Effect of Adenosine 5′-Triphosphate on Hypersensitive Death of Potato Tuber Cells Infected by Phytophthora infestans

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We thank Y. Nishibe and Y. Umemura for generously supplying experimental materials. This research was supported by research grants 936004 and 148040 from the Ministry of Education, Japan.

Accepted for publication 6 December 1977.

ABSTRACT

NOZUE, M., K. TOMIYAMA, and N. DOKE. 1978. Effect of adenosine 5′-triphosphate on hypersensitive death of potato tuber cells infected by Phytophthora infestans. Phytopathology 68: 873-876.

The hypersensitive death of cells in aged potato tuber disks infected by an incompatible race of *Phytophthora infestans* is delayed by treatment with 0.1 mM 2,4-dinitrophenol (2,4-DNP) or sodium azide. This inhibition of cell death was reversed by addition of ATP, but not by ADP. Cells infected by a compatible race survived for a long time, and ATP had no effect on the length of survival. Little effect of 0.1 mM 2,4-DNP, sodium azide, or 1 mM ATP on penetration and intracellular hyphal growth of an incompatible race (race 0) of *P. infestans* was observed. These results indicate that ATP might affect host processes during hypersensitive death of cells of aged potato tuber disks infected by the incompatible race. However, ATP had only a minor effect on the occurrence of hypersensitive death of cells of fresh tuber disks. This suggested that the lack of potential of fresh disks to react hypersensitively to infection by the incompatible race was not due to deficiency of ATP.

Additional key words: potato late blight.

Cells of potato tuber disks exposed to air for more than 20 hr before inoculation (aged disks) die rapidly after infection by an incompatible race of *Phytophthora infestans*. The earliest cell death occurs within 30 min after penetration (3, 4, 11). On the other hand, hypersensitive cell death in fresh potato-tuber disks occurs much later (6, 11). Thus, cells of fresh disks lack the potential to react hypersensitively to infection by the incompatible race, but they acquire this potential within about 20 hr after the preparation of disks. Potato cells infected by a compatible race of *P. infestans* may survive for about 2 days (11).

In a previous paper (5), it was reported that de novo protein synthesis was necessary for cells of the freshly cut surfaces of potato tubers to acquire the potential for hypersensitivity. However, de novo protein synthesis was not necessary for the occurrence of hypersensitive cell death after the potential had developed (1, 5).

With the latter cells, the rapid occurrence of cell death was suppressed by treatment with the respiratory enzyme inhibitors, 2,4-dinitrophenol (2,4-DNP) (2, 10), sodium azide (11, 12, 13) and sulfhydryl (SH)-reagents (2). From these results, hypersensitive cell death appeared to be related to the metabolic activity of host cells.

In the present paper, we report on (i) reversal by adenosine 5′-triphosphate (ATP) of the inhibition by 2,4-DNP and sodium azide of hypersensitive cell death of aged potato-tuber disks infected by the incompatible race of *P. infestans*, and (ii) the effect of ATP on the hypersensitive response of fresh disks.

00032-949X/78/000149$03.00/0

MATERIALS AND METHODS

Plant materials and inoculation.—Tubers of potato cultivar Rishiri carrying the R_1 gene for late blight resistance were stored in a cold, dark room at 4 C until used. Tuber disks, 14 mm in diameter and 4-mm thick, prepared as reported previously (5), were incubated in a plastic box containing wet filter paper in a dark moist chamber at 18 C until they were inoculated with either race 0 (incompatible) or race 1 (compatible) of *P. infestans*. Zoospore suspensions were prepared as reported previously (5), and their concentrations were adjusted to 1.2 to 1.3×10^6/ ml by dilution. Fifty μliters of the zoospore suspension was placed on the cut surface of each disk, and the disks then were incubated in a plastic box at 18 C in a dark room.

Treatment with 2,4-dinitrophenol and sodium azide.—Twelve disks of potato tuber tissue were submerged with the inoculated surface down in 25 ml aqueous solution of 0.1 mM 2,4-dinitrophenol (Katayama Chemical Co., Nagoya, Japan), sodium azide (Nakarai Chemical Co., Kyoto, Japan), or in distilled water for 10 min. This treatment began either 1.5 hr after inoculation or 30 min before inoculation.

Addition of adenosine 5′-triphosphate (ATP).—One hundred μliters of the sodium salt of ATP (Boehlinger Mannheim Japan Co., Katayama Chemical Co., Nagoya) in 0.05 M phosphate buffer at pH 7.0 was applied to the inoculated surfaces of potato tuber disks 3 hr after inoculation. Phosphate buffer was used instead of ATP as the control.

