Plant Physiol. (1991) 95, 990–996
0032-0889/91/95/0990/07/$01.00/0

Received for publication July 18, 1990
Accepted November 17, 1990

Carrier-Mediated Uptake of Abscisic Acid by Suspension-Cultured *Amaranthus tricolor* Cells

Jacqueline Bianco-Colomas, Philippe Barthe, Monique Orlandini, and Marie Thérèse Le Page-Degivry*

Laboratoire de Physiologie végétale, Université de Nice-Sophia Antipolis, Parc Valrose, 06034 Nice, Cedex, France

ABSTRACT

Abscisic acid (ABA) uptake by *Amaranthus tricolor* cell suspensions was found to include both a nonsaturable component and a saturable part with K_m of 3.74 ± 0.43 micromolar and an apparent V_{max} of 1.5 ± 0.12 nanomoles per gram per minute. These kinetic parameters as well as the uptake by intact cells at 0°C or by frozen and thawed cells, are consistent with operation of a saturable carrier. This carrier-mediated ABA uptake was partially energized by ΔpH: it increased as the external pH was lowered to pH 4.0; it decreased after the lowering of the ΔpH by the proton ionophore carbonylcyanide-*m*-chlorophenylhydrazone or after the altering of metabolically maintained pH gradient by metabolic inhibitors (KCN, oligomycin). The carrier is specific for ABA among the plant growth regulators tested, is unaffected by (RS)-*trans*-ABA and was inhibited by (S)-ABA, (R)-ABA, and also by the ABA analog LAB 173711.

Transmembrane transport is fundamental to the intercellular transport and the distribution of hormones between intracellular compartments. From the many studies of auxin transport using tissues, cells or vesicle preparations, the available evidence suggests a model that includes permeation of the neutral lipophilic form of the weak acid (IAAH) through the lipid bilayer, a saturable symport of the auxin anion (IAA^-) with protons and a passive efflux of anion via a specific saturable anion channel. Some studies suggest that a saturable binding component may contribute to the total uptake by vesicle system (7). For the other weakly acidic hormones (ABA, GAs) simple diffusion of their hydrophobic neutral species and saturable high affinity uptake carriers have also been described but they have received considerably less attention (1–4, 20 for ABA; 9, 22, 23 for GAs).

As far as ABA is concerned, diffusion of the undissociated species was first demonstrated for chloroplasts by Heilmann *et al.* (12) and later for mesophyll cells by Kaiser and Hartung (13). In addition to the diffusive path, Astle and Rubery (1, 2) have demonstrated the existence of carrier-mediated uptake restricted to the apical regions of roots of runner bean seedlings and in suspension-cultured *Phaseolus coccineus* L. cells (3). Data of Daie and Wyse (8) did not substantiate carrier-mediated uptake but indicated the presence of an energy requiring component in source and sink tissue of sugar beet. Both a diffusive and an energy-dependent component were also involved in translocation of maternal ABA to the developing embryo in French bean (17). The purpose of this study was to investigate the ABA transport characteristics in suspension-cultured *Amaranthus tricolor* L. cells.

MATERIALS AND METHODS

Radiochemicals and Chemicals

Radiochemicals were obtained from the Radiochemical Centre, Amersham, (U.K.). A racemic sample of (RS)-[2-^{14}C] ABA (204 GBq·mol^{-1}, 99% radiochemical purity) was stored in darkness as an ethanolic solution at 2°C (a complete list of abbreviations used in this paper can be found in Table I). For experimental use, labeled ABA was diluted in Na phosphate/citrate buffer (pH 5.0) supplemented with 2% (w/v) sucrose. Nonradioactive (RS)-ABA, 2,4-D, IAA, kinetin, and gibberellic acid were purchased from Sigma. Some compounds were generous gifts from different laboratories: (S)-ABA and (R)-ABA, the natural and unnatural enantiomer (26), from Dr. F. Kienzle, Hoffman La Roche, Basel; (RS)-*trans*-ABA from Dr. P. Wareing, LAB 173711 from Dr. W. Rademacher, BASF, Limburgerhof, RFA. DMSO solutions of CCCP, ethanolic solutions of oligomycin, and aqueous solutions of KCN were made up as required.

