Cylindrical inclusion bodies of wheat streak mosaic virus and three other potyviruses only self-assemble in mixed infections

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Potyviruses produce cylindrical inclusions (CIs) in the cytoplasm of infected cells. Immunogold labelling and electron microscopy of embedded and sectioned wheat and maize cells doubly infected by different potyviruses revealed no mixing of inclusion proteins in CIs. The viruses were wheat streak mosaic virus (WSMV), agropyron mosaic virus and hordeum mosaic virus, in wheat, and WSMV and maize dwarf mosaic virus in maize. The three viruses in wheat were indistinguishable morphologically and in ultrastructural features but can be separated by serology and host range. The absence of phenotypic mixing in CIs showed that in the presence of CI proteins of other potyviruses, assembly was either highly virus-specific, or that no opportunity existed for CI proteins to assemble into hybrid CIs in mixed infections.

Introduction

In infected plant cells all potyviruses induce cylindrical inclusions (CIs) composed of a protein of $M_r$ 68K to 72K, specified by the viral genome (Edwardson, 1966; Brakke et al., 1987; and see Matthews, 1981; Dougherty & Hiebert, 1980a, b; Quiot-Douine et al., 1986; Dougherty & Carrington, 1988). CI proteins of some potyviruses are serologically related (Purcifull et al., 1973), but most are immunologically distinct.

Hordeum mosaic virus (HorMV) and agropyron mosaic virus (AgMV) are potyviruses morphologically indistinguishable from wheat streak mosaic virus (WSMV) (Slykhuis & Bell, 1966). HorMV and AgMV are more closely related to WSMV than to other potyviruses. Maize dwarf mosaic virus (MDMV) is transmitted by aphids.

It was of interest to see whether CIs of potyviruses would form hybrid structures in cells doubly infected by two different potyviruses. CI proteins of WSMV did not co-assemble with those of MDMV in doubly infected maize but the reverse was not tested (Brakke et al., 1987). It is reported here that CI proteins of the potyviruses investigated do not co-aggregate in vivo with CI proteins of another potyvirus to an extent that is detectable by immunogold labelling of doubly infected cells.

Methods

Hosts and viruses. Wheat plants (Triticum aestivum L. var. Michigan Amber) were manually inoculated with AgMV (American Type Culture Collection, ATCC, PV75) or HorMV (ATCC, PV81) or with WSMV (ATCC, PV57). Other wheat plants (var. Centurk) were doubly inoculated with AgMV (ATCC, PV75) and HorMV. Doubly infected plants were also obtained by simultaneous inoculation of WSMV and AgMV or WSMV and HorMV. Alternatively, inoculation with WSMV was done when symptoms appeared in plants which had been inoculated with AgMV or HorMV. This was done usually 4 to 5 days after the first inoculation. MDMV-B was received from S. Jensen (this department) and manually inoculated into the inbred maize line South Dakota P2. When symptoms appeared (after 4 to 5 days), plants were superinfected with WSMV. Superinfected plants were tested for the presence of WSMV 5 to 7 days later. The presence of each virus in double infections was confirmed by immunoelectron microscopy (Lin, 1984). Plants were grown in pasteurized compost in a greenhouse at 25 to 30°C. Leaf tissue samples were taken from young emerging leaves of plants 7 to 14 days after inoculation.

Electron microscopy. Tissue pieces were fixed and embedded in London Resin White as described previously (Langenberg, 1985).

Immunolabelling. Rabbit or rat antisera were used as a primary label at a dilution of 1/200 to 1/400 in 4% normal goat antiserum in 0.1 M-K$_2$HPO$_4$-citrate pH 7.2 containing 0.05% sodium azide. The preparation of rabbit antiserum to CI protein of WSMV has been reported (Brakke et al., 1987). CI proteins of HorMV and AgMV were prepared in the same way and antibodies were raised by intramuscular injection of rabbits or rats with the proteins emulsified in complete Freund's adjuvant. Antiserum to CI protein of MDMV-B was received from S. Jensen (this department). Antisera were absorbed with an equal volume of crude juice (1 g tissue/ml water) of the host plant before dilution and used as a label for ultrathin sections. Gold-labelled goat anti-rabbit or anti-rat IgG was prepared exactly as described previously (Lin & Langenberg, 1983). The two-step method of labelling was employed. Thin sections were then stained with uranyl acetate and lead citrate for 15 and 1 min respectively and viewed in a Zeiss EM10A at 60 kV.
Results

The symptoms produced in wheat, and the morphology and ultrastructural appearance of cells infected with HorMV, AgMV and WSMV, are so similar that they cannot be differentiated. No special features other than those already described in the literature on the ultrastructure of infection with each of these viruses was noticed in double infections of WSMV with AgMV, HorMV and MDMV. All doubly infected plants, however, showed more stunting and a more severe yellow mosaic than plants infected with a single virus as previously noted by Slykhuis & Bell (1966).

