Conditioning of Parsley (Petroselinum crispum L.) Suspension Cells Increases Elicitor-Induced Incorporation of Cell Wall Phenolics

Heinrich Kauss*, Rochus Franke, Karin Krause, Uwe Conrath, Wolfgang Jeblick, Bernhard Grimmig, and Ulrich Matern

FB Biologie der Universität Kaiserslautern, Postfach 3049, D-6750 Kaiserslautern, Germany (H.K., R.F., K.K., U.C., W.J.); and Institut für Biologie II der Universität Freiburg, Schänzlestrasse 1, D-7800 Freiburg, Germany (B.G., U.M.)

The elicitor-induced incorporation of phenylpropanoid derivatives into the cell wall and the secretion of soluble coumarin derivatives (phytoalexins) by parsley (Petroselinum crispum L.) suspension cultures can be potentiated by pretreatment of the cultures with 2,6-dichloroisonicotinic acid or derivatives of salicylic acid. To investigate this phenomenon further, the cell walls and an extracellular soluble polymer were isolated from control cells or cells treated with an elicitor from Phytophthora megasperma f. sp. glycinea. After alkaline hydrolysis, both fractions from elicited cells showed a greatly increased content of 4-coumaric, ferulic, and tyrosol. The pretreatment effect was most pronounced at a low elicitor concentration. Its specificity was elaborated for coumarin derivatives (phytoalexins) by parsley 4-hydroxybenzoic acid, as well as 4-hydroxybenzaldehyde and 2,6-dichloroisonicotinic acid or derivatives of salicylic acid, which were liberated from cell walls upon alkaline hydrolysis and for “lignin-like” cell wall polymers determined by the thioglycolic acid method. It was shown that 3-chlorosalicylic acid that conditioning most likely improves the signal transduction leading to the activation of genes encoding phenylalanine ammonia lyase and 4-coumarate: coenzyme A ligase. The conditioning thus sensitizes the parsley suspension cells to respond to lower elicitor concentrations. If a similar mechanism were to apply to whole plants treated with 2,6-dichloroisonicotinic acid, a known inducer of systemic acquired resistance, one can hypothesize that fungal pathogens might be recognized more readily and effectively.

The interaction of plants with fungal pathogens and the resulting defense reactions of the plant occur initially at single cells and are experimentally difficult to synchronize. For biochemical and physiological studies, therefore, model systems of fungal elicitor preparations and wounded plant tissues or suspension-cultured cells have become customary. Initially, the defense responses studied successfully in this way were mainly the production of soluble fungitoxic “phytoalexins” (Hahlbrock and Scheel, 1989; Bowles, 1990). Subsequently, and with increasing frequency, the elicitor induction of callose deposition (Kauss, 1987, 1990) and the formation of covalently linked cell wall phenolics were also studied (Barber et al., 1989; Bruce and West, 1989; Graham and Graham, 1991). These reactions are generally thought to correspond in the whole plant to defense reactions occurring locally at the infection site.

Another defense strategy of plants became evident from studies of the so-called systemically acquired resistance, in which a signal derived from an initial infection spreads over adjacent tissues, or even throughout the plant, where it induces in a developmental fashion the production of certain “pathogenesis-related proteins” (Bowles, 1990; Raskin, 1992). Some of these pathogenesis-related proteins have been identified as enzymes of obvious utility in the direct defense against invading fungi, e.g. chitinase, 1,3-β-glucanase, and peroxidase (Ward et al., 1991), but the function of many of these proteins remains unknown. If, for example, the increase in apoplastic peroxidase plays a role in defense-related polymerization of cell wall phenolics, it has to be assisted by an increased biosynthesis and export of phenylpropanoid substances. Cytological studies, e.g. those in cucumber (Hammerschmidt and Kuc, 1982; Kovats et al., 1991), also indicate that additional defense reactions operating at the level of the cell wall have to be considered to explain the phenomenon of systemically acquired resistance. It appeared feasible, therefore, to create a model system that facilitates investigation of elicitor-triggered responses thought to correspond to the local defense strategy in combination with responses caused by inducers of systemically acquired resistance.

