Effect of Infection by *Phytophthora infestans* and Treatment with Zoosporial Water-Insoluble Component on Leakage of $^{86}\text{Rb}^+$ from Potato Tuber Cells

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西村範夫*・富山宏平**・道家紀志**：ジャガイモ塊茎細胞の $^{86}\text{Rb}^+$ 漏出に対する
疫病菌感染および疫病菌遊走子不溶性成分の影響

Abstract

Potato tuber disks, 15 mm in diameter and 1 mm thick, which had absorbed $^{86}\text{RbCl}$, were inoculated by incompatible and compatible races of *Phytophthora infestans*. At intervals, leakages of $^{86}\text{Rb}^+$ from the infected disks were determined. The leakage of $^{86}\text{Rb}^+$ was accelerated by infection with the incompatible race in an early stage of infection, at which hypersensitive death of the infected cells had not yet occurred. The acceleration rate was not great, but statistically significant. There was, on the contrary, no difference in leakage of $^{86}\text{Rb}^+$ between control and the disks infected by the compatible race. $^{86}\text{Rb}^+$ -labelled disks were immersed for 10 min in a suspension of water-insoluble zoosporial component (C-fr, sugar content, 30 $\mu\text{g}/\text{ml}$). At intervals, leakages of $^{86}\text{Rb}^+$ from the treated disks were determined. The results showed that C-frs of both the compatible and incompatible races reduced greatly the release of $^{86}\text{Rb}^+$. Effects of infection opposite to that of C-fr suggested that the latter, as it is, may not be the very cause of the hypersensitive response in potato late blight, or that C-fr may not induce the proper response of hypersensitivity, when the disks were treated by the method used in the present experiments.

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Introduction

In previous papers^{4,5}, we reported that uptakes of ^3H -L-leucine and ^{32}P by potato tuber disks have been already reduced by inoculation with either incompatible or compatible races of *Phytophthora infestans* before penetrations were observed under a light microscope. In the stage at which the parasites have penetrated into host cell but the hypersensitive death of the penetrated cells have not yet occurred, the uptakes of ^3H -L-leucine and ^{32}P were already reduced greatly by infection by the incompatible race but not by the compatible one. There was little difference in the leakage of ^3H -L-leucine and ^{32}P from the disks among those infected by the incompatible and compatible races and non-infected ones. On the contrary, uptake of $^{86}\text{Rb}^+$ by the disks was accelerated

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by infection with the incompatible race in the early period of infection, but not by the compatible race⁴⁾.

In the experiments reported here, we examined the effect of infection by the incompatible and compatible races of *P. infestans* on the leakage of $^{86}\text{Rb}^+$ from the cells of potato tuber disks. The effect of water-insoluble zoospore component (C-fr), the "hypersensitivity-like phenomena inducing factor"^{4,5,6)}, on the leakage of $^{86}\text{Rb}^+$ was also investigated.

Materials and Methods

Preparation of plant materials and inoculation. The potato cultivar Rishiri having R₁-gene was used. Tuber disks 15 mm in diameter and 1 mm thick were prepared as described previously⁶⁾. After being aged for about 20 hr at 18 C, both sides of the disks were sprayed with distilled water to eliminate air bubbles, excess water was blotted off with filter paper and then used for the experiments.

Race 0 and race 1, 2 of *P. infestans* were used. The former is incompatible and the latter compatible with the potato cultivar Rishiri. The zoosporangia suspension obtained from the cut surfaces of tubers of potato cultivar Irish Cobbler was filtered through No. 2 Toyo filter paper and washed twice with distilled water; then the zoosporangia were resuspended in distilled water containing $2 \times 10^{-4}\text{M}$ CaCl_2 . The suspension was allowed to germinate at about 10 C. One side of the isotope-labelled disk was inoculated with zoospore suspension ($5\text{--}6 \times 10^5$ spores/ml).

