**Introduction**

Plants exhibit a variety of inducible defense responses against infection by pathogens. From the chemical point of view, such responses consist of diverse changes in secondary metabolism, resulting in the syntheses of antifungal compounds (Kuč, 1995), as well as of polymeric compounds which serve as a physical barrier against intrusion by pathogens (Matern et al., 1995).

In potato (*Solanum tuberosum*), the chemical defense responses are characterized by the induction of sesquiterpenoid phytoalexins such as rishtin (Katsui et al., 1968; Tomiyama et al., 1968), and hydroxycinnamoyl amide compounds, which contain tyramine and octopamine as the amine donors (Clarke, 1982). While the former phytoalexin accumulation has been observed only in tuber tissue (Rohwer et al., 1987), the latter is related to phenylpropanoid metabolism following pathogen infection in a tissue-independent manner (Clarke, 1982; Keller et al., 1996), and thus appears to be of more concern. Although these amide compounds show no practical antifungal activity themselves (unpublished data), it has been proposed that they are deposited into cell walls and utilized for physical reinforcement of the wall (Clarke, 1982; Facchini et al., 1999; Keller et al., 1996; Negrel et al., 1996).

An accumulation of hydroxycinnamoyl amides in potato was first observed in the tuber tissue infected with the late blight fungus, *Phytophthora infestans* (Clarke, 1982). Accumulation also occurs in leaves infected with *P. infestans* (Keller et al., 1996) and in suspension-cultured cells which have been treated with an elicitor preparation from the same fungus (Pi elicitor) (Schmidt et al., 1998). Recently, we reported that the treatment of tuber tissue with laminarin, a β-1,3-glucooligosaccharide derived from *Laminaria digitata*, causes an accumulation in a manner similar to the Pi elicitor, and the saccharide-nature of the elicitor may be responsible for the induction of the amide compounds (Miyagawa et al., 1998). The hydroxycinnamoyl amides in potatoes are thought to be synthesized from a hydroxycinnamoyl CoA ester and the corresponding amine precursors. The enzyme which catalyzes this cin-

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**Abbreviations:** CoA, coenzyme A; P-Co, N-p-coumaroyloctopamine; CHX, cycloheximide; 4CL, 4-hydroxycinnamic acid:CoA ligase; PAL, phenylalanine ammonia lyase; THT, hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl)transferase; TyrDC, tyrosine decarboxylase.
Induction of N-p-coumaroyloctopamine in Potato

Materials and Methods

Plant material

All potato tubers (Solanum tuberosum cv. En-iwa) used in this study were stored at 4 °C for a minimum of 6 months following harvesting. The internal part of the tuber was cut into disks (8 mm diameter, 2 mm thick), and washed with water for 30 min. Washed disks were incubated at 18 °C in the dark under wet conditions for 24 h prior to treatment with an aqueous solution of laminarin.

Chemicals

Laminarin from Laminaria digitata was purchased from SIGMA Chemical Co. Laminariheptaose was purchased from Seikagaku Co., JAPAN. Cycloheximide was purchased from Nacalai Tesque Co., JAPAN. p-CO and N-feruloylcoptopamine were synthesized as described by Negrel et al. (1993). p-Coumaroyltyramine and N-feruloyltiamine were prepared according to the methods of Villegas and Brodelius (1990). p-Coumaroyl CoA was prepared by transesterification of hydroxycinnamoyl-N-hydroxysuccinimide esters as described by Stöckigt and Zenk (1975). [2,3,4,5,6-5H] L-phenylalanine and [2,6-2H] L-tyrosine were purchased from ISOTEC, USA.

Quantification of phenolic amide compounds

10 μl of an aqueous laminarin solution (1.0 mg/ml) was applied to potato tuber disks, followed by incubation for 24 h. Three disks were combined, weighed, and extracted with 2.5 ml of 2% acetic acid (aq.) at 100 °C for 10 min. These extracts were centrifuged at 3000 rpm for 10 min, followed by HPLC analysis of the supernatant (column: Wakosil 5C18, 4.6 x 150 mm, solvent: 40% methanol in water containing 0.1% H₃PO₄, flow rate: 0.8 ml/min, detection: UV 310 nm).

