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Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid

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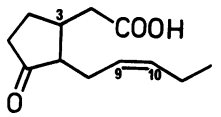
ABSTRACT **Fungal cell walls and fragments thereof (elicitors) induce the formation of low and high molecular weight defense compounds in plant cell suspension cultures. This induced synthesis requires a signal molecule transmitting the message between the elicitor plant cell wall receptor and gene activation. We demonstrate in this study that *cis*-jasmonic acid is rapidly synthesized in plant cell cultures of diverse taxonomic origin (gymnosperms and mono- and dicotyledonous plants) after challenge with a fungal elicitor preparation. The rapid decline of *cis*-jasmonic acid in some of these tissues is attributed to rapid metabolism of this pentacyclic acid. The induction of alkaloids by several different molecules provoking the elicitation process is strictly correlated with the synthesis of jasmonates. Elicitation leads to a rapid release of α-linolenic acid from the lipid pool of the plant cell. α-Linolenic acid and 12-oxophytodienoic acid, the formation of which is also induced, are known to be distant precursors of jasmonic acid. We assume *cis*-jasmonic acid and its precursors to be the signaling molecules in the elicitation process.**

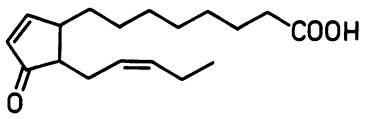
Evidence is accumulating that jasmonic acid (JA) and its derivatives are an integral part of a general signal transduction system (1) that must be present between the elicitor–receptor complex (2) and the gene activation process responsible for the induction of enzyme synthesis (3) that leads to the formation of low molecular weight defense compounds (phytoalexins). Jasmonates have been shown to induce the synthesis of proteinase inhibitor proteins in response to wounding and pathogen attack (1, 4) and to trigger the formation of phytoalexins such as flavonoids, alkaloids, terpenoids (5) and their biosynthetic enzymes (5, 6), and thionins, polypeptides with antifungal activity, in barley (7). More general effects of jasmonates on plant systems, such as the induction of senescence, tendril coiling, and tuber formation, have been summarized recently (8).

Cell suspension cultures are a convenient model to study the effect of elicitors (e.g., microbial cell walls or fragments thereof) on secondary plant metabolism. It has recently been shown that suspension cultures of *Rauvolfia canescens* rapidly synthesized JA after treatment with fungal cell wall fragments (5). Indeed, even exogenously applied jasmonate, in the absence of elicitor and in all suspension cultures tested (36 species), induced the synthesis of specific low molecular weight compounds (5). Jasmonate, therefore, mimics the action of an elicitor in all cell culture systems so far investigated (5). Furthermore, jasmonate and its precursor, 12-oxophytodienoic acid (PDA), induced transcriptional activation of genes involved in secondary metabolite formation (6). These compounds bearing a cyclopentanone ring system are most likely the sought-after signal transducers of the elicitation process.

In this communication, we report that cell cultures of a wide taxonomic distribution (gymnosperms and mono- and dicotyledons) all respond to elicitor treatment with a rapid increase of *cis*-JA, which has the cis conformation of the side chains at C-3 and C-7. In one case (*Rauvolfia serpentina*), the *cis*-JA precursor PDA was induced as well. The *de novo*-synthesized jasmonate was determined by a sample preparation procedure and gas chromatography/mass spectrometry (GC/MS) to be (9Z)-*cis*-JA and evidence was presented that this JA species racemizes to its trans epimer subsequent to its formation in the tissue. We also report that *Eschscholtzia californica* cell suspension cultures, when allowed to metabolize exogenously supplied [1-^{14}C]linolenic acid, rapidly release free α-linolenic acid from their lipid pool upon elicitation. The release of this fatty acid could feed the jasmonate cascade and thus give rise to *cis*-JA. *cis*-JA and/or its precursor, PDA, are most likely second messengers in the signal chain leading from the elicitor–receptor complex (2) to the formation of low and high molecular weight defense compounds (1, 4, 5).