Water-insoluble zoospore component (C-fr). The method to extract C-fr was essentially the same as reported previously⁶⁾, and solubilized by the method employed by Lisker and Kuć²⁾. The zoospores were homogenized for 5 min with 20 mM acetate buffer at pH 4.5 using a teflon homogenizer and centrifuged at $20,000 \times g$ for 30 min. The precipitate was suspended in 50 mM borate buffer (pH 8.8), boiled for 1 hr and centrifuged at $20,000 \times g$ for 30 min. The supernatant was dialyzed against ten liters of distilled water two times and finally against tris-HCl buffer at pH 7.4. Concentration of the solution was regulated so that each of the water-insoluble zoospore components (C-fr) of both the races contained 30 μg sugar/ml. Sugar content was measured by the method of Dubois *et al.*¹⁾

Treatment with $^{86}\text{RbCl}$. Fifteen pieces of the tuber disks were immersed in 30 ml of $^{86}\text{Rb}^+$ (0.5 $\mu\text{Ci/ml}$) and shaken for 10 min. $^{86}\text{Rb}^+$ (sp. activity 4.17 mCi/ml) was purchased from New England Nuclear Corp. After being rinsed with water, the disks were washed by shaking in water for 5 min. After washing three times, the excess water was blotted off with filter paper. On one side of the washed disk was inoculated zoospores of either race 1, 2 or race 0. The inoculated disks were incubated at 18 C for 90 min. After the inoculation fluid was blotted off with filter paper, square wet filter papers (1.5 $\times$ 1.5 cm) were placed on the surface of the $^{86}\text{Rb}^+$ absorbed disks. Five sheets of the square filter papers were exchanged for a new ones at each interval of 30 min, and they were put into 10 ml of RbCl (10 mM) and kept overnight. Radioactivity of 1 ml of RbCl solution was determined in the same way as described previously⁶⁾.

In another series of experiments, 5 pieces of the tuber disks, which had absorbed $^{86}\text{Rb}^+$, were immersed in 10 ml of C-fr solution and shaken for 10 min. At each interval

of 10 min, the C-fr solution was exchanged for a new one, and radioactivity of each 1 ml of C-fr solution was determined.

In both the experiments to test the effect of infection and C-fr solution, the control disks were treated with distilled water containing $2 \times 10^{-4} \text{M}$ CaCl_2 .

Determination of total radioactivity absorbed. Five pieces of the tuber disks were ground in a mortar and pestle, and the homogenate was centrifuged at $20,000 \times g$. Approximate total radioactivity was calculated from that of the supernatant. In a previous paper⁴⁾, we showed an evidence that the radioactivity of the supernatant may give approximate total radioactivity absorbed by the tuber disks.

Results and Discussion

Cumulative leakages of radioactivity from the $^{86}\text{Rb}^+$ -labelled disks infected by *P. infestans* are shown in Fig. 1. In these experiments, approximate total radioactivity of $^{86}\text{Rb}^+$ absorbed by the disks was 2.8×10^5 cpm and 2.6×10^5 cpm in 1st and 2nd experiments, respectively. Infection of the disks with the incompatible race of *P. infestans* accelerated the leakage of $^{86}\text{Rb}^+$ from the tuber disks in an early stage of infection, at which the death of the infected cells had not yet occurred. The acceleration rate was not great, but statistically significant. The acceleration increased later. No acceleration of the

leakage was found in the disks inoculated with the compatible race as compared with that of the uninfected disks. In present experiments, penetration by both the incompatible and compatible races began to occur in some cells 2 hr after inoculation. Hypersensitive death of the cells infected by the incompatible race began to occur in some cells about 3 hr after inoculation, and death of about 50 % of the infected cells occurred about 8 to 9 hr after inoculation. The death of the cells infected by the compatible race did not occur, while the present experiments were carried out^{4,5)}.

