Effect of Coronatine on the Metabolism of Phenolics in the Discs of Potato Tuber

Yōsuke Mino*, Ryutarō Sakai*, Kenji Uchino* and Toshiyuki Sasabuchi*

Abstract

Alterations of phenolics in potato tuber discs treated with coronatine, a pathotoxin produced by *Pseudomonas syringae* pv. *atropurpurea*, were examined in relation to the changes in some oxidase activities involved in their metabolism. The content of phenolics was higher in coronatine treated discs than in non-treated discs. This was also true for the levels of polyphenol oxidase, peroxidase and ascorbate oxidase activities, their increases by coronatine treatment being markedly inhibited by cycloheximide or actinomycin D. An isoperoxidase induced specifically by coronatine treatment was recognized by isoelectric focusing electrophoresis. Among substrates tested, only o-diphenols were oxidized by polyphenol oxidase, and o- and p-diphenols (except guaiacol) by peroxidase irrespective of treatment. Researches on the physiological activities induced by coronatine were discussed to be useful for the elucidation of host-parasite interaction.

(Received May 19, 1980)

Introduction

It is widely accepted that the accumulation of phytoalexins and phenolics in the plant tissues is involved in the increase in disease resistance induced in the tissues by the infection of phytopathogens. But we have only a few studies on whether the disease resistance is elicited by physical injury or chemical substances such as pathotoxins. During the work on the hypertrophy response of potato tuber tissue by coronatine, an extracellular toxin produced by *Pseudomonas syringae* pv. *atropurpurea* (Reddy and Godking) Young *et al.*, we have found that the browning is markedly accelerated in the tissue by this toxin. The present study was attempted to examine the variations in phenolic content, together with several oxidases related to their metabolism in potato tuber discs and get a clue to elucidate some roles of coronatine in the disease resistance as model system.

Materials and Methods

Plant material. The tubers of potato (*Solanum tuberosum* L. cv. Danshaku) cultivated at Hokkaido Agricultural Experiment Station in 1978 were stored at 4°C and used...
at need. The cylinders (2 cm in diameter) were prepared by punching out the tuber tissues with a cork borer and then cut into slices (0.5 mm in thickness) with a microtome. After the discs were washed for 10 min in running tap water, the excess water on the surface was sucked up with filter papers.

**Coronatine treatment.** Twenty microliters of 20% acetone solution containing 0.1 mM coronatine were applied on one side of a disc. A control disc was also treated with 20% acetone solution. These coronatine-treated and non-treated discs, respectively, were put on a moistened filter paper in a petri dish (9 cm in diameter) and incubated at 22°C.

**Determination of phenolics.** Twenty discs were homogenized in 10 ml of cold acetone for 5 min using a prechilled mortar and pestle, followed by filtration through filter paper. One milliliter of the filtrate was made up to 10 ml with deionized water. The content of phenolics in the diluted solution was determined by the method of Folin-Denis.8

**Enzyme preparation.** The residue on the filter paper was washed thoroughly with cold acetone and dried at room temperature. The acetone powder thus obtained was dissolved in 15 ml of deionized water, followed by centrifugation at 12,000 × g for 10 min at 0°C. The supernatant solution was used as crude enzyme preparation.

**Assay of enzyme activity.** Polyphenol oxidase: The reaction mixture consisted of each of 1 ml of enzyme solution, phosphate buffer (0.1 M, pH 6.5) and catechol solution (1 mM). After incubation at 30°C for 20 min, the absorbance (420 nm) of oxidized product was determined using a spectrophotometer (Shimazu UV 140). Peroxidase: The reaction mixture consisted of 0.5 ml of enzyme solution, 3 ml of acetate buffer (0.2 M, pH 6.0), 0.5 ml of 0.8% H2O2 solution and 1 ml of 1% guaiacol solution. After incubation at 30°C for 10 min, the absorbance (470 nm) of oxidized guaiacol was determined. Ascorbate oxidase: The activity was measured using a Warburg apparatus. Two milliliters of the enzyme solution (diluted with an equal volume of the phosphate buffer) were put into a main chamber and 0.2 ml of Na-ascorbate (10 mM) into a side arm in a flask, and the activity was measured as the amount of oxygen consumed.

