Amino acid conjugates of jasmonic acid induce jasmonate-responsive gene expression in barley (*Hordeum vulgare* L.) leaves

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Abstract Leaves of barley (*Hordeum vulgare* L. cv. *Salome*) treated with jasmonic acid (JA), its methyl ester (JM), or its amino acid conjugates exhibit up-regulation of specific genes and down-regulation of house-keeping genes. This transcriptional regulation exhibits several specificities. (i) The (−)-enantiomers are more active, and conjugates are mainly active if they carry an L-amino acid moiety. (ii) The various JA-responsive genes respond differentially to enantiomeric and chiralic forms. (iii) Both JA and its amino acid conjugates exhibiting no or negligible interconversion induce/repress genes.

Key words: Amino acid conjugates of jasmonic acid; *Hordeum vulgare*; Jasmonic acid signalling; Jasmonate-induced protein; Stereospecificity

1. Introduction

Biologically active forms of jasmonates, in particular (−)-jasmonic acid (Fig. 1, (−)-JA, **1**), and its volatile methyl ester (JM) have been identified in all plant species studied so far [1]. Furthermore, (−)-JA (**1**) was shown to occur conjugated with a variety of amino acids in higher plants [2,3]. Several environmental stresses like wounding [4–6], electric current application [7], osmotic stress [8], mechanical stress [9] or local burning [7] lead to an endogenous rise of jasmonates, thereby inducing synthesis of specific proteins like proteinase inhibitors in tomato or vegetative storage proteins in soybean (for review cf. [10]). A similar rise of jasmonates occurs upon elicitation of cell suspension cultures leading to phytoalexin synthesis [11,12]. Interestingly, JA amino acid conjugates were also found to increase in response to environmental stress. In wounded potato leaves they represent up to 15% of all jasmonate compounds [13], and in osmotically stressed barley leaves, (−)-JA (**1**) and JA conjugated with valine, leucine or isoleucine accumulate simultaneously [8,14]. Thus, the question arises on signalling qualities of JA (**1, 2**) and its amino acid conjugates.

Jasmonate-induced events such as the biosynthesis of glucosinolates [15], ethylene formation [16], tendril coiling [9], or the emission of volatiles [17] were also induced upon treatment with coronatine which can be regarded as an analogue of JA amino acid conjugates [18]. Coronatine is a phytotoxin produced by several pathovars of *Pseudomonas syringae*, and is up to 100-fold more active than JA (**1, 2, 8**) or JM (**7**) in several JA-responsive events [9,19].

A coronatine-insensitive mutant of *Arabidopsis thaliana* is also insensitive to JA (**8**) [20]. Like coronatine, amino acid conjugates of 1-oxo- and 1-hydroxy-indane4-carboxylic acid induce emission of volatiles in leaves of the Lima bean *Phaseolus lunatus* [18]. Furthermore, all these compounds induce pin2 expression in tomato leaves without endogenous rise of jasmonates [21]. Due to structural similarities between coronatine, JA amino acid conjugates and indanone amino acid conjugates these data suggest that JA conjugation is an important step in the signal transduction of a jasmonate-responsive event [18]. However, most of the indanone derivatives which induce emission of volatiles in the Lima bean or pin2 expression in tomato leaves were inactive in the JA-responsive touch-mediated tendril coiling of *Bryonia dioica* (Weiler, pers. commun.). This calls for differences in the molecular specificity of signals among different plant species and raises the following questions:

1. Do JA amino acid conjugates act downstream of (−)-JA (**1**) as a terminal signal or do both act without interconversion to each other?
2. Is there a structural specificity in the action of JA (**1, 2**) and JA amino acid conjugates with respect to the enantiomeric forms of the JA moiety on the one hand, and the chirality of the amino acid moiety on the other hand?
3. Do the various JA-responsive genes respond identically to different stereospecific forms of JA and their amino acid conjugates?

These questions are addressed here using GC/SIM-MS analysis of endogenous jasmonates and expression studies on various JA-responsive genes of barley leaves.

2. Materials and methods

2.1. Synthesis of JA–isoleucine conjugates

JA conjugates were chemically synthesized by reaction of (±)-JA (**8**) with the corresponding enantiomers of isoleucine using the mixed anhydride method [22]. The resulting diastereomeric pairs were completely separated by RP-HPLC giving the *N*-[(−)-jasmonoyl]- and *N*-[(+)-jasmonoyl]-conjugate, respectively, in either L- or D-configuration [22].