In a previous report⁴⁾, infection by the incompatible race was shown to accelerate the influx of $^{86}\text{Rb}^+$ into the infected cell. Therefore, the present and previous⁴⁾ results indicated that the infection accelerated both influx and efflux of $^{86}\text{Rb}^+$ of the disks. These results showed that the effect of infection by the incompatible race on $^{86}\text{Rb}^+$ -influx was opposite to those of ^3H -L-leucine and ^{32}P . $^{86}\text{Rb}^+$ has usually been used as a tracer of K^+ . However, to use $^{86}\text{Rb}^+$ as a tracer, specific activity of $^{86}\text{Rb}^+$ should be high and the chemical amount of Rb^+ should be kept at a minimum relative to K^+ ⁸⁾. As the present experimental condition may satisfy the necessary condition, $^{86}\text{Rb}^+$ can be looked upon as tracer of K^+ in the present experiments.

In the next experiments, effect of C-fr on the leakage of $^{86}\text{Rb}^+$ was tested (Fig. 2).

Fig. 1. Effect of infection by race 0 and race 1, 2 of *Phytophthora infestans* on cumulative leakage with time of $^{86}\text{Rb}^+$ from tuber disks of potato cultivar Rishiri.

●: control, ○: race 0, ×: race 1, 2

Fig. 2. Effect of water-insoluble zoosporeal component (C-fr) of race 0 and race 1, 2 of *Phytophthora infestans* on cumulative leakage with time of $^{86}\text{Rb}^+$ from tuber disks of potato cultivar Rishiri.
●: control, ○: race 0, ×: race 1, 2

Approximate total radioactivities in the tuber disks were 3.8×10^5 cpm and 3.9×10^5 cpm in the 1st and 2nd experiments, respectively. C-frs of both the incompatible and compatible races inhibited the leakage of $^{86}\text{Rb}^+$ from the disks. C-fr of race 1, 2 inhibited more strongly the leakage than that of race 0. However, the difference between both the C-frs is taken to be due to the difference in the preparations of C-fr used in these experiments, and not due to the difference in physiological activity related directly to the pathogenicity.

In previous studies^{4,5,6}, we reported that C-fr had an effect similar to the infection by the incompatible parasite on absorption of ^3H -L-leucine and ^{32}P by the tuber disks. The present experimental

results, however, showed that infection by the incompatible race had a different effect on the leakage of $^{86}\text{Rb}^+$ from that of C-fr. Their opposite effects suggested a possibility that C-fr, as it is, may not be the very thing which cause the hypersensitive response in potato. As another possibility, it suggests that the method of treatment with C-fr in the present experiment may not induce the proper hypersensitive response, even if C-fr contains a substance inducing the hypersensitive response.

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和文摘要

ジャガイモ塊茎細胞の $^{86}\text{Rb}^+$ 漏出に対する疫病菌感染
および疫病菌遊走子不溶性成分の影響

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$^{86}\text{Rb}^+$ を吸収させたジャガイモ塊茎ディスクに *Phytophthora infestans* の非親和性レース (race 0) および親和性レース (race 1, 2) を接種し、接種面からの $^{86}\text{Rb}^+$ の漏出量を測定した。非親和性レース接種の場合には過敏細胞死が起る前にわずかであるが確実に漏出が促進され、親和性レースの場合にはその影響はみられなかった。前報告⁴⁾の結果から、 $^{86}\text{Rb}^+$ では ^3H -L-leucine および ^{32}P の場合と異なり非親和性レースの感染初期に吸収および漏出がともに促進されると考えられる。一方、疫病菌遊走子の酢酸緩衝液 (pH 4.5) 不溶性成分 (C分画)⁶⁾ を塊茎ディスクに処理すると、 $^{86}\text{Rb}^+$ の漏出は阻害される。非親和性レース感染により $^{86}\text{Rb}^+$ の漏出が促進されるので、C分画と感染ではその影響が異なる。これらの結果はC分画が厳密な意味での過敏反応そのものではないことを示しているか、あるいはここで用いられた方法ではC分画が感染と同じ宿主の反応を誘導できないことを示すと考えられる。