Several lines of evidence suggest that salicylic acid is involved in the establishment of systemically acquired resistance (Malamy and Klessig, 1992; Raskin, 1992). Physiological

---

1 Supported by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

* Corresponding author; fax 49-631-205-2998.
studies, however, indicate that this substance is not the primary systemic signal in cucumber (Rasmussen et al., 1991). Whether other possible systemic signaling substances, such as jasmonate (Staswick, 1992) or peptides like systemin (Pearce et al., 1991), are serving as the primary in vivo signal remains to be shown. Nevertheless, DCIA was shown to mimic the systemic resistance induction process in various plants (Métraux et al., 1991; Ward et al., 1991). Preincubation of parsley suspension cultures with DCIA was recently shown to enhance the sensitivity of the cells toward a fungal elicitor inducing the secretion of coumarin derivatives (Kauss et al., 1992b). Salicylic acid gave similar, but less pronounced, effects in this system. The enhancement effect was also found for two enzymes involved in coumarin synthesis, namely, phenylalanine ammonia lyase and S-adenosyl-l-methionine:xanthotoxol O-methyl transferase. Similarly, the elicitor-induced synthesis of mRNA specific for the former enzyme and for 4-coumarate:CoA ligase was also enhanced upon preincubation of the parsley cells with DCIA or salicylic acid (Kauss et al., 1992b). These results suggested that unknown components of the elicitor signal perception/transduction pathway are rate-limiting in the cells under normal growth conditions but are increased upon conditioning the cells with DCIA.

The present report extends this study in three directions. It explores the elicitor-induced incorporation of monomeric and polymeric phenolics into the cell wall of parsley cells, it documents that other chemicals structurally related to salicylic acid are even more potent than DCIA for cell conditioning.

MATERIALS AND METHODS

Parsley Cell Suspension and Its Handling

The origin and growth conditions for the dark-grown parsley (Petroselinum crispum) cell-suspension culture were as described by Kauss et al. (1992b). Inoculation from a 6-d-old culture was performed using 20 to 25 mg cells (fresh weight) per mL. For the conditioning experiments, the cultures were divided after 3 d when they had reached 50 to 70 mg of cell wet weight per mL of suspension, and the appropriate culture was performed using 20 to 25 mg cells (fresh weight) of cells were added to 5 mL of 1 N NaOH, overlaid with N2, and treated on a roller mixer as above in the dark at room temperature for about 20 h. The pH was adjusted to about 2.5 with 6 N HCl, and the phenolics were extracted twice with 5 mL of ethyl acetate. The solution was evaporated and solubilized in 60 to 300 μL of methanol, and aliquots of 20 μL were subjected to HPLC. Semipreparative hydrolysis of the cell wall (3.0 g of dry cell wall powder) was carried out in 1 N NaOH (100 mL) at 80°C for 1 h followed by an additional 1 h at room temperature. The resulting suspension was acidified, and phenolics were extracted as described above.

Analytical separations were carried out at a flow rate of 1 mL min⁻¹ on a Lichrosorb 100 RP-18 (5 μm) column (Merck, Darmstadt, Germany) in 5% (v/v) acetic acid (solvent A) and 20% (v/v) acetic acid plus 25% (v/v) acetonitrile (solvent B), using a linear gradient from 7 to 55% solvent B during the first 20 min and from 55 to 99% solvent B during the subsequent 10 min, with detection at 275 nm. The gradient was held at 99% solvent B for 5 min. Routinely, only one aliquot of each extract was analyzed, and these values are given in this report. So for the separation of several aliquots from the same extract was between ±3% for 4-coumaric acid and ±8% for 4-hydroxybenzaldehyde.

