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Uptake of Jasmonic Acid and Related Compounds by Mesophyll Protoplasts of the Barley Leaf

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Abstract. Jasmonic acid (JA) permeates the plasma membrane of mesophyll cells by diffusion as the lipophilic undissociated JAH molecule probably without the participation of a saturable uptake component. The mesophyll plasma membrane is nearly impermeable to the JA anion. The permeability coefficients of JA and several JA derivatives (its methyl ester (JAMe), 7-iso-cucurbic acid (7-iso-CA), 6-epi-7-iso-cucurbic acid (6-epi-7-iso-CA), and both stereoisomers of the JA leucine conjugate ((+)-JA-Leu and (−)-JA-Leu)) were determined and used in a simplified mathematical model to predict stress-dependent JA redistribution between cytosol and apoplast in comparison with ABA. The redistribution of JA takes place similar to ABA; however, its velocity is much higher because of the high JA membrane permeability. When the permeability coefficients for the mesophyll plasma membrane are plotted double-logarithmically against the ratio of the distribution coefficient and the molecular ratio to the power of 1.5 ($K_D M_r^{-1.5}$), two straight lines result for two different classes of compounds. The permeability coefficients of JA conjugates are lower than that of the free acid by approximately one order of magnitude, but they are still significantly higher than that of ABA.

(−)-Jasmonic acid (JA; Fig. 1) detected as senescence promoter (Ueda and Kato 1980) and growth inhibitor (Dathe et al. 1981) belongs to a group of native growth regulators obviously involved in stress response (cf. Parthier et al. 1992). Besides JA its isomer (+)-7-iso-jasmonic acid (7-iso-JA, Fig. 1) their methyl ester, amino acid conjugates and analogues with modifications at the side chains and at the cyclopentanone ring occur naturally (cf. Sembdner et al. 1989, 1990; Sembdner and Parthier, 1993). In some bioassays, 7-iso-JA and the methyl esters are biologically more active than JA (Meyer et al. 1984). Several amino acid conjugates of JA are able to promote efficiently the senescence of barley leaf segments also (Herrmann et al. 1987). The biological activity of the diverse JA analogues differs significantly, and nothing is known about the primary site of action. Furthermore, the xylem saps of different plant species contain remarkably high levels of acidic JA conjugates detectable by a radioimmunoassay (1–10 μg JA-equivalents per milliliter xylemsap); thus, they might be a favored transport form of JA (W. Hartung and W. Dathe, unpublished). For this reason, the re-uptake of JA and conjugates by plant cells from the extracellular medium seems to have high biological significance. Thus, we exposed uniform mesophyll protoplasts to the incubation medium in order to avoid the measured uptake results from total tissue with different cell types and layers. Only with protoplasts are we able to determine exactly the permeability coefficients because the membrane surface can be estimated accurately. From the uptake of these compounds by mesophyll protoplasts, we determined their permeation properties in comparison to the corresponding values of abscisic acid (ABA) and other phytohormones.

Environmental conditions (acidic air pollutants, drought stress, etc.) are known to alter the intracellular pH gradients in leaves. Hartung et al (1988) found drought stress-induced alkalinization of the leaf apoplast that might even be accompanied by a moderate acidification of the cytosol (Daeter and Hartung, 1990). The now flatter pH gradient across

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the plasma membrane is believed to be responsible for the redistribution of acidic compounds (e.g., the stress hormone ABA). Jasmonates are involved in cellular stress responses, too, and are described as modulators of protein synthesis and accumulation (Sembdner and Parthier 1993). To estimate how JA distributes between mesophyll cytosol and apoplast with changing compartmental pH values, the physicochemical properties and the P_s values were put into a simplified whole-leaf model developed to calculate compartmental distribution and redistribution of ABA (Hartung and Slovik 1991).