2.2. Separation of the JA enantiomers

Racemic JA (**8**) obtained by alkaline hydrolysis of (±)-JM (**7**) (Firmenich, Geneva, Switzerland) was chromatographically resolved on the chiral stationary phase Nucleodex β-PM [23]. The purity of the baseline separated enantiomers (−)-JA (**1**) and (+)-JA (**2**) was analyzed by measurement of their chiroptical properties.

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2.3. Plant material, application of jasmonate compounds, RNA extraction and Northern blot analysis

Primary leaves of 7-day-old seedlings of barley (*Hordeum vulgare* L. cv. *Salome*) were used in all experiments. Growth of seedlings, application of jasmonate compounds, RNA extraction and Northern blot analysis was performed as described recently [24].

For Northern blot analysis the following cDNA probes, all of them isolated from JM (7) treated barley leaves, were used: pHvJ3015 which codes for the jasmonate-induced protein of 23 kDa JIP-23 [25], pJRG5 which codes for a caffeic acid 0-methyltransferase [26], pJRG1 which codes for a lectin-like protein of rice roots [27] and cDNAs of not further characterized jasmonate-responsive genes (*JRG*) (JRG10, JRG12) [26].

2.4. Extraction and processing of plant material for jasmonate determination

Leaf segments (1 g fresh weight (f.wt.)) were frozen in liquid nitrogen and stored at −20°C prior to analyses. Leaf tissues were ground in liquid nitrogen and treated with 4 ml of 80% (v/v) methanol at 4°C for 20 h. For determination of jasmonates, [$^2H_6$]-JA (**8**) was added as an internal standard in appropriate amounts [28]. After centrifugation, the supernatants were subjected to an ion exchange chromatography on DEAE-Sephadex A-25. First, each cartridge filled with 3 ml of activated gel was eluted with 6 ml of methanol and 6 ml of 0.05 N methanolic acetic acid. Subsequently, fractions eluting with 7 ml of 1 N methanolic acetic acid were concentrated in vacuo and further purified on a Sep-Pak C_{18} cartridge (Merck, Darmstadt, Germany). The cartridges were pre-equilibrated with 10% (v/v) methanol in 0.2% (v/v) aqueous acetic acid. After treatment with 5 ml of the solvent, jasmonates were eluted with 6 ml of 80% (v/v) acidic methanol. The eluate was concentrated and finally delivered to a LiChrospher RP-18 column (250×4 mm, 5 μm). The analyses were performed on an HPLC-Set (KNAUER, Berlin, Germany) using a mobile phase consisting of 50% (v/v) methanol in 0.2% (v/v) aqueous acetic acid at a flow rate of 1 ml min^{-1}. UV detection was done at 210 nm. Fractions corresponding to the retention volumes of authentic JA (**1**, **2**) were combined.

For GC/SIM-MS analyses, the JA (**1**, **2**) eluates were treated with ether containing diazomethane. The JA (**1**, **2**) contents per gram f.wt. were calculated using a calibration curve of the internal standard [$^2H_6$]-JA (**8**). The intensities of the molecular ion at *m/z* 230 for the deuterated reference and the molecular ion at *m/z* 224 for the non-labeled compound were recorded [28]. The capillary GC/MS measurements were carried out according to the procedure reported previously [14].

2.5. Immunocytochemistry

Pieces of leaf segments treated for 24 h with water, 5×10^{-5} M (−)-JA (**1**) or (+)-JA-L-Ile (**4**) at the conditions described above, were fixed with 3% paraformaldehyde in phosphate-buffered saline and embedded in polyethylene glycol as described [29]. Sections (2 μm) mounted on poly-L-lysine-coated slides were immunolabelled with anti-JIP-23, a rabbit polyclonal monospecific antibody raised against the recombinant protein produced in *Escherichia coli* HMS 174 pLysE using the vector pJC40 and the insert pHvJ3015, was used in a dilution of 1:5000 as primary antibody and goat anti-rabbit IgG-fluorescein isothiocyanate (FITC) conjugate (SIGMA) as secondary antibody. After staining with 0.1 μg ml^{-1} 4,6-diamidino-2-phenylenediamine (DAPI), sections were enclosed in para-phenylenediamine and examined with a Zeiss 'Axioplan' epifluorescence microscope using the proper filter combinations. Micrographs were recorded on Kodak 'Elite 400' films.