Semipreparative separation of monomeric phenols was accomplished on a Lichrosorb RP18 (5 μm) column (Knauer, Bad Homburg, Germany) in 15:84:1 (v/v/v) acetonitrile:water:acetic acid (solvent A) and 40:59:1 (v/v/v) acetonitrile:water:acetic acid (solvent B), using a linear gradient from 0 to 100% solvent B in solvent A during 25 min at a flow rate of 0.9 mL min⁻¹, with detection at 280 nm. For identification, the elution of individual compounds was monitored by photodiode array spectroscopy (Waters 990, Eschbach, Germany) over the range 240 to 450 nm.

For the determination of lignin-like polymers, the cell walls from 1 g of cells were treated with thioglycolic acid, and the resulting derivatives were solubilized with NaOH and precipitated with HCl as described by Graham and Graham (1991). The precipitate was solubilized in 5 mL of 0.5 N NaOH and photometrically quantified at 320 nm.

Identification of Phenolic Monomers

Major compounds collected from semipreparative HPLC were identified by photodiode array absorption, mass, and 1H-NMR spectroscopies. Electron impact mass spectra were
recorded for samples introduced via a direct inlet on a Finnegan MAT 312 spectrometer, at a source temperature of 220°C and an ionization energy of 70 eV (Institut für Organische Chemie und Biologie, Universität Freiburg, Germany). Chemical ionization in methane was used for confirmation of large ions. 1H-NMR spectra were recorded on a Bruker WM 300 spectrometer (300 MHz; Max-Planck-Institut für Immunbiologie, Freiburg-Zähringen, Germany).

RESULTS

Treatment of parsley suspension cells with fungal elicitor renders them slightly brown and induces a drastic increase in the amount of phenolic material which, after alkaline hydrolysis of the cell walls, can be partitioned into ethyl acetate and separated by HPLC. A rather broad peak eluting between 24 and 36 min was not further considered but may include oligomeric or polymeric phenolics. Identification of individual monomeric substances from the materials eluting early was accomplished by a combination of UV, MS, and NMR spectroscopy after semipreparative isolation under adjusted HPLC conditions. In this way, a series of chemically related substances was identified (Fig. 1). The most prominent HPLC signals were assigned to 4-coumaric, ferulic, and 4-hydroxybenzoic acids, as well as 4-hydroxybenzaldehyde and vanillin. The aldehydes were easily recognized by their hypsochrome-shifted UV absorption in comparison to cinnamic acids, as well as by the downfield singlet resonance of the aldehyde proton (9.38 and 9.41 parts million⁻¹) detected by 1H-NMR spectroscopy. Mass spectral fragmentation fully supported the identification, and the identity of all of these compounds was further confirmed by cochromatography with authentic reference samples on analytical RP18-HPLC.

Preparative HPLC revealed the additional presence of two minor phenolics in the alkaline hydrolysates, which eluted earlier (Rt 7.05 and 7.2 min) than 4-hydroxybenzoic acid (Rt 8.85 min). These compounds were identified as phényl ethanols correlating in their ring substitution to 4-coumaric and ferulic acids. 2-(4-Hydroxyphenyl)-ethanol ("tyrosol"), identified initially by its mass spectral fragmentation, was compared to a commercial sample by the NMR data (Pouchert, 1983) and chromatographic mobilities. 2-(4-Hydroxy-3-methoxyphenyl)-ethanol ("methoxytyrosol") was identified by its corresponding mass spectral fragmentation only.

Data concerning the quantitative composition of the major

Determination of Secreted Coumarin Derivatives

Coumarin derivatives in the 1:100- or 1:1000-diluted supernatant were determined after 24 h as recently described (Kauss et al., 1992b), but the relative fluorescences (excitation 335 nm, emission 398 nm) measured were converted to the values given by multiplying them by the dilution factor and dividing by 10⁵.

