Effect of Infection by *Phytophthora infestans* on Uptake of ^3H -leucine, ^{32}P and ^{86}Rb by Potato-Tuber Tissue

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西村範夫*・富山宏平* : ジャガイモ塊茎スライスの ^3H -ロイシン, ^{32}P
および ^{86}Rb の吸収におけるジャガイモ疫病菌感染の影響について

Abstract

Aged tuber slices of potato cultivars Rishiri (R_1 -gene) and Irish cobbler (r-gene) were inoculated with race 0 (incompatible with Rishiri and compatible with Irish cobbler) and race 1 (compatible with both cultivars) of *Phytophthora infestans*. After inoculation, they were treated, at intervals, with ^3H -leucine, $\text{H}_3^{32}\text{PO}_4$ and $^{86}\text{RbCl}$ solution for 50 min, and then washed and homogenized. Uptake of ^3H -leucine, and ^{32}P into the $2 \times 10^4 \times g$ supernatant fraction of the homogenates of the slices (Rishiri and Irish cobbler) infected by both races were already lower than those of non-infected ones 1.4 hr after inoculation, when most of encysted zoospores had germinated but not yet penetrated the host-cell wall. From about 2.4 hr after inoculation, when about 20 % of the appressoria had begun to penetrate, the rates of uptake of ^3H -leucine and ^{32}P began to decrease more rapidly in the incompatible host-parasite combination than in those of compatible combinations: At this stage, no death of the infected cells has occurred yet even in the incompatible combination. In the case of ^{86}Rb ion, on the contrary, the rate of uptake was higher in slices infected by the incompatible race than in non-infected slices or slices infected by the compatible race at 18 C. At 10 C, however there was little difference among the rates of uptake in the non-infected slices and slices infected by incompatible and compatible races. The most plausible explanation of these results is that the physiological activity of host-plasmalemma and/or the metabolic activity is altered by infection with the incompatible race in an early period of infection when no host cell death has occurred. From these results it was presumed that the alteration of physiological activity of plasmalemma and/or metabolism of host caused by recognition of infection by an incompatible race of *P. infestans* might occur almost simultaneously with penetration.

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Introduction

Microscopic and cinephotomicrographic observations suggested that the cytoplasmic response of a potato cell to invasion by an incompatible race 0 of *Phytophthora infestans* was different from that by a compatible race just after or just before penetration^{2,6)}. The host cytoplasm aggregated at the site of penetration by the incompatible race before the first sign of penetration could be recognized under an optical microscope, and the cytoplasmic aggregate seemed to persist at the site of the penetration. On the contrary, in the case of penetration by a compatible race, the

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cytoplasmic aggregate occurred occasionally at the penetration site, but it often streamed away and disappeared. Cinephotomicrographic observations showed that, in an initial period of infection, the outline of the penetration hyphae of the incompatible race was hardly recognized in the aggregate of cytoplasm, while that of the compatible race was usually easy to recognize under a microscope, even in the aggregate of host cytoplasm. These observation suggested that there may be some interaction between the host plasmalemma and the parasite almost simultaneously, just before or just after penetration. Electron microscopic observation⁴⁾ also suggested that the ultrastructure of the cytoplasm of potato cells underwent changes during infection by an incompatible race of *P. infestans*. To test this assumption, the effects of infection by compatible and incompatible races of *P. infestans* on the electric potential of tuber slices were determined³⁾. The results suggested that alteration of plasmalemma was caused by infection by both compatible and incompatible races, and the alteration was greater in the case of incompatible combinations than that of compatible ones.

Numerous papers have reported permeability changes of host-plasmalemma caused by infection of fungal or bacterial parasites or fungal and bacterial toxins^{8,9)}. Rather few studies, however, have been done on the permeability changes of the infected cell itself in an initial period of infection, as, in most of the diseases, infected tissue consisted of both infected and non-infected cells and the infection process was not synchronized. Thatcher (1942) showed by a plasmolysis method that permeability of susceptible potato cells is increased by infection by *P. infestans*.⁵⁾ The experiments, however, were done only with a compatible combination and the permeability change was observed about a day after inoculation. In the experimental model system used in the present experiments, the infection process is relatively well-synchronized and most cells of the tissue are infected. The data obtained from experiments using this experimental model system seemed to give information about physiological activities of plasmalemma and/or metabolic activities of an infected cell.