Plant Material

The suspension cultures originated in our laboratory from a callus culture derived from a hypocotyl explant of *Amaranthus tricolor* L. var *bicolor ruber* Hort. seedling and maintained on a medium of Murashige-Skoog (21) supplemented with 2,4-D and kinetin (6). Cells were grown at 23 ± 1°C under 16 h d photoperiod (fluorescent light) on a shaker

Table I. *Abbreviations Used in This Paper*

(S)-ABA, natural form, (+)-S-ABA
(R)-ABA, (−)-S-ABA
ABA, (RS)-ABA, racemic mixture of (+)-S-ABA and (−)-S-ABA
[2-^{14}C]ABA, (RS)-[2-^{14}C]ABA
PA, phaseic acid
DPA, dihydrophaseic acid
BHT, 2-6-di-*t*-butyl-4-methylphenol
CCCP, carbonylcyanide-*m*-chlorophenylhydrazone
C_e, extracellular total concentration
C_i, intracellular total concentration
pH_e, external pH
pH_i, intracellular pH
pH_v, vacuolar pH
pH_c, cytoplasmic pH

(Biolafitte, type AS 850) operating at 110 rpm. All experiments were performed with 5-d-old suspensions (exponential growth phase). Cells were collected on a glass filter, rinsed with distilled water, and resuspended at a density of 50 g fresh weight L^{-1} in 2% (w/v) sucrose; they were kept for at least 1 h at the temperature of experiments in a shaker prior to the start of incubations.

Incubation Procedures

The incubation procedures were based on Astle and Rubery (3). Two different incubation procedures were used according to whether the uptake rate of [2-^{14}C]ABA was studied as a function of the incubation time or of the [2-^{14}C]ABA concentration. When the time course of uptake was followed, 3 mL samples were removed from an incubation of 50 mL gently stirred at 23°C. In the second type of experiment, in which each individual measurement was obtained from a single incubation, 2 mL of cell solutions (stock cell suspensions) were transferred to vials containing 1 mL of buffered medium to initiate incubations performed at 0, 17, 23, or 27°C, in a reciprocal shaker (33 strokes·min^{-1}). Uptake was initiated by addition of radiolabeled ABA. In both cases the incubation mixture was composed of 2% (w/v) sucrose, Na phosphate (0.2 M)/citrate (0.1 M) buffer over the range from pH 3.0 to 7.0, and radiolabeled substrate plus other additives as described in the text. Uptake was terminated by rapid filtration under reduced pressure through a Whatman glass microfibre filter disc (2.5 cm in diameter). Cells were maintained under suction for 15 s before removal from the filter, weighed, then placed in a vial containing 5 mL aqueous scintillation fluid. After a minimum of 16 h with periodic shaking, when extraction of radioactivity was complete, samples were counted using a SL Intertechnique Liquid Scintillation Spectrometer.

Extraction of Radioactivity from Cells and TLC

The cells were rinsed on a buchner funnel and then immerged in liquid N_2. The material was ground in a mortar with chilled 80% methanol containing an antioxydant, BHT (0.45 mM). The homogenate was stirred for 2 h at 4°C and centrifuged for 5 min at 2000*g*. The pellet was reextracted twice with the same volume of cold 80% methanol. After the last centrifugation, about 98% extraction of radioactivity was found in aqueous methanolic supernatants. The supernatants were collected, evaporated under reduced pressure at 40°C, and chromatographed on Merck precoated Silica-Gel F254 plates developed in the solvent system used by Zeevaart and Milborrow (27): toluene-ethyl acetate-acetic acid (50/30/4; v/v). Each TLC was divided into half-R_F zones and the radioactivity counted (5): the solvent system allowed ABA (R_F 0.47) to be well separated from its oxidation metabolites: PA (R_F 0.33) and DPA (R_F 0.22), and from its conjugates (esters and glucosides) which remained on the start line.

Free Space Volume

Free space volumes were determined by equilibrating cells with radioactive mannitol, a nonpermeant compound, and quantifying the radioactivity associated. Two milliliters of stock cell suspension (100 mg of cells) were incubated with 1 mL of Na phosphate (0.2 M)/citrate (0.1 M) buffer (pH 5.0) with 2% sucrose and 12 KBq of D-[1-^{14}C]mannitol (2.22 GBq·$mmol^{-1}$, 99% radiochemical purity, Amersham, England).

