Signal Transduction

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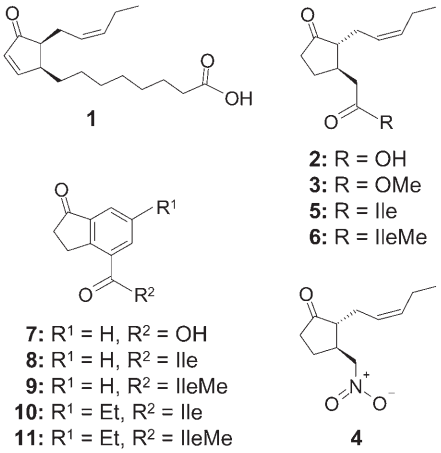
Structural Requirements of Jasmonates and Synthetic Analogues as Inducers of Ca^{2+} Signals in the Nucleus and the Cytosol of Plant Cells**

*Agnes Walter, Christian Mazars, Mathias Maitrejean, Jörn Hopke, Raoul Ranjeva, Wilhelm Boland, and Axel Mithöfer**

Dedicated to Professor Jürgen Ebel

Octadecanoid-derived phytohormones, such as jasmonates and 12-oxophytodienoic acid (OPDA; **1**) as well as their synthetic analogues (Scheme 1), induce various physiological



Scheme 1. Structures of jasmonate-related natural and synthetic compounds.

responses in tissues and cell cultures of different plant species.[1,2] Although jasmonates are known to mediate abiotic and biotic stress including wounding, and pathogen and herbivore attack, there is only limited knowledge about jasmonate-induced signaling pathways that initiate subsequent cellular responses. Circumstantial and indirect evidence suggests that changes in the concentration of free Ca^{2+} in the cytosol might be induced downstream of the jasmonate signal.[3,4] Such data may extend to jasmonate signaling the role of Ca^{2+} as a ubiquitous second messenger that mediates a wide variety of cellular processes in plants.[5,6] Specific Ca^{2+} signatures in the cytosol as well as in the nucleus are described for plant cells after stimulation with pathogen-derived elicitors or osmotic stress.[7,8] In order to investigate calcium responses in both compartments upon treatment with jasmonates and synthetic functional mimics, in the present study transgenic tobacco cells, carrying the Ca^{2+}-sensing protein aequorin either in the cytosol or nucleoplasm, were employed.[9]

We found that two of the naturally occurring compounds, OPDA (**1**), the biosynthetic precursor of jasmonic acid (JA; **2**), and JA itself, induced transient Ca^{2+} signals in a concentration-dependent manner in both the cytosol ($\Delta[Ca^{2+}]_{cyt}$) and the nucleus ($\Delta[Ca^{2+}]_{nuc}$). However, the Ca^{2+} patterns differed sharply in terms of kinetics and response amplitude. OPDA (**1**) induced a rapid $[Ca^{2+}]_{cyt}$ increase up to 1 μM within the first 30 s after application (Figure 1a) followed by a strong $[Ca^{2+}]_{nuc}$ increase (Figure 1b); which was ten times higher than $\Delta[Ca^{2+}]_{cyt}$ and delayed by only 15 s. To our knowledge, such a dramatic increase in $[Ca^{2+}]_{nuc}$ has never been described for plant cells. The concentration of the signal compound necessary for 50% of the induced response (EC_{50}) was determined to be (0.57 ± 0.06) mM for the cytosol and (0.43 ±

