiary structure in tobacco (N' gene), pepper (L^1 gene), and eggplant. **A**, Table showing elicitor activities of mutant CPs; ΔN (deletion of residues 1 through 14), ΔC (deletion of residues 148 through 158), CP25 (deletion of residues 102 through 119), amino acid substitutions W17R and F48S. + = hypersensitive response (HR); - = no HR observed. **B**, Alpha carbon tracing of the TMV CP. Residues highlighted in white show location of amino acid deletions in the ΔN , ΔC and CP25 constructs; those in black show locations of substitutions W17R and F48S.

of the four α -helices to properly associate. Both of these substitutions knocked out the elicitor activity of the P20L CP in all three hosts. Taken together, these data indicate that structural maintenance of the core four-helix bundle, a fundamental feature of all tobamovirus CPs, is essential for CP recognition in these hosts. However, residues at either end of the helical bundle contribute differentially to the recognition process.

To compare CP surface features involved in these recognition responses a set of 26 previously constructed P20L CP mutants was used to infect pepper (L^1 gene) (Table 1). Eggplant was excluded from these experiments due to difficulties in obtaining consistent infections from transcribed viral RNA. The P20L CP mutants used in this study contain single amino acid substitutions designed to alter surface-exposed residues at locations throughout the CP molecule. Each P20L CP mutant was examined for its ability to elicit the pepper HR at both 25 and 29°C. Three HR phenotypes were observed: temperature-insensitive substitutions, which had no effect on the HR at either temperature; temperature-sensitive substitutions, which inhibited HR elicitation at 29 but not 25°C; and knockout substitutions that inhibited the HR at both temperatures. Of the 26 substitutions examined in this study, 12 resulted in the conversion of the temperature-insensitive phenotype of the P20L CP to either temperature sensitive or knockout in pepper. Seven of these substitutions—E50K, K53I, R71L, D77N, T81R, L84C,

and D88I—were located within or directly adjacent to the right face of the α -helical bundle. Two other substitutions that resulted in temperature-sensitive phenotypes, Q34R and Q38A, were located within the short hairpin loop connecting the left- and right-slewed α -helices. Together, these same nine substitutions produced similar HR phenotypes in tobacco (Table 1; Taraporewala and Culver 1996). This suggests that the surface structure along the right face of the α -helical bundle along with features of the hairpin loop are essential for recognition in both pepper and tobacco. However, a one-to-one correspondence in the HR phenotypes of these substitutions in both pepper and tobacco was not always observed (Table 1). For example, substitution R71L produces a knockout phenotype in tobacco but has only a temperature-sensitive phenotype in pepper. Furthermore, two other substitutions located within the right face of the α -helical bundle, Y72F and P78G, produced temperature-sensitive phenotypes in tobacco but had no effect on the HR phenotype in pepper.

Three substitutions, G32Y, D116N, and I133L, also disrupted CP recognition in pepper, resulting in either temperature-sensitive or knockout HR phenotypes (Table 1). Interestingly, these substitutions were located outside the right face of the α -helical bundle and had no effect on the HR phenotype in tobacco (Taraporewala and Culver 1996). Thus, the ability of these substitutions to disrupt CP recognition in pepper but not in tobacco suggests that surface features of the CP other than those along the right face of the α -helical bundle also contribute significantly to the induction of the L^1 HR in pepper. Twelve other substitutions created outside of the right face of the α -helical bundle had no effect on the recognition of CP in either tobacco or pepper. Taken together, these results indicate that structural features within the right face of the α -helical bundle are involved in both N' and L^1 gene recognition (Fig. 3). However, for L^1 gene recognition, specific regions outside of the right face of the α -helical bundle also contribute to the recognition process.

Previous studies have identified CP residues critical for HR elicitation in pepper by natural isolates of pepper mild mottle tobamovirus (PMMV). The PMMV CP is closely related to the TMV CP and shares 72% amino acid identity (Alonso et al. 1991). A variation in residue 138 was found to control the ability of the PMMV-I CP to act as an HR elicitor in *C. chinense* (L^3 gene) (Berzal-Herranz et al. 1995). Likewise, variations at both positions 43 and 50 in the CP of strain PMMoV-Ij also controlled HR elicitation in L^3 pepper (Tsuda et al. 1998). It is interesting to note that these residues lie within or close to regions identified as important for the L^1 gene recognition of the TMV CP. Within the TMV CP, residue 138 has an orientation and location similar to that of residue 133, and, when mutated, knocks out recognition by the L^1 gene HR. Residues 43 and 50 fall directly within the right face of the α -helical bundle shown to be critical for recognition in both L^1 and N' HRs. This suggests that the CP features controlling elicitor activity in L^3 pepper appear also to be similar to those essential for recognition in L^1 pepper and N' tobacco.