Measurement of Intracellular pH with Chemical Probes

The technique is based on the preferential diffusion through the membranes of the undissociated molecules of radiolabeled probes (16).

The "overall intracellular pH" (14) was measured using the lipophilic weak acid, benzoic acid, as a probe. Two milliliters of stock cell suspension (100 mg of cells) were incubated with 1 mL of Na phosphate (0.2 M)/citrate (0.1 M) buffer (pH 5.0) with 2% sucrose (w/v) and 36 KBq of [carboxyl-^{14}C]benzoic acid (2.1 GBq·$mmol^{-1}$, 98.5% radiochemical purity, Amersham, England). Metabolism of [^{14}C]benzoic acid was determined after chromatography of an aliquot of the extracts on silicagel plates developed in isopropanol-ammonia-water (8/1/1, v/v); it was negligible after 5 or 10 min incubation and did not exceed 13% of total benzoic acid absorbed after a 3-h incubation.

Vacuolar pH was measured in the same way using 49 KBq of the weak base [7-^{14}C]benzylamine (2.15 GBq·$mmol^{-1}$, 99% radiochemical purity, Amersham, England) as a probe (15). Metabolism of [^{14}C]benzylamine was determined after chromatography of an aliquot of the extracts on silicagel plates developed in ethyl acetate-water-acetic acid (60/60/20, v/v); no degradation product was observed even after a 5-h incubation.

These probes dissociated outside and inside the cell according to the pH of the compartments. The intracellular and extracellular total concentrations of the probe were measured at the equilibrium. The corresponding accumulation ratio was a simple function of the extracellular pH, intracellular pH, and pKa of the probe, as shown first by Waddel and Butler (25).

The cytoplasmic pH value is derived from the pH_i, taking into account the pH_v and the relative volume of the cytoplasm (14). In spite of its limitations (16), this method gave a good estimation of the mean value of pH_c of a population and of its variations due to chemical treatments.

Replication

All experiments were performed at least three times. For more replicates, statistical data are given.

RESULTS

Intracellular pH of *Amaranthus* Cells and Its Variations

In the 5-d-old suspensions used for all the experiments, cells were in the middle of the exponential phase; they were therefore small, actively dividing and often grouped in small aggregates (diameter less than 0.3 mm).

Free space volume, determined by equilibrating cells with radioactive [^{14}C]mannitol gave an estimate of about 20% at diffusion equilibrium. The uptake of the weak lipophilic acid probe, benzoic acid, was rapid and the equilibrium was reached within 10 min; its accumulation expressed as the ratio

Table II. *Changes in Intracellular pH after Addition of Different Metabolic Inhibitors*

Values are the means ± SE (n = 6).

	Vacuolar pH	Cytoplasmic pH
Control	5.18 ± 0.02	7.09 ± 0.02
CCCP (10 μM) with 20 min preincubation	5.65 ± 0.05	6.44 ± 0.11
Oligomycin (10 μM) with 20 min preincubation	5.19 ± 0.01	6.76 ± 0.03
KCN (1 mM) with 60 min preincubation	5.16 ± 0.01	6.91 ± 0.06

of concentration of radioactivity inside the cells (C_i) to that in the external medium (C_e) was then 11.21 ± 0.7. Applying the equation of Waddell and Butler (25), gave a pH_i estimate of 6.07 ± 0.02 (10 replicates).

The uptake of benzylamine was slower and the equilibrium was reached only after 1 h. Vacuolar pH could be thus estimated to be 5.18 ± 0.02 (10 replicates).

Cytoplasmic pH could be calculated from these two components and was estimated to be 7.09 ± 0.02 in these control cells.

The effects of various metabolic inhibitors on the different components of the cell pH were also studied (Table II). The proton ionophore CCCP affected both vacuolar and cytoplasmic pH. It caused an increase of vacuolar pH and a decrease of cytoplasmic pH; *i.e.* it tended to reduce the pH gradient between the different compartments but did not eliminate it within the 20 min preincubation period. KCN and oligomycin lowered only the cytoplasmic pH.

