Coat protein gene sequences of two cucumber mosaic virus strains reveal a single amino acid change correlating with chlorosis induction

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The coat protein genes of two chlorosis-inducing strains of cucumber mosaic virus (CMV) were compared by nucleotide sequence analysis. The predicted amino acid sequences of the encoded coat proteins were compared with those of two other chlorosis-inducing and four mosaic-inducing CMV strains. Overall, the sequences were highly conserved, with more than 95% amino acid sequence identity between any two strains. However, a proline is present at amino acid 129 of all the mosaic-inducing strains, whereas that position is occupied by either a serine or a leucine in the coat proteins of all the chlorosis-inducing strains. The correlation of chlorosis induction and a substitution for proline with leucine or serine at amino acid 129 suggests that this residue is the determinant of chlorosis induction.

Many strains of cucumber mosaic virus (CMV) are known and they are phenotypically distinguishable by host range and symptom induction in various indicator plants (Kaper & Waterworth, 1981). Typical symptoms of CMV infection of tobacco plants include a light-green, dark-green mosaic, distortion and stunting; in contrast, several CMV strains induce a bright yellow chlorosis. A study by Rao & Francki (1982) using pseudorecombinant viruses has shown linkage between the chlorosis-inducing phenotype of M-CMV and RNA 3. This has been confirmed using a pseudorecombinant virus constructed with infectious cDNA-derived transcripts of RNA 1 and RNA 2 of Fny-CMV (a mosaic-inducing strain), and RNA 3 of M-CMV (Shintaku & Palukaitis, 1990). This pseudorecombinant virus induces chlorotic symptoms in tobacco plants which are indistinguishable from those induced by M-CMV. The chlorosis-inducing determinant of M-CMV RNA 3 has been mapped further by molecular recombination between cDNA clones of Fny-CMV and M-CMV RNA 3 (Shintaku & Palukaitis, 1990). This study demonstrated that the domain that controls the chlorosis/mosaic-inducing symptom phenotype is downstream of the cDNA SalI (RNA 3 nucleotide 1296) site, and upstream of the cDNA XhoI (RNA 3 nucleotide 1838) site. This domain is completely within the coat protein gene coding region (which extends from RNA 3 nucleotides 1258 to 1913). Further, a comparison of the predicted amino acid sequences of the coat proteins of Fny- and M-CMV revealed only eight differences, seven of which are between the cDNA SalI and XhoI sites (Shintaku & Palukaitis, 1990).

Like Fny-CMV, the mosaic-inducing strains O-CMV, D-CMV and C-CMV have been reported to induce the typical light-green, dark-green mosaic in tobacco plants (Lakshman & Gonsalves, 1985; Takanami, 1981; Marchoux et al., 1975). M-CMV, Price's no. 6-CMV (P6-CMV), Fulton's C-CMV (FC-CMV) and Y-CMV (in the absence of its satellite RNA) have all been reported to induce bright yellow chlorosis in tobacco plants (Takanami, 1981; Mossop et al., 1976; Fulton, 1950; Price, 1934).

To determine whether a correlation exists between a particular amino acid substitution in the coat protein and the chlorosis-inducing phenotype in tobacco plants, the coat protein genes of the chlorosis-inducing CMV strains FC-CMV and P6-CMV were sequenced, and the amino acid sequences were deduced. The putative coat protein amino acid sequences of these strains were compared with those of other chlorosis-inducing (M- and Y-CMV) as well as mosaic-inducing (Fny-, O-, D- and C-CMV) strains (Owen et al., 1990; Hayakawa et al., 1988, 1989; Cuozzo et al., 1988; Quemada et al., 1989).

FC-CMV and P6-CMV viral RNAs were a gift from Dr Peter Palukaitis (Cornell University). The coat protein genes were sequenced by the dideoxynucleotide chain termination method using avian myeloblastosis virus reverse transcriptase as described (Mierendorf & Pfeffer, 1987). Six primers, complementary to different regions of the coat protein gene and the 3' non-translated region of RNA 3, were used in the sequencing reactions. These primers were complementary to nucleotides 1434
to 1450, 1533 to 1548, 1633 to 1651, 1692 to 1707, 1811 to 1826.

The nucleotide sequences of the coat protein genes of FC-CMV and P6-CMV show very few differences from each other or those of the six other CMV strains analysed (Fig. 1), with over 97% sequence identity between any two strains. Thus, even though P6-CMV and FC-CMV were isolated from different locations within the USA at an interval of 20 years (Fulton, 1950; Price, 1934), they show very little evidence of third codon position alterations, suggesting that the nucleotide sequence itself may be important in various CMV RNA-host component interactions.

The predicted amino acid sequences of the coat proteins of FC-CMV and P6-CMV were compared with those of the two other chlorosis-inducing and the four mosaic-inducing CMV strains (Fig. 2). Overall, the sequences are highly conserved with more than 95% amino acid sequence identity between any two strains. There is a distinct and consistent difference at amino acid 129 between the coat proteins of the chlorosis- and mosaic-inducing CMV strains. All of the coat proteins of the chlorosis-inducing CMV strains in this study have a serine (FC- and Y-CMV) or leucine (M- and P6-CMV) at amino acid 129, whereas the mosaic-inducing strains have a proline at this position. A substitution of any amino acid for proline results in significant effects on the secondary structure of the polypeptide, and the association of this substitution with chlorosis induction strongly suggests that this position is the determinant of chlorosis induction.

The substitution of either a leucine or a serine for proline is determined by nucleotide substitutions at different positions. It is therefore likely that chlorosis induction is not determined by a particular nucleotide substitution, but rather by the structural alteration in the coat protein caused by the replacement of the proline at

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**Fig. 1.** Alignment of the nucleotide sequences of the coat protein genes of some mosaic-inducing (Fny-, C-, D- and O-CMV) and chlorosis-inducing (M-, Y-, FC- and P6-CMV) CMV strains. Nucleotides identical to the Fny-CMV coat protein gene sequence are indicated by dashes.

**Fig. 2.** Alignment of the predicted amino acid sequences of the coat proteins of some chlorosis- and mosaic-inducing CMV strains. The four strains shown in boxes are chlorosis-inducing CMV strains. Identical amino acids are indicated by dashes.
position 129. Whether the coat protein alone, coat protein and viral RNA, or virions derived from such coat protein subunits are responsible for the induction of chlorosis remains to be determined.

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References


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