Materials and Methods

Plant Material and Protoplast Isolation

Mesophyll protoplasts were isolated from barley leaves (*Hordeum vulgare* L. cv. Salomé, grown for 8–10 days at 25°C, 8 h darkness, 16 h light). The abaxial epidermis was removed and the leaf segments (about 4 cm, leaf tip removed) were put on a digestion medium (modified to Kaiser et al 1982) containing 0.7% Cellulase ("Onozuka R-10," Serva, Heidelberg, Germany), 0.5% Macerozyme R-10 (Serva) and 0.2% bovine serum albumin (Serva) in a cell protoplast washing medium (CPW: 1 mM $MgSO_4$, 10 mM $CaCl_2$, 1 μM KJ, 0.1 μM $CuSO_4$, 0.2 mM KH_2PO_4, 1 mM KNO_3) according to Frearson et al. (1973) with 7% sorbitol (CPW 7) at pH 5.5. The segments were incubated at 30°C for 2 h in darkness. After optimum digestion, the medium was carefully removed and the protoplasts were released by shaking the tissue gently in CPW 7. The protoplasts were filtered through a nylon sieve (150 μm mesh width) and collected over some drops of Ficoll medium (5% Ficoll, Pharmacia, Uppsala; CPW: 0.4 M saccharose) and centrifuged (4 min, 100*g*). The supernatant was discarded, protoplasts laid over the Ficoll medium were carefully collected, suspended in a Betaine buffer (0.45 M Betaine (Fluka, Neu-Ulm), 20 mM KCl, 3 mM $CaCl_2$, 1 g/L polyvinyl-pyrrolidone (Serva), and 20 mM MES (Serva; pH 5.5–6.5) or HEPES (Fluka, Neu-Ulm; pH 7.0–8.0), depending on the desired pH value) and centrifuged (10 min, 100*g*). The protoplasts were collected, resuspended in the Betaine buffer, and centrifuged again (2 min, 100*g*). The pellet was taken up in the appropriate Betaine buffer to give about 3×10^6 protoplasts/ml. Protoplasts were counted using a Fuchs-Rosenthal-chamber and their diameter was measured. Assuming a perfect spherical shape of the protoplasts, the area of the plasma membrane and the protoplast volume was calculated from the average protoplast diameter that was determined to be 35 μm.

Uptake Experiments

The protoplast suspension (965 μl) was preincubated for 20 min with 15 μl ^{3}H-labeled water (~250,000 dpm/ml). Then, 20 μl of the ^{14}C-labeled compounds were added to give a final concentration of 1×10^{-4} M in the case of JA, JAMe, and the CA analogues or 3×10^{-5} M in the case of the JA conjugates. The uptake of the radioactive compounds was stopped first after 30 s and then at intervals of 45 s by subjecting 100 μl of the protoplast suspension to centrifugation through 100 μl of a preformed silicon oil layer (AP100:AR20S = 1:1, Voitländer, Kronach). The intact protoplasts were collected above 5 μl Percoll (Sigma, St. Louis, MO, USA). The vial tip was cut off above the protoplasts, put into a scintillation vial, and measured for ^{14}C and ^{3}H. To determine the specific radioactivity, we measured 100 μl of the protoplast suspension without centrifugation. From the ^{3}H-label within the protoplasts, the specific radioactivity and the average protoplast volume we were able to calculate the exact number of protoplasts in each sample. Each experiment at a defined pH value was performed twice the same day with protoplasts of the same preparation, and was repeated at three different days.

Determination of the Dissociation Constant (pK_a), the Distribution Coefficient (K_D), the Uptake Rate, and the Permeability Coefficient (P_s)

The pK_a values of JA (1 mg), CA analogues (1 mg), and JA-Leu (0.5 mg) were ascertained by titration with 0.1 N NaOH. The added NaOH volume was plotted against the changing pH value of the aqueous solution (1 ml) and the pK_a value resulted from the turning-point of the titration curve.

The distribution coefficients were determined in octanol/water at pH 6.0 (20 mM MES) and pH 7.0 (20 mM HEPES) according to Gimmler et al. (1981). The uptake rates (mol × protoplast^{-1} × min^{-1}) were calculated from the linear part of the uptake curve.

The P_s values of the undissociated molecules were calculated according to Gimmler et al. (1981) using the uptake rates, the particular pH value, the average protoplast surface (3.848×10^{-9} m^2) and the pK_a value of the specific compound (Table 1).

Radiochemicals

Diluted ^{3}H-labeled water (about 5 kBq/mmol) was purchased from Amersham & Buchler (Braunschweig). All ^{14}C-labeled compounds applied were synthesized.