Specific mutations within the U1 CP that disrupt CP-CP interactions can confer elicitor activity to the U1 CP in *N. sylvestris* (N' gene) (Culver et al. 1994). We have speculated that by interfering in the normal self-association of the CP these substitutions alter its aggregation profile, leading to

Table 1. Effects of TMV (tobacco mosaic tobamovirus) CP (coat protein) substitutions on the tobacco N' and pepper L^1 HRs (hypersensitive responses)

Location in CP	Substitution ^a	N' gene HR ^{b,c}	L^1 gene HR ^b
N terminus	P7S	TI	TI
	F10Y	TI	TI
	S15A	TI	TI
Beta Sheet	K53I	TS	TS
	T59N	TI	TI
	D66A	TI	TI
	Y70F	TI	TI
	R71L	KO	TS
	Y72F	TS	TI
LS Helix	P20L	TI	TI
	G32Y	TI	TS
Short Loop	Q34R	TS	TS
RS Helix	Q38A	TS	TS
	E50K	TS	TS
RR Helix	D77N	TS	TS
	P78G	TS	TI
	T81R	TS	TS
	L84C	TS	TS
	D88I	TS	TS
	T89A	TI	TI
Long Loop	N91A	TI	TI
	E95Q	TI	TI
	P102L	TI	TI
	D109A	TI	TI
	D116N	TI	TS
LR Helix	E131Q	TI	TI
	I133L	TI	KO

^a All substitutions were made at surface-exposed residues in the N' elicitor CP P20L. See Taraporewala and Culver (1996) for detailed descriptions of these mutations. Numbers indicate amino acid position in the TMV CP sequence.

^b Mutants were assayed for their ability to elicit the HR at both 25 and 29°C: TI, temperature insensitive; TS, HR elicitation at 25 but not 29°C; KO, knock-out phenotype in which no HR was observed at either temperature.

^c Data previously reported by Taraporewala and Culver (1996).

threshold levels of a form of CP (monomer, dimer, trimer, etc.) that expose the elicitor active site for recognition by the host component. In support of this hypothesis it has been shown that specific CP mutations known to enhance the ability of the CP to self-associate can ameliorate the ability of elicitor CPs to induce the HR in *N. sylvestris* (Culver et al. 1994; Lu et al. 1996). In this study, CP mutations that enhance CP-CP interactions were examined for their effect on the ability of the U1 CP to elicit the HR in pepper (*L*¹ gene) and eggplant. CPs E50Q and E95Q/E97Q/D109N were designed to negate repulsive intersubunit carboxylate groups whose electrostatic interactions help drive virion disassembly (Culver et al. 1995; Lu et al. 1996). The effect of these substitutions on CP aggregation is an enhanced ability of the protein to self-associate into long, virion-like rods even in the absence of viral RNA. Interestingly, substitutions E50Q and E95Q/E97Q/D109N abolished the ability of the CP to function as an elicitor in eggplant but not in pepper. In comparison, substitutions E50Q or E95Q/E97Q/D109N did not affect the inability of tobacco to recognize the wild-type U1 CP. Thus, CP quaternary structure appears to have a greater effect on CP recognition by tobacco and eggplant than on CP recognition by pepper. This may also

suggest that there is a difference in the aggregate form (monomer, dimer, trimer, etc.) of CP that is recognized by these hosts. Analytical ultracentrifugation studies indicate differences in the size distributions of small CP aggregates between elicitor and nonelicitor CPs of the *N'* gene HR (Toedt et al., *in press*). Thus, different CP aggregates could serve as the active elicitor species in different hosts.

In summary, the structural maintenance of the CP's α -helical bundle appears to be essential for HR elicitation in tobacco (*N'* gene), pepper (*L*¹ gene), and eggplant, while differences in recognition appear to result from how these hosts perceive CP surface features and/or quaternary configurations. The similarities by which these hosts recognize the CP suggest a functional relationship between these genes. Since these plants are members of the family Solanaceae, many of which serve as TMV hosts, the possibility exists that these genes are not only functionally related, but structurally homologous as well. Evidence from cloned *R* genes supports this possibility in that homologues of known *R* genes have been identified in diverse plant species (Baker et al. 1997). However, the cloning and characterization of the tobacco *N'*, pepper *L*¹, and eggplant genes will be necessary to further investigate their similarities.

Fig. 3. Positions of substituted residues in the TMV (tobacco mosaic tobamovirus) CP (coat protein) and their elicitor activities in tobacco (*N'* gene) and pepper (*L*¹ gene). Substituted residues are numbered. **A**, Locations and effects of substitutions mapped previously for the tobacco *N'* gene (Taraporewala and Culver 1996). **B**, Locations and effects of these same substitutions on the pepper *L*¹ gene. Black, knock-out substitutions; gray, temperature-sensitive substitutions; white, temperature-insensitive substitutions.

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