Feeding experiments with [2,3,4,5,6-5H] L-phenylalanine and [2,6-2H] L-tyrosine

A 10 μl aliquot of 10 mM [2,3,4,5,6-5H] L-phenylalanine or [2,6-2H] L-tyrosine dissolved in 0.1 M Tris[tris(hydroxymethyl)aminomethane]HCl buffer (pH 7.5) was applied on a tuber disk following treatment with a 10 μl aliquot of laminarin solution (1.0 mg/ml). This treatment was repeated at six hour intervals after the initial appli-
cation. After 20 h p-CO was extracted as described above, and a 1.0 ml aliquot of the extract was evaporated in vacuo. The dried extracts were redissolved in 100 μl of methanol, and analyzed by LC-APPI-MS. Positive ion-spray ionization mass spectra measurements were carried out using a Parkermerer-Sciex API-165 instrument (ion-spray voltage: 5 kV, orifice voltage: 30V, nebulizer gas, curtain gas: nitrogen) combined with a Shimadzu LC-10A HPLC system. (column: Cosmosil 5C18, 4.6 × 150 mm, solvent: 40% methanol in water containing 0.1% acetic acid, flow rate: 0.8 ml/min).

Enzyme assays

A 10 μl aliquot of an aqueous laminarin solution (1.0 mg/ml) was applied to potato tuber disks which were then incubated for a specified time.

Phenylalanine ammonia lyase (PAL)

Three disks (about 0.5 g) were homogenized with sea sand in five volumes of 0.1 mM sodium phosphate buffer (pH 7.5) containing 2-mercaptoethanol (14.4 mM). The homogenate was centrifuged at 12,000×g for 10 min, and the supernatant was desalted on a PD-10 column. The protein fraction was used as the crude enzyme extract. The reaction mixture consisted of 60 μl of crude enzyme extract and 600 μl of 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM L-phenylalanine as the substrate. The mixture was incubated at 30 °C for 60 min, and the reaction was quenched by the addition of 60 μl of 1N HCl. After the addition of methanol (720 μl), the amount of trans-cinnamic acid produced was determined by HPLC (column: Wakosil 5C18, 4.6 × 150 mm, solvent: 70% methanol in water containing 0.1% acetic acid, flow rate: 0.8 ml/min, detection: UV 280 nm).

4-Hydroxycinnamic acid:CoA ligase (4CL)

Three disks (about 0.5 g) were homogenized with sea sand in five volumes of 0.1 mM sodium phosphate buffer (pH 7.5) containing 2-mercaptoethanol (14.4 mM) and glycerol (25% v/v). The homogenate was centrifuged at 12,000×g for 10 min and the supernatant was used as the crude enzyme extract. Aliquots (0.15 ml) of crude extract were incubated at 30 °C in a solution composed of 0.3 ml of 5.0 mM p-coumaric acid, 0.75 ml of 20 mM ATP containing 20 mM MgCl2, and 0.2 mM KH2PO4-NaOH buffer (pH 7.5). The reaction was initiated by adding of 0.3 ml of 1.0 mM CoA and the suspension was incubated for 10 min. 4CL activity was determined from the increase in absorbance at 333 nm using a molar extinction coefficient of 2.2 × 104 as described by Zenk et al. (1980).

Hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl)transferase (THT)

Three disks (about 0.5 g) were frozen in liquid N2 and homogenized with sea sand in five volumes of 0.1 mM sodium phosphate buffer (pH 7.5) containing 2-mercaptoethanol (14.4 mM). The homogenate was centrifuged at 12,000×g for 10 min, and the supernatant was used as the crude enzyme extract. The reaction mixture contained 10 μl of 500 μM p-coumaroyl CoA, 10 μl of 10 mM octopamine hydrochloride, 70 μl of 0.1 mM sodium phosphate buffer (pH 8.0) and 10 μl of crude enzyme extract. The mixture was incubated at 30 °C for 10 min, and the reaction was quenched by the addition of 20 μl of acetic acid. p-CO levels were quantified by HPLC (column: Wakosil 5C18, 4.6 × 150 mm, solvent: 40% methanol in water containing 0.1% H3PO4, flow rate: 0.8 ml/min, detection: UV 310 nm).