Time Dependence of [2-^{14}C]ABA Uptake at pH 5.0 and Metabolism of ABA

The uptake of [2-^{14}C]ABA (2.7 μM, pH 5.0) was followed over 30 min at 23°C. At the initial point (5 s) some association of radioactivity with the cells was observed which was unaffected by the temperature of the incubation. This was assumed to represent uptake into the free space and was routinely substracted. Uptake was linear up to 15 min and then increased more slowly.

The fraction of absorbed [2-^{14}C]ABA that remained unmetabolized after different periods of incubation was determined by analysis of methanolic extracts. As shown in Table III, after 5 min incubation (2.7 μM, pH 5.0), conjugated metabolites and oxidative products contributed no more than 2.5% of the extracted ^{14}C. This percentage increased slowly during the first half-hour. The conjugation of ABA appeared of minor importance whatever the duration of incubation, while the major part of ABA metabolism was associated with oxidative products, mainly with DPA. Taking metabolic conversions into account, the average internal ABA concentration in cytosol exceeded the external concentration after about 2 to 3 min. Uptake times of 5 min were therefore routinely used to obtain the initial rate.

Table III. *Changes in Metabolism of [2-^{14}C]ABA with Times of Incubation*

Values are the means ± SE (n = 5).

Incubation Times	Distribution of Radioactivity			
	Nonmetabolized ABA	Oxidation products		Conjugated metabolites
		PA	DPA	
min		%		
5	97.5 ± 0.3	0.4 ± 0.1	1.1 ± 0.2	1 ± 0.1
10	97 ± 0.4	0.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
15	96.5 ± 0.3	0.5 ± 0.2	1.7 ± 0.2	1.3 ± 0.3
30	95 ± 0.6	0.8 ± 0.2	3.2 ± 0.4	1 ± 0.1
60	91 ± 0.5	1.2 ± 0.2	6.2 ± 0.3	1.6 ± 0.2

Concentration Dependence of [2-^{14}C]ABA Uptake

Uptake of [2-^{14}C]ABA at 23°C and pH 5.0 was studied as a function of its concentration. The total uptake of [2-^{14}C]ABA increased linearly up to a concentration of about 10 μM; then it slowed for higher concentrations (Fig. 1a). This result was consistent with a biphasic system: an unsaturable component could be plotted graphically as a straight line passing through the origin; a saturable component could be derived by subtracting the unsaturable component from the total uptake.

This biphasic system could be approached by another type of experiment. When increasing concentrations of nonradioactive ABA were added simultaneously to a low concentration of [2-^{14}C]ABA (2.7 μM), uptake of [2-^{14}C]ABA was inhibited; it decreased sharply at first as ABA concentration rose to 10 μM and then almost leveled off above 10 μM (Fig. 2). This result showed again that, at low concentration, saturable uptake occurred in parallel with a linear nonsaturable component, while at higher concentrations when the saturable component ceased to increase, the diffusible component predominated. This approach is useful for the experimental determination of the linear nonsaturable component. Indeed in the presence of a large excess of nonradioactive ABA (33

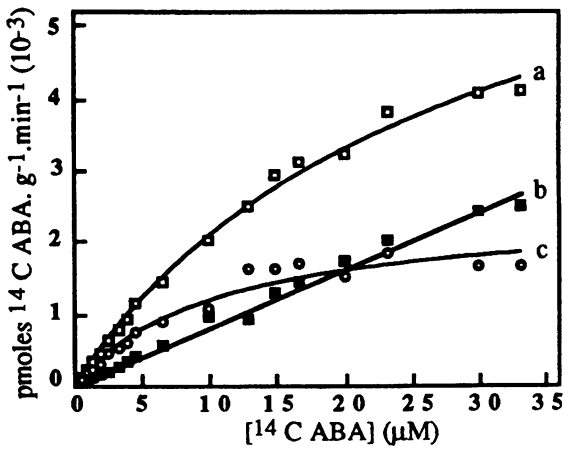

Figure 1. Concentration dependence of uptake of [2-^{14}C]ABA (23°C, 5 min incubation) in the absence (a) or in the presence (b) of an excess of nonradioactive ABA (33 μM). The saturable component (c) could be calculated as the difference between uptake with and without nonradioactive ABA.

