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METHYL JASMONATE CONDITIONS PARSLEY SUSPENSION CELLS FOR INCREASED ELICITATION OF PHENYLPROPANOID DEFENSE RESPONSES

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SUMMARY: Pre-incubation of suspension-cultured parsley cells with methyl jasmonate greatly enhances their ability to respond to fungal elicitors by secretion of coumarin derivatives. The effect is most pronounced at relatively low elicitor concentration and also observed for the incorporation of esterified hydroxycinnamic acids and of "lignin-like" polymers into the cell wall. These three responses correspond to defense reactions induced locally when a fungal pathogen attacks plant cells. In contrast, the conditioning of parsley cells by the signal substance methyl jasmonate is reminiscent of the developmental nature of systemic acquired resistance and renders the cells more effective for the elicitor-induced local defense reactions.

Suspension-cultured parsley cells are a valuable model for the elucidation of pathogen defense reactions that occur in response to a local infection of plant cells by a fungal pathogen (1). The crude elicitor preparation from fungal cell wall contains a glycoprotein which induces transcription of various genes encoding enzymes required for the synthesis of soluble secreted coumarin derivatives ("phytoalexins") and of phenolic compounds covalently linked to plant cell wall constituents (1).

Another defense strategy of plants is the "systemic acquired resistance" that is caused by an initial infection by a pathogenic microorganism and leads within a few days to the development of resistance in adjacent tissues or plant organs (for citations see refs. 2-5). In this case, an unknown signal derived from the first local infection spreads systemically over the plant and induces, in a developmental process, the synthesis of "pathogenesis related proteins". The function of many of these proteins remains unknown but some have been identified as enzymes of obvious use in the direct defense of invading fungal pathogens, e.g. chitinase, 1,3-ß-glucanase or peroxidase.

It is known that 2,6-dichloroisonicotinic acid, a synthetic non-toxic plant protection agent, can short-cut the systemic acquired resistance induction process (3). This substance was recently shown to increase the sensitivity of parsley suspension cells towards fungal elicitors (5). Similar but less pronounced effects were found for salicylic acid (5) which plays a role in the development of systemic acquired resistance (4) but is thought not to be the primary systemic signal in cucumber (2). The plant lipid derivative methyl jasmonate resembles mammalian eicosanoids in structure and biosynthesis and has been implicated as an

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endogenous signalling substance in various plant responses (for citations see refs. 6-8). In this report we investigate a new aspect of methyl jasmonate action which may link the local and the systemic defense strategies in plants.

MATERIALS AND METHODS

Origin, growth and handling of parsley suspension cultures as well as the preparation of elicitor from *Phytophthora megasperma* f. sp. *glycinea* (Pmg) were as described (5). Briefly, cultures were routinely supplied at day 3 after subculture with methyl jasmonate, 24 h later with elicitor and then the responses determined 24 h later. Secreted coumarins were determined fluorometrically, and the values given are scaled by a factor of 10^{-2} (5). Alternatively, coumarin derivatives were extracted with chloroform from 2 ml of the growth medium, solubilized in 2 ml of methanol and the concentration determined photometrically at 320 nm (9).

Cell walls were prepared from 2 g cells (fresh weight) as described (10), hydrolyzed in 1 N NaOH for 24 h and the hydrolyzate acidified. The free hydroxycinnamic acids were partitioned into ethylacetate and separated by HPLC at a flow rate of 1 ml min^{-1} on a Lichrospher 100 RP-18 (5 μm) column (Merck, Darmstadt, Germany) with detection at 275 nm. Solvent A was 5% (v/v) acetic acid and B was 20 % (v/v) acetic acid + 25 % (v/v) acetonitrile. Components were eluted with a linear gradient from 7 % to 55 % solvent B over the first 20 min and from 55 % to 99 % solvent B over the subsequent 10 min. The gradient was held at 99 % solvent B for 5 min. Alternatively, the cell walls from 1 g cells were treated with thioglycolic acid, the resulting derivatives of "lignin-like" polymers precipitated and their concentration photometrically determined at 320 nm in 4 ml of 0.5 N NaOH (11).

Chemically synthesized methyl jasmonate containing several stereoisomers was generously supplied by Firmenich GmbH (Kerpen, Germany). The substance was added in 20 μl ethanol per 20 ml of suspension; the controls received the same final concentration of solvent.