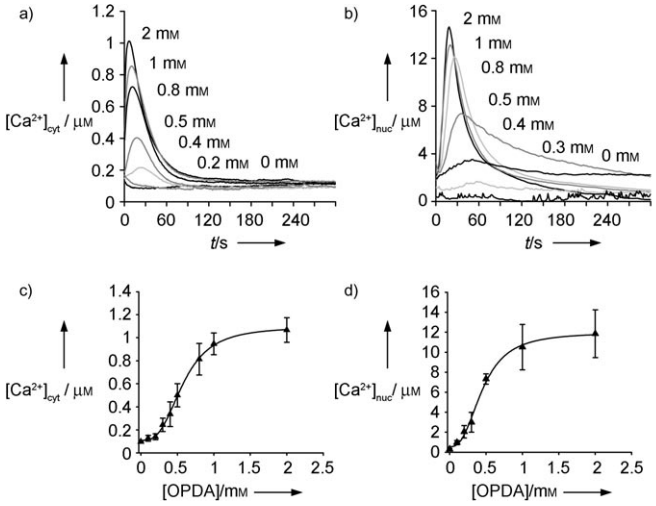


Figure 1. Changes in the Ca^{2+} concentration in the a) cytosol ($\Delta[Ca^{2+}]_{cyt}$) and b) nucleoplasm ($\Delta[Ca^{2+}]_{nuc}$) of tobacco cells induced by different concentrations of OPDA; determination of EC_{50} values for OPDA-induced changes in c) $\Delta[Ca^{2+}]_{cyt}$ and d) $\Delta[Ca^{2+}]_{nuc}$. Traces represent Ca^{2+} responses obtained in three independent experiments.

[*] Dipl.-Biol. A. Walter, Dr. M. Maitrejean, Dr. J. Hopke, Prof. Dr. W. Boland, Priv.-Doz. Dr. A. Mithöfer
Max-Planck-Institut für Chemische Ökologie
Bioorganische Chemie
Hans-Knöll-Strasse 8, 07745 Jena (Germany)
Fax: (+3641) 571-256
E-mail: amithoefer@ice.mpg.de
Dr. C. Mazars, Prof. Dr. R. Ranjeva
Signaux et Messages Cellulaires chez les Végétaux
UMR CNRS/UPS 5546
24 Chemin de Borde Rouge, BP 42617 Auzeville
31326 Castanet-Tolosan (France)

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0.08) mM for the nucleoplasm (Figure 1c,d). JA (**2**) also induced a fast $\Delta[Ca^{2+}]_{cyt}$ within the first minute (Figure 2a) with an $EC_{50} = (1.13 \pm 0.21)$ mM. In contrast, the induced Ca^{2+} signature in the nucleus (Figure 2b) ($EC_{50} = (0.87 \pm$

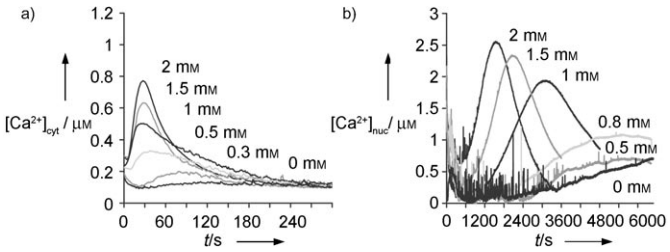

Figure 2. Changes in the Ca^{2+} concentration in the a) cytosol ($\Delta[Ca^{2+}]_{cyt}$) and b) nucleoplasm ($\Delta[Ca^{2+}]_{nuc}$) of tobacco cells induced by different concentrations of JA. Traces represent Ca^{2+} responses obtained in three independent experiments.

0.05) mM) was different from $\Delta[Ca^{2+}]_{cyt}$ because this response started only after about 20 min, and the peak maxima shifted strongly with time depending on the JA (**2**) concentration used. Although the EC_{50} values seem to be quite high, they are in the same range as needed for the induction of volatile emissions in lima bean.[10] Moreover, it is not clear how much of the exogenously applied compounds can enter the cells. Compared to the action of OPDA (**1**), the JA-induced $\Delta[Ca^{2+}]_{nuc}$ did not exceed 2.5 μM and showed also different kinetics. These clear-cut differences in the kinetics of $\Delta[Ca^{2+}]_{nuc}$ strongly suggest the involvement of different signal transduction processes initiated by JA (**2**) and OPDA (**1**). Interestingly, OPDA (**1**) has been described to induce individual cellular responses that only partially overlap with jasmonate-induced responses, also indicating the existence of signaling pathways different from those of JA (**2**).[11]

Next, we addressed the question of whether biologically active jasmonates such as the methyl ester of JA (MeJA; **3**)[1] and the JA–isoleucine conjugate (JA-Ile) (**5**)[12] were able to elicit intracellular $[Ca^{2+}]$ changes. We could not detect any induction of $\Delta[Ca^{2+}]$ upon treatment with MeJA (**3**) up to a concentration of 2 mM, neither in the cytosol nor in the nucleus, which indicates that any hydrolysis of MeJA (**3**) in the cells does not occur fast enough to result in the initiation of Ca^{2+} signals by released JA (**2**). The JA-Ile conjugate (**5**) had no effect on $[Ca^{2+}]_{cyt}$ but was as active as JA (**2**) in the induction of $\Delta[Ca^{2+}]_{nuc}$ (Figure 3b), revealing a modulation of the JA activity by conjugation with an amino acid. The methyl ester of JA-Ile (**6**) was inactive in both compartments, similar to MeJA (**3**).

