Inhibitory Action of Methionine upon the Barley Powdery Mildew (Erysiphe graminis f. sp. hordei) 2. Electron Microscopy of Primary Haustoria, Hyphae and Conidia on Barley Leaves treated with L-Methionine

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Abstract

Effects of L-methionine on the barley powdery mildew (Erysiphe graminis f. sp. hordei) were studied by electron microscopy. Barley leaves were treated with L-methionine (8 x 10^{-3} M aqueous solution) 24 hr before inoculation with the fungus. Primary haustoria formed in the treated leaves were examined under an electron microscope 24, 58, 96 and 168 hr after inoculation. At early stages of infection, no degeneration was observed in the cytoplasm of the haustoria. Their sheath membranes were covered with electron-dense materials and their sheath matrix also contained electron-dense materials different from the former. At later stages, the haustorial content increased in electron-density, and large, more densely stained deposits were found in the cytoplasm. Epidermal cells of barley leaves treated with L-methionine before inoculation with the fungus usually became necrotic, accumulating electron-dense granules in the vacuoles. Since this phenomenon has been observed in leaves of barley varieties resistant to the barley powdery mildew, the inhibitory action of L-methionine on the fungus is thought to be due to the induction of resistant reaction in barley leaves by L-methionine treatment. In hyphae of the fungus on barley leaves treated with L-methionine, degeneration of cell organelles could not be found, but large vacuoles with electron-dense deposits could. When barley leaves were inoculated with the fungus and treated with L-methionine 96 hr after inoculation, abnormal conidia showing different stages of degeneration (partial expansion of cell wall, aggregation of the cytoplasm, and disappearance of the aggregated materials) were collected 72 hr after treatment. (Received August 29, 1977)

Introduction

Several amino acids have been reported to show the chemotherapeutic activity against certain fungal diseases of plants. These amino acids have also been proved to be those which are not involved in the normal nitrogen metabolism of plants. An only exception to this is the inhibitory action of L-methionine on

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plant diseases caused by obligate fungi 1,2,3). In a previous paper we reported that growth of the barley powdery mildew (Erysiphe graminis f. sp. hordei) was remarkably inhibited on barley leaves treated with L-methionine1). According to light microscopic studies L-methionine did not show the action during the infection process (conidium germination, appressorium formation and penetration), but did after the formation of primary haustoria. The fungus on barley leaves treated with L-methionine produced abnormal primary haustoria which showed a small number of elongated projections suggesting a marked decrease in the absorbing activity. When barley leaves were treated with L-methionine immediately after the beginning of conidium formation, a long chain consisting of twenty or more conidia was formed on a conidiophore. In this paper we describe details of ultrastructural changes found in the barley powdery mildew (race 9 of E. graminis f. sp. hordei) on barley leaves treated with L-methionine.

Materials and Methods

The barley variety used in this study was “Golden Melon”, a variety susceptible to race 9. The seeds were sown in sterilized vermiculite in pots, which were kept for 2 weeks under the condition of 25°C and 60% relative humidity. Subsequently primary leaves of 6 cm long were cut and placed in petri dishes containing \(8 \times 10^{-3}\) M L-methionine solution. Fresh conidia used as inoculum were collected from barley plants infected with the fungus after removing old conidia by blowing 48 hr ago. Leaves in the petri dishes were inoculated with these conidia by use of a brush. The inoculated leaves were collected 24, 58, 96 hr and 7 days after inoculation. Small pieces from the inoculated leaves were fixed at 0°C for 3 hr in 5% glutaraldehyde in 0.1 M phosphate buffer pH 7.0 containing 0.2 M sucrose. After rinsing in the same buffer overnight, the leaf pieces were post-fixed in 1% osmium tetroxide in Dulton buffer at 0°C for 4 hr. Then they were rinsed for 10 min. in 0.1 M phosphate buffer three times. After dehydration in a graded series of ethyl alcohol, they were embedded in Epon 812. Thin sections were post-stained in uranyl acetate for 20 min followed by lead citrate for 5 min and observed under an electron microscope (JEM 100-S).

Results

Haustoria

Control: Primary haustoria continued to grow for 4-5 days after inoculation, becoming mature ones having about ten projections on each side of the central body1). It has been reported that the haustoria differ with age in their size, cell contents, sheath membrane and matrix1,4). So we firstly observed the primary haustoria formed in untreated barley leaves 24, 58, 96 hr and 7 days after inoculation.

Young haustoria 24 and 58 hr after inoculation were shown to have a cell wall thinner than that in hyphae and conidia of the same fungus (Plates I-A, B, C and V-A, B). The wall appeared to be so granular and amorphous that it could not be discerned from the sheath matrix (Plate I-C). The cytoplasm was surrounded by a plasmamembrane showing a marked wavy appearance. It contained mitochondria with tubular cristae, lipid bodies, many ribosomes and small vacuoles (Plate I-C). The sheath membrane consisted of inner and outer layers, and the former was
thicker than the latter. Outside the sheath membrane many vesicles were observed (Plate I-C). The sheath membrane enveloping the projections showed a marked wavy appearance, and the host cytoplasm accumulated around it (Plate I-C). The sheath matrix appeared to be amorphous and contained many electron-dense particles (Plates I-A, B and II-A).