RESULTS

Parsley cell suspension cultures respond to fungal elicitor by the synthesis and secretion of coumarin derivatives (Fig. 1). When the elicitor was given simultaneously with methyl jasmonate, there was some increase in secretion of coumarin derivatives; although, addition of 5 μM methyl jasmonate without elicitor results after an overall incubation time of 48 h in

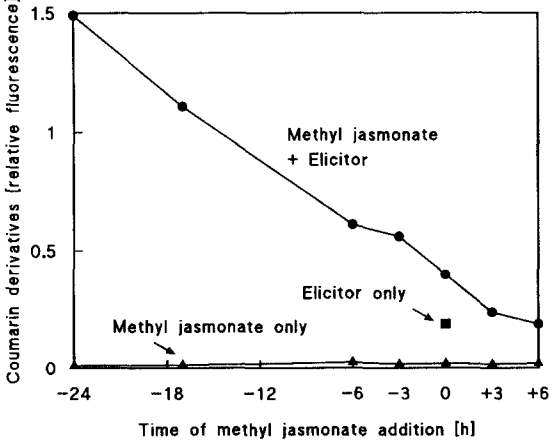

Fig. 1. Cooperation of methyl jasmonate (5 μM) and fungal elicitor (4 μg ml^{-1}) in the secretion of coumarin derivatives. The time of elicitor addition was defined as zero and the coumarin derivatives determined 24 h later.

minimal induction (Fig. 1). The effect of methyl jasmonate on elicited coumarin secretion is, however, dramatically increased when the cultures are pre-incubated with methyl jasmonate (Fig. 1). This indicates that methyl jasmonate conditions the cells for enhanced elicitor response. The pre-conditioning effect is most pronounced at relatively low elicitor concentrations and is nearly saturated at 5 μM methyl jasmonate (Fig. 2A). Cells pre-incubated with methyl jasmonate require an optimal elicitor concentration for secretion of coumarin derivatives (Fig. 2A). In some experiments the pre-incubated cells treated with 20 or 40 μg ml^{-1} elicitor secreted even considerably less coumarins than the control cells (data not shown). Under the conditions employed, the induction of coumarin derivatives by methyl jasmonate without elicitor increased in a dose-dependant manner but was rather low even at 100 μM, compared to the high secretion found on sequential application of methyl jasmonate and elicitor (Fig. 2, compare ordinates of A and B).

Measurement of the secreted mixture of coumarin derivatives by the fluorescence assay monitors mainly blue-fluorescent compounds (5). We have measured, therefore, also the overall A_{320} of a chloroform extract. This method is thought to monitor coumarin derivatives more generally (9) and resulted in similar methyl jasmonate effects (data not shown).

Cells pre-incubated with 5 μM methyl jasmonate and subsequently incubated with elicitor also exhibited an increased incorporation of esterified ferulic and p-coumaric acids and of "lignin-like" polymers into the cell walls (Fig. 3). In this case, the methyl jasmonate effect was also pronounced at a high elicitor concentraton (20 μg ml^{-1}, Fig. 3), in contrast to secreted soluble coumarins (Fig. 2A). On strong stimulation of the phenylpropanoid pathway the hydroxycinnamic acids produced appear to become preferentially esterified to cell wall polymers. This reaction proceeds in endomembrane vesicles presumably derived from the Golgi apparatus of parsley cells, employing the respective CoA-thioesters as substrates and endogenous polysaccharide acceptors (12). The activity of the corresponding transferase is not

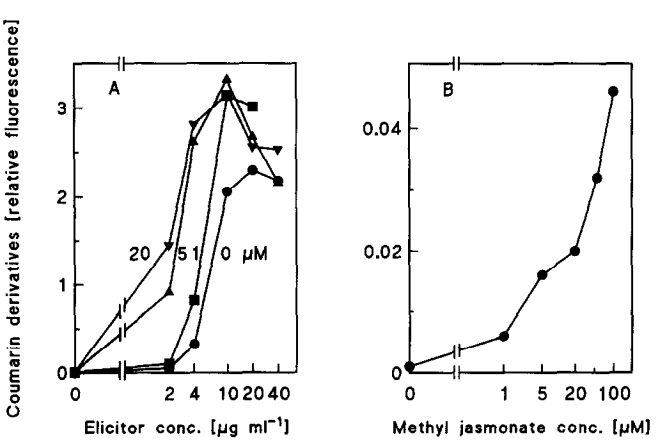

Fig. 2A. Elicitor dose-response of parsley cells pre-incubated with various concentrations of methyl jasmonate. Methyl jasmonate (controls = 0 μM,●; 1 μM,■; 5 μM,▲; 20 μM,▼) was added 24 h before elicitor and coumarin derivatives determined 24 h after elicitor addition.
Fig. 2B. Coumarin derivatives induced in the same cell population by various concentrations of methyl jasmonate without elicitor. Overall incubation time with methyl jasmonate was 48 h. Note the greatly expanded scale of the ordinate.