Microscopic observations.—Microscopic observations were made by the method described in a previous paper (5). To measure the length of intracellular hyphae, we

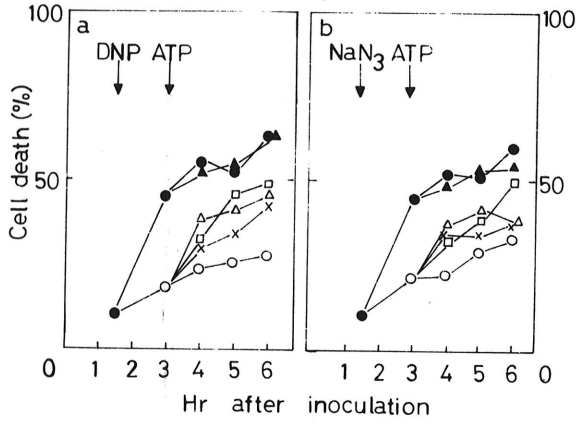

Fig. 1-(a, b). Effect of treatment of aged potato tuber tissue disks with 2,4-dinitrophenol (2,4-DNP) **a)** or sodium azide (NaN_3) **b)** and adenosine triphosphate (ATP) on hypersensitive death of cells infected by the incompatible race 0 of *Phytophthora infestans*. Legend:•=aged disks were first treated with H_2O 1.5 hr after inoculation, then with 0.05 M phosphate buffer at pH 7.0; ▲=treated first with H_2O, then with 1.0 mM ATP in 0.05 M phosphate buffer at pH 7.0; o=treated first with 0.1 mM 2,4-DNP or NaN_3, then with phosphate buffer; ×=treated first with 2,4-DNP or NaN_3, then with 0.5 mM ATP in phosphate buffer; Δ=treated first with 3,4-DNP or NaN_3, then with 1 mM ATP in phosphate buffer; and □=treated first with 2,4-DNP or NaN_3, then with 5.0 mM ATP in phosphate buffer.

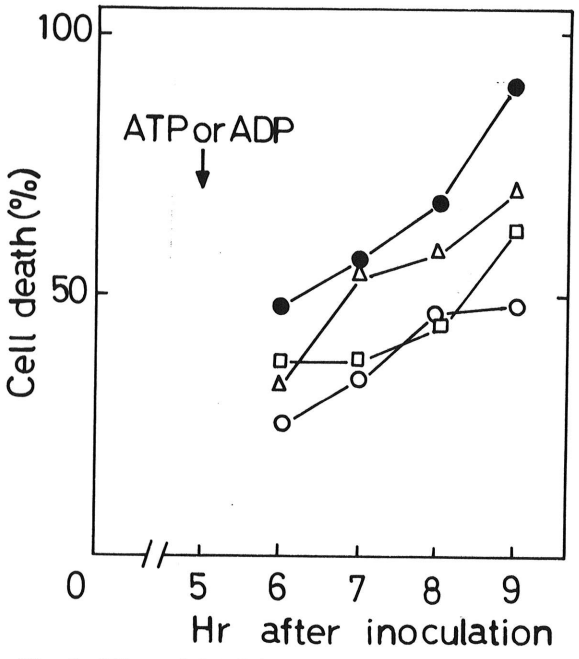

Fig. 2. Effect of 2,4-dinitrophenol (2,4-DNP), adenosine triphosphate (ATP), and adenosine diphosphate (ADP) on hypersensitive death of potato-tuber disks infected by the incompatible race 0 of *Phytophthora infestans*. The aged disks were treated with 0.1 mM 2,4-DNP for 30 min before inoculation. Inoculated disks were treated with 1 mM ATP (Δ) or ADP (□) in 0.01 M phosphate buffer at pH 6.5 or the same buffer alone (o) 5 hr after inoculation. As a control, other disks were treated with water before inoculation and then treated with phosphate buffer (•).

fixed the inoculated tissue with FAA (70% ethanol:formalin:acetic acid, 40:2.6:1, v/v). Most of the experiments were repeated three or more times, and about 100 infected cells were observed in each experiment.