Tyrosine decarboxylase (TyrDC)

A crude enzyme extract was prepared by the same procedure as described for the PAL assay. The reaction mixture contained 90 μl of the crude enzyme extract, 30 μl of 10 mM L-tyrosine, 30 μl of 1.0 mM pyridoxal-5’-phosphate, and 150 μl of 0.1 mM sodium phosphate buffer (pH 7.5). The mixture was incubated at 30 °C for 60 min and after the addition of 30 μl of 1N HCl to quench the reaction, the induced tyramine was converted to dansyl tyramine by a modification of the method described by Smith and Davis (1985). An 100 μl of the reaction mixture was added to an aliquot (200 μl) of saturated Na2CO3 and 400 μl of 7.5 mg/ml dansyl chloride in acetone in sealed vials. And the vials were incubated at 60 °C for 60 min in the dark. Following the addition of 100 μl of a proline solution (100 μg/ml), the mixture was incubated for an additional 30 min under identical conditions. The mixture was extracted with 800 μl of ethyl acetate, followed by removal of the organic
phase under a steady stream of \( \text{N}_2 \). The residue was resuspended in 500 \( \mu \text{l} \) methanol, filtered through a PTFE filter (0.45 \( \mu \text{m} \), ADVANTEC), and analyzed by HPLC (column: Wakosil 5C18, 4.6 \( \times \) 150 mm, gradient system (methanol : water (0.1% phosphate)): from 60 : 40 to 95 : 5 in 27 min, flow rate: 1.0 ml/min, temperature: 35 °C, detection fluorescence: excitation 365 nm, emission 510 nm). Dansyl tyramine was eluted at 19.2 min under these conditions.

**Protein quantification**

The protein content in the crude extract was determined according to the methods of Bradford (1976) using bovine serum albumin as a standard.

**Quantification of cell deposited p-CO**

Cell wall fractions of potato tuber disks were prepared as described Keller et al. (1996). The cell wall fraction (a white powder) was suspended in 2.0 ml of 0.3 \( \text{n} \) sodium methoxide in 80% methanol (aq.), and stirred continuously under \( \text{N}_2 \) for 2 h at 80 °C followed by 1 h at room temperature. The hydrolysate was acidified by the addition of 50 \( \mu \text{l} \) 85% \( \text{H}_3\text{PO}_4 \) (aq.) and centrifuged for 10 min at 3000 rpm. Following removal of the supernatant *in vacuo*, the residue was resuspended in 1.0 ml of water and extracted twice with 2.0 ml of diethyl ether. The extract was dried and redissolved in 20% methanol (aq.), 20 \( \mu \text{l} \) of which was used for HPLC analysis (column: Wakosil 5C18, 4.6 \( \times \) 150 mm, gradient system (methanol : water (0.1% phosphate)): from 20 : 80 to 80 : 20 in 50 min, flow rate: 0.8 ml/min, temperature: 35 °C, detection: UV 310 nm). p-CO, which was eluted at 22.6 min under these conditions, was identified by co-chromatography with an authentic sample and detected via the pseudomolecular ion \((m/z: [\text{M+H}]^+ 300)\) by LC-API-MS.

**Results**

**Composition of phenolic amides induced by laminarin**

To date, four kinds of compounds (N-\( p \)-coumaroyloctopamine (p-CO), N-feruloyloctopamine, N-\( p \)-coumaroyltiramine, N-feruloyltiramine; Fig. 1) have been described as inducible phenolic amides in potato tuber tissue (Clarke, 1982). The changes in the amount of these compounds were analyzed, in order to characterize the features of the elicitor response. As shown in Fig. 2, the amounts of all four hydroxycinnamoyl amides were increased in laminarin treated tuber disks, reaching maximum levels after 24 h. No induction of these amides was observed in control disks. Among these compounds, the increase in the level of p-CO was greatest, with levels reaching 200 nmol/g tissue.

