# Hydrogen Peroxide as a Dynamic Trigger for Phytoalexin Production

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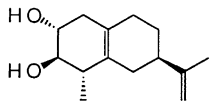
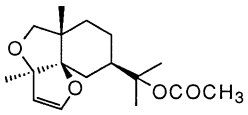
(Received November 29, 1994)

Potato tuber tissues, when infected with *Phytophthora infestans* or treated with arachidonic acid, generated $H_2O_2$ which then elicited accumulation of the phytoalexins. The plant tissues, when treated with $H_2O_2$, induced phytoalexin production, indicating that $H_2O_2$ is a dynamic substance for triggering the phytoalexin production in potato.

Potato phytoalexins,[1] represented by rishitin (**1**) and phytuberin (**2**), which are elicited by some substances designated as inducers or elicitors, have been demonstrated to be involved in a defense mechanism against disease.[2,3] While various exogenous elicitors have been generally known,[4] there are few reports of exact isolation of an endogenous elicitor,[5] which has been deduced to exist in plants. In view of the fact that phytoalexins generally accumulate at the site of infection, it is presumed that phytoalexin elicitation may be correlated with active oxygen species, generated from interaction of diseased plants with air. We propose herein that $H_2O_2$ is a dynamic trigger substance for phytoalexin production.



**1**




**2**

$H_2O_2$ was measured by a method using N,N-dimethyl-1,4-phenylenediamine and horseradish peroxidase (HRP).[6] The oxidized cation radical was monitored by an absorption of 515 nm, where the concentration of $H_2O_2$ was proportional to the absorbance within the range from 0.2 μM to 3.9 μM . When aged slices of potato tubers of cultivar Rishiri (resistant) were infected with race 0 of *Phytophthora infestans*, the red color was detected on the surface of the slices, indicating that $H_2O_2$ was generated on the diseased potato slices. In addition, generation of $H_2O_2$ was accompanied by accumulation of **1** (Figure 1). Gradual accumulation of **1** would be attributed to inhibition of further metabolism,[7] which was caused by necrosis of the cells with $H_2O_2$. Arachidonic acid as a chemical agent, which was isolated from the cell wall of *P. infestans*,[8] was then incubated with aged potato slices instead of the pathogen (Figure 2). The amount of $H_2O_2$ was maximum (ca. 2.0 nmol/slices) after 4 h, when **1** started to accumulate. The findings indicated that $H_2O_2$ was generated from the host plant itself but not from the fungus.

The amount of $H_2O_2$ detected from plant tissues upon infection with the fungus would correspond to the difference between the amount generated and that consumed for phytoalexin elicitation as well as decomposed by catalase or peroxidase in the tissues. In fact, when aged potato slices (Rishiri) were applied with catalase (10μM and 40μM) for 1 h and were then incubated with arachidonic acid for 24 h, each accumulated amount of **1** decreased to 1/4 and 1/16, respectively, compared to that in the case of catalase-unapplied slices. The experiments reveal that phytoalexin production is inhibited or slowed during infection if $H_2O_2$ generation is inhibited.

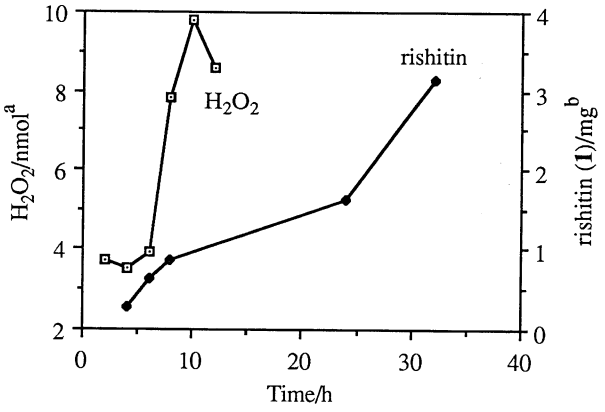



**Figure 1.** The amount of $H_2O_2$ generated from and **1** accumulated in potato slices (Rishiri) inoculated with *P. infestans* (race 0). Each value represents the average of three experiments. a) These values denote the concentration of $H_2O_2$ generated per 10 slices of potatoes. b) These values show the weight of rishitin (**1**) per kg of potato slices.