RESULTS

Effect of adenosine 5′-triphosphate (ATP) on hypersensitive death of potato tuber cells treated with 2,4-dinitrophenol (2,4-DNP) or sodium azide.—Aged disks of potato tuber tissue were inoculated with the incompatible race 0 of *Phytophthora infestans* and treated with 2,4-DNP or sodium azide, both of which were followed by different concentrations of ATP (Fig. 1). As previously reported, the time necessary for hypersensitive cell death was prolonged by 2,4-DNP (10) or sodium azide (11, 12), but it was shortened by the addition of ATP (Fig. 1). There was little difference among the effects of 0.5, 1.0, and 5.0 mM ATP. Addition of ADP, however, did not reverse the inhibition of hypersensitive cell death by 2,4-DNP (Fig. 2). In the case of infection by the compatible race 1, the host cells survived for a long time. Addition of ATP had no effect on the survival time of host cells infected by the compatible race (Fig. 3). Sodium azide or 2,4-DNP also had no effect on the survival time of cells of aged disks infected by the compatible race. The differences in percentage cell death between aged disks treated with 2,4-DNP alone or with 2,4-DNP followed by ATP are statistically significant at $P<0.05$ according to the analysis of variance. With disks treated with sodium azide alone or sodium azide followed by ATP, the differences are statistically significant at $P<0.01$.

Effect of adenosine triphosphate (ATP) on hypersensitive cell death in fresh disks.—Fresh tuber disks inoculated with the incompatible race were treated with 1 mM ATP 3 hr after inoculation (Fig. 4). The percentage of infected cells not treated with ATP that died

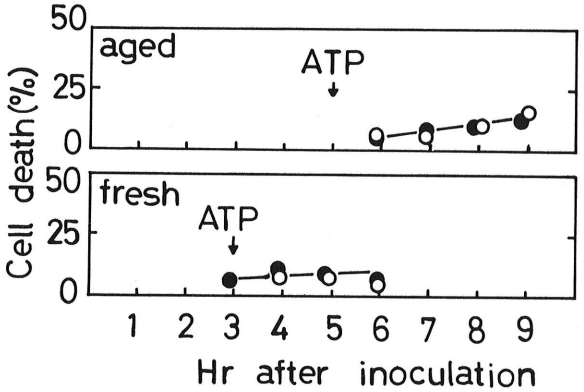

Fig. 3. Effect of adenosine triphosphate (ATP) on the reaction of potato tuber cells to infection by the compatible race 1 of *Phytophthora infestans*. Inoculated aged disks were treated with either 1 mM ATP dissolved in 0.01 M phosphate buffer at pH 6.5 (o) or the same buffer alone (•) for 45 min, beginning 5 hr after inoculation. In the case of fresh disks, they were treated with 1 mM ATP dissolved in 0.05 mM phosphate buffer at pH 7.0 (o) or the same buffer alone (•) beginning 3 hr after inoculation.

was very much lower in fresh disks than in aged disks. Treatment with ATP increased the number of dead cells very slightly.

Repeated treatment of aged disks with 2,4-dinitrophenol (2,4-DNP) or sodium azide.—When aged disks were treated with 2,4-DNP or sodium azide before being inoculated with race 0 or soon after inoculation, the occurrence of hypersensitive cell death was delayed. In this experiment the inoculated cells were first treated with 2,4-DNP or sodium azide 1.5 hr after inoculation and then treated again with the same reagent 5 hr after inoculation. Hypersensitive cell death was inhibited by the first treatment, but the gradual increase of cell death, which occurred later, could not be inhibited by the second treatment.

Effect of 2,4-dinitrophenol (2,4-DNP), sodium azide, and adenosine triphosphate (ATP) on Phytophthora infestans.—Treatments with 0.1 mM 2,4-DNP, sodium azide, and 1.0 mM ATP had little effect on penetration and intracellular hyphal growth of *P. infestans* (Table 1).

DISCUSSION

It is clear that ATP, but not ADP, reversed the inhibition by 2,4-DNP or sodium azide of hypersensitive cell death of aged potato tuber tissue disks infected by the incompatible race of *P. infestans*. Treatment with ATP had no effect on the response of host cells to infection by the compatible race. Sodium azide, 2,4-DNP, and ATP had little effect on penetration and intracellular hyphal growth. Sodium azide and 2,4-DNP are oxidative enzyme inhibitors and uncouplers of phosphorylation, respectively, and they inhibit ATP generation. All these facts strongly suggest that ATP might be involved in hypersensitive cell death. These results suggest that the hypersensitive cell death is not simply the collapse of an infected cell, but an energy-requiring vital response to infection.