All these results strongly suggested high specificity of the perception systems transducing the initial jasmonate signals into appropriate Ca^{2+} signatures. This encouraged us to analyze structure–activity relationships in more detail, and we included synthetic 1-oxoindanoyl-L-isoleucine conjugates known to mimic jasmonates.[2] The only compound besides OPDA (**1**) and JA (**2**) that showed Ca^{2+}-inducing activity in both compartments was 1-oxoindane-4-carboxylic acid (**7**) (Figure 3a,b). The 1-oxoindanoyl moiety of the conjugates was previously reported to be a building block lacking

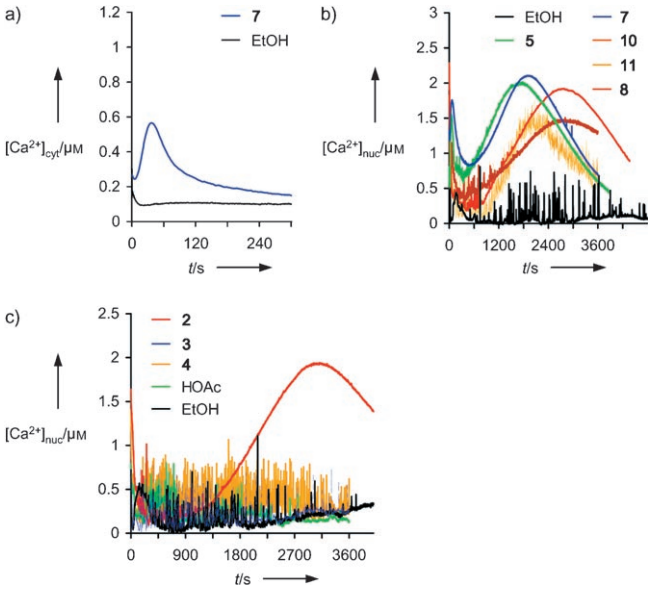

Figure 3. Changes in the Ca^{2+} concentration a) in the cytosol ($\Delta[Ca^{2+}]_{cyt}$) of tobacco cells induced by 1 mM of compound **7**; b) in the nucleoplasm ($\Delta[Ca^{2+}]_{nuc}$) of tobacco cells induced by 1 mM of compounds **5**, **7**, **8**, **10**, **11**; c) in the nucleoplasm ($\Delta[Ca^{2+}]_{nuc}$) of tobacco cells induced by 1 mM of compounds **2**–**4** and acetic acid (HOAc); ethanol (EtOH) was used as solvent control. Traces represent Ca^{2+} responses obtained in three independent experiments.

biological activity;[13] nevertheless in this system its activity resembles that of JA (**2**). Congruent with the results obtained for JA-Ile (**5**), the isoleucine conjugate of **7**, 1-oxoindanoyl-L-isoleucine (**8**), as well as the substituted 6-ethyl-1-oxoindanoyl-L-isoleucine (**10**) proved to be active only in the nucleus (Figure 3b), whereas 1-oxoindanoyl-L-isoleucine methyl ester (**9**) was completely inactive. Taken together, these results disclose two structural qualities of jasmonates that seem to be pivotal for the induction of Ca^{2+} signatures. First, the compounds lost their activity on $[Ca^{2+}]_{cyt}$ by conjugation with isoleucine but retained the activity on $\Delta[Ca^{2+}]_{nuc}$. Up to now, only the activation of JA (**2**) by formation of JA-Ile (**6**) has been reported.[12] We show for the first time a partial loss of JA activity by conjugation with an amino acid. Second, all compounds including the conjugates became completely inactive by esterification of the carboxy group. Interestingly, the methyl ester of **10**, 6-ethyl-1-oxoindanoyl-L-isoleucine methyl ester (**11**), was the only compound observed that was able to induce a $\Delta[Ca^{2+}]_{nuc}$ although no free carboxy group was present in the molecule (Figure 3b). The ethyl group, the substituent at C6, confers the ability to overcome the prevention of the Ca^{2+}-inducing activity caused by esterification, which correlates with the generally enhanced biological activity of **11** compared to that of **9**.[2]