Chemicals and mRNA Analysis

Preparation of a crude elicitor fraction from P. megasperma f. sp. glycinea was as described by Kauss et al. (1992b). The crude elicitor preparation was solubilized in water at 2 mg mL⁻¹ on a weight basis and autoclaved. DCIA was kindly supplied by H. Kessmann (Ciba-Geigy, Basel, Switzerland). All other chemicals were purchased from Sigma (Deisenhofen, Germany), Aldrich (Steinheim, Germany), or Fluka (Ulm, Germany). Deuterated solvents of gold label quality (Aldrich) were used for 1H-NMR spectroscopy.

The specific mRNAs were analyzed as described by Kauss et al. (1992b) but using a 4-fold higher amount of 32P-labeled cDNAs for hybridization. The dot blots were cut out and subjected to liquid scintillation counting.

Figure 1. Structural relationships of monomers liberated from the cell walls of elicitor-treated parsley cells upon alkaline hydrolysis.
monomers liberated by alkali from the cell walls and the extracellular polymer are given in Table I. The time course of appearance after elicitor addition was very similar for all of the substances listed in Table I. As the example 4-hydroxybenzaldehyde from the cell walls (Fig. 2) shows, a small initial increase is observed about 6 h after addition of the elicitor, followed by a greater linear increase during the next 18 h. A similar time course of all the monomeric phenolic derivatives (Table I) was also observed in the ethanol-precipitated extracellular polymer from the spent growth medium (data not shown). Although the phenolics were induced in any experiment performed, there was considerable variation among individual experiments with regard to the relative amounts of the various substances found, possibly due to unavoidable slight variations in growth conditions. Therefore, we report representative experiments, but each type of experiment was performed three to six times. For instance, in cell walls 4-coumaric acid and 4-hydroxybenzaldehyde occur in similar amounts in Table I, whereas the content of the latter substance is relatively more prominent in Figure 4 (control cells, 40 μg mL⁻¹ elicitor). Similarly, 4-hydroxybenzoic acid and the respective aldehyde ranged from an equal concentration (data not shown) to a 2- (Table I) or even 4-fold higher value for the aldehyde (data not shown). The extracellular soluble polymer in some experiments yielded about the same amount of the phenolic monomers as the walls from the corresponding cells (Table I), whereas in other experiments only about a quarter of the amount was found (data not shown).

When the parsley cell suspension was preincubated with 20 μM DCIA for 1 d and challenged afterward with elicitor (4 μg mL⁻¹), the peaks of all monomeric phenolics recovered on HPLC were greatly increased in comparison with cells treated with only elicitor (compare B and C in Fig. 3). Nevertheless, it should be noted that all peaks, including 4-hydroxybenzaldehyde, are also present in small amounts in nonelicited control cells (Fig. 3A); although they are too low to show up in Figure 4. The effects of DCIA conditioning are especially pronounced at low elicitor concentrations, both for the secreted coumarin derivatives and for all monomeric phenolics liberated from the cell wall under alkaline conditions (Fig. 4). At elevated elicitor concentrations the dose-response curve for secreted coumarin derivatives slightly decreases (Fig. 4A). This is apparent only and is due to the fact that the convenient fluorescence assay overestimates some blue-fluorescent components of the coumarin mixture, which decrease in proportion on high stimulation of the cells.

The ability of different substances to condition parsley cells was investigated in detail with coumarin secretion as the assay (Table II). The two commercial monochlorosalicylic acids were more potent than either salicylic acid or DCIA. In addition, 2,6-dihydroxybenzoic acid exhibits some potency, whereas other isomers of dihydroxybenzoic acid, and 3- and

![Table 1. Elicitor-induced increase in phenolic products liberated by alkaline hydrolysis from parsley cell walls and from the ethanol-precipitated extracellular soluble polymer](image)

Cultures were used 6 d after inoculation, supplied with elicitor (40 μg mL⁻¹), and incubated for further 24 h. Preparation of cell walls, alkaline hydrolysis, and HPLC separation of the monomeric phenolic substances are described in "Materials and Methods." HPLC detection limit was 0.1 nmol g⁻¹ fresh weight. For notes on the variability between experiments, see the text.