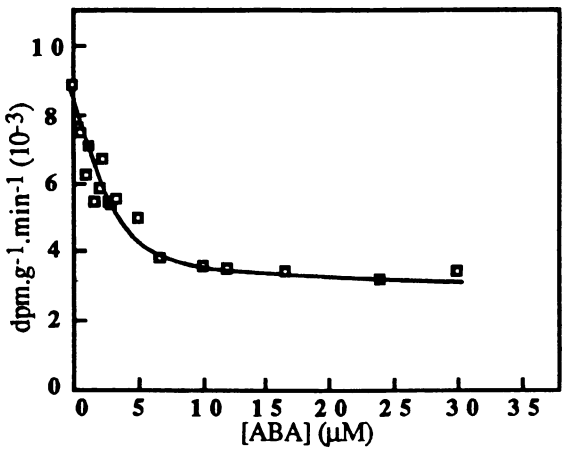

Figure 2. Effect of increasing concentrations of nonradioactive ABA on uptake of [2-^{14}C]ABA (2.7 μM) at pH 5.0, 23°C, after a 5 min incubation.

μM), the diffusible component alone was detectable (Fig. 1b); the saturable component (Fig. 1c) could then be deduced by comparing the uptake of [2-^{14}C]ABA in the absence or in the presence of this excess of nonradioactive ABA. This method was used to determine the respective contributions of the two components throughout the rest of this work.

The double reciprocal plot of specific uptake and [2-^{14}C] ABA concentrations allowed the kinetic parameters of the saturable component to be calculated (Fig. 3). At pH 5.0, K_m = 3.74 ± 0.43 μM and V_{max} = 1.5 ± 0.12 nmol·g^{-1}·min^{-1}. In the light of these results, further experiments, performed in order to determine the properties of this saturable component, used routinely a [2-^{14}C]ABA concentration of 2.7 μM. Uptake by frozen-and-thawed cells over a 5 min incubation was considerably reduced (about 27 pmol per gram of fresh weight). Uptake by intact cells at 0°C was lowered to about 13 to 14% of its value at 23°C (Table IV). In this case, both

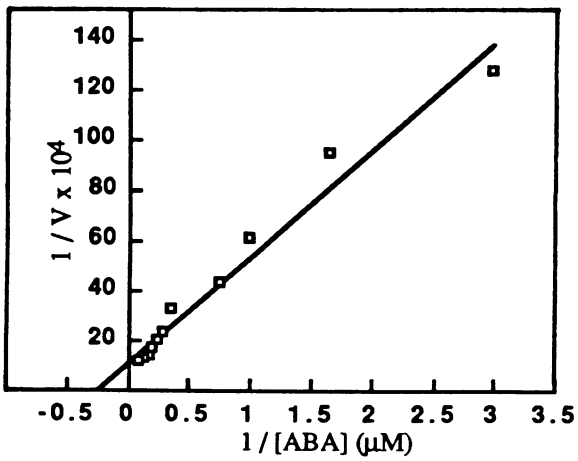

Figure 3. Double-reciprocal plots of specific uptake and [2-^{14}C]ABA concentration.

Table IV. *Comparison of the Total Uptake of [2-^{14}C]ABA (2.7 μM [pH 5.0], 5 min Incubation) and Its Diffusive and Saturable Components at 0 and 23 °C*

Results are expressed in pmol·g^{-1}·min^{-1}. The uptake at 0°C was also expressed in percentage of that at 23°C.

	Experiment 1		Experiment 2	
	23°C	0°C	23°C	0°C
Total uptake	477	68.5 (14%)	712.4	94.6 (13%)
Diffusion	236	26.4 (11%)	227.6	24.9 (11%)
Saturable component	241	42.2 (17%)	484.8	69.7 (14%)

diffusive and saturable components were affected to about the same extent (11 and 14–17%, respectively).

pH-dependence of [2-^{14}C]ABA Uptake

Figure 4 (curve a) shows a marked dependence of [2-^{14}C] ABA uptake on external pH. Uptake at pH 3 was more rapid than at higher pH. Concerning the nonsaturable component (curve b) obtained in the presence of 33 μM nonradioactive ABA, the pH-dependence of [2-^{14}C]ABA uptake showed the typical sigmoidal curve expected for uptake of a lipophilic weak acid with a midpoint about 4.5, comparable to the pK of ABA.