cis-JA



PDA

MATERIALS AND METHODS

Plant Cell Cultures. Suspension cultures were obtained from the departmental culture collection. Cells of *Agrostis tenuis*, *E. californica*, *Phaseolus vulgaris*, and *R. serpentina* were cultivated in Linsmaier and Skoog medium (9). Cultures of *Taxus baccata* were grown in the medium described in ref. 10 modified with 0.2% NZ-amine, 10 μM 2,4-dichlorophenoxyacetic acid, 3 μM indole acetic acid, 3 μM naphthaleneacetic acid, and 1 μM kinetin on a gyratory shaker (100 rpm) at 24°C in continuous light (650 lux) for 7 days. Cells were harvested under sterile conditions by suction filtration, 150 g (fresh weight) was suspended in 800 ml of fresh medium in 1-liter Fernbach flasks, and growth was continued for 3 days. For jasmonate analysis, cells were elicited with yeast elicitor (5) at 250 μg/ml of cell suspension (*E. californica*; 50 μg/ml), and 60 ml of suspension was removed at the times indicated. Control cells were treated in an identical manner except that H_2O was added instead of elicitor. *Lactobacillus* elicitor is a 36-kDa glycoprotein isolated from *Lactobacillus casei* (11). Chemically synthesized heptaglucan elicitor (12) was a kind

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Abbreviations: PDA, 12-oxophytodienoic acid; PFB, pentafluorobenzyl; GC/MS, gas chromatography/mass spectrometry; JA, jasmonic acid.

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gift from Bayer, Leverkusen, Germany. Bacitracin was purchased from Serva. Vanadyl sulfate was from Merck. Actinomycin D and all other elicitors were from Sigma.

Jasmonate Determinations. Suspension cells [60 ml; 0.6 g (dry weight)] grown as described above were removed under sterile conditions, rapidly filtered, and shock-frozen in liquid N_2. To the frozen cells were added 100 ng of 9,10-dihydro-JA (5) (as internal standard, found not to occur in cell cultures tested), 10 ml of saturated NaCl solution, 0.5 ml of 1 M citric acid, and 25 ml of diethyl ether containing 0.005% butylated hydroxytoluene as antioxidant. The mixture was homogenized for 3 min with a high-performance disperser (Ultra-Turrax T 25 at 24,000 rpm, IKA-Werk, Staufen, Germany). After centrifugation (10 min at 2000 × *g*), the ether phase was removed and the aqueous layer was extracted a second time with 25 ml of ether/0.005% butylated hydroxytoluene. The combined ether extracts were applied to an aminopropyl solid-phase extraction glass column (Macherey & Nagel; 500 mg in 3 ml). The column was washed with 5 ml of chloroform/isopropanol, 2:1 (vol/vol), and material was eluted with 7 ml of ether/acetic acid, 98:2 (vol/vol). The sample was taken to dryness at 50°C under a stream of nitrogen. The residue was dissolved in 200 μl of chloroform/diisopropylethylamine, 1:1 (vol/vol), and derivatized 60 min at 50°C with 10 μl of pentafluorobenzyl (PFB) bromide. The derivatization mixture was then dried under a stream of nitrogen, and the residue was taken up in 5 ml of *n*-hexane and added onto a silica solid-phase extraction column (Macherey & Nagel; 500 mg in 3 ml). Material was eluted with 7 ml of *n*-hexane/ether, 2:1 (vol/vol). The sample was concentrated under a stream of nitrogen to 50 μl, and 1 μl was analyzed by GC/MS [Varian 3400 gas chromatograph linked to a Finnigan MAT quadrupole SSQ 700 mass spectrometer; J & W Scientific DB-1 column (15 m × 0.25 mm); linear He flow at 23 cm/s; column temperature step gradient, 100°C for 0.5 min, 100–180°C at 20°C/min, 180–300°C at 30°C/min, and 300°C for 5 min; chemical ionization using NH_3; electron potential, 70 eV]. Retention times of the PFB esters were as follows: (9*E*)-*trans*-JA, 7.30 min; (9*Z*)-*trans*-JA, 7.37 min; *trans*-9,10-dihydro-JA, 7.41 min; (9*Z*)-*cis*-JA, 7.52 min; *cis*-9,10-dihydro-JA, 7.55 min; α-linolenic acid, 11.12 min; PDA isomers, 12.37 min, 12.44 min, and 13.18 min. For routine quantitative analysis, negative fragment ions corresponding to $[M\text{-}PFB]^-$ (JA isomers, m/z = 209; 9,10-dihydro-JA isomers m/z = 211, linolenic acid m/z = 277, and PDA isomers m/z = 291) were measured in the selected ion monitoring mode. Because of partial isomerization during the work-up procedure, the peaks of isomeric compounds were usually integrated together. For further proof of the identity of PFB-jasmonate, additional GC/MS runs were performed in the (less sensitive) electron impact mode and the fragmentation pattern was compared with that of authentic standard. The mass spectrum of *cis*-PFB-JA, isolated after an elicitation experiment (from *A. tenuis*) showed the same fragment ion intensities as isolated *trans*-PFB-JA and was identical to a synthetic control derivative: (electron impact, 70 eV) m/z 390 (50) (M^+), 322 (14), 209 (9), 191 (23), 181 (100), 151 (22), 141 (55), 131 (13), 107 (14), 95 (29), 83 (26), 67 (16), 55 (23).

