Importance of the C-terminal domain of soybean mosaic virus coat protein for subunit interactions

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The potyvirus coat protein (CP) is involved in aphid transmission, cell-to-cell movement and virus assembly, not only by binding to viral RNA, but also by self-interaction or interactions with other factors. In this study, a number of CP mutants of Soybean mosaic virus (SMV) containing deletions and site-directed mutations were generated and cloned into yeast two-hybrid vectors. Interaction was confirmed by the expression of reporter genes, including HIS3, ADE2 and MEL1, in yeast strain AH109. Deletion of the C-terminal region of the CP caused loss of the CP–CP self-interaction ability detected in CP mutants with the C-terminal region. Alanine substitution at the amino acid positions R190, E191, E212, R245, H246 and R249 disrupted CP–CP interaction, whereas substitutions at the amino acid positions R188, D189, D198, K205, K218 and D250 did not. These results indicate that the C-terminal region of SMV CP may contain a domain(s) or amino acids required for CP–CP interaction and virus assembly.
The interactions, confirmed by growth on SD/agar media and with respect to galactosidase activity. In contrast, the C-terminal deletion mutants of CP (CP ΔF3 BD and CP ΔF3 AD) lost their ability to self-interact; the co-transformed yeast with C-terminal deletion mutant plasmids did not grow on SD media and showed no x-galactosidase activity (see Supplementary Table S1, available in JGV Online). These results suggest that the F3 region was not only necessary, but also sufficient, for CP–CP self-interaction. The C-terminal region (amino acid residues 170–256) of SMV-G7H CP contained crucial amino acid(s) or domain(s) responsible for CP self-interaction. Potentially imperfectly folded SMV-G7H CP fragments translated from truncated cDNA seemed to provide active structures that were sufficient for full or partial CP self-interaction.

The CP-deletion analysis allowed us to focus on the C-terminal region of the SMV CP. Alanine-substitution mutations were introduced in the C-terminal region of the SMV CP to verify the specific amino acid domain(s) required for CP–CP self-interaction. Both positively and negatively charged amino acids on the C-terminal region of SMV-G7H CP were substituted with alanine (Fig. 2a; Supplementary Table S2, available in JGV Online). Mutagenesis was performed by using PCR with mutagenic megaprimers (Picard et al., 1994). Alanine-substituted mutants were fused to the yeast two-hybrid vector pAS2-1 that encodes the GAL4 DNA-binding domain. To confirm the interactions, colonies from 12 pairs of alanine-substituted mutant (ASM) CPs and wt CPs were restreaked on SD agar medium supplemented with X-a-Gal reagent and liquid-cultured in YPDA broth for quantitative analysis of enzyme activity (Yeast Protocol Handbook, Clontech). Chromatic reactions produced by x-galactosidase activity from co-transformed yeast cells corresponded exactly to the results of the SD agar-medium assay (Fig. 2b). Of the 12 ASM CPs, six mutants retained the ability to interact with wt CP, but the other mutants lost that ability. Alanine substitution at the amino acid positions R190, E191, E212, R245, H246 and R249 disrupted CP self-interaction, whereas substitutions at R188, D189, D198, K205, K218 and D250 did not. Mutants with underlined t-test values were considered to have mutational effects from the substitution of each amino acid residue relative to the wt CP at a significance level of 

\[ \text{P} = 0.05 \]

these mutants were also grouped separately from the non-underlined mutants by Duncan’s multiple-range test (Fig. 2d). Expression of ASM CPs in yeast cells was also verified. The CPs from all ASMs were the same size as the wt CPs and were detected successfully (Fig. 2e).

