The degradation of potato virus M (PVM) particles in plant cells

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Abstract

Degradation of potato virus M particles was observed in the cells of *Solanum tuberosum*, *Solanum rostratum*, *Lycopersicon esculentum* and *Lycopersicon chilense* plants infected with this virus. PVM particles found in the cytoplasm of infected parenchyma cells grouped together in the form of inclusions, often found near the tonoplast. The ends of the virus particles and the tonoplast came into close contact. Cytoplasmic protrusions containing PVM particles, reaching into vacuoles were formed in those places. In addition to a large central vacuole, small vacuoles were observed in cells containing PVM particles. Various stages of degradation of cytoplasmic protrusions were observed both in the large and small vacuoles.

Key words: potato virus M, degradation of particles, cytoplasmis, protrusions, vacuole

INTRODUCTION

The infection of plant cells by a virus is a dynamic and complex process. Its essence is the replication of the virus using the plant's cellular apparatus. This requires the switching of the cell's metabolism to the synthesis of the proteins and nucleic acids required by the virus for its replication, at the expense of compounds characteristic for and vital to the cell's own life processes. Complete virus particles, which are foreign proteins in the cell's cytoplasm, are formed as the result of these processes. This complicated replication mechanism and the accumulation of viral inclusions in the cytoplasm disturbs the cell's autoregulatory mechanisms and leads to various reactions on the part of the plant, at least part of which may be taken as defence reactions.

The potato virus M (PVM) multiplies in the parenchyma of plants and it is there that the cytological changes related to this process are observed

(Rudziński-Langwald 1978, 1986). At the same time, changes in the cells of such hosts as *Solanum tuberosum*, *Solanum rostratum*, *Lycopersicon esculentum* and *Lycopersicon chilense* are observed that do not result directly from viral replication itself, but are a reaction of the cells of various species to the presence of PVM particles in them.

The objective of this paper is to present the defensive reactions of cells of various species to the presence of PVM particles.

MATERIAL AND METHODS

The cytological studies were conducted on leaf fragments from: *Solanum tuberosum* (L.), *Solanum rostratum* (Dunal), *Lycopersicon esculentum* (Mill) and *Lycopersicon chilense* (Dun.). The plants were mechanically inoculated with potato virus M (PVM) isolate M55a. Samples were taken 1, 2, 4 and 6 weeks after inoculation; healthy, not-inoculated plants were used as controls. The samples were fixed in 3% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer, pH 7.2 for 4 hours at room temperature. This was followed by postfixation in 2% osmium tetroxide for 2 hours at 4°C. The material was dehydrated in rinsing concentration of ethyl alcohol (10-70%) acetone (70-100%) and propylene oxide. The samples were embedded in Epon 812 and sectioned and stained with uranyl acetate and lead citrate. The material was observed in a JEM 100 C electron microscope.

RESULTS

The species *S. tuberosum*, *S. rostratum*, *L. chilense* and *L. esculentum* are hosts for potato virus M. PVM particles were found in the basic cytoplasm of cells of infected plants. Initially, they were random clusters. Four to 6 weeks after inoculation, PVM particles were observed grouping together in the cytoplasm of infected cells near the tonoplast of the cell's vacuoles (Figs. 1-3). After only 2 weeks following inoculation, small vacuoles were seen to form in cells in which virus particles were observed. The gathering of bunches of virus particles near the tonoplast of these vacuoles, as well as near that of the large, central vacuole is partially understandable, since the motion of the cytoplasm in these places may be slower. It seems that many inclusions adhere relatively loosely to the tonoplasts mentioned above (Fig. 2). However, there are types of inclusions that touch the tonoplast with one end or are directly near to it (Fig. 3). It seems that the contact of the virus particles with the tonoplast is more durable than it would be from just simple adhesion. This is concluded from occasional observations of damage to tonoplasts. Fragments of such tonoplasts protrude into the vacuole and the PVM particles that adjoin it move along with that fragment of the membrane. This suggests that there is a permanent connection between the end of the PVM particle and the surface of the tonoplast (Fig. 4).