**Isoelectric focusing electrophoresis.** Two milliliters of enzyme solution mentioned above were dried in vacuo. The powder obtained was suspended in 200 µl of deionized water. A portion (50 µl) was subjected to electrophoresis for 90 min at 4°C. Gel was prepared by the method of Catslmpoolas.2 Electric current was 2 mA per column. The detection of peroxidase activity was carried out according to the method of Shinshi and Noguchi.11

**Results**

**Variations in the content of phenolics**

As shown in Fig. 1, though the content of phenolics increased by only cutting,5 coronatine-treated discs were higher in their contents than in non-treated discs during the period tested.

**Variations in enzyme activities**

The level of polyphenol oxidase activity increased rapidly in coronatine-treated discs, while gradually in non-treated discs (Fig. 2). The level of peroxidase activity was also
Fig. 1. Variations in the content of phenolic compounds in the discs of potato tuber. Open and closed circles indicate control and coronatine treatment, respectively. Phenolic content is expressed as absorbance at 700 nm.

Fig. 2. Variations in polyphenol oxidase activity in the discs of potato tuber. Open and closed circles indicate control and coronatine treatment, respectively. The activity was expressed as the absorbance at 420 nm by catechol oxidized.

Fig. 3. Variations in peroxidase activity in the discs of potato tuber. Open and closed circles indicate control and coronatine treatment, respectively. The activity was expressed as the absorbance at 470 nm by guaiacol oxidized.

Fig. 4. Variations in ascorbate oxidase activity in the discs of potato tuber. Open and closed circles indicate control and coronatine treatment, respectively. The enzyme activity was expressed as the amount of oxygen uptake (µl).
higher in coronatine-treated discs than in non-treated discs during the first 2 days, but attained the same level after 3 days in both treated and non-treated discs (Fig. 3). The level of ascorbate oxidase activity was little altered by coronatine treatment or cutting during the first 1 day, but thereafter increased markedly in coronatine-treated discs, while slowly in non-treated discs (Fig. 4).

**Effect of inhibitors for protein synthesis on the phenolic content**

As shown in Fig. 5, the increase in the content of phenolics in the discs treated with coronatine (cf. Fig. 1) was inhibited to a great extent by cycloheximide or actinomycin D, suggesting that the increase in phenolics was brought about by *de novo* syntheses of the enzymes concerned.

**Effect of inhibitors for protein synthesis on the levels of oxidases**

In Table 1 were summarized the effects of cycloheximide or actinomycin D on the syntheses of the oxidases in the discs. The increases in polyphenol oxidase, peroxidase and ascorbate oxidase activities in sliced potato tuber treated with coronatine

Table 1. Effect of cycloheximide and actinomycin D on the increases in polyphenol oxidase, peroxidase and ascorbate oxidase activities in sliced potato tuber treated with coronatine

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cycloheximide</th>
<th>Actinomycin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenol oxidase</td>
<td>96.0&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>102.0</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>75.0</td>
<td>87.5</td>
</tr>
<tr>
<td>Ascorbate oxidase</td>
<td>95.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

<sup>a)</sup> Figures given are inhibition (%). Inhibition (%) was calculated from the following formula: \([(A-B)/A] \times 100\), where A and B stand for the increment in the enzyme activity by coronatine treatment and that in the presence of an inhibitor, respectively.
Table 2. Substrate specificity of polyphenol oxidase and peroxidase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Polyphenol oxidase</th>
<th>Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day (C)</td>
<td>3 day (T)</td>
</tr>
<tr>
<td></td>
<td>0 day (C)</td>
<td>3 day (T)</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Vanillin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catechol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

The concentration of substrate tested was 1 mM. Both enzyme activities were detected by coloration of substrate oxidized (+). (C) and (T) indicate control and coronatine treatment, respectively. The reaction conditions were the same as those in Figs. 2 and 3, respectively.

oxidase in the discs by coronatine treatment (Figs. 2-4) were repressed within the range of 75-100% by cycloheximide or actinomycin D, suggesting that all these enzymes were for the most part newly synthesized from free amino acids.

*Isoelectric focusing electrophoresis of peroxidase*

As shown in Fig. 6, only two bands of isoperoxidases (A and B) were detected at the outset and their activities increased with the time after cutting. A new band (C) appeared in the discs 2 days after cutting. Another band (D) was detected only in the discs treated with coronatine.