To confirm SMV CP–CP self-interaction in vitro, we performed an in vitro binding experiment using a full-length SMV CP ORF clone inserted into pET25b harbouring the His-tag sequence and [35S]Met-labelled SMV CP synthesized...
by \textit{in vitro} translation. The T7 promoter-linked SMV CP ORF fragment was used for \textit{in vitro} translation. Four representative alanine-substituted mutants (R189A, E191A, K218A and R245A) were also amplified with the same primers for an additional pull-down assay to show the specific binding of wt CP. \textit{In vitro}-translated products were separated by electrophoresis and successful synthesis of target proteins was observed (Fig. 3a). The estimated mass of the \textit{in vitro}-translated SMV CP (33 kDa) band was the greatest on the gel; smaller bands at about 30 and 25 kDa were considered to be CP derivatives. Additional proteins smaller than the expected size were also detected in the final elution products (Fig. 3a). These CP derivatives seemed to maintain the self-interaction ability despite
being incomplete, possibly because the derivatives were not much smaller than the wt CP and therefore did not lose a significant degree of their function. Translation products from 13 potential initiation sites in the SMV CP were expected to be approximately 29, 25, 17, 15, 10 and 3 kDa, corresponding exactly to the blotted protein bands. With the exception of the 3 kDa protein, these derivatives still contained the six identified amino acid sites and probably retained the ability to bind in vitro. A column that was not loaded with the expressed pET25b CP was used as a negative control. Almost all in vitro-translated CP was detected in the final eluted solution, which contained the initially bound His-tagged SMV CP (Fig. 3b, lane 1). Subsequently, four CP ASMs (R189A, K218A, E191A and R245A) were used for in vitro binding with His-tagged SMV CP. Two mutated CPs (R189A and K218A) that showed active interaction in vivo also interacted with His-tagged SMV CP (Fig. 3b, lanes 2 and 4, respectively). In contrast, two additional mutated CPs (E191A and R245A) that showed negative interaction in vivo were not bound to the His-tagged SMV CP (Fig. 3b, lanes 3 and 5, respectively). To ensure that the SMV CP–CP self-interaction was specific, an unrelated Potato virus X (PVX) CP expressed similarly in Escherichia coli (Kwon et al., 2005) was also used for in vitro binding. The His-tagged PVX CP was initially bound, and in vitro-translated SMV CP was loaded. In vitro-translated SMV CP did not bind to the His-tagged PVX CP (Fig. 3b, lane 6), suggesting that the SMV CP–CP self-interaction was specific.

The CP alignment of the SMV strains and closely related potyviruses revealed that the highly conserved amino acids were located on the C-terminal half of the CP. Noticeably, the C-terminal region (170–256 aa) was predicted to have strong helices, whereas the other regions did not. Although the N-terminal region of the SMV CP was also predicted to have several short helices, the amino acid sequences were not well conserved among the SMV strains compared with those in the C-terminal region (Supplementary Figure, available in JGV Online). The highly conserved DAG motif, located near the N terminus of the CP of aphid-transmissible potyviruses, was present in residues 10–12 of SMV-G7H [Supplementary Figure, panel (a)]. This motif may be an inevitable mediator in aphid transmission (Blanc et al., 1997; Flasinski & Cassidy, 1998; Peng et al., 1998). When virus particles were dispersed by aphids through interaction with the HC-Pro dimer (Guo et al., 1999), the HC-Pro dimer connected to the CP through the DAG motif; the surface-exposed N-terminal region is thought to be necessary to facilitate this interaction (López-Moya et al., 1999). If the N-terminal region is provisionally considered as an element for transmission only, the possibility of C-terminal involvement in CP self-interaction for virus assembly increases.

More research is needed to distinctly divide the domains or key amino acids required for assembly and to examine the other exposed regions facing inward that facilitate genomic RNA binding and/or other functions. In addition, assembled homodimeric CP units have been shown to require further assembly to form their final flexuous-rod morphology (Riechmann et al., 1992). This finding suggests that at least one of the three domains interacts in a different manner with preformed CP self-interacting units. When the SMV-G7H CP was aligned with those of other SMV strains as well as those of other selected potyviruses, the amino acid composition of the three helices and the one extended-strand region was almost identical (data not shown). In the present study, alanine substitutions that disrupted CP self-interaction were found on six residues and were roughly located in two regions (aa 190–212 and 245–249) of the SMV CP. These regions include previously reported key amino acid residues mentioned above and may also be potential regions containing additional key residues.

Further research is required to reveal these residues and to elucidate the role(s) of each C-terminal-region helix in self-interaction and in the assembly of SMV CP.

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References