The PDA increase was measured using 9,10-dihydro-JA as internal standard because of the present lack of isotopically labeled PDA. Since PDA is a relatively unstable molecule in comparison to dihydro-JA, the determined concentrations of PDA represent minimal values.

Methyl jasmonate [racemic mixture containing 90.6% (9*Z*)-*trans*-methyl jasmonate, 9.2% (9*Z*)-*cis*-methyl jasmonate, and 0.2% (9*E*)-*trans*-methyl jasmonate as determined by GC] was obtained when previously available from Serva. JA was prepared by alkaline hydrolysis of the methyl ester. 9,10-Dihydro-JA was synthesized from JA by catalytic hydrogenation with Pd/charcoal (5).

Alkaloids were determined as described (5).

Linolenic Acid Metabolism. Seven-day-old cells of *E. californica* were harvested under sterile conditions by filtration and 45 g (fresh weight) was suspended in 250 ml of fresh Linsmaier and Skoog medium (9). After 3 days of cultivation (24°C; continuous light, 650 lux; gyratory shaker, 100 rpm), 1-ml aliquots of the suspension culture were transferred under sterile conditions into wells of a Nunc multiculture dish (24 wells) and each was fed with 0.1 μCi of [1-^{14}C]linolenic acid (53.9 mCi/mmol; 1 Ci = 37 GBq; Amersham). After 14 hr of incubation on a reciprocal shaker at 140 strokes per min, cells were elicited with 50 μg of yeast elicitor per ml of cell suspension (control cells were treated in an identical manner except that the same amount of water was added instead of elicitor). At the indicated time intervals, the contents of each well were transferred into Eppendorf vials and centrifuged (3 min in an Eppendorf centrifuge), and the pellets were washed with 1 ml of medium. After a second centrifugation, 1-ml aliquots of chloroform/methanol, 1:2 (vol/vol), were added to the pellets, which were then extracted at room temperature for 15 min as described (13). After addition of 0.4 ml of 0.1 M KCl, the mixtures were centrifuged again, and aliquots of the resulting chloroform phase (40,000 cpm) were subjected to TLC [Polygram silica gel plates, Macherey & Nagel; solvent system, *n*-hexane/diethyl ether/acetic acid, 80:20:1 (vol/vol) (14, 15); linolenic acid R_f = 0.42]. Radioactivity was quantitated with a Tracemaster 20 automatic TLC-linear analyzer (Berthold, Wildbad, Germany).