In this paper, we report uptake of ³H-leucine, ³²P and ⁸⁶Rb by tuber slices in an initial period of infection by *P. infestans*.

Materials and Methods

Preparation of tuber slices and inoculation. The potato cultivars Irish cobbler (r) and Rishiri(R₁) were used. Tubers were stored in a cold room at 4 C until used. Tissue cylinders 15 mm in diameter were cut out from central parenchymatous tissue with a cork borer. Slices 1 mm thick were cut from the cylinders with a microtome and washed for 5-10 min in running tap water, rinsed with distilled water, blotted with a filter paper, and incubated at 18 C in a moist condition for 24 hr.

Two races of *P. infestans* (race 0 and race 1) were used. The former is incompatible with Rishiri and compatible with Irish cobbler whereas the latter is compatible with both cultivars. Zoosporangial suspensions were obtained from mycelial mats growing on the cut surfaces of fresh tubers of Irish cobbler. Zoosporangia were collected on Toyo filter paper NO. 2, washed with distilled water, resuspended in distilled water containing 2×10^{-4} M CaCl₂, and incubated at 4 C. The zoospore suspension was centrifuged at $300 \times g$ for 2 min to eliminate the ungerminated zoosporangia and then adjusted to a concentration of $65-70 \times 10^5$ /ml. One side of each tuber slice was sprayed with the zoospore suspension, and the slices

were incubated at 18 C. Microscopic observation showed that most cells of the slices were infected by one or more encysted zoospores.

Microscopic observation. Microscopic sections were periodically cut from the inoculated slices, and observed under an optical microscope. Percentage penetration by the parasite was determined and the time from inoculation to penetration was calculated from the time course of penetration. The microscopic sections were immersed in 1 M sucrose solution containing 10^{-6} M neutral red. The cells which could not be plasmolyzed and/or could not be stained with neutral red were taken as dead cells.

Uptake of ^3H -leucine, ^{32}P and ^{86}Rb . To determine the effect of concentration of these compounds on their uptake by the tuber slices, the surfaces of cut tuber slices were first sprayed with distilled water to eliminate air bubbles. After blotting off excess water with filter paper, the inoculated surfaces were immersed in 2 ml of substrate solution for 50 min at 18 C. The substrate solution for leucine-experiments consisted of 60 μg of chloramphenicol, L-leucine-4,5- ^3H (2 μCi) and 0.1, 0.2, 0.5, 1.0, 2.0 or 3.0 mM L-leucine. The substrate solution for ^{32}P -experiments consisted of 60 μg of chloramphenicol, $\text{H}_3^{32}\text{PO}_4$ (2 μCi) and 0.25, 0.5, 1.0, 2.0, 4.0 or 8.0 mM Na-K-phosphate buffer (pH 5.5). The substrate solution for ^{86}Rb -experiments consisted of 60 μg chloramphenicol, $^{86}\text{RbCl}$ (2 μCi) and 1, 10, 25 or 50 mM RbCl . To test the effect of infection on uptake of compounds, the following experimental conditions were employed. The inoculated surfaces of slices were immersed in 2 ml of substrate solution in a petri dish containing 60 μg of chloramphenicol, L-leucine-4,5- ^3H (2 μCi) and 1 mM L-leucine or 2 ml of substrate solution containing 60 μg of chloramphenicol, $\text{H}_3^{32}\text{PO}_4$ (2 μCi) and 2 mM Na-K-phosphate buffer (pH 5.5) or 2 ml of substrate solution containing 60 μg of chloramphenicol, $^{86}\text{RbCl}$ (2 μCi) and 25 mM RbCl . After treatment with radioactive compounds, the slices were washed three times with 10 ml of cold distilled water for 5 min and then excess water was blotted off with filter paper. These slices were then frozen at -20°C . The frozen slices were homogenized by grinding in a mortar for 2.5 min with 4 volumes of cold 50 mM phosphate buffer (pH 7.7) containing 8 mM β -mercaptoethanol. The homogenate was centrifuged at $2 \times 10^4 \times g$ for 20 min. One ml of the supernatant was placed in a vial and mixed with 10 ml of triton X-100 scintillation fluid (toluene scintillator, toluene : triton X-100, 2 : 1, v/v). The radioactivity was measured using a Packard Tri-Carb liquid scintillation spectrometer Model 3320 (Packard Inc.). All experiments were repeated two or three times, and averages of the experiments are shown in each figure and table.