^{14}C-JA and ^{14}C-JAMe: For the synthesis of ^{14}C-JA, we used the fungus *Botryodiplodia theobromae* Pat. (syn *Lasiodiplodia theobromae* Griff. et Maubl.), isolated as a phytopathogenic strain from rose plants grown in the green house (Böhmer and Kiewnick 1989). The fungus was kindly supplied by Dr. Böhmer, Landwirtschaftskammer Rheinland, Pflanzenschutzamt, Bonn. This fungal strain synthesizes JA similarly to another strain of *Botryodiplodia theobromae* isolated from grapefruit (Miersch et al. 1987a), but the ratio of (+)-JA/(−)-JA is significantly lower from the beginning of fermentation (W. Dathe, unpublished). The fermentation was performed as described by Miersch et al. (1987a). Two Fernbach flasks (each 100 ml) were inoculated with the fungal strain and simultaneously with 2.5 ml [2-^{14}C]acetic acid (sodium salt, 37 MBq, 1.91 GBq/mmol, 1.6 mg; Amersham & Buchler) at pH 5.4. After 3 days (surface culture, darkness, 30°C) the culture broth of both flasks was filtered (pH 4.7; mycel: 1.2 g dry weight), partitioned with $CHCl_3$ at pH 3.0, dried with Na_2SO_4, evaporated to dryness, and chromatographed on a DEAE-Sephadex A-25 column (45 × 1.1 cm) with a discontinuous gradient of acetic acid in 80% methanol (Gräbner et al. 1976). Fractions eluted with 0.25/0.5 N acetic acid corresponding to JA were combined, evaporated, and further purified by column chromatography (20 × 1.3 cm) on LiChroprep RP-18 (40–63 μm, Merck, Darmstadt, Germany) at 0.3 kp/cm^2 eluted with methanol:acetic acid (0.2%) = 60:40. The JA fraction eluted at 44–50

Table 1. Physicochemical properties of JA and analogues in comparison to ABA (Hartung and Slovik, 1991).

Parameter	JA	ABA	JAMe	7-iso-CA	6-epi-7-iso-CA	(+)-JA-Leu	(−)-JA-Leu
Molecular ratio (M_r)	210	264	224	212	212	323	323
Dissociation (20°C)							
pK_a	4.50	4.75	—	4.50	4.50	4.15	4.1
Distribution coefficients[a]							
K_D (mol)	57.8	20.5	6.8	24.7	9.0	206	230
K_D (ion)	0.077	0.0085					
Relative ion conductance[b]							
p_{rel} (from K_D)	1.33×10^{-3}	4.15×10^{-4}					
Collander terms							
K_D (mol)$M_r^{-1.5}$	0.019	0.0048	0.0020	0.008	0.029	0.036	0.040
Permeability coefficients calculated from uptake experiments at pH 6.0							
P_S ($\times 10^{-6}$ m s^{-1})	3.24	0.02[c]	0.05	2.67	0.98	0.23	0.30

[a] K_D denotes the distribution coefficient (octanol:water). K_D is calculated from experimental data assuming that only the neutral molecule species is soluble in octanol. K_D (mol) denotes the K_D for the undissociated molecule species, K_D (ion) of the corresponding phytohormone anion (Hartung and Slovik 1991).

[b] p_{rel} is the anion permeability of a membrane relative to its permeability to the neutral hormone molecule species, calculated as K_D(ion)/K_D(mol) (Hartung and Slovik 1991).

[c] P_S of the *Spinacia* mesophyll plasma membrane according to Gimmler et al. (1981).

ml was further purified by TLC on silica gel GF_{254} in $CHCl_3$: ethyl acetate:acetone:acetic acid = 40:10:5:1 (solvent system 1) separating JA (R_f = 0.75) from cucurbic acid (R_f = 0.50), a JA-related compound yielding 7.15 mg ^{14}C-(−)-JA/^{14}C-(+)-7-iso-JA (9:1, determination by capillary GC, column:FS-OV-225-CB, 0.25 µm, 0.32 mm i.d., 25 m, 160°C; detector:230°C; (−)-JAMe:R_t = 5.77 min, (+)-7-iso-JAMe:R_t = 6.87 min) at 6.29 MBq/mmol (^{14}C-incorporation rate: about 0.58%) and 0.5 mg ^{14}C-cucurbic acid (5.56 MBq/mmol). In order to get ^{14}C-JAMe, we methylated an aliquot of the ^{14}C-JA sample with diazomethane.