Identification of Radioactive Linolenic Acid. *Linolenic acid p-bromophenacyl ester.* The TLC spot migrating with the same R_f as linolenic acid in the solvent system *n*-hexane/diethyl ether/acetic acid, 80:20:1 (vol/vol), was eluted with chloroform. The sample was dried under a stream of nitrogen and derivatized with a solution of 2 mg of *p*-bromophenacyl bromide in 100 μl of chloroform/diisopropylethylamine, 9:1 (vol/vol), for 2 hr at 50°C. Unlabeled linolenic acid *p*-bromophenacyl ester (300 μg) was added to the sample and chromatographed in dichloromethane/*n*-hexane, 1:1 (vol/vol). The *p*-bromophenacyl derivative (R_f, 0.14) was well separated from excess *p*-bromophenacyl bromide (R_f, 0.34) and eluted. Specific activity was 57,684 cpm/μmol, as determined by scintillation counting of an aliquot and UV absorbance at 255 nm, ε = 16151. The derivative was rechromatographed in hexane/ether/diisopropylamine, 80:20:1 (vol/vol), and eluted from the plate (R_f, 0.48); specific activity was 57,284 cpm/μmol. After a third chromatography in *n*-hexane/ethyl acetate/acetic acid, 70:30:1 (vol/vol) (R_f, 0.91), the specific activity, 57,411 cpm/μmol, still remained constant. The chemical ionization MS (isobutane) gave m/z 475, 477 $(M+H)^+$.

Stearic acid phenacyl ester. Labeled linolenic acid *p*-bromophenacyl ester from the experiment described above was diluted with 150 mg of unlabeled compound and catalytically hydrogenated with Pd/Al_2O_3 in 20 ml of chloroform/methanol/diisopropylethylamine, 10:10:0.5 (vol/vol), for 1 hr at room temperature. The sample was dried, dissolved in ether, and passed through a silica solid-phase extraction column to remove diisopropylethylamine hydrobromide. The etheral solution was dried and the residue was crystallized from methanol to give 37 mg of stearic acid phenacyl ester—m.p. 64–66°C [literature value, 69°C (16)]; specific activity, 688 cpm/μmol upon cooling. Crystallization was repeated two times yielding a constant specific activity of 696, e.g., 691 cpm/μmol. The chemical ionization MS (isobutane) gave m/z 403 $(M+H)^+$.

RESULTS AND DISCUSSION

If the jasmonates or compounds of the jasmonate cascade (6, 17) are really the long-postulated signal compounds (18)

mediating the response between the elicitor–receptor complex (2) and gene activation (5, 6), then taxonomically diverse and nonrelated plant species should upon elicitation respond with an increased synthesis of JA, as has recently been documented for only one species, *R. canescens* (5). Cell cultures of each of the taxonomic subdivisions Gymnospermae (*Taxus*), monocotyledons (*Agrostis*), and dicotyledons (*Eschscholtzia* and *Phaseolus*) were challenged separately with an aqueous cell wall preparation of yeast. In all cultures tested, the JA concentration increased within 30 min after exposure to the elicitor (Fig. 1). Depending on the plant species, the maximum concentration of the free acid was reached after 90–240 min. The absolute amount of JA was strictly dependent on the species and varied between ≈1000 ng/g (dry weight) (*Agrostis*) and only 40 ng/g (dry weight) (*Taxus*). Careful analysis demonstrated that the yeast cell wall elicitor did not contain jasmonate or its precursors in free or bound form. Moreover, it was observed that the jasmonate concentration in the nonelicited control tissues varied between 3 ng/g (dry weight) (*Eschscholtzia*) and 10 ng/g (dry weight) (*Agrostis*) depending on the tissue, but in each case the individual endogenous jasmonate levels were almost constant during the entire experiment. GC/MS analysis of the peak fraction (90 min) of elicited *A. tenuis* cells revealed the correct mass spectrum for the PFB derivative of JA in the electron impact and in the negative and positive chemical ionization mode. However, analysis of jasmonate from unelicited *Agrostis* cells showed two peaks corresponding to the *trans*-PFB-JA and *cis*-PFB-JA epimer in a ratio of 9:1 in the mass chromatogram. This fact indicates an epimerization at thermodynamic equilibrium to the above ratio during extraction of the plant material, presuming an enantiomerically pure biosynthesis of the compound. After elicitation, a concentration increase of both epimers was observed, but now leading to a trans/cis ratio of ≈1:1 (an observation that is rather typical for all cell cultures so far analyzed and is not restricted to *A. tenuis* cell cultures). To minimize epimerization during the work-up procedure, the derivatization method was changed to avoid alkaline conditions. Sample preparation was conducted exactly as described above, except that the sample was methylated with excess etheral diazomethane for 10 min (5) and all steps were performed at room temperature. GC/MS analysis of endogenous JA as the methyl ester in *A. tenuis* cells, 80 min after addition of yeast elicitor, showed a 1:3 ratio of trans/cis epimers, in contrast to unelicited cells where the ratio was again 9:1. Electron impact mass spectra of the methyl ester of endogenously synthesized *cis*-JA was identical with that of authentic standard (5). These results demonstrated that jasmonate synthesized under the influence of elicitor appears to be the cis epimer (9*Z*)-*cis*-JA, although partial epimerization takes place during sample preparation. Interestingly, in all unelicited cells so far analyzed, endogenous jasmonate is an epimeric mixture near thermodynamic equilibrium, indicating that epimerization has already occurred in the plant. In elicited cells, *cis*-JA seems to be biosynthesized *de novo* and not to be released from endogenous storage molecules in which conjugated JA most likely would be at the epimeric equilibrium.