Leakage of radio-isotopes. Tuber slices, the surfaces of one side of which had been sprayed with distilled water to eliminate air bubbles and blotted with filter paper, were placed with the water-sprayed surface down for 50 min in the radioisotope solutions described previously. After treatment, the slices were washed three times, blotted and then inoculated with a zoospore suspension of race 0 and race 1. At intervals, each tuber slice was shaken, without wiping off the inoculation fluid, in 10 ml of distilled water for 20 min in a cold room at 8 C. After discarding the slices, each 1 ml of the water was placed in a vial and mixed with 10 ml of triton X-100 scintillator. The radioactivity was measured using a Packard Tri-Carb liquid scintillation spectrometer.

Results

Time for germination, penetration and cell death

Fig. 2. Time from inoculation of potato tuber slices with zoospores of *P. infestans* to germination of encysted zoospores, penetration of cell walls and hypersensitive cell death of the infected cells. Potato tubers of Rishiri were used. A: germination of encysted zoospores, B: penetration of host cell walls, C: hypersensitive cell death. ●: incompatible race, x: compatible race.

Measurements were made of the time from inoculation to germination of encysted zoospores, from inoculation to penetration and from inoculation to hypersensitive cell death (Fig. 2). Most of the encysted zoospores germinated within 3 hr after inoculation. Penetration of host cell walls by the appressoria began 1.5 hr after inoculation. Fifty percent of the appressoria penetrated the cell wall within about 3 hr and most of them had penetrated within 4.5 hr after inoculation. The host cells infected by the incompatible race began to die from 3 hr after inoculation and about 50 % of the infected cells had died by about 9 hr after inoculation.

Effect of concentration of the compounds on their uptake.

Characteristic uptakes of L-leucine, orthophosphate and Rb ion into $2 \times 10^4 \times g$ of supernatant of the tuber slices are shown in Fig. 3. The uptakes of L-leucine, P and Rb were linearly proportional to the concentration of each compound, when they were applied at higher concentrations than 1 mM of L-leucine, 2 mM of P or 25 mM of Rb, respectively.

Effect of infection on 3H -leucine and ^{32}P uptake

Uptake of radioactivity from 3H -leucine into $2 \times 10^4 \times g$ supernatant of tuber slices

either non-infected or infected by incompatible or compatible races of *P. infestans*

Fig. 3. Relation between amount of labelled compounds incorporated into the $2 \times 10^4 \times g$ supernatant of potato tuber slices and concentrations of the compounds applied to the slices.

Fig. 4. Effect of infection of potato tuber slices by race 0 and race 1 of *P. infestans* on the uptake of radioactivity from ^3H -leucine into the 2×10^4 g supernatant fraction of the slices. A: Irish cobbler, B: Rishiri. ●: non-infected, ○: infected by race 0 and ×: infected by race 1.

are shown in Fig. 4. No significant difference was observed between slices infected by incompatible and compatible races 1.4 hr after inoculation, but uptake into the infected slices was lower by about 25 % than that of non-inoculated slices except for Irish cobbler infected by race 0 at this time. From 2.4 hr after inoculation, the rate of uptake began to decrease in both incompatible and compatible combinations, but the rate of decrease was greater in the former. Not much decrease was observed, however, in the non-infected slices. From 5.4 hr after inoculation, the rate of uptake into 2×10^4 g of supernatant became almost constant. Similar results as with ^3H -leucine were

obtained with ^{32}P (Fig. 5). Uptake of ^{32}P into 2×10^4 g supernatant of the slices inoculated by both race 0 and race 1 were already lower than those of non-inoculated slices 1.4 hr after inoculation. At this time, there was no difference in the rate of uptake between the slices inoculated by race 0 and race 1. After that, the rate