(+)-JA-^{14}C-Leu and (−)-JA-^{14}C-Leu (Fig. 1): The synthesis of JA-Leu-conjugates was performed similarly as described for the corresponding nonlabeled compounds (Kramell et al. 1988), but we used the methyl ester ((S)-[U-^{14}C]-leucine methyl ester (7.82 GBq/mmol)-Lachema, Prag) instead of the free acid. The reaction of (±)-JA (14.5 mg, 0.069 mmol) and (S)-[U-^{14}C]-leucine methyl ester (total radioactivity: 1.85 MBq) diluted with 4.01 mg inactive leucine methyl ester resulted in a mixture of the stereoisomeric leucine derivatives of (+)-JA and (−)-JA. The diastereomeric pair obtained could be baseline resolved by HPLC (Schneider et al. 1989). The chromatography was carried out isocratically on Hibar RT (Merck) (containing LiChrospher 100 RP-18 (5 µm, 200 × 4.0 mm, I.D.) with methanol:water = 40:60, 1.0 ml/min, using a Raytest-detector set at 210 nm. Under these conditions, the derivative (+)-JA-(S)-[U-^{14}C]-LeuOMe (R_t = 15.8 min) is eluted before (−)-JA-(S)-[U-^{14}C]-LeuOMe (R_t = 18.8 min). The specific radioactivity was determined to be 32.56 MBq/mmol for both specimens. In order to get the free acid of the conjugate, an aliquot of each diastereomeric ester was hydrolysed (1 N NaOH, 24 h, room temperature) and the aqueous phase partitioned at pH 3–4 with $CHCl_3$. The acidic derivatives were further purified by HPLC on Hypersil RP-8 (5 µm, 200 × 4 mm, I.D.) with methanol:acetic acid (0.2%) = 40:60, 1.0 ml/min, detection at 210 nm yielding (−)-JA-(S)-[U-^{14}C]-Leu at R_t = 63.5 min and (+)-JA-(S)-[U-^{14}C]-Leu at R_t = 42.5 min.

^{14}C-6-epi-7-iso-CA and ^{14}C-7-iso-CA (Fig. 1): For the synthesis of these CA analogues, we used an aliquot of ^{14}C-JA produced by *Botryodiplodia theobromae* (see above). After reduction with $NaBH_4$ (Dathe et al. 1991), the reaction products were purified by HPLC on LiChroCART (Merck) containing LiChrospher 100 RP-18 (5 µm, 125 × 4.0 mm, I.D.) with methanol:ace-

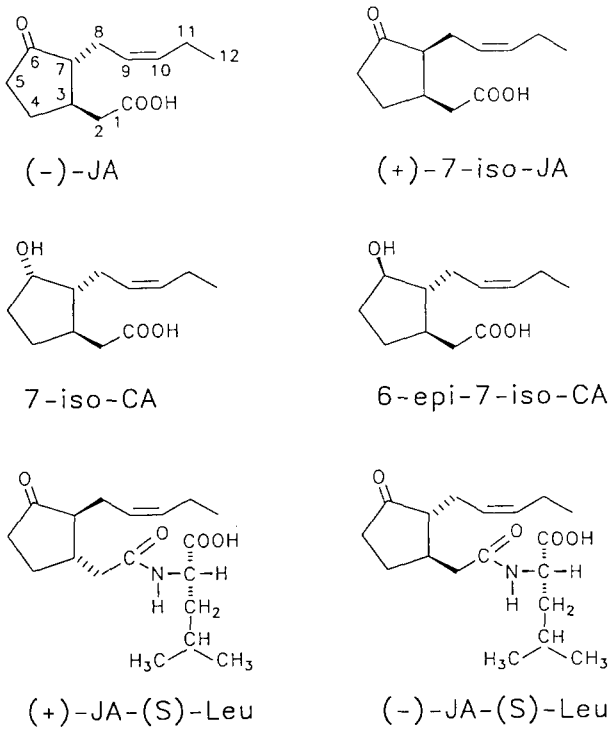

Fig. 1. Structures of jasmonates used in the uptake experiments.

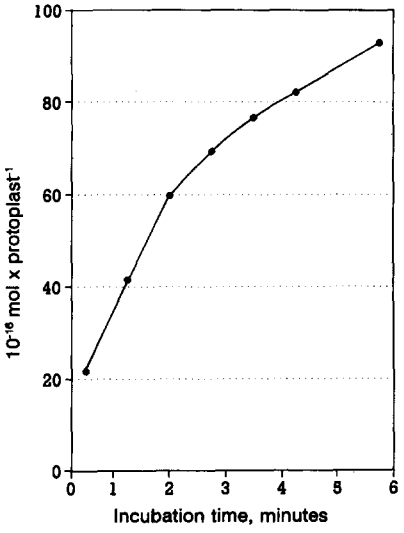

Fig. 2 Time-dependent uptake of JA by barley mesophyll protoplasts (1×10^{-4} M JA, pH 6.0). The uptake rate was calculated only from the linear part of the curve.