**Incorporation [2,3,4,5,6-\( ^5\text{H} \)] l-phenylalanine (phe-\( d_5 \)) and [2,6-\( ^2\text{H} \)] l-tyrosine (tyr-\( d_2 \)) into p-CO**

In order to clarify the biosynthetic origins of p-CO which was most abundantly accumulated in the potato tuber tissue treated with elicitor as de-
scribed above, the incorporation of \( \text{L-phenylalanine and L-tyrosine was examined using stable-isotope labeled compounds.} \)

Following treatment of the potato tuber disk with the labeled compounds and a laminarin solution (1.0 mg/ml) for 20 h, the incorporation rates were analyzed by LC-API-MS. Table I shows the relative intensities of the observed pseudomolecular \( p\text{-CO} \) ion \((m/z\ 300 \ [M+H^+])\) and its isotope ions. While the exogenous application of \( \text{phe-d}_5 \) significantly increased the intensity of the \( p\text{-CO-d}_4 \) ion \((m/z\ 304)\), a corresponding increase in \( p\text{-CO-d}_8 \) ion \((m/z\ 308)\) was not observed. Since one deuterium is thought to be replaced by a hydroxyl group, these results indicate that phenylalanine was incorporated into only one of the \( p\text{-coumaroyl-} \) and octopamine moieties that constitute \( p\text{-CO}. \)

Similarly, the application of \( \text{tyr-d}_2 \) resulted in an increase in the ion intensity from \( p\text{-CO-d}_2 \) \((m/z\ 302)\), but no increase in that from \( p\text{-CO-d}_4 \) \((m/z\ 304)\). On the other hand, when \( \text{phe-d}_5 \) and \( \text{tyr-d}_2 \) were applied simultaneously, an increase in the \( p\text{-CO-d}_6 \) \((m/z\ 306)\) ion intensity was clearly observed, in addition to those from \( p\text{-CO-d}_4 \) and \( p\text{-CO-d}_2 \). These results strongly suggest that \( \text{l-phenylalanine and l-tyrosine are, respectively, incorporated into the different moieties of \( p\text{-CO in the elicited potato tuber tissue.} \)

**Activation of four enzymes responsible for \( p\text{-CO} \) biosynthesis by treatment with laminarin**

The results of feeding experiments showed that \( p\text{-CO} \) accumulated in potato tuber tissue is synthesized by the pathway shown in Fig. 3. In this pathway, phenylpropanoid-metabolism-related enzymes such as \( \text{PAL and 4CL} \) are involved in the synthesis of the \( p\text{-coumaroyl moiety, TyrDC for that of octopamine-moiety, and THT for the formation of amide bond.} \)

Thus, the effects of laminarin treatment on the activities of these biosynthetic related enzymes were examined. The time-dependent changes in each enzyme activity are shown in Fig. 4. All enzyme activities in the control tissue increased to some extent in the absence of elicitor treatment. In particular, THT and 4CL activities were increased to 102.8 and 46.2 \( \mu \text{katal / mg protein}, \) respectively. Laminarin treatment caused an additional transient activation of these enzymes. While the maximal levels of THT and 4CL activities reached about twice those of control, the activity of \( \text{PAL} \) was 3 times the control and that of \( \text{TyrDC} \) was 5 fold greater than control levels. Following maximal induction, all enzyme activities declined to control levels within 48 h after treatment.

CHX \((10 \ \mu \text{g/ml})\) inhibited the activation of \( \text{PAL, THT and TyrDC in the laminarin-treated tuber disks, with the activities decreasing to that of control levels (Table II).} \)

Concomitantly, the accumulation of \( p\text{-CO in the tuber tissue was inhibited by CHX in a dose dependent manner, and the level of \( p\text{-CO decreased to 1/12 that of the positive control at a concentration of 25 \mu\text{g/ml, as shown in Fig. 5.}} \)

These results indicate that laminarin activates de novo protein biosynthesis, leading to the accumulation of \( p\text{-CO.} \)

**Effects of the elicitor concentration on enzyme activity**

To investigate the effects of the elicitor concentration on the enzyme activities, an aliquot of various concentration of laminarin was treated on potato tuber disks for 16 h and the activities of \( \text{PAL, 4CL, THT and TyrDC were determined.} \)

The activities of these enzymes were increased in a dose dependent manner up to 1.0 mg/ml (Fig. 6). Beyond this laminarin concentration, the activity of each enzyme reached a plateau and no increase was observed up to 10.0 mg/ml. These profiles were in good agreement with that observed for the relationship between the accumulated amount of \( p\text{-CO in potato tuber disks and laminarin concen-}

**Table I. Intensity of the molecular ion peak in the mass spectrum of \( N-p\text{-coumaroyloctopamine produced by potato tuber tissues fortified with [2,3,4,5,6-2H] L-phenylalanine (phe-d}_5] \) and [2,6-2H] L-tyrosine (tyr-d}_2].**