Fig. 5. Effect of infection of potato tuber slices by race 0 and race 1 of *P. infestans* on the incorporation of ^{32}P into the 2×10^4 g supernatant of the slices. A: Irish cobbler, B: Rishiri. ●: non-infected, ○: infected by race 0 and ×: infected by race 1.

of uptake began to decline faster in infections with the incompatible race than with the compatible race. The rate of uptake became almost constant 5.4 hr after inoculation. In the case of Irish cobbler, there was no difference between the pattern of ^{32}P uptake of the slices infected by race 0 and race 1.

In contrast to the uptake of ^3H -leucine and ^{32}P , uptake of ^{86}Rb into the $2 \times 10^4 \times g$ supernatants of the tuber slices of Rishiri infected by race 0 and race 1 gave a different result (Table 1). With tuber slices of Rishiri, ^{86}Rb uptake was greatest with slices infected by the incompatible race in the case of experiments carried out at 18 C. At 10 C, however, little difference was observed.

Table 1. Uptake of ^{86}Rb into the $20,000 \times g$ supernatant of potato tuber slices (Rishiri) infected by race 0 and race 1 of *P. infestans* and non-infected slices

	3.4 hr after inoculation		5.4 hr after inoculation	
	18 C	10 C	18 C	10 C
Non-infected	2.55	1.65	2.43	1.66
Infected by race 0	3.64	1.75	3.16	1.41
Infected by race 1	2.92	1.68	2.51	1.57

($\times 10^4$ c. p. m.)

Leakages of ^3H -leucine or ^{32}P

Little leakage of radioactivity of preabsorbed ^3H -leucine and ^{32}P from the non-infected tuber slices was observed after the treated slices were washed once or twice, but, with ^{86}Rb , the radioactive compound continued to leak from non-infected slices even after repeated washing (Fig. 1). Under the present experimental conditions, leakages of preabsorbed ^3H -leucine from the tuber slices of Rishiri were not changed by infection with race 0 and race 1 in an early stage of infection (Fig. 6). Similar results were obtained with ^{32}P . These results suggested that the decrease of the uptake was not due to the leakage of ^3H -leucine and ^{32}P .

Fig. 1. Radioactivities of each 10 ml of water used for repeated washing of the tuber slices treated with ^3H -leucine (A), ^{32}P (B) and ^{86}Rb (C). Bar (U) indicates amount of radioactivities absorbed into the $2 \times 10^4 \times g$ supernatant of tuber slices.

Fig. 6. Effect of infection by race 0 and race 1 of *P. infestans* on the leakage of radioactive substance from the potato-tuber slices fed with ^3H -leucine. The total radioactivity in the $2 \times 10^4 \times g$ supernatant of the slices was 2.1×10^5 dpm soon after treatment with ^3H -leucine.

Discussion

Uptakes of ^3H -leucine and ^{32}P into $2 \times 10^4 \times g$ supernatant fraction of the tuber slices were greatly reduced by infection with both compatible and incompatible races of *P. infestans*. The rate of uptake was already lower in the slices infected by both the compatible and incompatible races than in the non-infected slices 1.4 hr after inoculation, when about 50 % of the encysted zoospores began to germinate and no penetration had occurred. Unpublished data showed that uptakes of ^3H -leucine were reduced by about 20 %, when the tuber slices of Rishiri were inoculated with zoospore suspensions which had been frozen at -20°C and then thawed. The reduction rate caused by inoculation was almost the same in slices treated with frozen and thawed suspensions of either race 0 or race 1 spores. This suggested that

the early reduction of uptake might be due to some unknown substances released from ruptured zoospores contained in zoospore suspension. The rate of uptake in the slices infected by the incompatible race became lower as compared with both the slices non-infected or infected by the compatible race 2.4 hr after inoculation when the appressoria began to penetrate. About 3.4 hr after inoculation, when most of the appressoria had penetrated the cell wall and almost all of the infected cells were still alive even in the incompatible combination, the rate of uptake was most distinctly reduced in the slices infected by the incompatible race as compared with the slices infected by the compatible race or non-infected.