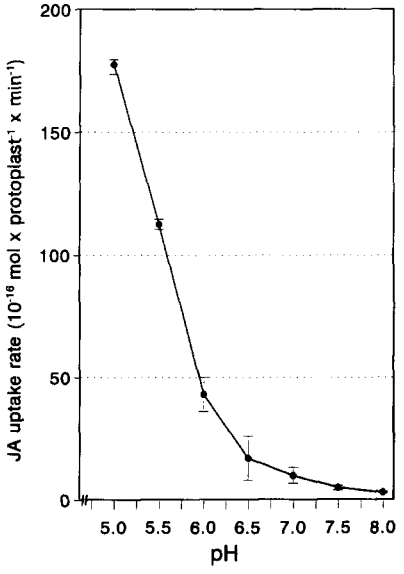

Fig. 3. pH-Dependent uptake of JA by barley mesophyll protoplasts. The compound was always applied at a concentration of 1×10^{-4} M. Each point is a mean of six experiments.

tic acid (0.2%) = 50: 50 (0.6 ml/min) yielding ^{14}C-6-epi-7-iso-CA at R_t = 8.45 min and ^{14}C-7-iso-CA at R_t = 10.40 min (both 6.29 MBq/mmol).

Metabolic Investigations

About 20 µl of ^{14}C-JA or ^{14}C-JAMe, respectively, were added to 1 ml protoplast suspension (pH 6.0). After 20 min of incubation, the suspension was centrifuged (2 min, 200*g*), the supernatant acidified to pH 3.0 and partitioned with $CHCl_3$. The pellet was resuspended in dest. water (1 ml) for 5 min, centrifuged, and the supernatant partitioned in the same way. Both chloroform extracts of ^{14}C-JA or ^{14}C-JAMe treated protoplasts were separated on TLC aluminium sheets coated with silica gel 60 F_{254} with concentrating zone (Merck) in solvent system 1 ($CHCl_3$:ethyl acetate:acetone:acetic acid = 40:10:5:1) or solvent system 2 (hexane:ethyl acetate:acetic acid = 60:40:1; JAMe) and analyzed by a radioscanner. Simultaneously, the $CHCl_3$ extracts of ^{14}C-JA were methylated and analyzed by capillary GC (conditions see above). These metabolic investigations were repeated three times with every compound.

Results

pH-Dependent Uptake of Jasmonic Acid

Jasmonic acid is taken up rapidly by isolated mesophyll protoplasts at pH 6.0. Uptake begins to reach equilibrium already after 3 min (Fig. 2). At a given JA concentration, the uptake rate at pH 7.0 is significantly lower compared to that at pH 6.0 and a further increase in the pH value to 8.0 keeps reducing the uptake rate (Fig. 3).

When the uptake rates of Fig. 3 are plotted against the corresponding JAH-concentrations (calculated according to the Henderson-Hasselbalch equation from the pH value of the medium and the given JA concentration), a linear relation can be observed (Fig. 4). A similar pH-dependent uptake can be observed with the two derivatives of cucurbic acid, 7-iso-CA and 6-epi-7-iso-CA. At low pH, both compounds are taken up with lower rates than JA; however, under alkaline conditions, the uptake rates are similar to that of JA (Fig. 5).

We have also investigated the uptake of JAMe and the JA conjugates (+)-JA-Leu and (−)-JA-Leu at pH 6.0 and 7.0. In contrast to free JA, the uptake of JAMe is not affected by the proton concentration of the medium. At both pH values tested, JAMe is taken up by the protoplasts at a rate of about 45×10^{-16} mol × protoplast^{-1} × min^{-1}. The uptake of the conjugates is very low (Fig. 5). Using TLC, HPLC, and GC, we have analyzed whether JA or JAMe were metabolized during the uptake experiments. In all cases, the protoplasts did metabolize neither JA nor JAMe within the experimental time (20 min). The radioactivity in both medium and protoplasts of the applied samples corresponded on TLC and HPLC to the authentic compound.

To clarify whether the two native JA isomers differ in their uptake rates, we analyzed the chloroform extracts of the medium and the protoplasts by GC. In both cases, we determined an identical JA/7-iso-JA ratio (9:1), indicating there is no favored uptake of either compound.