3. Results

3.1. JA conjugates stereospecifically induce JIP gene expression

Starting with the enantiomeric pair (−)-JA (**1**) and (+)-JA (**2**) (Fig. 1), the corresponding L- and D-isoleucine conjugates were chemically synthesized. The ability of these four conjugates as well as (−)-JA (**1**), (+)-JA (**2**) and (±)-JM (**7**), to induce various JA-responsive genes in barley leaf segments was tested by floating on the respective solutions. An initial

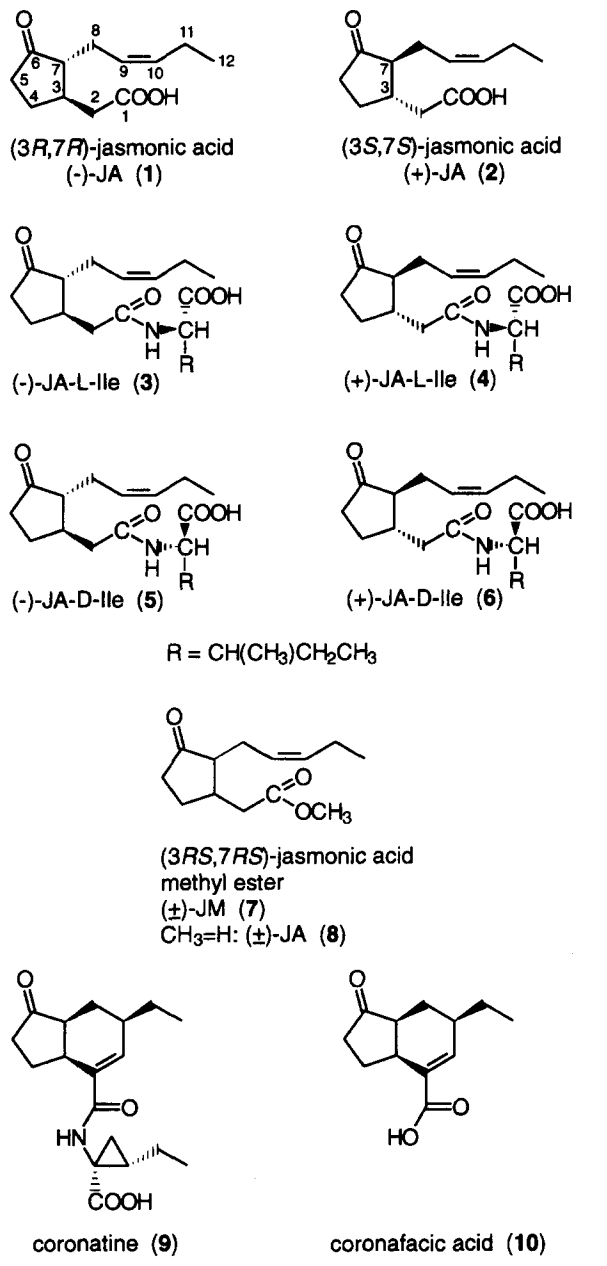

Fig. 1. Structures of (−)-JA (**1**), (+)-JA (**2**), (−)-JA-L-Ile (**3**), (+)-JA-L-Ile (**4**), (−)-JA-D-Ile (**5**), (+)-JA-D-Ile (**6**), (±)-JM (**7**), (±)-JA (**8**), coronatine (**9**) and coronafacic acid (**10**).

recording of time dependence of accumulation of JIP-23 mRNA in response to each compound revealed a steady increase reaching a maximum at about 24 h as shown for JM (**7**) in Fig. 2A. Therefore, we compared dose–response relationships at 24 h (Fig. 2B). The following structure activity relationships were observed:

1. JIP-23 mRNA accumulated in response to both (−)-JA (**1**) and (+)-JA (**2**), but the (−)-JA (**1**) was more active than (+)-JA (**2**) by at least one order of magnitude. In case of JRG1, JRG5, JRG10 and JRG12 this preference was higher.
2. The activity of (−)-JA (**1**) and (+)-JA (**2**) was similar to that of the respective L-isoleucine conjugates.
3. Both, the (−)-JA-L-Ile (**3**) and the (+)-JA-L-Ile (**4**) in-

duced expression of genes coding for JIP-23, but in the tested concentration no or only weak expression of the JRGs was found by (+)-JA-L-Ile (**4**), or (+)-JA (**2**).

4. Up to a concentration of 5×10^{-5} M D-isoleucine conjugates (**5, 6**) did not lead to accumulation of mRNAs of JIP-23, JRG1, JRG10 and JRG12 or that coding for JIP-23, but led to JRG5-mRNA accumulation.
5. Down-regulation of genes like that coding for rbcL occurred by JA (**1, 2**) and its L-isoleucine conjugates (**3, 4**). The (−)-form of the D-isoleucine conjugate (**5**) was also active.

These data show that induction and repression of genes by conjugates depends on stereospecificity of the JA moiety as well as the amino acid moiety. However, this specificity differs for different genes.