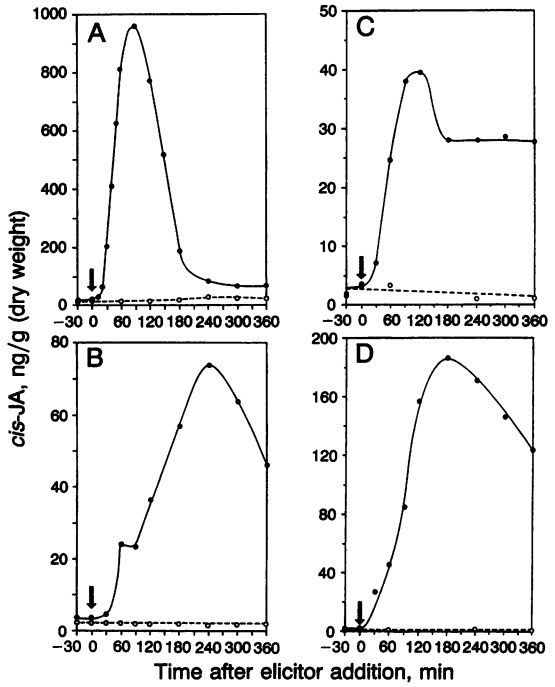

FIG. 1. Induction of JA by addition of yeast cell wall elicitor (250 μg/ml; e.g., 50 μg/ml for *E. californica*) to different cell suspension cultures: *A. tenuis* (*A*), *E. californica* (*B*), *T. baccata* (*C*), *P. vulgaris* (*D*). Arrow indicates the point of elicitor addition. ●, Elicitor-treated cultures; ○, untreated cultures.

After having established the correct relative diasteriomeric form of JA synthesized in response to the elicitation process, the influence of the yeast cell wall elicitor concentration on jasmonate synthesis was determined. *E. californica* cell suspension cultures were challenged with the cell wall elicitor at 1–1000 μg/ml. JA content was analyzed 120 min (see Fig. 1*B*) after onset of elicitation. As shown in Fig. 2, the synthesis of JA was clearly dependent on the elicitor concentration. Elicitation did not cause an all or nothing formation of JA but rather demonstrated saturation kinetics. JA accumulated in the cells in a dose-dependent manner and was clearly saturated with respect to elicitor concentration. It should be noted that even the addition of >1 μg of elicitor per ml of cell suspension results in a reliable stimulation of JA formation.