Fig. 1. A fragment of *L. chilense* parenchyma cell 4 weeks after inoculation. Inclusions of virus particles (V) seen in the cytoplasm among vacuoles. 42 000 $\times$. Fig. 2. A fragment of a leaf parenchyma cell of *L. esculentum* 6 weeks after inoculation. Numerous virus particles (V) gathered in inclusions are visible in the cytoplasm along with vacuoles. 36 000 $\times$. Fig. 3. A fragment of an *S. tuberosum* leaf parenchyma cell 4 weeks after inoculation. PVM particles are adhering by their ends to the tonoplast of the central vacuole. The close contact with the tonoplast (eg. arrow) is visible. 84 000 $\times$. Fig. 4. A fragment of an *S. tuberosum* parenchyma cell. The damaged tonoplast shifted into the inside of the vacuole is visible. The PVM particles are adhering to the tonoplast (arrow). 38 000 $\times$

Fig. 5. Fragment of an *S. rostratum* leaf parenchyma cell from an area adjoining the necrosis (4 weeks after inoculation). Large protrusions of the tonoplast containing several PVM particles each visible on the border of the vacuole. $74\,000\times$. Fig. 6. An *L. chilense* cell 6 weeks after inoculation. A finger-like protrusion containing PVM particles into the vacuole is visible. Around it are other protrusions of the tonoplast. $98\,000\times$. Fig. 7. A fragment of an *L. esculentum* cell 4 weeks after inoculation. In the upper part of the picture there is a protrusion containing PVM particles. $54\,000\times$. Fig. 8. An *S. tuberosum* cell 6 weeks after inoculation. A cytoplasmic protrusion containing virus particles. $64\,000\times$. Fig. 9. A fragment of a leaf parenchyma cell of *L. chilense* 4 weeks after inoculation showing a cytoplasmic protrusion containing PVM particles. $84\,000\times$

Fig. 10. A fragment of an *L. chilense* leaf parenchyma cell 6 weeks after inoculation. Visible are the small vacuoles containing cytoplasmic protrusions. $38\,000\times$. Fig. 11. An enlargement of the previous photograph, showing the upper left vacuole. At the bottom of the photograph, protrusions of the tonoplast into the vacuole. Visible inside the vacuole are cross sections of cytoplasmic protrusions. In the protrusion (asterisk), a well-preserved tonoplast and visible outline of a PVM particle. Two upper protrusions with less distinct contours. $84\,000\times$. Fig. 12. Enlargement of Fig. 10 showing a fragment of the right vacuole. A cross section of a protrusion with three well-preserved virions visible in the upper right-hand corner. Protrusions in different stages of disintegration visible in the lower corner. $84\,000\times$. Fig. 13. A fragment of an *L. esculentum* cell 4 weeks after inoculation. A cytoplasmic protrusion containing PVM particles visible in one of the vacuoles. $38\,000\times$

Fig. 14. A fragment of an *L. chilense* cell 6 weeks after inoculation. Dark, flaky contents in vacuoles and a cytoplasmic protrusion (arrow). 38 000 $\times$. Fig. 15. An enlargement of the previous photograph. Dark, homogenous deposits visible in the vacuole. In the upper part — a vesicle containing the remains of a membrane surrounding a dark deposit. 84 000 $\times$. Fig. 16. A fragment of an *L. esculentum* parenchyma cell 4 weeks after inoculation. Visible in the vacuole of the cell is a cytoplasmic protrusion surrounded by a membrane, containing PVM particles and flaky concretions. 36 000 $\times$. Fig. 17. A fragment of an *L. chilense* cell 6 weeks after inoculation. Visible in the central vacuole are protrusions surrounded by a membrane, containing virus particles and dark, flaky deposits. 38 000 $\times$

Fig. 18. A fragment of an *S. rostratum* cell from the edges of a necrosis on a leaf. In the upper part of the picture, characteristic clusters of short endoplasmic reticulum cisterns are visible in the cytoplasm, among which single PVM particles (asterisk) are seen. A virus particle (V) inclusion is visible in the lower part of the photograph. Various stages of disintegration of cytoplasmic protrusions are visible in the vacuole. 28 000 $\times$

Fig. 19. A fragment of an *L. esculentum* cell 4 weeks after inoculation. Visible are fusing vacuoles, the lower has dark deposits. PVM particles are visible in the cytoplasm. 19 000 $\times$. Fig. 20. Fragments of an *S. tuberosum* cell 6 weeks after inoculation. Three fusing vacuoles are visible. 19 000 $\times$. Fig. 21. A fragment of an *L. chilense* cell 6 weeks after inoculation. The process of fusing vacuoles. 12 800 $\times$

Finger-like protrusions of the tonoplast reaching into the vacuole are formed at the points where the PVM particles come into contact with the tonoplast. These invaginations envelop the PVM particles along with the surrounding cytoplasm (Figs. 5-9). Cytoplasmic protrusions into the vacuole are thus formed. Analysis of the electron micrographs indicates that the contact of the virus particles with the tonoplast stimulates the formation of these protrusions. This contact is maintained while the protrusion is formed (Figs. 5-8). Initially, these protrusions are enveloped by an undamaged tonoplast and the cytoplasm inside contains well-preserved particles (Figs. 16 and 17). It seems that at least several PVM particles in the cytoplasmic protrusion adhere by one of their ends to the tonoplast.