Similar results were found for the corresponding valine and phenylalanine conjugates. The latter were less active than aliphatic amino acid conjugates (data not shown). Furthermore, (−)-JA conjugates containing non-proteinogenous amino acids such as ε-amino-*n*-caproic acid or β-alanine as well as chiralic amino alcohols such as L-isoleucinol, L-leucinol, or L-valinol are not able to induce synthesis of JIP-23 (data not shown).

3.2. JA conjugates act directly without previous cleavage

The ability of JA conjugates to induce or to repress gene expression can be due to (i) release of JA by conjugate cleavage, (ii) increase of JA biosynthesis induced by conjugates, (iii) action as JA conjugate directly.

In order to find out which of these possibilities occurs in barley, GC/SIM-MS analysis was performed with the acid and neutral fraction of extracted leaf segments floated on water, (−)-JA-L-Ile (**3**) and (+)-JA-L-Ile (**4**), respectively. A time-dependent increase of free (−)-JA (**1**) up to only 1.2 nmol per gram f.wt. was observed after application of the (−)-derivative, but no significant amount of (+)-JA (**2**) was detectable upon treatment with the (+)-derivative (Fig. 3A). To detect the amount of JA (**1, 2**) released from conjugates but metabolized into the corresponding methyl ester during the treatment, we analyzed the neutral fraction (Fig. 3B). Again, only (−)-JA-L-Ile (**3**) released JA which was found to be finally methylated. Considering the dose–response curves shown in Fig. 2B, it seems unlikely that the minute amounts of (−)-JA (**1**) and (±)-JM (**7**) detected are sufficient to induce *JIP* gene expression. Under the conditions used, no methyl ester was detectable as a volatile (W. Boland, pers. commun.).

The direct activity of JA amino acid conjugates is also supported by the fact that coronatine, a molecular mimic of the conjugates [18], is highly active, whereas the JA-like half of this compound, coronafacic acid, is inactive (Fig. 2A). Upon treatment of barley leaves with coronatine the endogenous content of jasmonates did not exceed 0.6 nmol per gram f.wt. detected for water-treated leaves.