<table>
<thead>
<tr>
<th>Product</th>
<th>Cell walls</th>
<th>Soluble polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial*</td>
<td>Controlb</td>
</tr>
<tr>
<td>4-Coumaric acid</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>4-Hydroxybenzaldehyde</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Content at time of elicitor addition.  
  b Content in control cells 24 h later.  
  c Elicitor-treated cells 24 h later.
Elicited Cell Wall Phenolics in Conditioned Parsley Cells

Figure 3. Analytical HPLC separation of monomeric phenolic products liberated upon alkaline hydrolysis from cell walls of elicitor-treated control cells and from cells preincubated with DCIA and then treated with elicitor. One-half of a parsley cell suspension was treated at d 3 with 20 μM DCIA, whereas the other half remained as a control. One-half of each of the DCIA-treated and control cell samples was supplied at d 4 with elicitor (4 μg mL⁻¹). Cell walls were prepared at d 5. The same portion of each extract was analyzed for control cells (A), control cells plus elicitor (B), and DCIA-treated cells plus elicitor (C). The chromatogram for DCIA-preincubated cells without elicitor was very similar to that in A. Peak 1, 4-Hydroxybenzoic acid; peak 2, 4-hydroxybenzaldehyde; peak 3, vanillin; peak 4, 4-coumaric acid; peak 5, ferulic acid. Tyrosol elutes in the descending flank of peak 1 in this analytical separation system.

Figure 4. Elicitor dose response for the secretion of soluble coumarins (A) and for various cell wall phenylpropanoid derivatives (B–D) from control cells (○) and from cells preincubated with DCIA (▲). The same conditions as in Figure 3 were used with the indicated concentrations of elicitor. rel. fl., Relative fluorescence.

4-hydroxybenzoic acids, were without significant effect. The degree of coumarin secretion at the low elicitor concentration of 4 μg mL⁻¹ varied among different experiments. The two extremes are given in Table II. Generally, the conditioning effect caused by the less potent chemicals (e.g. isonicotinic acid) was best observed in cell batches with low coumarin secretion by control cells treated with 4 μg mL⁻¹ of elicitor (experiment I), whereas it was less obvious in cell batches

Table II. Elicitor-induced secretion of coumarin derivatives in parsley suspension cells conditioned with various substances chemically related to dichloroisonicotinic and salicylic acids

<table>
<thead>
<tr>
<th>Acid Used for Preincubation</th>
<th>Secreted Coumarin Derivatives</th>
<th>Exp. I</th>
<th>Exp. II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>relative fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>0.03</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Isonicotinic</td>
<td>0.27</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>DCIA</td>
<td>0.97</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Salicylic (2-hydroxybenzoic)</td>
<td>0.13</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic</td>
<td>0.11</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>4-Chlorosalicylic</td>
<td>1.36</td>
<td>1.90</td>
<td></td>
</tr>
<tr>
<td>5-Chlorosalicylic</td>
<td>2.67</td>
<td>2.31</td>
<td></td>
</tr>
<tr>
<td>3,5-Dichlorosalicylic</td>
<td>1.67</td>
<td>1.76</td>
<td></td>
</tr>
<tr>
<td>2,6-Dihydroxybenzoic</td>
<td>0.13</td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

Under the conditions used, no significant difference from the controls was observed with nicotinic; N-methyl nicotinic; 2,3-, 2,5-, 3,4-, 3,5-dihydroxybenzoic; or 3- and 4-hydroxybenzoic acid (data not shown).
exhibiting a comparatively high secretion under these conditions (experiment II).

The most effective compound, 5-chlorosalicylic acid, was further tested to determine its potency to condition parsley cells for increased elicited incorporation of lignin-like polymers into the cell walls (Fig. 5). The elicitor dose-response curve for these polymers shows, again, an increase for the conditioned cells, especially at low elicitor concentrations (Fig. 5).