Immunolabelling

Antiserum to the 66K CI protein of WSMV did not react in ultrathin sections with CI proteins of AgMV, HorMV or MDMV, nor did antiserum to CIs of the latter three react with CIs of WSMV in thin sections (data not shown). However, antiserum to AgMV-CI also labelled CIs of HorMV in tissue infected by HorMV only (Fig. 1). The reverse was not tested.

Serial sections of wheat tissue doubly infected by AgMV and WSMV were labelled with antiserum to AgMV-CI (Fig. 2) or WSMV-CI (Fig. 3). No CIs could be detected that labelled with both antisera. Partial label was entirely absent. Inclusions labelled with either one antiserum or the other. The same results were obtained in mixed infections of HorMV and WSMV.

Fig. 4 shows results of an ultrathin section of tissue infected with HorMV and WSMV that was labelled simultaneously with rat antiserum to HorMV-CI (small gold label, small arrows) and rabbit antiserum to WSMV-CI (large gold label, large arrows). Goat anti-rabbit IgG did not react with rat IgG in prior tests (data not shown). WSMV-CI also did not aggregate or assemble with MDMV-CI in doubly infected cells. Serial sections labelled with antiserum to WSMV-CI (Fig. 5) or antiserum to MDMV-CI (Fig. 6) show that only homologous CIs are labelled. Here also, no partial co-assembly of CI proteins could be detected. There was no evidence of phenotypic mixing.

Treatment with antiserum to CIs of all four viruses used here failed to show aggregates or deposits of excess CI proteins that were not assembled into characteristic CIs. Non-specific label was low.

Discussion

Evidence is presented that the WSMV-CI protein is not serologically related to CI proteins of AgMV, HorMV or MDMV. The reverse was also true. However, some cross-reactivity was noted between antiserum to AgMV-CI and HorMV-CIs (Fig. 1). In mixed infections, CIs of two viruses can appear in the same cell but co-aggregation or assembly of CI proteins to form hybrid CIs was not detected. AgMV and HorMV showed some cross-reactions with antisera to AgMV-CI, although at least the 3' end of the RNAs of AgMV and HorMV differ substantially (N. L. Robertson & R. C. French, unpublished results). They are, therefore, still considered distinct although related viruses. This agrees with findings of Slykhuis & Bell (1966).

It was surprising that no deposits of excess CI proteins could be located anywhere in the cell. Excess capsid protein deposits of many viruses are known although this has not been reported for any potyvirus.

Specificity in assembly of CIs must be very high in the presence of other potyvirus CI proteins very similar in size and function. CIs were often found intermingled and touching one another in the same cell. Except for the immunolabel it was sometimes difficult to distinguish where one CI stopped and the next one began (Fig. 4). It can be assumed that CIs of co-infecting viruses were assembled simultaneously in the cell since newly emerging leaves of doubly infected plants were used as the study material.
Non-co-aggregation of CI proteins

Fig. 2 and Fig. 3. Two serial sections of a cell infected by both AgMV and WSMV labelled either with antibodies to AgMV-CI (Fig. 2, top) or WSMV-CI (Fig. 3, bottom). Those CIs labelled by one antiserum are not labelled by the other.
Fig. 4. HorMV- and WSMV-infected cell labelled simultaneously with rat antibodies to HorMV-CI (small arrows, small gold label) and rabbit antibodies to WSMV-CI (large arrows, large gold label). No phenotypic mixing of HorMV and WSMV CIs is detectable.

Fig. 5 and Fig. 6. Serial sections of a cell infected by WSMV and MDMV labelled with antiserum to WSMV-C1 (Fig. 5, left) or to MDMV-CI (Fig. 6, right). Those CIs which label with one antiserum are not labelled by the other.
Mixing of similar capsid proteins of co-infecting viruses is rather common, as is genomic masking (see Matthews, 1981). Since phenotypic mixing of CIs was not found, it may be that the opportunity to form hybrid CIs does not present itself. Based on the absence of phenotypic mixing of CIs, it is predicted that the opportunity for phenotypic mixing or genomic masking of virions will also be rare or absent in the potyvirus group.

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References


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