In fresh disks, in which the potential to react hypersensitively to infection by the incompatible race had not yet developed (5), ATP had only a small effect on the time of hypersensitive cell death. This suggests that some unknown important physiological factors other than ATP had not yet developed in the fresh disks. As reported previously (5), de novo protein synthesis is necessary for cells of the freshly cut surface of tubers to acquire the potential to react hypersensitively. Treatment of the aged disks with 2,4-DNP and sodium azide 1.5 hr after inoculation delayed the occurrence of hypersensitive cell death, but could not prevent it. Two treatments with these compounds, at different times after inoculation, had no effect on the final process of hypersensitive cell death. These results suggest that 2,4-DNP or sodium azide could not entirely prevent the incompatible relationship between host and parasite once it was established.

Some SH-reagents, including the high-molecular-weight, dextran-bound *p*-chloromercuribenzoic acid, inhibit hypersensitive cell death in aged disks infected by the incompatible race (2). These results provided evidence that some SH-reagents may act on the plasma membrane, resulting in inhibition of hypersensitive cell death. However, the present experiments suggest that 2,4-DNP might inhibit hypersensitive cell death by uncoupling the ATP-generating system. Sodium azide also may affect hypersensitive cell death by affecting the generation of ATP. It has been reported that exogenously supplied ATP could reverse the changes in water permeability of plant cell reduced by treatment with respiration inhibitors (8, 9). Similarly, reduction of sucrose translocation by 2,4-DNP is restored by exogenously supplied ATP (7). In these reports, the authors suggested that exogenously added ATP might alter the physiological activity of the cell membrane. Further investigation is necessary to

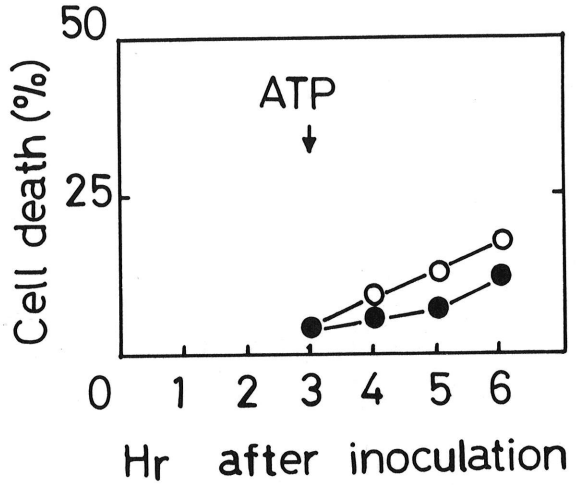

Fig. 4. Effect of addition of 1 mM adenosine triphosphate (ATP) on hypersensitive cell death in fresh potato tuber disks infected by the incompatible race 0 of *Phytophthora infestans*. The disks were treated with ATP 3 hr after inoculation. Legend: o=ATP in 0.05 M phosphate buffer at pH 7.0; and •=the same buffer alone.

TABLE 1. Effect of 2,4-dinitrophenol (2,4-DNP), sodium azide (NaN_3), and adenosine triphosphate (ATP) on the length of intracellular hyphae of the incompatible race 0 of *Phytophthora infestans* in cells of potato tuber tissue disks

Treatment[a]	Length of intracellular hyphae at inoculation plus: 4.5 hr (μm)	6.0 hr (μm)
Water-phosphate buffer[b]	28.6 ± 13.5[d]	40.9 ± 14.9
Water-ATP[c]	27.6 ± 11.0	38.7 ± 11.8
NaN_3-phosphate buffer[b]	24.4 ± 11.7	42.0 ± 18.6
NaN_3-ATP	29.5 ± 11.9	41.8 ± 16.3
2,4-DNP-Phosphate buffer[b]	27.2 ± 14.1	40.9 ± 15.0
2,4-DNP-ATP	28.7 ± 10.0	35.5 ± 12.9

[a]Treated with 0.1 mM 2,4-DNP or sodium azide 1.5 hr after inoculation, and then with 1 mM ATP 1.5 hr later.

[b]Treated with 0.05 M phosphate buffer pH 7.0 as a control.

[c]The ATP was dissolved in 0.05 M phosphate buffer at pH 7.0.

[d]Means of measurements from about 100 cells per disk, two replicate disks, in two separate experiments ± the standard deviation of the mean.

determine whether exogenously supplied ATP may affect the process of hypersensitive death of cells through a direct effect on cell membranes.

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