R. canescens cell cultures elicited with methyl jasmonate have shown (5) the strongest induction of metabolite (raucaffricine) formation among all cell cultures tested. Now *R. serpentina* cells were, in addition, challenged with yeast elicitor and the JA level was monitored for 12 hr (Fig. 3). As expected, the JA content of the control cells was constantly low [8 ng/g (dry weight)] over that time period and in accord with other species (Fig. 1). Elicitation with the yeast cell wall preparation leads to a rapid induction of *cis*-JA with a maximum within 30 min after the beginning of the experiment. The *cis*-JA content declined during the next 90 min but, with slight fluctuations, kept a relatively high (70% of maximum) level for the next 10 hr. GC/MS analysis revealed a

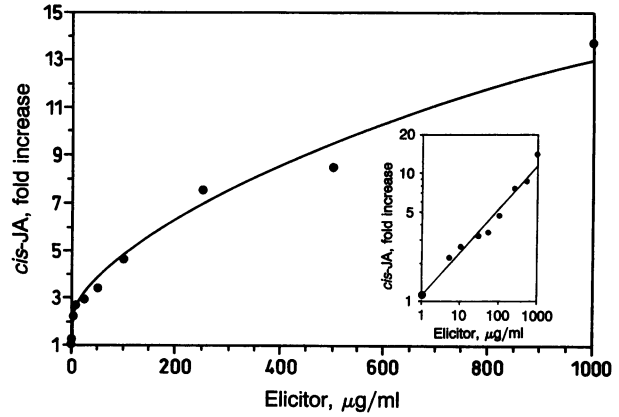

FIG. 2. Increase of JA 2 hr after addition of yeast cell wall elicitor to *E. californica* cell suspension cultures. The culture in the absence of elicitor yields JA at 3 ng/g (dry weight).

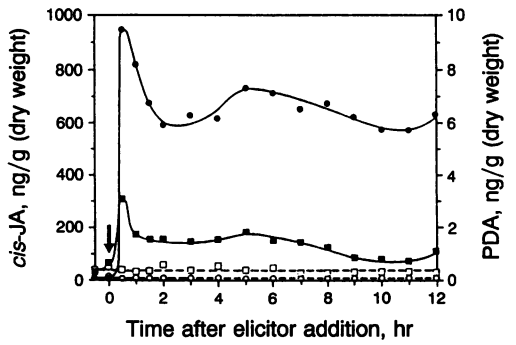

FIG. 3. Induction of endogenous pentacyclic compounds by addition of yeast elicitor (250 μg/ml) to cell suspension cultures of *R. serpentina*. Changes in the abundance of JA (●, ○) and PDA (■, □) in elicited (●, ■) and nonelicited (○, □) cells are shown. Arrow indicates the point of elicitor addition.

second compound that exactly mimicked the time course of JA but was 82 mass units higher than the JA fragment ion. MS analysis showed the characteristic ions at m/z 291 $(M\text{-}PFB)^-$ of the PFB-derivatized PDA (negative chemical ionization, NH_3) and at m/z 307 $(M+H)^+$ of the diazomethane-derivatized PDA (positive chemical ionization, isobutane), corresponding exactly to the retention times of enzymically synthesized (19) isomeric PDA derivatives. The exact match of the *cis*-JA and PDA formation kinetics, however, suggested that PDA is the precursor of *cis*-JA, as was postulated (17), the PDA molecule being first reduced and then exposed to three subsequent β-oxidation cycles thus yielding *cis*-JA. The analytical method to determine the onset of PDA formation after elicitation is not yet sensitive enough to decide whether PDA is synthesized prior to *cis*-JA as postulated (17).

We reported previously that not only cell wall preparations of *Penicillium* but also the cyclopeptide antibiotics bacitracin, colistin, and polymyxin B stimulated benzo[*c*]phenanthridine alkaloid synthesis in *E. californica* cell suspensions to exactly the same extent as the yeast elicitor (20). In contrast, none of the classical elicitors (21) tested, such as heptaglucan, vanadyl sulfate, actinomycin, c-AMP, or phosphomycin, stimulated alkaloid production (20). These results were now used to investigate whether compounds that lead to alkaloid synthesis in *E. californica* cell cultures also induce JA synthesis whereas compounds that do not are inactive. Table 1 shows that all compounds with a strong elicitation potential on alkaloid biosynthesis invariably also provoke the synthesis of jasmonate (maximal *cis*-JA concentrations were determined 4 hr after elicitation) prior to the onset of alkaloid formation. In contrast, compounds without effect on alkaloid synthesis did not induce jasmonate formation. Elicitation with yeast and *Lactobacillus* cell wall preparations leads to considerably different magnitudes of jasmonate and alkaloid induction. The difference cannot yet be explained. Nevertheless, one can conclude from Table 1 that the elicitation phenomenon correlates with jasmonate induction.