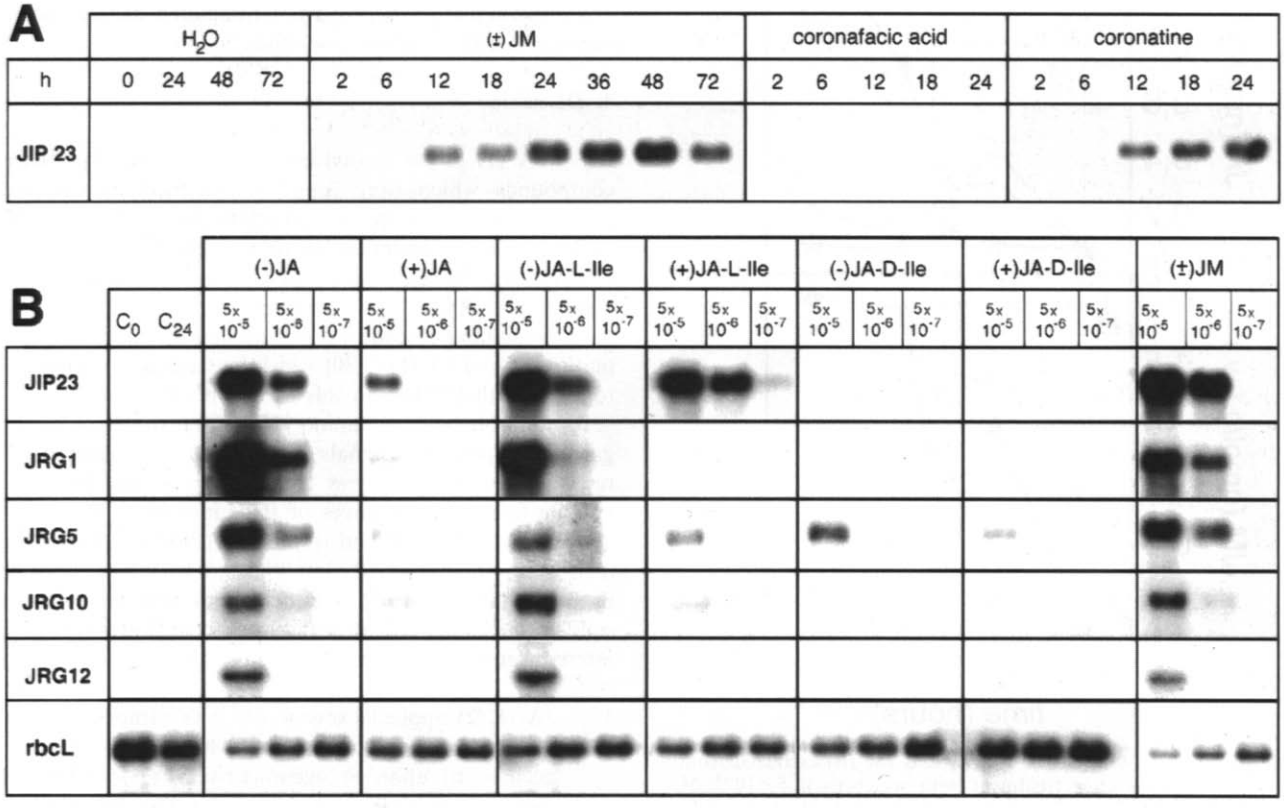

Fig. 2. Effects of (−)-JA (**1**), (+)-JA (**2**), (±)-JM (**7**) and the corresponding L- and D-amino acid conjugates as well as coronatine (**9**) and coronafacic acid (**10**) on the accumulation of transcripts of JRG1, JRG5, JRG10, and JRG12 or transcripts coding for JIP-23 or the large subunit of Rubisco (rbcL). Barley leaf segments were floated for different times (A) or for 24 h (B) on water (A and C_{24} in B) or solutions of different concentrations of the jasmonate compounds indicated (B). For A 45 μM (±) JM (**7**), 0.1 μM coronatine (**9**) and 250 μM coronafacic acid (**10**) were used. Total RNA (20 μg per lane, checked for loading by tracing rRNA under UV light) was separated electrophoretically, and Northern blot analysis was performed with DIG-labelled cDNA probes. Identical filters were used subsequently with the different probes.

3.3. JA acts without conversion into conjugates

To detect whether JA (**1, 2**) is active only after its conjugation with amino acids, we analyzed the amount of JA amino acid conjugates formed upon floating of leaf segments on water, or solutions of 5×10^{-5} M (+)-JA (**2**), 5×10^{-5} M (−)-JA (**1**), respectively (Fig. 4). As a control for the detectability of JA amino acid conjugates formed intracellularly leaves were also treated with 1 M sorbitol solution which is known to lead to accumulation of JA amino acid conjugates [14]. After a 24 h treatment with (−)-JA (**1**) or (+)-JA (**2**), no amino acid conjugate was detectable (Fig. 4, line B, C) suggesting lack of conjugation under these conditions. In contrast, JA conjugates were found upon osmotic stress (Fig. 4, line A).

3.4. Is there a tissue-specific expression of jasmonate-responsive genes induced by (+)-JA-L-Ile (4) or (−)-JA (1)

The data described so far suggest that JA (**1, 2**) and JA-Ile (**3, 4**) induce gene expression without being converted into each other. Such an individual signalling by both compounds might occur in the same cell or might be reflected by a tissue-specific expression in response to each signal. As revealed by an immunocytochemical approach (Fig. 5), JIP-23 visualized

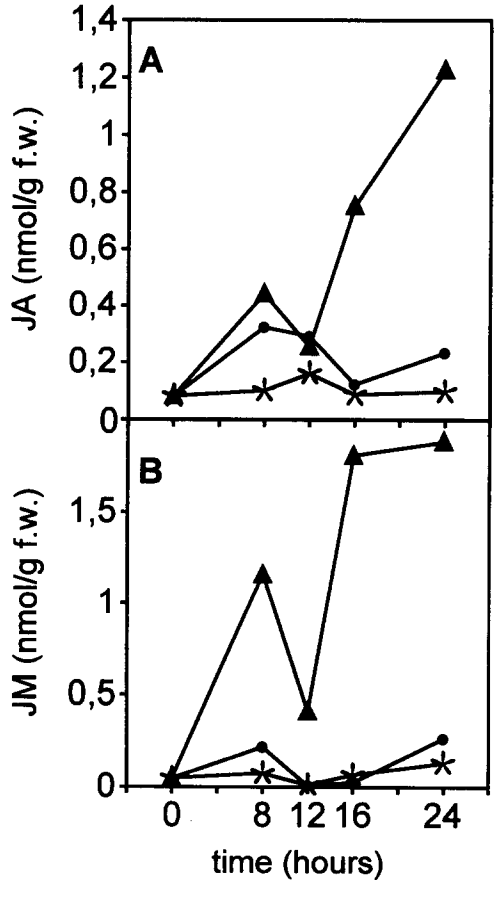

Fig. 3. GC/SIM-MS analysis of JA (A) and JM (B) extracted from barley leaf segments after treatment with water (×), 5×10^{-5} M (+)-JA-L-Ile (●) and 5×10^{-5} M (−)-JA-L-Ile (▲). At the times indicated, samples of 1 g f.wt. were taken for methanolic extraction, were separated by HPLC into an acid and a neutral fraction which were subsequently subjected separately to GC/SIM-MS analysis as indicated in Section 2. One example is given from three independent experiments exhibiting similar kinetics at slightly different absolute values due to differences in the biological material.