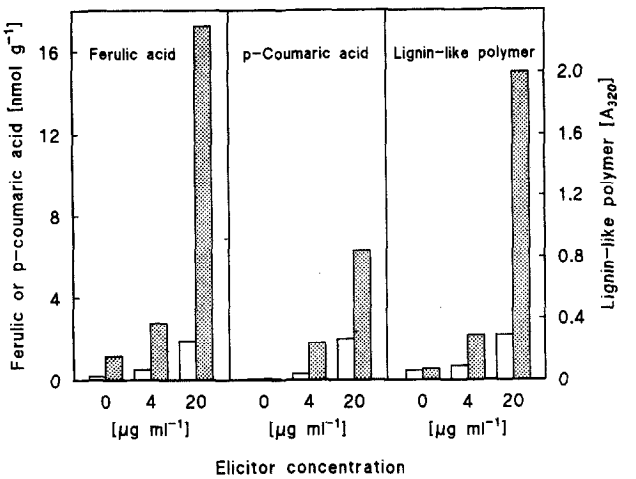

Fig. 3. Influence of pre-incubation with methyl jasmonate on elicitor-induced incorporation esterified ferulic and p-coumaric acids as well as "lignin-like" polymers into the cell walls of parsley cells. Control cells: white columns. Cells pre-incubated for 20 h with 5 μM methyl jasmonate: dotted columns.

considerably increased in elicitor-treated cells (A. Kohler and H. Kauss, unpublished results). This indicates that the hydroxycinnamoyl transferase is constitutive and not rate-limiting for the synthesis of the polysaccharide exporting covalently linked hydroxycinnamic acids.

DISCUSSION

Methyl jasmonate was recently shown to directly induce the intracellular accumulation of a wide spectrum of soluble secondary metabolites in numerous plant suspension cultures (8). Distinct effects were observed in that report at $\geq$100 μM methyl jasmonate. In the present report, the direct induction of coumarin secretion by methyl jasmonate is minimal (Figs. 1 and 2) and the most striking effect requires a pre-incubation period with low methyl jasmonate concentrations followed by treatment with a fungal elicitor (Fig. 1). This is reminiscent of the developmental nature of systemic acquired resistance (2-4) and indicates that the cells were sensitized to the fungal elicitor glycoprotein. At the level of whole plants this would imply, on the one hand, that lower concentrations of fungal elicitors could be perceived by the infected cells and would result in a timely and more effective production of defense substances derived from the phenylpropanoid pathway. Early recognition of fungal pathogens is known from cytological studies to correlate with resistance in e.g. the barley/powdery mildew interaction (13). On the other hand, the induction of methyl jasmonate synthesis by a fungal elicitor in suspension-cultured plant cells (8) might correspond in the whole plant to the generation of the systemic signal during the initial infection that induces the acquired resistance.

The conditioning effect was observed for secreted coumarins (Figs. 1 and 2) as well as for esterified hydroxycinnamic acids and "lignin-like" cell wall polymers (Fig. 3). Thus, the general phenylpropanoid metabolism appears to be affected. This is in accordance with the observation that the effects caused by pre-incubation of parsley cells with 2,6-dichloroisonico-

tinic acid are also manifested at the level of translation with the synthesis of mRNA specific for phenylalanine ammonia lyase and 4-coumaroyl:CoA ligase (5). It remains to be directly shown whether this applies also for methyl jasmonate. The observation that the conditioning renders the parsley cells more sensitive to low concentrations of the fungal elicitor suggests an improved cellular signal perception/transduction system. In parsley cells, early manifestations of elicitor action are an influx of Ca^{2+}, efflux of K^+ and a presumably associated internal acidification (14, 15) as well as changes in the phosphorylation status of proteins (16). Whether these events are also affected by methyl jasmonate conditioning remains to be shown.

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