As postulated for the wounding response in plants (1, 4, 22), tendril coiling (23), and the elicitation phenomenon (5), jasmonate is most likely formed from α-linolenic acid (17) that is possibly derived from plasma membranes. It has been observed (5) that elicitation increased not only the JA but also the linolenic acid content in *E. californica* cell cultures. To quantitate this response, α-[*carboxyl*-^{14}C]linolenic acid was applied to these cells. The position of the label guaranteed that only intact linolenic acid and immediate derivatives, but not jasmonate, etc., would be quantitated. β-Oxidation of linolenic acid or 3-oxo-2-(2′-pentenyl)cyclopentaneoctanoic acid would yield carboxyl-labeled acetyl-CoA, which would

Table 1. Elicitation of JA and benzophenanthridine alkaloid accumulation by potential biotic and abiotic elicitors in cell cultures of *E. californica*

Compound	Concentration, mg/ml	Fold increase over control (water): Jasmonate after 4 hr	Fold increase over control (water): Alkaloid after 24 hr
Active elicitor			
Yeast	0.05	14.0	8.5
Lactobacillus	0.1	30.0	4.5
Polymyxin B	0.04	5.5	3.0
Bacitracin	2	4.5	4.5
Colistin	0.1	3.0	3.0
Inactive compound			
Actinomycin D	0.1	1.0	1.0
Dibutyryl-cAMP	0.1	1.0	1.0
Phosphomycin	0.1	0.5	0.5
Vanadyl sulfate	0.025	0.5	1.5
Heptaglucan	0.1	0.9	1.1

Average control values: JA, 1.9 ng/g (dry weight) of cells; total alkaloid, 700 μg/g (dry weight) of cells.

be further metabolized and disappear into the background noise. The α-[1-^{14}C]linolenic acid applied to *E. californica* cells was almost quantitatively incorporated into lipids within 14 hr. Only trace amounts of free linolenic acid were detectable after TLC analysis. Addition of elicitor at 50 μg/ml of suspension to the labeled cells and further incubation for 6 hr resulted in a significant increase (up to 15%) of the total label in a compound chromatographically indistinguishable from free α-linolenic acid (Fig. 4*B*), which could not be observed after addition of water in control experiments (Fig. 4*A*). This ^{14}C-labeled compound was isolated, converted to the *p*-bromophenacyl ester, diluted with unlabeled ester, and rechromatographed twice in different solvent systems. The specific activity was constant already after the first step. The labeled derivative was catalytically hydrogenated to stearylphenylacyl ester and, after two recrystallizations, again did not change in specific activity. Therefore, the compound liber-

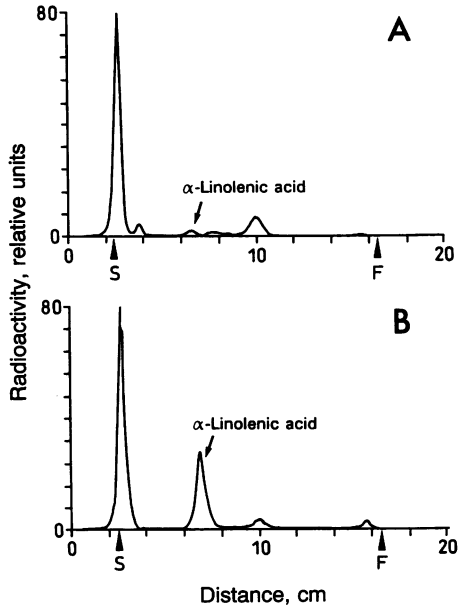

FIG. 4. Radio-TLC of total lipid extracts of *E. californica* cell suspension cultures. Cells were preincubated for 14 hr with α-[1-^{14}C]linolenic acid and treated with water (*A*) or yeast elicitor at 50 μg/ml (*B*) for an additional time period of 6 hr prior to extraction. S, start; F, front.

