Differences in the subcellular localization of tobacco mosaic virus and cucumber mosaic virus movement proteins in infected and transgenic plants

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Our study reveals differences in the way that tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) movement proteins (MPs) partition with cellular components separated into four fractions from different aged leaves of infected and transgenic plants. Immunoblot analyses showed that TMV and CMV MPs associated predominantly with components in the cell wall fractions from leaves of transgenic plants. In infected tissue, highest levels of TMV MP were found in the organelle fractions from young and middle-aged leaves whereas most of the CMV MP was found in the detergent wash of the cell wall fraction. These results remained consistent even when plants were doubly infected with TMV and CMV. These results imply that MPs of plant viruses from different taxonomic groups differentially associate with subcellular components and that MP produced by a viral infection is targeted to additional subcellular destinations than MP produced in transgenic plants.

The distribution of plant virus movement proteins (MPs) within a plant cell can be analyzed by differential centrifugation of leaf extracts. Moser et al. (1988) showed the presence of tobacco mosaic tobamovirus (TMV) MP in cell wall and organelle fractions of inoculated leaves, but did not detect significant amounts of MP in the soluble fraction and cell wall/Triton wash. Deom et al. (1990) found a majority of extractable TMV MP in cell wall fractions of old leaves of transgenic plants expressing the TMV MP gene. Vaquero et al. (1994) found cucumber mosaic cucumovirus (CMV) MP in cell wall fractions from transgenic plants, and Burman et al. (1994) localized CMV MP to cell wall fractions from infected plants. Cell wall locations of the CMV and TMV MPs in transgenic plants have been used to deduce the potential functions of MPs (Deom et al., 1987; Vaquero et al., 1994).

The previous studies of CMV and TMV MPs have used similar, but not identical, methodologies and have demonstrated the presence (or absence) of a single viral MP in a particular fraction. It is difficult to compare the independent results of the subcellular fractionation study of one MP to those for another because each study employs a different fractionation method. We studied CMV and TMV MPs in infected and transgenic leaf tissue and used a single extraction and fractionation protocol to make comparisons between the subcellular localization of different MPs.

Transgenic tobacco plants lines 274 and 277 express a TMV MP gene (Deom et al., 1987), and transgenic tobacco plants line 3a-3 express a CMV-Fny (serogroup 1) MP gene (Kaplan et al., 1995). Nontransformed tobacco plants were used as controls or were inoculated with TMV U1 or CMV-Pu (serogroup 1). Leaves from four virus-infected plants of each nontransformed line and four plants of each mock-inoculated transgenic line were harvested 15 days post-inoculation (p.i.). Inoculated leaves from each treatment were pooled, as were progressively younger leaf pairs above the inoculated leaf (+1 and +2 = leaf pair 1, etc., +1 being the oldest leaf). The fractionation methods and buffers we used were the same as described (Deom et al., 1990). A mixture of proteinase inhibitors was omitted from the grinding buffer because it had had no noticeable effect on the accumulation of the TMV MP and was not used in prior studies (Deom et al., 1990). Leaf homogenates were centrifuged at 1000 g to get a cell wall pellet (P1) and supernatant (S1) fraction. P1 was washed in grinding buffer containing 2% Triton X-100. Washes were pooled to give the Triton wash fraction (T) [not described by Deom et al. (1990)]. The remaining P1 fraction was boiled in guanidine buffer to help solubilize membrane-bound proteins (CW). The S1 fraction was centrifuged at 30000 g to give a pellet and supernatant (S30). The pellet was boiled in guanidine, and the extracted proteins were termed the P30 fraction.
Equivalent amounts of protein from each fraction were separated using SDS-PAGE and were blotted to membrane. Membrane was treated with TMV MP antisera (Gafny \textit{et al.}, 1992) or with CMV MP antisera (Kaplan \textit{et al.}, 1995), then treated with an alkaline phosphatase-conjugated anti-rabbit antibody and developed. The presence of viral coat protein was also determined in each S30 fraction (Cooper \textit{et al.}, 1995). Inverse optical densities (IOD) representing the relative amount of MP in each sample were determined with a Sci-Scan 5000 densitometer (United States Biochemical). IOD was adjusted for the percentage of soluble protein in each fraction derived from 1 g of leaf tissue. None of the 27 kDa TMV MP product reported by Deom \textit{et al.} (1990) was detected with the antibody we used, so measurements were taken from the 30 kDa product. CMV products of molecular mass 31 kDa and 35 kDa, similarly described by Kaplan \textit{et al.} (1995), were resolved in extracts from infected and transgenic tissue. Measurements were taken from the 31 kDa product which was more abundant. No attempts were made to estimate absolute amounts of MPs or to compare total amounts of CMV MP to TMV MP.