According to preliminary experiments in which total radioactivity incorporated from ^3H -leucine into slices was determined with a sample oxidizer (Packard model 306 Tri-Carb) 3.4 hr after inoculation, 3.4, 5.5 and 6.2 % of the total radioactivity of preabsorbed ^3H -leucine of slices non-infected, infected by race 0 and infected by race 1, respectively, were found in the $2 \times 10^4 \times g$ pellets. From these results, it was presumed that reduction in the rate of uptake of ^3H -leucine into the $2 \times 10^4 \times g$ supernatant fraction caused by infection with the incompatible race might be due to reduction in absorption of the labelled compounds by the potato cells. As previously described, most of the cells of the inoculated surface of the tuber slices were infected by the parasite and the labelled compounds were applied on this side. The infection process was almost synchronized (Fig. 2). These results suggested, therefore, that infection by the incompatible race might reduce influx of L-leucine through plasmalemma of infected cells because of alteration of permeability of plasmalemma and/or reduction of metabolic activities (Wheeler 1976)⁹⁾. The experiments with ^{32}P also suggested reduction of influx of ^{32}P through potato plasmalemma.

In the case of ^{86}Rb , on the contrary, uptake into the $2 \times 10^4 \times g$ supernatant was increased by infection, especially with the incompatible race. A similar result was reported with sweet potato root infected by *Ceratocystis fimbriata*¹⁾. The reason why with ^{86}Rb , results different from the cases of ^{32}P and ^3H -leucine was obtained is unclear. Further experiments are necessary to make clear this point.

From these results, it was presumed that the alteration of physiological activity of host plasmalemma caused by recognition of infection by an incompatible race of *P. infestans* might occur almost simultaneously with penetration.

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和文摘要

ジャガイモ塊茎スライスの ^3H -ロイシン, ^{32}P および ^{86}Rb の吸収におけるジャガイモ疫病菌感染の影響について

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ジャガイモ品種リシリ (R_1 -gene) およびダンシャク (r -gene) の塊茎を厚さ 1 mm のスライスにし、24時間、18°C中に静置した。ジャガイモ疫病菌 race 0 または race 1 を接種し、一定時間後に ^3H -ロイシン, $\text{H}_3^{32}\text{PO}_4$ または $^{86}\text{RbCl}$ を50分間、接種面から吸収させた。磨砕した後、 $20,000\times g$ 上清部の放射能活性を測定した。接種1.4時間後に ^3H -ロイシンおよび ^{32}P の吸収量は無接種区に比較して約25%低下した。この時間に、ほとんどの遊走子は発芽し始めているが宿主細胞には侵入していなかった。接種2.4時間後から ^3H -ロイシンおよび ^{32}P の吸収量は、親和性の組み合わせに比較して非親和性の組み合わせで顕著に低下した。この時間に非親和性の組み合わせにおいても宿主の細胞死はほとんど起きていなかった。 ^{86}Rb の吸収では、非親和性菌を接種したスライスの吸収量は非感染および親和性菌に感染したスライスより高かった。また10°Cで吸収させると差はほとんどなくなった。

^3H -ロイシンによる予備実験の結果から $20,000\times g$ 上清部の放射能活性をスライスへの取り込み量とみなすことができる。以上の結果は感染初期(侵入菌糸の貫入とほとんど同時)に宿主原生質膜が感染の影響を受けることを示す。また、親和性、非親和性の認識が侵入菌糸の貫入とほとんど同時におこなわれていることを示していると考えられる。また ^{86}Rb の結果は ^3H -ロイシンおよび ^{32}P の場合と異なるが、その理由は不明である。