The difference between the measured curves in the presence (curve b) and absence (curve a) of nonradioactive ABA gives the pH dependence of the saturable component (curve c). A slight maximum appeared at pH 4.0. The largest relative contribution of the saturable component to net uptake occurred at pH 5.0.

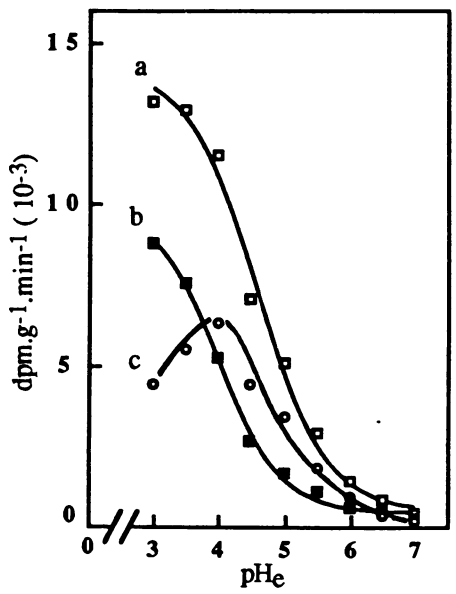

Figure 4. Dependence on pH of uptake of [2-^{14}C]ABA (2.7 μM, 23°C, 5 min incubation) in the absence (a: total uptake) or in the presence (b: diffusive component) of an excess of nonradioactive ABA (33 μM). Carrier-mediated uptake (c) was calculated as the difference between uptake with and without non-radioactive ABA.

Table V. *Temperature Effect on the Total Uptake of [2-^{14}C]ABA (2.7 μM [pH 5.0], 5 min Incubation) and on its Diffusive and Saturable Component*

Q_{10} was determined after measurements at 17 and 27°C.

Expt.	Effect of Temperature (Q_{10} between 17 and 27°C)		
	Total uptake	Nonspecific component	Specific component
1	1.65	1.21	1.86
2	1.57	1.38	1.82
3	1.6	1.28	1.79

Temperature and Metabolic Inhibitors Effects

To test for the presence of energy-dependent transport, ABA uptake was measured at 17 and 27°C. For these experiments, cells were maintained 1 h at the incubation temperature prior to addition of labeled ABA (Table V). Regarding the total uptake, the Q_{10} between 17 and 27°C was about 1.6. The Q_{10} for the passive component determined after adding an excess of nonradioactive ABA, was about 1.29 and the Q_{10} for the specific saturable component was 1.85, suggesting the presence of an energy-dependent component.

To determine if the uptake required metabolic energy, we have also used inhibitors of mitochondria function (oligomycin, KCN) and CCCP, a molecule known to be capable of affecting the pH gradient. The proton ionophore CCCP, added at 10 μM without preincubation, markedly inhibited total [2-^{14}C]ABA uptake which was reduced by 38 to 45% after 5 min incubation and even 65% after 20 min incubation. If it was applied with a 20 min preincubation (Table VI), the diffusive component was only reduced by 11% while the carrier-mediated component was almost totally inhibited (93%). KCN (1 mM) was not very effective if added without preincubation. After 1 h preincubation, it inhibited more than 90% of the total uptake; specific uptake was then totally inhibited. Oligomycin (10 μM), applied with a 20 min preincubation was strongly effective; total uptake was lowered to about 20%, the specific component was totally inhibited (97%); the diffusive component was affected to a lesser extent and was only reduced by 38%.

Table VI. *Effect of Metabolic Inhibitors on the [2-^{14}C]ABA Total Uptake and on its Two Components*

Values are the means ± SE (n = 6).

