Detection of Viral Proteins in Cytopathic Structures in Cowpea Protoplasts Infected with Cowpea Mosaic Virus

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SUMMARY

Infection of cowpea (Vigna unguiculata) cells or protoplasts with cowpea mosaic virus is accompanied by the appearance of characteristic cytopathic structures. A major constituent of these cytopathic structures, represented in thin sections by electron-dense material, was shown to contain viral non-structural proteins by its reaction with antisera to non-structural proteins and their detection with colloidal gold-labelled Protein A. With this same technique virus particles were found to accumulate mainly in the cytoplasm.

The genome of cowpea mosaic virus (CPMV) consists of two positive-stranded RNAs which are separately encapsidated in identical protein shells. Both RNAs (denoted B and M RNA) have a small protein (VPg) linked at their 5' end, are polyadenylated and possess a single long open reading frame (for a recent review see Goldbach & Van Kammen, 1985). Fig. 1 schematically represents how the large polyproteins encoded by these RNAs are processed into functional polypeptides. Infection with CPMV is accompanied by the appearance of characteristic cytopathic structures (Fig. 2; Assink et al., 1973; De Zoeten et al., 1974). These structures consist of amorphous electron-dense material and numerous membranous vesicles. Autoradiography performed on sections of isolated cytopathic structures treated with [3H]uridine provided evidence that the replication of the CPMV RNAs was associated with these vesicles (De Zoeten et al., 1974), but the nature of the electron-dense material has remained unknown.

In CPMV-infected cells, large amounts of virus particles are produced (up to 1 mg per g of leaves). Also most of the non-structural proteins are easily detectable in infected protoplasts by labelling with [35S]methionine (Rezelman et al., 1980). In an effort to locate sites within the infected cell where these structural and non-structural viral proteins accumulate we used an immunocytochemical technique in which binding of specific antisera was detected by its reaction with Protein A labelled with colloidal gold.

The preparation of Protein A-gold complexes with 7 nm diameter gold particles and immunogold labelling were as described by Van Lent & Verduin (1986). The antisera used for the experiments described in this paper have been characterized for their reactivity with CPMV-specific proteins present on protein blots. The anti-24K protein serum recognized the 24K protein and also all proteins containing the 24K sequence, namely the 170K, 110K and 84K proteins (Fig. 1; Wellink et al., 1987). Anti-170K serum recognized the 170K, 110K and 87K proteins (Dorssers et al., 1984). Anti-VPg serum, obtained after injection of a rabbit with synthetic VPg (Jaegle et al., 1987), reacted with 170K, 84K and 60K proteins on a protein blot (J. Wellink, unpublished data). The anti-CPMV serum was obtained by injecting a rabbit with purified CPMV components. Gamma globulins (IgG) were purified from the antisera by affinity chromatography on columns of Sepharose CL-4B-Protein A. Cowpea mesophyll protoplasts were isolated, inoculated with CPMV components or RNA and cultured under...
Fig. 1. Model for the expression of the CPMV RNAs. The open reading frames in both RNAs are indicated by white bars. Proteins are represented as single lines and VPg as a black square. All proteins shown are produced upon translation in vitro of the CPMV RNAs. However, with the exception of the 105K, 95K and 58K proteins, all of these proteins can also be found in CPMV-infected protoplasts. The positions of the translational start and stop codons are indicated. Cleavage sites are as follows: O, Gln–Met; V, Gln–Gly; ▽, Gln–Ser.

Fig. 2. Section of cowpea mesophyll protoplast, fixed with aldehydes and osmium tetroxide at 24 h after inoculation with CPMV. Cytopathic structures consisting of amorphous electron-dense material (ED) and membranous vesicles (Vs) are present in the cytoplasm: N, nucleus. Bar marker represents 300 nm.

continuous light as described by Rezelman et al. (1980) and Maule et al. (1980). At different times after inoculation (0, 12, 24 and 48 h) samples of these protoplasts were fixed with glutaraldehyde/paraformaldehyde and osmium tetroxide, dehydrated and embedded in methacrylate. To allow post-embedding immunogold labelling, similar samples were fixed with
glutaraldehyde/paraformaldehyde and subsequently dehydrated and embedded at low temperature in Lowicryl K4M as described by Van Lent & Verduin (1986).

In sections of aldehyde- and osmium-fixed protoplasts infection was first recognized at 12 h after inoculation by the presence of amorphous electron-dense material in the cytoplasm. No vesicles were found at this time. At 24 (Fig. 2) and 48 h post-inoculation cytopathic structures consisting of electron-dense material and an increasing number of vesicles had developed. These structures were not observed in mock-inoculated protoplasts. Similar results were reported by Rezelman et al. (1982). Sections of aldehyde-fixed and Lowicryl K4M-embedded protoplasts showed that cell organization and organelles were preserved, but preservation of membranes was poor due to extraction during the embedding procedure. In sections of protoplasts, isolated at 12, 24 and 48 h after inoculation and treated with anti-24K IgG and Protein A–gold complex, gold label was exclusively located on the electron-dense material of the cytopathic complex (see Fig. 3). The intensity of labelling of the electron-dense material did not vary noticeably with infection time. Similar results were obtained with anti-VPg serum (data not shown) and anti-170K serum (Fig. 4). No gold label could be detected in or near the vesicles of the cytopathic complex or in the cytoplasm, the nuclei, the chloroplasts or the mitochondria when antisera against the non-structural proteins were used (Fig. 3, 4). However, with anti-CPMV IgG, Protein A–gold complex label was found throughout the cytoplasm of protoplasts isolated 24 and 48 h after inoculation (Fig. 5). A similar distribution of virus particles was reported for another comovirus, red clover mottle virus, in pea leaf cells (Tomenius et al., 1983). No gold labelling was observed in sections of mock-inoculated protoplasts treated with anti-24K, -170K, -CPMV and Protein A–gold complex or in CPMV-infected protoplasts treated with preimmune serum and Protein A–gold complex.
Fig. 4. Section of cowpea mesophyll protoplast, embedded in Lowicryl K4M at 48 h after inoculation with CPMV, and incubated with anti-170K IgG and Protein A-gold complex. Gold label is mainly present over the electron-dense material (ED) but not over and between the vesicles (Vs). C, chloroplast; V, vacuole. Bar marker represents 300 nm.
The results obtained with the antisera against the 24K and 170K proteins and VPg show that the electron-dense material from the CPMV-induced cytopathic structure contains CPMV B RNA-encoded non-structural proteins. So far we have not been able to determine whether all B RNA-encoded proteins are present in this structure, since the three antisera used for these experiments each react with a set of overlapping proteins (Fig. 1). The vesicles that are also present in the cytopathic structure have been implicated in viral RNA replication (De Zoeten et al., 1974), but it remains to be established whether the electron-dense material also fulfills a function in this process. It has been proposed that the viral proteins active in the replication of the CPMV RNAs are only able to synthesize one RNA strand (Van Kammen & Eggen, 1986). It is therefore possible that after use proteins accumulate in the electron-dense material.

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REFERENCES


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