In older haustoria 7 days after inoculation, the cell wall became thicker than that of young ones (Plates I-C and II-B). The haustorial contents became so electron-dense that cell organelles except vacuoles and lipid bodies could not be discerned (Plate II-B). The sheath matrix was occupied by more densely stained materials, being difficult to be distinguished from the sheath membrane (Plate II-B). The host cytoplasm around the haustoria became also electron-dense, and many vesicles containing electron-dense granules developed (Plate II-B).

Treatment with L-methionine: Barley leaves were treated with $8 \times 10^{-3}$ M L-methionine solution 24 hr before inoculation. The primary haustoria formed in the treated leaves were examined under an electron microscope 24, 58 and 96 hr after inoculation.

In haustoria 24 and 58 hr after inoculation, the cell wall was twice or three times as thick as that of the control (Plates I-C and III-A, B). Their cell wall consisted of a single electron-lucent layer and did not contain electron-dense granules like those found in the control (Plates III-A, B). In the cytoplasm any noticeable change was not observed except that cristae of mitochondria decreased in number compared with those of the control (Plates I-C and III-C). The sheath membrane consisted of a two-layered unit membrane which was a little higher in the electron density than that of the control (Plates I-C and III-C). The haustorial wall was connected with the sheath membrane and showed no vesicular profiles which were found numerous in the control (Plates I-C and III-C). The sheath membrane around the projections did not show the remarkably wavy structure like that found in the control (Plates I-C and III-C). The sheath matrix was electron-light at some part, and that of old haustoria contained an electron-dense material (Plates II-B and III-B). The similar material was also found in the sheath matrix around the projections (Plate III-C).

The primary haustoria 96 hr after inoculation were found to be covered with a more densely stained material, and cell organelles except vacuoles containing deposits could hardly be discerned in them (Plate IV-A, B).

In leaves treated with L-methionine before inoculation, the epidermal cells usually became necrotic, containing a round, dark-stained nucleus and vacuoles with electron-dense granules (Plate IV-C). The mesophyll cells showed no noticeable changes in the cytoplasm except swollen endoplasmic reticula and increased electron-density followed by degeneration in tonoplasts (Plate IV-D). These observations could not be obtained in leaves which were not treated with L-methionine before inoculation or those which were treated with L-methionine but not inoculated.

**Hyphae and Conidia**

Control: Hyphae from colonies 58 hr after inoculation were observed under an electron microscope. Conidia to be examined were collected from leaves 7 days after inoculation, put on agar layer, and treated by double fixation.

The cell wall of hyphae consisted of two layers, and electron-dense materials adhered to the outer layer (Plate V-A). The plasmamembrane attached to the inner layer, and mitochondria, ribosomes, lipid bodies, glycogen granules and small vacuoles were observed in the cytoplasm (Plate V-A). Hyphal cells near appres-
sorium contained a large number of mitochondria which appeared to be larger than those in other hyphal cells (Plate V-A, B).

The cell wall of conidia consisted of two layers. They were an electron-dense inner layer and an electron-lucent outer layer. Electron-dense materials adhered to the outer layer as observed in hyphal cells (Plate VI-A). Conidal cells contained mitochondria, lipid bodies, ribosomes and glycogen granules in the cytoplasm (Plate VI-A). Vacuoles were usually small in size, containing electron-dense granules (Plate VI-A). The glycogen granules were larger in number in conidia than in hyphae. They were distributed throughout the cytoplasm or formed aggregates (Plate VI-A).

Treatment with L-methionine: Barley leaves were treated with $8 \times 10^{-3}$ M L-methionine solution 24 hr before inoculation, and 58 hr after inoculation the hyphae were collected from colonies on the leaves and observed under an electron microscope. In another experiment, barley leaves were treated with L-methionine 96 hr after inoculation and conidia were collected from the treated leaves 72 hr after the treatment. They were fixed and stained according to the same procedure as described for the control.

The cell wall of the hyphae did not show any noticeable change different from that of the control (Plates V-A, B, C, D). The hyphal cell usually had a large central vacuole surrounded by the cytoplasm (Plate V-C). The electron-density of the cytoplasm was higher than that of the control, and cell organelles other than mitochondria and lipid bodies could not be discerned (Plate V-D). Electron-dense deposits of irregular shapes were found in the large vacuole (Plate V-C).

Different degrees of structural changes were observed in the conidia. Firstly, the cell wall of the conidia partially expanded out, resulting in the separation of the cell wall followed by some degeneration of the cytoplasm. And some organelles became visible (Plate VI-B). Secondly, organelles in the conidial cells disorganized, granulated, and became aggregated masses (Plate VI-C). Finally, the electron-dense granules disappeared, and vacuolation was found in the conidia (Plate VI-D).