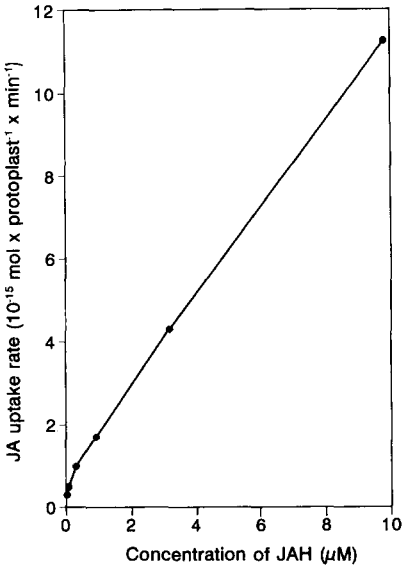

Fig. 4. JAH concentration-dependent uptake of JA by barley mesophyll protoplasts. The uptake rates of the experiments of Fig. 2 were plotted against the external JAH concentration calculated from the actual pH value of the medium and the pK_a of JA (4.5).

Permeability Coefficients (P_s) of the Plasma Membrane for JA Derivatives

The linear uptake rates within the first 2 min were used to calculate permeability coefficients. The permeability of the mesophyll plasma membrane is highest to JA, followed by 7-iso-CA and 6-epi-7-iso-CA. Compared with JA, the plasma membrane permeability to JA conjugates is lower by a factor of 7–12. The lowest P_s value was determined for JAMe (Table 1).

The permeability coefficients were plotted according to Collander (1954) against the ratio of the distribution coefficients and the molecular ratio to the power of 1.5 (Fig. 6; filled symbols). As a result, we see two straight lines with different slopes for two different classes of solutes, the free acids and the acidic and neutral conjugates. When the P_s values of the *Valerianella* mesophyll plasma membrane for various compounds (Baier 1990, W. Daeter unpublished results; open symbols) are added to this Collander plot, both straight lines seem to be completed. Note the relatively low permeability of the mesophyll plasma membrane to ABA compared with those of the jasmonates; even JA conjugates permeate the plasma membrane easier than ABA.

pH-Dependent Redistribution of JA Between Apoplast and Cytosol

Hartung and Slovik (1991) described a simplified mathematical model which shows the redistribution of ABA and other plant hormones when pH gradients between leaf cell compartments change due to drought stress. Because of its physicochemical properties [high P_s, low anion conductivity of the membrane, estimated as described by Hartung and Slovik (1991, Table 1)], JA behaves similar to ABA. Because of the high membrane conductance for JA, however, in this model the pH-dependent JA redistribution would occur much more rapidly than ABA redistribution (Fig. 7).

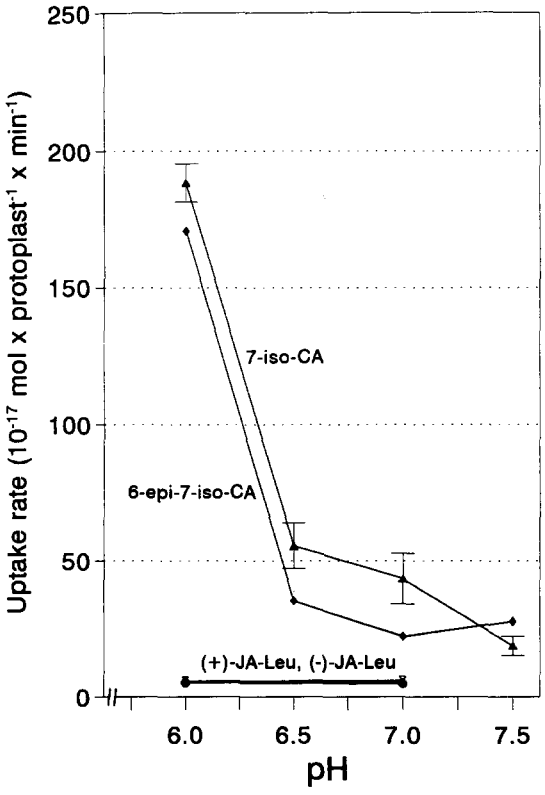

Fig. 5. pH-Dependent uptake of CA analogues (1×10^{-4} M) and JA conjugates (3×10^{-5} M) by barley mesophyll protoplasts. Each point is a mean of at least four experiments.