Deom \textit{et al.} (1990) detected highest levels of MP in the CW fractions from old leaves of transgenic plants with the TMV MP. Like Deom \textit{et al.} (1990), we found the highest levels of MP in the CW fractions from 274 plants (Fig. 1a, b). High levels of MP were detected in the CW of old leaves even as total amounts of soluble protein decreased. Unlike Deom \textit{et al.} (1990) who detected MP in P30 and S30 fractions of 277 plants, we could not detect MP in the three other fractions for line 274 plants. We attribute our differing resolution to the specificity of the TMV MP antibody we used and MP expression levels between lines.

We found high levels of CMV MP in CW fractions from leaves of all ages of 3a-3 plants (Fig. 1c, d). High levels of MP remained in the CW from older leaves even as the total amounts of soluble protein decreased. CMV MP was also detected in the P30 fraction. Vaquero \textit{et al.} (1994) found CMV MP in all fractions of young leaves and in CW and T fractions of old leaves of transgenic plants. By contrast, we did not see a broad distribution of MP across fractions from young leaves. Additionally, CMV MP has been found in the 100000 g supernatant of young leaves from 3a-3 plants (Kaplan \textit{et al.}, 1995). However, we were not able to detect the CMV MP in our S30 fraction. We attribute these discrepancies to the different methods used for purification and detection and the variation between plant lines.

We tested the possibility that an MP could accumulate to higher levels or be distributed in different subcellular locations when it was a product of viral infection rather than being constitutively expressed in transgenic plants.
Fig. 2. Relative accumulation of TMV MP (a, b) and CMV MP (c, d) in subcellular fractions of extracts from different leaves of plants from two separate experiments. Plants from each experiment were sampled 15 days p.i. Position 1 represents the inoculated leaf and subsequent positions represent increasingly younger leaf pairs above the inoculated leaf. Ordinate values represent the measured inverse optical density of detected MP on each immunoblot per g of fresh leaf tissue. ■, CW; □, P30; △, S30; ◊, T. There was no detectable MP in the S30 or T fractions from plants inoculated with TMV.

Instead of detecting the highest levels of TMV MP in the CW, as in transgenic plants, we detected the greatest levels of MP in the P30 fraction (Fig. 2a, b). Whereas Moser et al. (1988) did not detect MP in the P30 fraction 5 days p.i., we were able to detect it 15 days p.i. There was no detectable MP in the S30 or T fractions. Lowest levels of total amounts of MP were detected in the leaves +1 and +2 positions above the inoculated leaf.

To determine if the amount of MP produced in a leaf was dependent upon viral infection, we quantified amounts of TMV coat protein in the S30 fraction. Low levels of coat protein were detected in leaves where low levels of total amounts of MP accumulated (not shown). Likewise, high levels of coat protein were detected in inoculated and upper leaves of the plants where high levels of total amounts of MP were detected. Thus, amounts of MP correlated with virus progression.

One set of TMV-infected plants was subjected to short, daily increments of severe summer temperatures (> 32 °C; not shown). However, MP levels were not reduced. TMV MP accumulation may not be adversely affected by high temperature (Wolf et al., 1991).