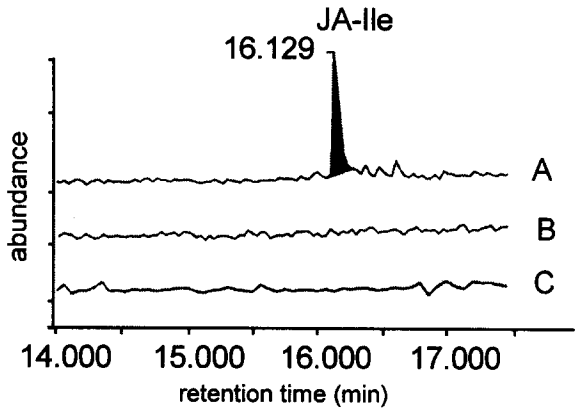

Fig. 4. Occurrence of JA conjugates after treatment with 5×10^{-5} M (−)-JA (**1**) (B), 5×10^{-5} M (+)-JA (**2**) (C) or 1 M sorbitol (A) solution for 24 h. The neutral fractions of the HPLC separation (cf. Section 2) was subjected to GC/SIM-MS analysis. One example is given from three independent experiments.

by the fluorescence of FITC-labelled antibodies was detectable within the mesophyll and the bundle sheath cells of barley leaves upon a 24 h treatment with (−)-JA (**1**) (Fig. 5a). The spatial pattern of the occurrence of JIP-23 in barley leaves treated with (+)-JA-L-Ile (**4**) for 24 h was identical to that of (−)-JA-treated leaves (Fig. 5b versus 5c). Within the epidermal cells, JIP-23 could be detected only upon a 48 h treatment (data not shown) presumably due to technical reasons. Possibly the fine cytoplasmic seam of epidermal cells requires obviously a higher degree of accumulated JIP-23 than the mesophyll cells to allow immunodetection.

4. Discussion

(−)-JA (**1**) or its methyl ester are ubiquitously occurring compounds which were found to function as signals for gene expression in response to various stresses like wounding, pathogen attack, water deficit or nutrient imbalance [10]. By studying wound-responsive gene expression in tomato leaves, Farmer and Ryan first gave strong arguments that a lipid-based signalling cascade via octadecanoids results in the formation of (−)-JA (**1**) [5,30] which was recently shown to act together with ethylene in this wound response [6].

In addition, also JA amino acid conjugates have to be regarded as potential signals because they (i) accumulate in response to osmotic stress [14], (ii) are strong inducers of volatile formation in leaves of the Lima bean [18], and (iii) are able to induce wound-responsive genes in tomato leaves [21]. Here we presented data that in barley the signalling properties of JA (**1, 2**) and its amino acid conjugates are similar, and both act with structural specificities and negligible interconversion into each other:

1. JA (**1, 2**) applied exogenously acts without conversion into amino acid conjugates. Although JA conjugates accumulate upon endogenous rise of (−)-JA (**1**) by osmotic stress [8,14], no JA conjugates were detectable after a 24 h JA treatment (Fig. 4). This may reflect that the source of JA (**1, 2**), added exogenously or formed endogenously, influences the ability of JA (**1, 2**) to be conjugated with amino acids. Although JA (**1, 2**) and its conjugates exhibited similar dose–response