Discussion

Our uptake experiments provide evidence that permeation and intracellular distribution of JA occur by similar mechanisms as in the case of ABA. JA is a weak acid with a pK_a value (4.5) comparable to that of ABA. From the octanol distribution data at different pH values, it was concluded that the relative anion conductivity of JA is extremely low (Table 1). The uptake experiments showed a pH dependence very similar to that reported earlier for ABA (Kaiser and Hartung 1981). Because of the low specific activity of the labeled material, it was not possible to investigate the concentration dependence of JA uptake. Therefore, we have plotted the external JAH concentration calculated from the JA concentration and the pH value using the Hender-

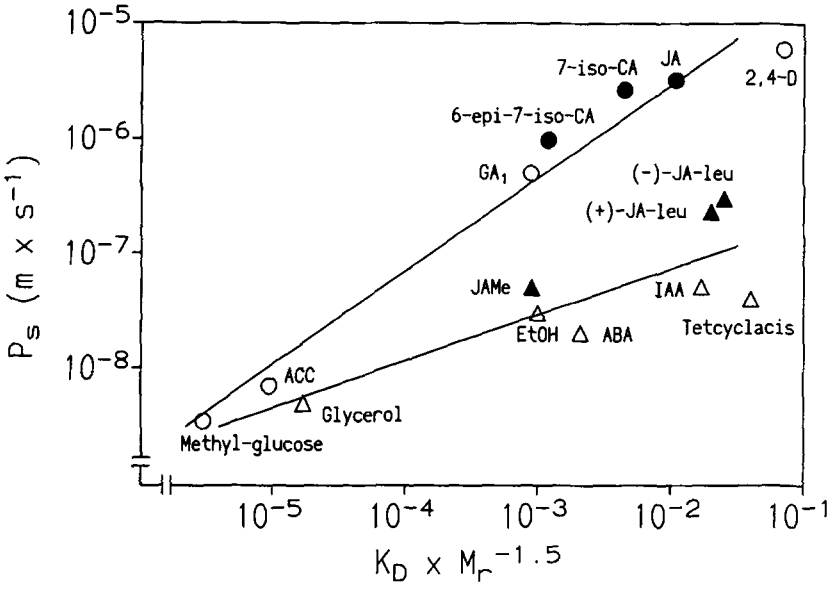

Fig. 6. The P_s values of the mesophyll plasma membrane for various compounds determined at pH 6.0 are plotted double-logarithmically against the $K_D M_r^{-1.5}$ ratio (Collander plot). The data for the filled symbols were determined experimentally with barley mesophyll protoplasts at pH 6.0. The open symbols represent data that were determined by Baier et al. (1990) using *Valerianella locusta* mesophyll cells. The P_s value of ^{14}C-Tetcyclacis was determined by Daeter (*V. locusta*, unpublished).

son-Hasselbalch equation against the uptake rate of the ^{14}C-labeled compounds. This plot resulted in a linear correlation between JAH concentration and JA uptake rate, indicating that only JAH is taken up. However, at alkaline pH, when the JAH concentration is very low, the measured uptake rates are slightly higher as they are expected in the case of diffusion. This may indicate a slightly higher plasma membrane permeability of JA at high pH values or an additional low permeability of the membrane for JA^- due to the relatively high JA concentrations we had to use in these experiments. Since the pH-dependent uptake curves of the CA derivatives tested look very similar, we concluded that uptake of CA analogues may occur by the same mechanism as JA.

In all experiments, we never found evidence for any JA metabolism. Similar observations were made by Loveys and Robinson (1987), who could not find any ABA metabolism in mesophyll protoplasts. Three explanations are possible. On the one hand, JA metabolism may be inhibited completely under conditions of osmotic stress, as it occurs in isolated protoplasts. On the other hand, one could conclude that JA metabolism is dependent on processes in the apoplast which could be associated to cell wall located proteins, to soluble enzymes within the apoplastic solution, or to proteins on the outer surface of the mesophyll plasma membrane which might be inactivated or removed by the protoplasting procedure. Another explanation would be a very low rate of metabolism leading to nondetectable amounts of JA metabolites within the experimental time.

To characterize the diffusion of the jasmonates, permeability coefficients of the mesophyll plasma membrane were determined. Compared to other naturally occurring acidic growth regulators, the permeability coefficient of JA is very high, even higher than that of the easily permeating auxin molecule. However, the P_s values might be slightly overestimated because some free chloroplasts might have been also present in the protoplast suspension, and the chloroplast envelope was shown to exhibit a higher membrane permeability to a number of compounds than does the plasma membrane (Heilmann et al. 1980). In the case of acidic jasmonates, we always assumed that the undissociated species is the only permeating molecule. At pH 6.0, the determined $P_s$value form the series: JA > 7-iso-CA > 6-epi-7-iso-CA > (−)-JA-Leu ≥ (+)-JA-Leu > JAMe (Table 1).