As found before with DCIA (Kauss et al., 1992b), preincubation with 5-chlorosalicylic acid also greatly increases the synthesis of mRNA specific for Phe ammonia lyase and 4-coumarate:CoA ligase induced by low elicitor concentration (Fig. 6).

DISCUSSION

Parsley suspension cultures have been intensively used as a model system in which to study details of the biochemistry and physiology of the elicitor-induced secretion of coumarin derivatives (Hahlbrock and Scheel, 1989). The active component in the crude fungal elicitor preparation used is a glycoprotein (Parker et al., 1991). In the present report we show that in parallel with coumarin secretion two other responses related to phenylpropanoid metabolism are also induced by the same elicitor preparation, namely, increases in the incorporation of phenolics recovered from the cell wall either as monomers after alkaline hydrolysis (Fig. 1, Table I) or as lignin-like polymers precipitated as thioglycolic acid derivatives (Fig. 5, control cells).

The ferulic and 4-coumaric acids found were most likely esterified to cell wall polysaccharides as shown for the primary cell wall of normal cells (Fry, 1986) and suggested for soybean cotyledons treated with a glucan-type elicitor (Graham and Graham, 1991). It appears possible that the 4-hydroxybenzoic acid derived from the elicited cell walls and from the extracellular polymer (Table I) was also esterified to polymers, but this has to be established. However, the type of binding of 4-hydroxybenzaldehyde, vanillin, tyrosol, and methoxytyrosol to cell wall polymers remains unclear. The two aldehydes were also liberated by alkali from normal graminaceous cell walls (Hartley and Keene, 1984) and from cell walls of elicited suspension-cultured cells of Lycopersicon peruvianum (Beimen et al., 1992). It was speculated that these aldehydes may be linked by their phenolic groups to cell wall polysaccharides, but experimental evidence was not provided (Hartley and Keene, 1984). The close chemical relationship of vanillin and 4-hydroxybenzaldehyde with ferulic and 4-coumaric acid (Fig. 1) suggests an alternative hypothetical possibility, namely, that the aldehydes are not present as such in the cell walls but are derived from alkali-labile derivatives of the respective hydroxycinnamic acids that are themselves esterified to polysaccharides. This might apply also to the related compounds tyrosol and methoxytyrosol (Fig. 1), which have not yet been reported in the context of the plant cell wall, although glycosidic tyrosols are known to be plant constituents (Le Totour and Didier, 1992). Although the aldehydes may function as “cell wall-derived phytoalexins,” no guess can be made about the eventual role of phenylethanols in disease resistance.

The lignin-like polymeric material recovered from the cell walls by the thioglycolic acid method is chemically ill defined. In our experiments (Fig. 5) as well as in those reported by, for example Barber et al. (1989), Bruce and West (1989), and Graham and Graham (1991), this material was also consid-
erably increased upon elicitor treatment. Evidence is accumulating that polymeric phenolic material may be bound by ester linkages within the cell wall, e.g., the lignin in the cell wall of grasses. Ether-linked ferulic acid has been reported in this material (Scalbert et al., 1985), and more recent results suggest that all etherified ferulic acid—at least 10% of the lignin monomers—is also ester-linked, presumably to polysaccharides (Lam et al., 1992). Fry (1984) has suggested that hydroxycinnamic acids esterified to two chains of cell wall polysaccharides may become cross-linked to oligomers and thus contribute to the resistance against wall-degrading enzymes. The latter property has indeed been shown for suspension-cultured soybean cells, in which phenolic cell wall material increases upon treatment with chitosan as an elicitor (Köhle et al., 1984).