CMV MP also differed in the way it accumulated in transgenic and infected leaves. Most of the detectable CMV MP in infected plants was found in the T fraction, which did not yield detectable amounts of MP in any of our other experiments (Fig. 2c, d). CMV MP was also detected in the S30 fraction from infected plants whereas S30 from transgenic leaves did not yield detectable amounts of CMV MP. Lower levels of MP were found in the CW and P30 fractions. Highest levels of total amounts of CMV MP were found in young leaves. The presence of low total amounts of MP in leaves just above the inoculated leaf corresponded with low levels of CMV coat protein in the S30 fraction. One set of CMV infected plants was also briefly subjected to temperatures > 32 °C (not shown). This did not affect the ratios of detectable MP between fractions.

In a separate experiment, we made subcellular fractions of leaves from plants doubly inoculated with TMV and CMV to test the ability of each MP to partition independently in the plant cell. Once again, the greatest amounts of TMV MP were found in the P30 fractions, and levels of TMV MP in each fraction seemed unaffected by the presence of CMV MP (not shown). Likewise, the presence of TMV MP did not affect the distribution or levels of CMV MP in any fraction. The highest levels of CMV MP were still detected in T fractions.

Our fractionation results can be interpreted to predict cellular components with which the TMV and CMV MPs are associated in transgenic and infected plants.
Fraction P1 can contain cell wall components, nuclei and plastids (Nivison et al., 1988; Stern & Newton, 1988). The remains of this fraction after Triton X-100 washing (CW) will contain nuclei stripped of outer membranes (Watson & Thompson, 1988), cell wall components and plasmodesmata core complexes (Turner et al., 1994). Urea is known to release the collar that surrounds the plasmodesmata, and the collar is near a region of callose deposits (Turner et al., 1994). The TMV MP may also interact with callose at the plasmodesmata (Wolf et al., 1991). Because the CMV MP and TMV MP were found in the CW fractions washed with Triton X-100 and treated with guanidine, a denaturant, the collar region or callose deposits could be an essential interactive area for both the CMV and TMV MPs. The nuclei or nuclear membranes probably do not accumulate much MP (Tomenius et al., 1987; Burman et al., 1994).

Triton X-100 solubilizes chloroplasts and thylakoids (Pascoe & Ingle, 1978) and the desmotubules and plasma membranes that are part of the plasmodesmata complex (Turner et al., 1994). Since CMV MP produced in infected plants was found extensively in the T fraction, we suspect that CMV MP is mostly associated with solubilized desmotubules and plasma membranes of the plasmodesmata core. Our observation that TMV MP was not found in this fraction from infected plants could point to a major difference between the biology of the TMV and CMV MPs. TMV MP could be less soluble than CMV MP, causing it to remain in the CW fraction. However, we found a large portion of the TMV MP from infected plants in P30, a fraction likely to contain Golgi body membranes and endoplasmic reticulum (Hanson et al., 1988). TMV MP has been located in cytoplasmic regions outside the nucleus where these organelles exist (Moshi et al., 1992).

The decreased levels of MP detected in CMV and TMV MP transgenic plants, when compared to their infected counterparts, may be due to different levels of expression. Even though transgenic plants accumulate less MP, they can complement viral movement. So, amounts of MP made constitutively must be sufficient for plasmodesmata size-exclusion limit modification. This implies that locations other than the plasmodesmata-area proper may be repositories for excess amounts of MP in infected plants, as suggested by Citovsky et al. (1993). Our result that the most MP was found in the youngest tissues where virus infection is most active supports this conclusion. These repositories could implicate the MP in secondary functions. Secondary MP functions can influence symptom development (Tsai & Dreher, 1993; Cooper et al., 1995) or virulence (Nejdat et al., 1991). Notwithstanding, the sites of MP accumulation do not necessarily correspond with sites of activity.

Our preliminary study is not an attempt to demonstrate an ideal extraction method for both TMV and CMV MPs, but our methodology is consistent for both MPs. This consistency is supported by the observation that both the TMV MP and CMV MP were distributed in leaves independently of each other in doubly infected plants. We conclude that MPs in infected plants have complex distributions and do not necessarily accumulate in the cell in the same manner as MPs expressed in transgenic plants at lower levels.

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References


Short communication


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