In order to specify the requirement of a free carboxy group in JA (**2**), 3-(nitromethyl)-2-((*Z*)-pent-2-enyl)cyclopentanone (JNO) (**4**) was employed. The NO_2 group of JNO (**4**) nearly the same size and electron distribution as the carboxy group of JA (**2**).[13] As shown in Figure 3c, JNO (**4**) was inactive, thus demonstrating that a carboxylate is absolutely necessary to induce an intracellular Ca^{2+} signature.

However, the presence of an organic acid, such as acetic acid, alone was not sufficient to trigger a Ca^{2+} response in the tobacco cells, indicating the need of more defined structural conditions (Figure 3c).

Recent data had established that calcium specifically induced gene expression to mount appropriate responses to external stimuli.[14] Interestingly, a number of the calcium-responsive genes are also induced by jasmonates, suggesting that jasmonate- and calcium-based signaling pathways may be linked at least partially.[15] Here, we observed that a transient increase in the concentration of the second messenger, Ca^{2+}, can be induced in the cytosol and the nucleoplasm of plant cells by certain jasmonates. With the newly discovered biological activity of 1-oxoindane-4-carboxylic acid (**7**), the synthetic compounds proved to be a perfect match for the jasmonates and revealed perceptible structure–activity relations: Only nonconjugated compounds with a free negatively charged carboxy group such as OPDA (**1**), JA (**2**), and 1-oxoindane-4-carboxylic acid (**7**), were able to induce Ca^{2+} signals in both compartments. Isoleucine-conjugated derivatives of **2** and **7** showed only Ca^{2+}-inducing activities in the nucleus, whereas the methyl esters of the isoleucine conjugates (**6, 9**) without any carboxy function were completely inactive except for 6-ethyl-1-oxoindanoyl-L-isoleucine methyl ester (**11**); thus, this last compound argues against a conceivable explanation that different uptake properties of all methyl esters might be the only reason for their inactivity. Further, it could be demonstrated that autonomous $\Delta[Ca^{2+}]_{nuc}$ may be generated independently of $\Delta[Ca^{2+}]_{cyt}$ in intact cells. Our results suggest the presence of at least two highly specific but different perception mechanisms and signaling pathways that are involved in the initiation of Ca^{2+} signatures in the cytosol and the nucleus of plant cells. The identification of these perception systems and the elucidation of downstream signaling events leading to the different calcium patterns are the next challenges.

Experimental Section

(±)-*cis*-12-Oxophytodienoic acid (**1**) was generously provided by O. Miersch and C. Wasternack, Halle, Germany. (−)-Jasmonic acid (**2**) and (±)-jasmonic acid methyl ester (**3**) were purchased from Sigma-Aldrich. The synthetic compounds were synthesized as previously described: 1-oxoindane-4-carboxylic acid (**7**),[16] 1-oxoindanoyl-L-isoleucine (**8**),[16] 1-oxoindanoyl-L-isoleucine methyl ester (**9**),[16] 6-ethyl-1-oxoindanoyl-L-isoleucine (**10**),[17] 6-ethyl-1-oxoindanoyl-L-isoleucine methyl ester (**11**),[17] jasmonic acid-L-isoleucine (**5**),[16] jasmonic acid-L-isoleucine methyl ester (**6**),[16] 3-(nitromethyl)-2-((*Z*)-pent-2-enyl)cyclopentanone (**4**).[18] Measurements of intracellular Ca^{2+} were performed with transgenic tobacco (*Nicotiana tabacum* L. cv. BY-2) cell lines expressing the Ca^{2+}-sensing protein apoaequorin either in the cytosol or in the nucleus.[9] Final solvent concentrations never exceeded 1% (v/v); each luminescence variation was evaluated with respect to the whole amount of reconstituted aequorin in the sample.[9]

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