	Inhibition		
	Total	Diffusive	Carrier-mediated component
		%	
CCCP (10 μM)			
Without preincubation	43.5 ± 3.1	5 ± 0.3	93 ± 1.8
With 20 min preincubation	65 ± 0.3	11 ± 0.5	93 ± 1.3
KCN (1 mM) with 60 min preincubation	91.3 ± 0.8	77 ± 4.1	100 ± 0.9
Oligomycin (10 μM) with 20 min preincubation	78.3 ± 2.3	38 ± 2.8	97 ± 0.9

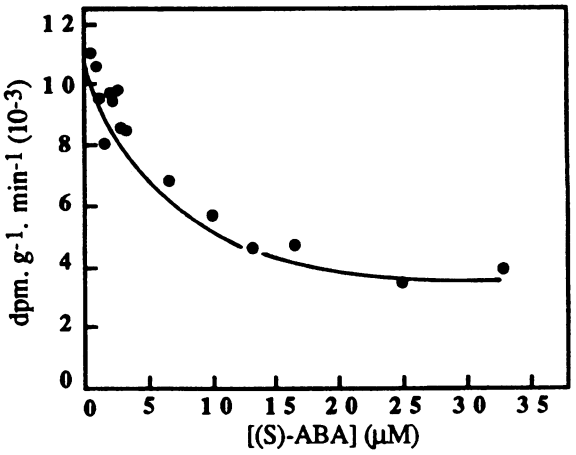

Figure 5. Effect of increasing concentrations of unlabeled (S)-ABA on [2-^{14}C]ABA uptake.

Carrier Specificity

To characterize the specificity of the transport process, and thus presumably of the carrier site itself, the ability of different chemicals to interact with the carrier was investigated.

The stereospecificity was tested by adding a range of concentrations of nonradioactive (S)-ABA (Fig. 5) or (R)-ABA enantiomer (Fig. 6) to the incubation media containing 2.7 μM (RS)-[2-^{14}C]ABA. Naturally occurring (S)-ABA, as well as its (R) enantiomer, inhibited (RS)-[2-^{14}C]ABA uptake to the same maximal extent as (RS)-ABA, *i.e.* to 70% of the total uptake (at 23°C [pH 5.0] and for a [2-^{14}C]ABA concentration of 2.7 μM) and with a K_m value of the same order: 3.74 μM ± 0.42 for (RS)-ABA, 3.8 μM for (S) enantiomer, 3.2 μM for (R) enantiomer. This similarity must be considered as significant since polyclonal antibodies (18) raised against (S)-

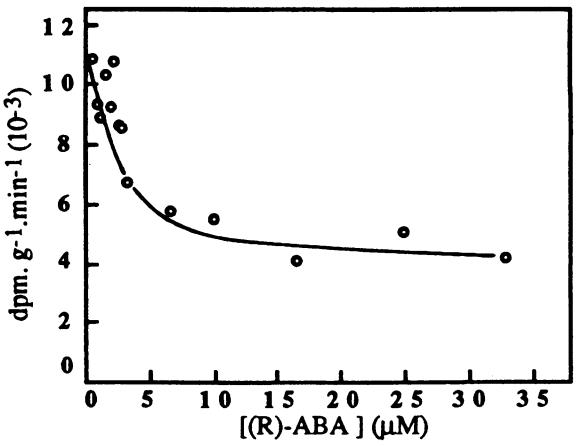

Figure 6. Effect of increasing concentrations of unlabelled (R)-ABA on [2-^{14}C]ABA uptake.

Table VII. *Effect of Various Compounds Added in High Concentration (33 μM) on the Total Uptake of [2-^{14}C]ABA (2.7 μM [pH 5.0], 23°C, 5 min Incubation)*

Values are the means ± SE (n = 6).

	Inhibition
	%
(RS)-ABA	73.3 ± 1.9
(S)-ABA	63 ± 2.1
(R)-ABA	67 ± 2.8
LAB 173711	45 ± 1.9
trans-ABA	8.5 ± 0.7
2,4-D	14 ± 0.3
IAA	12 ± 0.4
Kinetin	6 ± 0.4
GA_3	2.5 ± 0.3

ABA showed less than 0.5% of cross-reactivity with the sample of (R)-ABA.

The terpenoid ABA analog LAB 173711 (24), added at 33 μM, reduced total uptake by 45% (Table VII); the diffusive component was unaffected and at this concentration, the carrier-mediated uptake of 2.7 μM of [2-^{14}C]ABA at 23°C and pH 5.0 was inhibited by 78%. Added at a high concentration (33 μM), the naturally occurring *trans*-ABA isomer did not significantly affect the uptake of [2-^{14}C]ABA. A similar absence of inhibition was also observed (Table VII) for the other plant growth regulators which were tested: 2,4-D, IAA, kinetin, GA_3 (33 μM).