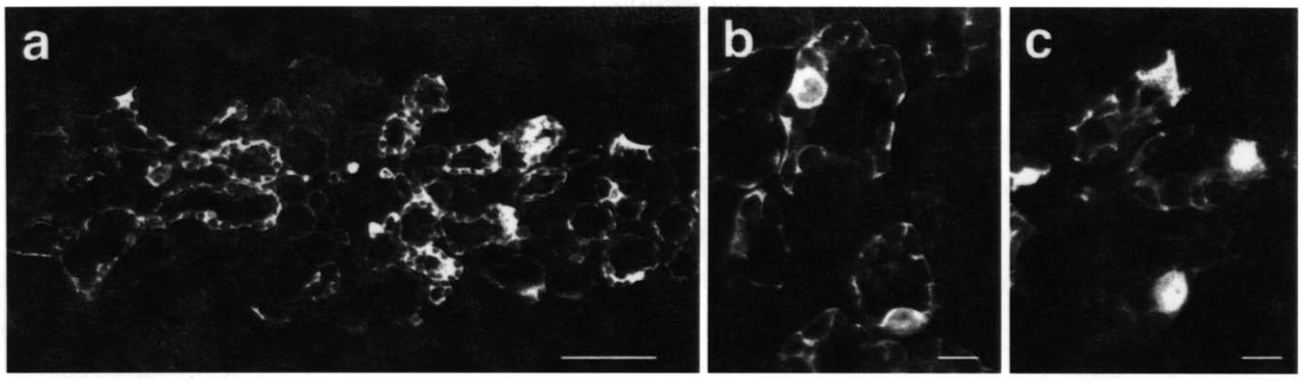

Fig. 5. Survey of the localization of JIP-23 in barley leaves treated with (−)-JA (**1**) or (+)-JA-l-Ile (**4**). The occurrence of JIP-23 is visualized by FITC-conjugated antibodies (a–c). a: Cross-section of a leaf treated with (−)-JA (**1**). The fluorescent label is visible in all mesophyll and bundle sheath cells. Bar represents 50 μm. b: Detail of (a), mesophyll cells. Note the label within the cytoplasm and nucleus. Bar represents 10 μm. c: Cross-section of a leaf treated with (+)-JA-L-Ile (**4**). Note the same distribution of label as in (b). Bar: 10 μm.

relationships (Fig. 2) it seems highly probable that JA (**1, 2**) is active per se since it is not converted into conjugates (Fig. 4). Furthermore, both compounds, JA as well as JA amino acid conjugates, induce gene expression in all living cells of a barley leaf (Fig. 5).

2. JA conjugates seem to act without cleavage to JA (**1, 2**) and the amino acid. Although some (−)-JA (**1**) is released from (−)-JA-L-Ile (**3**) as analyzed by GC/SIM-MS, the high activity in terms of gene expression (Fig. 2) found for the non-hydrolyzable (+)-JA-L-Ile (**4**) suggests that conjugates are active per se. This is in accordance with the fact that coronatine known to be a molecular mimic of amino acid conjugates [18], is highly active, whereas its building block coronafacic acid is inactive (Fig. 2A). The activity detected upon coronatine treatment is not caused by an endogenous rise of jasmonates. This supports recent data obtained with tomato [21]. Furthermore, the absence of JA (**1, 2**) upon (+)-JA-L-Ile (**4**) treatment (Fig. 3) indicates that JA biosynthesis is not induced by this compound. Apparently, JA amino acid conjugates occur in cells lacking a cleaving enzyme, or the conjugates are inaccessible for cleavage. The only organism from which a JA conjugate cleaving amidohydrolase could be purified so far, the fungus *Botryodiplodia theobromae*, does not accumulate JA conjugates [31].

In addition to the well-studied JA-responsive events [10,32], activity of JA amino acid conjugates was recently shown for the above mentioned volatile formation [18], the phytoalexin synthesis [33] and wound-responsive events [21]. Here, we show that JA (**1, 2**) and its conjugates function without being converted into each other. Thus, it is highly improbable that JA amino acid conjugates are storage forms of JA in barley leaves as described for other plant hormones as well as species [34]. As analyzed by uptake measurements with isolated barley mesophyll protoplasts, it is highly improbable that the structural specificities of enantiomeric and chiralic forms found here simply reflect uptake specificities, e.g. the (−)- and (+)-forms of JA amino acid conjugates exhibited similar if not identical rates of uptake [35].

The specificities found for the configuration of JA (**1, 2**) and the chirality of the amino acid moiety of the conjugates are in accordance with previously discussed requirements for biological activity [18,33], but also emphasize differences between systems. Most interestingly, in barley leaves structural specificities of JA compounds differ with respect to the expression of different genes: (i) JIP-23 mRNA accumulates to higher levels in response to the (+)-enantiomers than most of the JRG-mRNAs (Fig. 2), and (ii) conjugates carrying a D-amino acid induce JRG5 only (and repress rbcL). Whether these specificities in JA signalling are caused by different affinities of one or several receptors or by cumulative effects in the signalling cascade, is unknown.