*Substrate specificity of polyphenol oxidase and peroxidase*

Substrate specificity of the two enzymes was shown in Table 2. Among substrates tested, only o-diphenols were oxidized by polyphenol oxidase, and o- and p-diphenols (except guaiacol) by peroxidase irrespective of coronatine-treated and non-treated discs.

**Discussion**

The levels of polyphenol oxidase, peroxidase and ascorbate oxidase activities were shown to be more elevated in the discs treated with coronatine than in non-treated discs (Figs. 2-4). But the phenolic content was also higher in the former discs as compared with the latter (Fig. 1) as was reported for the leaves of rice plant treated with ophiobolin, a toxin produced by *Cochliobolus miyabeanus*. An explanation for this is that phenolics are actively synthesized in the discs treated with coronatine in excess of the amount of phenolics oxidized by the enzymes concerned. This may be endorsed by the fact that the increase in phenolics induced by coronatine was not recognized in the presence of cycloheximide or actinomycin D (Table 1). The increasing rates of both polyphenol oxidase
and peroxidase activities were higher in coronatine-treated discs than in non-treated discs during the first 2 days, but the situation was thereafter reversed (Figs. 2 and 3). This may be explained in terms of the shortage of materials for the enzyme syntheses, since the discs of 0.5 mm in thickness were used in this experiment. There is however a possibility that the effectiveness of coronatine is nullified through its decomposition in the discs at the later stage.

Many workers have reported that various enzymes are induced in plant tissues by cutting as were the cases for polyphenol oxidase, peroxidase and ascorbate oxidase in our experiments (Figs. 2–4). The activities of these enzymes increased in coronatine-treated discs in excess of those induced only by cutting (Figs. 2–4). Nakamura and Oku reported that polyphenol oxidase is activated in the leaves of rice plant by ophiobolin treatment. But in this study the increases in these enzyme activities were shown to be markedly inhibited by cycloheximide or actinomycin D (Table 1). This suggests that these increased activities are mainly derived from de novo syntheses of the enzymes concerned.

Novacky and Wheeler demonstrated that some of isoperoxidases are induced in the leaves of a susceptible variety of oat by low concentration of victorin, but not in a resistant variety. In our experiments an isoperoxidase, which was qualitatively different from the isoperoxidase induced by cutting (Fig. 6), was shown to be elicited in the discs by coronatine treatment. Asada et al. reported that lignin in the healthy tissue of Japanese radish root is different from that in the diseased tissue infected by Peronospora parasitica and they stated that the different lignins seem to be synthesized by different isoperoxidases in the healthy and diseased tissues, respectively. We are however uncertain about the roles of an isoperoxidase induced specifically in potato tuber tissue by coronatine treatment.

Phenolics, together with some enzymes involved in their metabolism, were increased in potato tuber discs by coronatine treatment as mentioned above. But coronatine is an extracellular toxin produced by Pseudomonas syringae pv. atropurpurea, the incitant of chocolate spot disease on Italian ryegrass (Lolium multilorum Lam.). It remains therefore unknown whether the present results imply the defense action of plants against parasites. Researches using host plants including resistant varieties will cast new light on the questions in this field.

Literature cited


和文摘要

ジャガイモ塊茎切片中のフェノール性物質の
代謝におよぼすコロナチンの影響

美濃洋輔・酒井隆太郎・内野賢二・笹淵俊幸

イタリアンライグラスかさ枯病菌, Pseudomonas syringae pv. atropurpurea, が生産する病原毒素, コロナチンでジャガイモ塊茎を処理し, フェノール性物質の代謝変動をそれに関与する2, 3酸化酵素の動態との関連において調べた。切片中のフェノール含量は切断のみによっても増加したが, コロナチン処理によりさらにそれを上回った。同様の現象がポリフェノールオキシダーゼ, バーオキシダーゼおよびアスコルビン酸酸化酵素にも認められた。さらにコロナチンにより誘導される上記酵素の増加は新たな蛋白質の合成に由来することが明らかにされた。切断のみによっては出現しないバーオキシダーゼのアイソサイムがコロナチンにより誘導された。調べた基質の中で, ポリフェノールオキシダーゼはオルトフェノール類のみを, バーオキシダーゼはオルトおよびパラジフェノール類のみ（グアヤコールを除く）を酸化した。