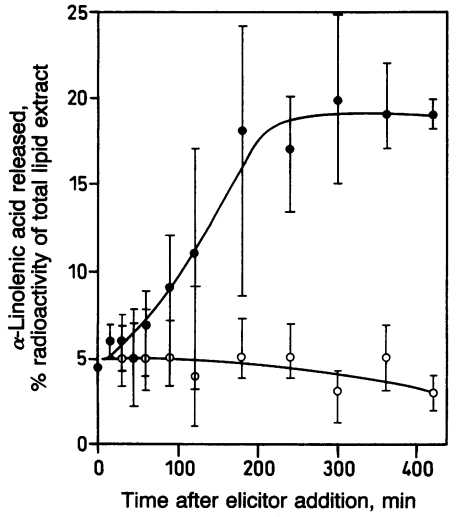

FIG. 5. Time course of free α-linolenic acid accumulation in yeast elicitor-treated (50 μg/ml) cell suspension cultures of *E. californica*. ●, Yeast elicitor-induced cultures; ○, untreated cultures.

ated from the labeled lipids under the influence of elicitor is unequivocally α-linolenic acid. We then investigated the time course of this α-linolenic acid liberation after elicitation. An average of four experiments using *E. californica* cell suspensions consisting of duplicate determinations throughout is depicted in Fig. 5. Free linolenic acid was detectable after a lag phase of 30 min only in the elicitor-treated *E. californica* cells. Maximal release of labeled linolenic acid was observed 130–180 min after elicitation. This level remained constant for the rest of the experiment. Most likely, only a part of the free linolenic acid pool is channeled into the jasmonate cascade, which yields maximal amounts of JA 4 hr after elicitation. Since exogenously supplied [^{14}C]linolenic acid was rapidly metabolized into different lipid classes (Fig. 4*A*) and the labeled linolenic acid pool formed under the influence of elicitor was constant for a considerable time, it is likely that this pool is situated in a compartment not accessible to further metabolism.

Elicitation of plant cell cultures with fungal elicitors led after 10–20 min to a rapid and intense synthesis of *cis*-JA in a variety of species belonging to both the gymno- and angiosperms (Fig. 1). Application of different amounts of elicitor induced a proportional increase in *cis*-JA synthesis that followed first-order kinetics. The JA precursor PDA showed the same induction kinetics as jasmonate (Fig. 3). Both JA and PDA have been shown (5, 6) to induce the synthesis of low molecular weight defense compounds. Simultaneously, the poly(A)$^+$ RNAs of several genes involved in secondary compound synthesis as well as the corresponding enzymes have been shown to accumulate (refs. 5 and 6, and T. M. Kutchan, personal communication). Evidence is, therefore, accumulating that indeed *cis*-JA and/or its precursor PDA, itself or possibly after degradation to jasmonate, are second messengers in the signal chain leading from the elicitor–receptor complex (2) to the formation of secondary compounds with ecochemical function. The compounds of the jasmonate cascade are formed from α-linolenic acid, as was convincingly shown (17). In analogy to the prostaglandin cascade in mammals, linolenic acid has been postulated to participate in a lipid-based signaling system that activates proteinase inhibitor synthesis in response to insect and pathogen attack (4). We have demonstrated here that elicitation of plant cell cultures led to release of α-linolenic acid (Figs. 4 and 5), which in turn could serve as precursor to (9*Z*)-*cis*-JA. Proof now emerges for the postulate that the members of the pentacyclic jasmonate family transmit the elicitor signal intracellularly via a lipid-based signaling system to induce low and high molecular weight defense compounds. These results support Ryan's insight of 20 years ago (24): "This response holds promise for designing new approaches to biological pest control."

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