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References

[1] Meyer, A., Miersch, O., Büttner, C., Dathe, W. and Sembdner, G. (1984) J. Plant Growth Regul. 3, 1–8.
[2] Bohlmann, F., Wegner, P., Jakupovic, J. and King, R.M. (1984) Tetrahedron 40, 2537–2540.
[3] Brückner, C., Kramell, R., Schneider, G., Schmidt, J., Preiss, A., Sembdner, G. and Schreiber, K. (1988) Phytochemistry 27, 275–276.
[4] Peña-Cortés, H., Fisahn, J. and Willmitzer, L. (1995) Proc. Natl. Acad. Sci. USA 92, 4106–4113.
[5] Bergey, D.R., Howe, G.A. and Ryan, C.A. (1996) Proc. Natl. Acad. Sci. USA 93, 12053–12058.
[6] O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyer, H.M.O. and Bowles, D.J. (1996) Science 274, 1914–1917.
[7] Herde, O., Atzorn, R., Fisahn, J., Wasternack, C., Willmitzer, L. and Peña-Cortés, H. (1996) Plant Physiol. 112, 853–860.
[8] Lehmann, J., Atzorn, R., Brückner, C., Reinbothe, S., Leopold, J., Wasternack, C. and Parthier, B. (1995) Planta 197, 156–162.
[9] Weiler, E.W., Kutchan, T.M., Gorba, T., Brodschelm, W., Niesel, U. and Bublitz, F. (1994) FEBS Lett. 345, 9–13.
[10] Creelman, R.A. and Mullet, J.E. (1997) Annu. Rev. Plant Mol. Biol. 48, 355–381.
[11] Gundlach, H., Müller, M.J. and Kutchan, T.M. (1992) Proc. Natl. Acad. Sci. USA 89, 2389–2393.
[12] Blechert, S., Brodschelm, W., Hölder, S., Kammerer, L., Kutchan, T.M., Mueller, M.J., Xia, Z.-Q. and Zenk, M.H. (1995) Proc. Natl. Acad. Sci. USA 92, 4099–4105.
[13] Harms, K., Atzorn, R., Brash, A., Kühn, H., Wasternack, C., Willmitzer, L. and Peña-Cortés, H. (1995) Plant Cell 7, 1645–1654.

[14] Kramell, R., Atzorn, R., Schneider, G., Miersch, O., Brückner, C., Schmidt, J., Sembdner and G., Parthier, B. (1995) J. Plant Growth Regul. 14, 29–36.
[15] Bodnaryk, R. and Yoshihara, T. (1995) J. Chem. Ecol. 21, 1735–1743.
[16] Greulich, F., Yoshihara, T. and Ichihara, A. (1995) J. Plant Physiol. 147, 359–366.
[17] Boland, W., Hopke, J., Donath, J., Nüske, F. and Bublitz, F. (1995) Angew. Chem. Int. Ed. 34, 1600–1602.
[18] Krumm, T., Bandemer, K. and Boland, W. (1995) FEBS Lett. 377, 523–529.
[19] Koda, Y., Takahashi, K., Kikuta, Y., Greulich, F., Toshima, H. and Ichihara, A. (1996) Phytochemistry 41, 93–96.
[20] Feys, J.F., Benedetti, C.E., Penfold, C.N. and Turner, J.G. (1994) The Plant Cell 6, 751–759.
[21] Dorans, A.M.I., Boland, W., Atzorn, R., Wasternack, C. and Bowles, D.J. (1997) J. Biol. Chem. (submitted).
[22] Kramell, R., Schneider, G. and Miersch, O. (1997) Chromatographia 45, 105–108.
[23] Kramell, R., Schneider, G. and Miersch, O. (1996) Phytochem. Anal. 7, 209–212.
[24] Hause, B., Demus, U., Teichmann, C., Parthier, B. and Wasternack, C. (1996) Plant Cell Physiol. 37, 641–649.
[25] Andresen, I., Becker, W., Schlüter, K., Burges, J., Parthier, B. and Apel, K. (1992) Plant Mol. Biol. 19, 193–204.
[26] Lee, J., Parthier, B. and Löbler, M. (1996) Planta 199, 625–632.
[27] Lee, J.E., Vogt, T., Hause, B. and Löbler, M. (1997) Plant and Cell Physiol. 38 (in press)
[28] Miersch, O. (1991) Z. Naturforsch. 56b, 1727–1729.
[29] Van Lammeren, A.A.M., Keijzer, C.J., Willemse, M.T.M. and Kieft, H. (1985) Planta 165, 1–11.
[30] Farmer, E.E. and Ryan, C.A. (1992) Plant Cell 4, 129–134.
[31] Hertel, S., Knöfel, H.-D., Kramell, R. and Miersch, O. (1997) FEBS Letters 407, 105–110.
[32] Wasternack, C. and Parthier, B. (1997) Trends Plant Sci. 2, 302–307.
[33] Tamogami, S., Rakwal, R. and Kodama, O. (1997) FEBS Letters 401, 239–242.
[34] Kleczkowski, K. and Schell, J. (1995) Crit. Rev. in Plant Sciences 14, 283–298.
[35] Dathe, W., Kramell, H.-M., Daeter, W., Kramell, R., Slovik, S. and Hartung, W. (1993) J. Plant Growth Regul. 12, 133–140.