Thus, the elicitor-increased incorporation of esterified hydroxycinnamic acids into the parsley cell wall would offer a greater possibility for cross-linking polysaccharides and/or for anchoring of phenolic polymers recovered as lignin-like material, which is found to be increased in the presence of elicitor (Fig. 5, control cells). It has been shown that the ferulic acid esters of presumed cell wall polymers are synthesized from the respective COA thioesters in parsley cell endomembranes (Meyer et al., 1991). Recent experiments show the same for other hydroxycinnamates esters of presumed cell wall polymers (A. Köhler and H. Kauss, unpublished data). These results suggest that the hydroxycinnamates esters of cell wall polysaccharides are formed in the Golgi apparatus and secreted, like all other matrix polysaccharides, as preformed macromolecules. Accordingly, the pattern of hydroxycinnamates esters in the extracellular polymer secreted by elicited parsley cells is similar to that in the cell wall (Table I). The analysis of this material, obviously representing a macromolecular carrier for covalently linked cell wall phenolics, is in progress.

The elicitor-induced production of secondary metabolites in suspension cultures is thought to correspond in the whole plant to defense responses occurring upon local attack of cells by pathogens. In contrast, the conditioning of parsley suspension cells by preincubation with DCIA and salicylic acid derivatives (Figs. 3–6, Table II) is reminiscent of the developmental nature of systemically acquired resistance observed with DCIA and salicylic acid in whole plants (Ward et al., 1991; Malamy and Klessig, 1992; Raskin, 1992). The observation that the conditioning with DCIA or salicylic acid results from an increase in the transcription of specific genes (Kauss et al., 1992b) has now been shown for 5-chlorosalicylic acid (Fig. 6). These results suggest that such conditioning improves unknown components of the system responsible for the reception and/or transduction of the signal “elicitor.” The parsley cells obviously become more sensitive to the elicitor and more effective in responding to elicitor treatment with phenylpropanoid responses that are considered to play a role in defense. It is tempting to speculate that at the level of attacked plant tissues, a similar improvement in the warning system of the cells would result in a timely and, therefore, more successful recognition of pathogens. This suggestion is consistent with the observation that pathogen-induced deposition of cell wall phenolics in cucumber plants correlates with systemically acquired resistance (Hammerschmidt and Kuc, 1982).

Chlorinated derivatives of isonicotinic and salicylic acid are more potent for the conditioning of parsley cells than the naturally occurring parent substance (Table II). The reason for this observation remains unclear. The chlorinated salicylic acids can also induce resistance against various pathogens of tobacco, tomato, and barley (Buchenauer and Fleischmann, 1992). It appears possible that these chlorinated substances are more permeable or more resistant to degradation by cellular enzymes. The position of chlorine in the salicylic acid derivative appears not to be crucial (Table II). That, nevertheless, a certain specificity is involved can be concluded from the finding that 2,6-dihydroxybenzoic acid exhibits activity similar to salicylic acid, whereas some respective isomers show essentially no effect (Table II). It appears of interest in this context that 2,6-dihydroxybenzoic acid is also active as a calorigene in an Arum lily, whereas respective isomers are inactive (Raskin et al., 1989). Because the results of Rasmussen et al. (1991) suggest that salicylic acid might not be the primary systemic signal, our observations raise the question whether other naturally occurring signaling substances are imitated by the substances active in conditioning of parsley cells (Table II). In this context the isolation of n-chloro-dihydroferulic acid from healthy camation stems and its increase upon fungal infection (Niemann et al., 1991) are clearly of interest. In addition, the natural plant signaling substance methyljasmonate (Staswick, 1992) has recently been used to condition parsley suspension cells for enhanced elicited coumarin secretion and incorporation of phenolics into cell walls (Kauss et al., 1992a). It now remains to be established whether this substance can also induce resistance in plant tissues.

ACKNOWLEDGMENTS

The authors thank J. Wörth (Institut für Organische Chemie und Biochemie, Universität Freiburg) and K. Himmelspach (Max-Planck-Institut für Immunobiologie, Freiburg-Zähringen) for MS and NMR spectra.

Received October 28, 1992; accepted February 26, 1993.

Copyright Clearance Center: 0032-0889/93/102/0459/08.

LITERATURE CITED