It also should be noted that P_s values of all JA derivatives, even those of the conjugates, are significantly higher than that of ABA. Thus, JA conjugates, which may be the transport form of JA within the xylem, can permeate the plasma membrane easily without being cleaved.

When P_s values are plotted double-logarithmically against the ratio of the octanol/water distribution coefficient and the molecular ratio to the power of 1.5 as described by Collander (1954), JA, 7-iso-CA, 6-epi-7-iso-CA, and the JA conjugates form two separate groups. They are completed to two straight lines of different slopes, when the P_s values determined by Baier (1990) and Daeter (unpublished) for the mesophyll plasma membranes are added. Gimmler and Hartung (1988) argued that sol-

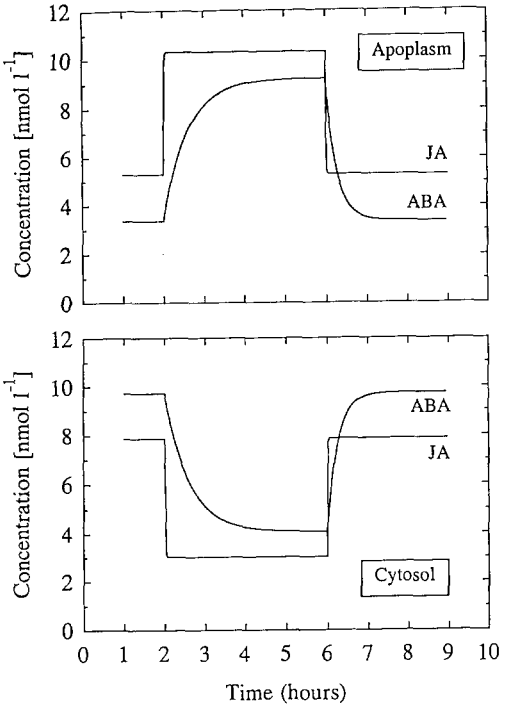

Fig. 7. The kinetics of the JA and ABA concentrations in the apoplasm and cytosol of mesophyll cells for stress-induced pH alterations calculated on the basis of the model of Slovik et al. (1992) and Slovik and Hartung (1992a,b). The simulation was started with an apoplastic and cytosolic pH (pH_{apo}, pH_{cyt}) of 6.5 and 7.3, respectively. After 2 h, the stress period was started by increasing pH_{apo} to 7.3 and decreasing pH_{cyt} to 7.15. After 6 h, the pH relations of an unstressed leaf were reestablished.

utes with high hydrogen bonding capacities form the straight line with lower permeability coefficients.

We have incorporated the physicochemical data of JA (Table 1) into a simplified mathematical model which predicts the stress-dependent redistribution of ABA between the cytosol and the apoplast of mesophyll cells. Especially due to the high permeability of the plasma membrane to JA and to the low anion conductivity, JA also behaves as a nearly perfect Henderson-Hasselbalch compound, similarly to ABA. However, stress-dependent distribution of JA is predicted to occur much more rapidly than that of ABA. The model also predicts an apoplastic JA concentration which is higher than the apoplastic ABA concentration. With the available assay techniques, we could not find measurable amounts of free JA in xylem and apoplastic saps (W. Hartung and W. Dathe, unpublished), whereas significant levels of JA conjugates were found. As long as more sensitive JA assays are not available, no clear conclusions about the real apoplastic JA concentrations in comparison with that predicted in the model can be drawn.

The higher biological effectivity of JAMe than JA in senescence promotion in barley leaf segments (Miersch et al. 1987b, Herrmann et al. 1987) does not correspond to the uptake rate of JAMe by protoplasts. However, the two JA conjugates are absorbed by protoplasts only to a very small extent without differences between both isomers (Fig. 5). While (+)-JA-Leu is inactive in senescence promotion, its naturally occurring stereoisomer (−)-JA-Leu (Schmidt et al. 1990) is nearly as active as JA (H.-M. Kramell, G. Herrmann, R. Kramell, and O. Miersch, unpublished results). Whether minute hydrolysis of the JA conjugates is a prerequisite for its biological activity is not completely clarified (H.-M. Kramell et al., unpublished results). Therefore, the differences in biological activity of various jasmonates cannot be explained by differences in the uptake rate across the plasma membrane.

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