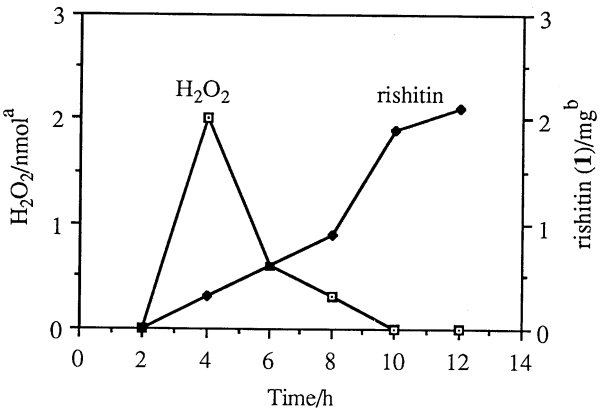



**Figure 2.** The amount of $H_2O_2$ generated from and **1** accumulated in potato slices (Rishiri) treated with arachidonic acid. Each value represents the average of three experiments. a) These values denote the concentration of $H_2O_2$ generated per 10 slices of potatoes. b) These values show the weight of rishitin (**1**) per kg of potato slices.

On the other hand, when aged slices of potato tubers were treated exogenously with an aqueous $H_2O_2$ solution, the following results were obtained (Table 1): (i) A large excess of $H_2O_2$ elicited accumulation of **1** and **2** in both Rishiri and Irish Cobbler, (ii) the detected amount of **1** depended on the concentration of $H_2O_2$ incubated, and (iii) at least 7.5 μmol/10 slices of $H_2O_2$ was required for formation of **1** when $H_2O_2$ was incubated *at a time*. Further experiments, using glucose-glucose oxidase

**Table 1.** Elicitation of phytoalexins in potato with $H_2O_2$ [a]

| Potato-cultivar | $H_2O_2$/μmol[b] | Metabolites/mg[c] rishitin (**1**) | phytuberin (**2**) |
|---|---|---|---|
| Irish Cobbler | 750 | 4.4 | 4.2 |
| | 75 | 1.8 | 0 |
| Rishiri | 750 | 6.8 | 6.0 |
| | 75 | 2.3 | 0 |
| | 7.5 | 0.2 | 0 |
| | 0.75 | 0 | 0 |

a) Various concentrations of aqueous solution of $H_2O_2$ were prepared, and each solution (15 ml) was incubated on the aged sliced potato tuber tissues (each 200 g) at 23 °C fo 48 h in the dark. b) These values reveal the concentration of $H_2O_2$ per 10 slices of potatoes. c) These values indicate the weight of rishitin (**1**) or phytuberin (**2**) per kg fresh weight of potato slices.

system,[9] were performed to apply $H_2O_2$ to the slices at a very low concentration *continuously*, because glucose is oxidized to glucono-δ-lactone with glucose oxidase, releasing $H_2O_2$ without generation of superoxide anion ($O_2^-$). When aged potato slices (Rishiri) were incubated with a glucose and glucose oxidase solution, the results indicated that continuous application of $H_2O_2$ even at a very low concentration (ca. 8 nmol/10 slices) could also elicit formation of **1** (0.02 mg/kg fresh wt.). It is emphasized that the concentration was essentially identical with that (ca. 10 nmol/10 slices) of $H_2O_2$ detected on the surface of the diseased potato slices inoculated with an incompatible pathogen (Figure 1). Thus, $H_2O_2$ was found to act as a dynamic trigger substance for phytoalexin formation in potato tubers.

$H_2O_2$ is known to be originated from triplet oxygen in air directly or via $O_2^-$ in nature. We have observed that application of arachidonic acid to potato slices led to production of **1** *without involvement of* $O_2^-$. The superoxide dismutase (SOD) was incubated with potato slices and immediately arachidonic acid was incubated as a stress to the slices. Resultingly, accumulation of **1** was not inhibited in the presence of SOD (20 h after incubation with or without SOD at 23 °C, **1** amounted to 0.90 or 1.07 mg/kg fresh wt., respectively). Moreover, when arachidonic acid was incubated with potato slices, generation of $O_2^-$ was not observed by reduction of cytochrome c. The results indicate that the $O_2^-$ appeared not to have any direct relation with formation of phytoalexin. In contrast with the hypothesis proposed by Doke,[10] these three observations indicate that $H_2O_2$, rather than $O_2^-$, should be a direct trigger of phytoalexin formation. Thus, we conclude here that $H_2O_2$ is one of the direct and general trigger substances for phytoalexin production in potato tissues. Herein we would like to define $H_2O_2$ as a dynamic trigger for phytoalexin production.

## References and Notes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