<table>
<thead>
<tr>
<th>( m/z )</th>
<th>control</th>
<th>phe-d(_5)</th>
<th>tyr-d(_2)</th>
<th>phe-d(_5) + tyr-d(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>301</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>302</td>
<td>6.0</td>
<td>6.0</td>
<td>25.6</td>
<td>18.3</td>
</tr>
<tr>
<td>303</td>
<td>3.4</td>
<td>3.4</td>
<td>6.9</td>
<td>6.2</td>
</tr>
<tr>
<td>304</td>
<td>2.4</td>
<td>34.2</td>
<td>2.4</td>
<td>30.6</td>
</tr>
<tr>
<td>305</td>
<td>2.4</td>
<td>8.2</td>
<td>2.4</td>
<td>8.0</td>
</tr>
<tr>
<td>306</td>
<td>2.2</td>
<td>2.6</td>
<td>1.2</td>
<td>9.1</td>
</tr>
<tr>
<td>307</td>
<td>3.5</td>
<td>6.8</td>
<td>3.3</td>
<td>10.4</td>
</tr>
<tr>
<td>308</td>
<td>3.4</td>
<td>3.8</td>
<td>3.2</td>
<td>5.3</td>
</tr>
</tbody>
</table>

(\%) Intensity of \([M+H^+]\) ion \((m/z: 300)\) was arbitrary set to 100\%.
Fig. 3. Proposed pathways for the biosynthesis of \( p \)-CO in potato tuber tissue. Two possible pathways are shown, in which \( p \)-CO was produced by the direct condensation between octopamine and the \( p \)-coumaroyl-CoA thioester (left pathway), and the oxidation of \( N \)-\( p \)-coumaroyltyramine (right pathway). PAL, phenylalanine ammonia lyase; 4CL, 4-hydroxycinnamic acid: CoA ligase; THT, hydroxycinnamoyl-CoA:tyramine \( N \)-(hydroxycinnamoyl)transferase; TyrDC, tyrosine decarboxylase. The steps marked with a question mark have not been established.

Fig. 4. Activation of PAL (A), 4CL (B), THT (C) and TyrDC (D) in potato tuber disks treated with laminarin. The disks were prepared 24 h prior to the treatment with an aqueous solution of laminarin (1.0 mg/ml). Closed circles represent activities of enzymes in disks treated with laminarin and open circles those of the control disks. The results are expressed as the mean of triplicate experiments ± SD.

Table II. Effects of cycloheximide (CHX, 10 \( \mu \)g/ml) on the laminarin induced activation of enzymes responsible for \( p \)-CO biosynthesis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Laminarin 1.0 mg/ml</th>
<th>Laminarin 1.0 mg/ml + CHX</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL</td>
<td>34.1 ± 1.8</td>
<td>120 ± 3.3</td>
<td>22.8 ± 0.5</td>
</tr>
<tr>
<td>THT</td>
<td>135 ± 3.8</td>
<td>235 ± 31.5</td>
<td>62.7 ± 7.7</td>
</tr>
<tr>
<td>TyrDC</td>
<td>13.8 ± 1.7</td>
<td>91.8 ± 13.4</td>
<td>16.7 ± 1.5</td>
</tr>
</tbody>
</table>

All enzyme activities were determined at 16 h after laminarin treatment. The results are expressed as the average of triplicates ± SE. PAL, phenylalanine ammonia lyase; THT, hydroxycinnamoyl-CoA:tyramine \( N \)-(hydroxycinnamoyl)transferase; TyrDC, tyrosine decarboxylase.

tration (Miyagawa et al., 1998), indicating that the activation of these enzymes are responsible for the accumulation of \( p \)-CO.
The activities of PAL, 4CL, THT and TyrDC 16 h after treatment with laminariheptaose (3.0 mg/ml) are shown in Table III. All the examined enzymes were activated to levels higher than the control. However in all cases, enzyme levels induced by laminariheptaose were less than those induced by laminarin. These results are consistent with the induction of p-CO in tuber disks by laminariheptaose and laminarin, and the effects of these two saccharides on potato tuber tissue are likely to be essentially the same.