Discussion

It has been known that L-methionine seems to suppress the disease development in plant diseases caused by obligate parasites which form haustoria in host cells\(^1,2,3\), but not in those caused by non-obligate parasites\(^5\). It has also been reported that L-methionine is not effective when it is applied to the parasites directly\(^3\). In a previous paper we reported that primary haustoria in barley leaves treated with L-methionine showed abnormal structures and their functions were greatly reduced\(^1\). These facts suggest that the normal metabolism of host plants become an unsuitable one for the growth of parasites by L-methionine treatment.

Results of our observations seem to support this suggestion. In barley leaves treated with L-methionine, primary haustoria at early stages were found to contain normal cell organelles like those in control leaves, but remarkable degeneration was found in sheath membranes and matrix. To the outside of the sheath membrane adhered electron-dense materials which appeared to originate from the degenerated cytoplasm of host cells. The same materials were found in epidermal cells of barley leaves which were inoculated with conidia after L-methionine treatment. Besides, the sheath matrix contained electron-dense matrix, but they were lower in electron density. Cell organelles could not be discerned in haustoria at later
stages of growth.

Kunoh\(^4\) reported such phenomena in leaves of barley varieties resistant to the barley powdery mildew. He considered that degeneration of haustoria was preceded by that of host cytoplasm, because it occurred after degeneration of sheath membrane and matrix. He stated that these phenomena were thought to be a kind of resistance due to the hypersensitivity of the invaded epidermal cells. Simons\(^7\) investigated the effects of two fungicides (oxycarboxin and benomyl) on the fine structure of the oat crown rust \((Puccinia coronata var. avenae)\), and reported that cell organelles in the haustoria degenerated primarily, but no sudden degeneration occurred in the cytoplasm of host cells invaded by the haustoria\(^7\). Richmond and Pring\(^6\) also examined the effects of benomyl on \(Botrytis fabae\), and observed the disorganization of fungal structures without any noticeable change in the cytoplasm of host cells. From these reports, together with our results, it is suggested that the inhibitory action of L-methionine on the barley powdery mildew may be due to the resistant reaction induced in host cells by L-methionine treatment.

Literature cited

Explanation of plates

Plate I ad II. Primary haustoria of *Erysiphe graminis* f. sp. *hordei* formed in untreated barley leaves.

Plate I. A. A primary haustorium in an epidermal cell, 24 hr after inoculation. × 9,500
B. Cross section of a haustorial neck in an epidermal cell, 24 hr after inoculation. × 27,000
C. A primary haustorium in an epidermal cell, 58 hr after inoculation. Note the wavy sheath membrane around a haustorial projection. × 23,000

Plate II. A. Oblique section of a primary haustorium in an epidermal cell, 96 hr after inoculation. × 23,000
B. A primary haustorium in an epidermal cell, 7 days after inoculation. Note the electron-dense content in the haustorium. × 7,200

Plate III and IV. Primary haustoria of *E. graminis* f. sp. *hordei* formed in barley leaves treated with L-methionine.

Plate III. A. Central body of a primary haustorium in an epidermal cell, 24 hr after inoculation. Note the thick wall and the electron-dense materials in the sheath matrix. × 22,000
B. A haustorial neck in an epidermal cell, 58 hr after inoculation. × 17,000
C. Haustorial projections in an epidermal cell, 58 hr after inoculation. Note the irregularly shaped sheath membrane. × 22,000

Plate IV. A. A primary haustorium in an epidermal cell, 96 hr after inoculation. × 8,900
B. A primary haustorium in an epidermal cell, 96 hr after inoculation. Note the large and electron-dense deposits. × 14,200
C. An epidermal cell in barley leaves treated with L-methionine and then inoculated with the fungus. Note the accumulation of electron-dense granules. × 10,200
D. A mesophyll cell in the same barley leaves. Note the tonoplast disorganization. × 17,000

Plate V. A. Cross section of a hypha on untreated barley leaves, 58 hr after inoculation. × 18,700
B. A hypha on untreated barley leaves, 58 hr after inoculation. Note that the cell near an appressorium is filled with cytoplasm. × 15,000
C. Cross section of a hypha on barley leaves treated with L-methionine, 58 hr after inoculation. Note the large deposits in a large central vacuole. × 24,300
D. Cross section of a hypha on barley leaves treated with L-methionine, 96 hr after inoculation. × 20,400

Plate VI. A. A mature conidium formed on untreated barley leaves. Note the glycogen granules. × 5,300
B-D. Conidia formed on barley leaves treated with L-methionine.
B. Note the outer expansion of the cell wall. × 4,300
C. Note the aggregated cytoplasm. × 5,900
D. Note the disappearance of most of the aggregated cytoplasm. × 4,300

Abbreviation

a, appressorium; bo, haustorial body; c, conidium; ch, channel; co, collar; cp, chloroplast; ec, epidermal cell; h, hypha; mc, mesophyll cell; n, haustorial neck; nu, nucleus; pr, haustorial projection; sb, secretory body; sm, sheath membrane; sma, sheath matrix; t, tonoplast; v, vacuole; w, host cell wall.
Plate IV

A

sma
pr
pr
bo
v

B

w
pr
bo
v
sma

C

w
nu
ec

D

v
ch
mc
t
Plate VI