The cytoplasmic protrusions lose contact with the cytoplasm, of which they were a part, due to narrowing that develops (Figs. 13 and 18). In this way, inside the vacuole, separate fragments of the cytoplasm become enveloped by a membrane that derives from the tonoplast. They contain minute amounts of cytoplasm and several to several-dozen PVM particles (Figs. 10-13). Sometimes cytoplasmic protrusions containing ribosomes are also observed (Figs. 7, 13 and 18).

The next stage in the degradation of PVM particles is the disintegration of the surrounding membrane and the entire contents of the protrusions. During the final stage, dark, homogenous deposits are formed which do not show any structure and are not surrounded by a membrane (Figs. 11, 12, 14, 15, 18). Numerous intermediate stages are observed, however, (Figs. 10-13 and 16 and 17) in which the surrounding membrane is still intact but its contents are partially disintegrated. Cytoplasmic protrusions of various stages of disintegration can be found in one vacuole (Figs. 11, 12 and 18).

Fusing of vacuoles can be observed in cells. The cytoplasmic bridges that exist between particular small vacuoles narrow, then tear apart, and in this way the vacuoles fuse. The fusing of small vacuoles with the central vacuole is also observed (Figs. 19-21).

DISCUSSION

Infection of plants with a virus leads to the formation of new virus particles inside the cells. In the first period following inoculation, the number of virus particles in the plant increases dynamically. However, after a certain time, the concentration of the virus begins to decrease. This phenomenon is known for several viruses, eg. potato virus A (Bartels 1954). A similar situation has been described for potato virus M (Bartels and Volk 1966).

Three weeks following inoculation, the virus concentration in tomato leaves is high in all of the leaves. In the later stages, this concentration begins to fall, starting from the lower leaves. Changes in the concentration of PVM particles in potato plants follow the same lines, although there are potato varieties in

which the PVM concentration remains high for very long periods of time (Bartels 1967).

It is seen from the above, that on cellular level, after a period of intense multiplication of the virus, expressed as the increase in the number of viral particles accumulated in the cytoplasm, a stage begins during which the number of particles does not increase, but even falls. Replication processes are inhibited. Analysis of electronmicrographs of cells infected with potato virus M at different times after inoculation confirm the rule given above.

During the early stages of infection, clusters of endoplasmic reticulum and ribosomes appear and are the probable sites of viral replication. In the later stages, they give way to inclusions of complete virons (Rudzińska-Langwald 1990). This does not, however, explain the fall in the number of particles in the cell, as observed starting three weeks after inoculation (Bartels and Volk 1966). Degradation of viral particles in vacuoles is observed in the plant cells 4 and 6 weeks after inoculation. This corresponds to the observed decrease in the concentration of viruses in plants infected with PVM as observed by Bartels and Volk (1966) and Bartels (1967).

Processes of lysis of virus particles in infected cells by cellular systems having just such a purpose have not hitherto been described. On the other hand, Cadilhac et al. (1972) observed increased autolytic activity in cells of plants infected with a virus from the polyvirus group. There were, however, no viruses in the lysosomes formed in the cells, in spite of the fact that autolysis occurring in the vacuoles of plant cells is known (Coulomb and Coulomb 1983). The process of entering the cytoplasmic protrusion by the long virus particles is very characteristic. It seems that the contact of the PVM particle with the tonoplast is the reason for forming the protrusion and the reason why the particles get into the invagination.

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Degradacja cząstek wirusa M ziemniaka (PVM) w komórkach roślin

Streszczenie

W komórkach roślin *Solanum tuberosum*, *S. rostratum*, *Lycopersicon esculentum* i *L. chilense* porażonych wirusem M ziemniaka stwierdzono procesy degradacji cząstek tego wirusa. Cząstki PVM występujące w cytoplazmie porażonych komórek miękkiszowych grupują się w inkluzje, które często obserwuje się przy tonoplaście. Dochodzi do bliskiego kontaktu między końcami cząstek wirusa i tonoplastem. W miejscach tego kontaktu tworzą się wypustki cytoplazmatyczne wnikaające do wakuoli, zawierające cząstki PVM. W komórkach zawierających cząstki PVM obserwuje się występowanie, oprócz dużej centralnej wakuoli, małych wakuol. Zarówno w dużej, jak i w małych wakuolach obserwuje się różne stadia degradacji wypustek cytoplazmatycznych.