Table III. Effects of laminariheptaose on the activity of enzymes responsible for p-CO biosynthesis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Laminarin 1.0 mg/ml</th>
<th>Laminariheptaose 3.0 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL</td>
<td>30.9 ± 5.7</td>
<td>74.0 ± 3.9</td>
<td>58.5 ± 1.2</td>
</tr>
<tr>
<td>4CL</td>
<td>45.5 ± 4.0</td>
<td>70.2 ± 3.5</td>
<td>54.3 ± 4.4</td>
</tr>
<tr>
<td>THT</td>
<td>135 ± 8.8</td>
<td>235 ± 31.5</td>
<td>208 ± 11.3</td>
</tr>
<tr>
<td>TyrDC</td>
<td>13.8 ± 1.7</td>
<td>62.7 ± 13.4</td>
<td>33.2 ± 1.3</td>
</tr>
</tbody>
</table>

All enzyme activities were determined at 16 h after laminarin treatment. The results are expressed as the average of triplicates ± SE. PAL, phenylalanine ammonia lyase; 4CL, 4-hydroxycinnamic acid; CoA ligase; THT, hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl)transferase; TyrDC, tyrosine decarboxylase.

Deposition of p-CO in cell wall

It has been proposed that phenolic amide compounds are produced for deposition in the cell wall, in order to form physical barriers to the attack by pathogens (Clarke, 1982; Facchini et al., 1999; Keller et al., 1996; Negrel et al., 1996). Thus, the amount of unextractable p-CO in cell walls was examined, in order to evaluate the exact activity of amide compounds biosynthesis. After the potato tuber tissue was thoroughly extracted by various solvents, the obtained cell wall fraction was hydrolyzed under alkaline conditions. The large amount of p-CO was recovered from the cell walls of laminarin treated tissue which reached levels as high as about 560 nmol/g tissue after 48 h (Table IV). In contrast, only small amounts of p-CO were recovered from the control cell wall fraction by the same procedure. This result indicates that neither soluble nor cell wall bound p-CO is hardly formed in the control tissues, so that almost
Table IV. Effects of the laminarin on the amounts of p-CO deposited in the cell walls of potato tuber disks.

<table>
<thead>
<tr>
<th>Time after laminarin p-CO (nmol/g fresh tissue)</th>
<th>Control</th>
<th>Laminarin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>treatment (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9.2 ± 2.4</td>
<td>5.4 ± 1.8</td>
</tr>
<tr>
<td>48</td>
<td>40.9 ± 10.8</td>
<td>562 ± 157</td>
</tr>
</tbody>
</table>

The results are expressed as the means of triplicates ±SD.

no biosynthesis of p-CO occurs in the absence of laminarin treatment, even though a significant activation of some biosynthesis-related enzymes had occurred.

Discussion

A variety of hydroxycinnamoyl amide compounds in plants have been documented (Chaves and Roque, 1997; Chen et al., 1998; Martin-Tanguy et al., 1978; Mühlenbeck et al., 1996; Yamamoto et al., 1991; Yoshihara et al., 1981). Among them, N-p-coumaroyloctopamine (p-CO), N-feruloyloctopamine, N-p-coumaroyltiyramine, and N-feruloyltiyramine (Fig. 1) have been found in potato tuber tissue (Clarke, 1982). Treatment with an aqueous solution of laminarin resulted in increased levels of all four amide compounds tested (Fig. 2). While the inductions of N-feruloyloctopamine, N-p-coumaroyltiyramine, and N-feruloyltiyramine did not exceed the level of 30 nmol/g fresh tissue, the changes in the level of p-CO were more drastic and reached 203.4 nmol/g of fresh tissue 24 h after application. This accumulation profile in tuber tissue was observed in several other potato cultivars tested (data not shown). The accumulation of similar phenolic amide compounds has been observed in some plants such as tobacco, tomato, and onion under stressed conditions (McLusky et al., 1999; Mühlenbeck et al., 1996; Negrel and Martin, 1984; Pearce et al., 1998), and in suspension-cultured potato cells treated with Pi elicitors (Schmidt et al., 1998). However, increases of octopamine amides of this magnitude have not been described to date and therefore might be characteristic of potato tuber tissue treated with β-1,3-glucooligosaccharides. It has been proposed that hydroxycinnamoyl amide compounds are deposited into the cell walls of the tuber tissues and utilized for physical reinforcement against pathogen infections (Clarke, 1982; Facchini et al., 1999; Keller et al., 1996; Negrel et al., 1996). In agreement with this theory, a large amount of p-CO was recovered from the cell wall of laminarin treated potato tuber tissue (Table IV).

Feeding experiments indicated that l-phenylalanine and l-tyrosine are precursors of p-CO biosynthesis (Table I). The results also showed that these precursors were separately incorporated into two components of p-CO, namely the p-coumaroyl and octopamine moieties. Since a p-coumaroyl moiety is often found as a component of secondary metabolites of the plant phenylpropanoid pathway originating from l-phenylalanine, the octopamine moiety of p-CO is likely to be of l-tyrosine origin. It has been suggested that tyramine amides of hydroxycinnamic acids are synthesized from the same precursors (Villegas and Brodelius, 1990).

On the basis of this information, we suggest possible pathways leading to p-CO biosynthesis (Fig. 3). However, the issue whether p-CO is formed by direct condensation between octopamine and the p-coumaroyl CoA thioester (left pathway in Fig. 3), or whether it is formed by the oxidation of N-p-coumaroyltiyramine (right pathway) remains unknown. As has been shown in our previous report, the octopamine moiety of p-CO in potato tuber has the S configuration (Matsuda et al. submitted), being opposite to that of neuroactive octopamine present in animals (Goosey and Candy, 1980; Midgley et al., 1989; Starratt and Bodnaryk, 1981). Work to further clarify the biosynthetic pathway of p-CO in potato tuber tissue is now in progress.

The treatment of potato tuber disks with an aqueous solution of laminarin caused an increase in enzyme activities related to the biosynthesis of hydroxycinnamoyl amide compounds, namely PAL, 4CL, THT and TyrDC (Fig. 4). The results herein are consistent with findings of suspension-cultured potato cells (Schmidt et al., 1998), in which the activation of these enzymes, except for 4CL, was demonstrated to occur as the result of treatment with an elicitor preparation from P. infestans. Treatment with laminariheptaose also caused a significant activation of these enzymes in tuber tissue (Table III), indicating that the β-1,3-glucosaccharide structure is an important factor.
for the elicitor activity. The observed activation by the oligosaccharide was thought to be the result of de novo protein biosynthesis, since it was strongly inhibited by CHX (Fig. 5, Table II). Among the enzyme activities studied, those of PAL, 4CL, and THT were found to be elevated prior to elicitor treatment (Fig. 3A, B, C) which was probably due to the wounding of the tuber tissue, as the result of the preparation of sample disks (Negrel et al., 1993). However, the activation of these enzymes in control tissues resulted in negligible production of either the soluble or cell wall bound forms of hydroxycinnamoyl amides (Fig. 2, Table IV), so that they appeared to be “idling”. By contrast, a significant level of activation of TyrDC in tuber tissue occurred only after treatment with laminarin (Fig. 3D). Recent work has demonstrated that mRNA of THT is specifically accumulated in potato suspension culture cell treated with Pi elicitor. However, the time courses of the enzyme activity in potato tuber tissue (Fig. 4) implied that TyrDC is more closely associated with the induced biosynthesis of hydroxycinnamoyl amides than other enzymes such as PAL and THT. The importance of TyrDC has also been shown in the hydroxycinnamoyltyramine biosynthesis of suspension cultured tobacco cells after treatment with a chitosan elicitor (Villegas and Brodelius, 1990).

It has been shown that plant defense responses are triggered both by tissue wounding and by chemical signal substances, or elicitors, of pathogen origin (Bögre et al., 1997; Díaz and Merino, 1998; Negrel et al., 1993). The elucidation of the signaling systems relevant to these stimuli in plants is a matter of great concern. Accordingly, the specific activation of TyrDC by laminarin in the wounded potato tissue observed in this study is of particular importance, since it suggests the presence of a signaling pathway stimulated by saccharide elicitors in potato which is independent of that for wounding. Future work will focus on elucidating the chemical basis of elicitor-active saccharides as well as its perception mechanisms in plants.

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treated cell suspension cultures and fungus-infected leaves of *Solanum tuberosum*. Phytochemistry **42**, 389–396.