DISCUSSION

In agreement with Astle and Rubery (1–3) with roots or cell suspensions, we found that ABA uptake by *Amaranthus tricolor* included both a diffusion of the undissociated species (ABAH) and a saturable component. The diffusive component showed the typical pH dependence curve expected for uptake of a lipophilic weak acid. It was very sensitive to temperature and was nearly completely inhibited at 0°C. Such a high degree of sensitivity was already shown by Kaiser and Hartung (13) in mesophyll cells of *Papaver* and by Astle and Rubery (3) in *Phaseolus coccineus* cells. This behavior contrasts with uptake of IAA (13) and probably reflects the lower mobility of ABA than IAA through lipid membranes, at least at low temperatures.

The very low [2-^{14}C]ABA "uptake" by frozen-and-thawed cells over a 5 min incubation at 23 or 0°C, and the failure of nonradioactive ABA to decrease this uptake, indicate that over the time scale of the present experiment, the saturable component represents carrier-mediated uptake at the plasmalemma rather than intracellular binding. The characteristics of the carrier-mediated transport appear very similar to those which were described by Astle and Rubery (3) in *P. coccineus* cells. As for bean root tissue or runner bean cells (20) the carrier is specific for ABA among the plant growth regulators tested and is unaffected by (RS)-*trans*-ABA. However the specificity for (S)-ABA demonstrated in bean root (20) and in *P. coccineus* cells (4) was not found in *Amaranthus* cells; naturally occuring (S)-ABA and its (R) enantiomer inhibited (RS)-[2-^{14}C]ABA uptake to the same maximal extent and with the same K_m. However, such observations do not necessarily furnish a definitive proof that (R)-ABA is an alternative substrate for the carrier but only that it interacts with the carrier. The ABA analog LAB 173711 (24) also inhibits carrier-mediated ABA uptake.

Concerning possible driving forces, carrier-mediated ABA uptake increased as the external pH was lowered to pH 4.0, so it may partially be energized by ΔpH. The energy required to transport ABA into cells can be furnished by an artificially imposed pH gradient across the membrane; in our usual experimental conditions the imposed pH gradient between the cytoplasmic pH 7.09 and the pH of incubation medium (5.0) was 2.09. When the proton ionophore CCCP was added, the lowering of this ΔpH (to 1.44) decreased uptake. This energy for ABA movement could also be provided in these intact cells by a metabolically maintained pH gradient; lowering of the incubation temperature or adding some metabolic inhibitors which altered the mitochondrial function also resulted in a decrease of uptake, concomitant with a lowering of the ΔpH.

However, it appeared (Table VI) that the saturable component was more sensitive than the diffusive component to the various metabolic inhibitors. Such differences have already been reported by other authors: in sugar beet leaves and roots, the partial inhibition observed with metabolic inhibitors suggested to Daie and Wyse (8) that the ABA uptake was a combination of both energy-dependent and passive transport and they routinely used CCCP to separate energy-dependent and passive uptake; Martin and Pilet (19) also showed in maize root segment, that only the saturable component of the auxin uptake was sensitive to FCCP. Our results showed that within the limits of pH_c observed under the influence of metabolic inhibitors, ABA dissociation is only slightly affected. With the lowest pH_c observed (6.44 with CCCP), 98% of the ABA was in dissociated form compared with 99.5% at the initial pH 7.1; it is not surprising therefore that variations in accumulation by diffusion should be only slightly modified. Moreover, the importance of the duration of preincubation in the presence of CCCP and of incubation in the presence of ABA should not be neglected; Felle (10) observed no acidification of the cytoplasm of *Riccia fluitans* rhizoid cells after 5 min treatment in the presence of 25 μM CCCP. Kaiser and Hartung (13) showed that in mesophyll cells of *Papaver,* the inhibition of the passive component of ABA by CCCP increased with time.

Furthermore, it must not be forgotten that cell suspensions represent a multicompartmented system; each compartment is differentially affected by chemical treatments. Our results showed that only CCCP affected both the cytoplasmic pH and the vacuolar pH. With vacuolar preparations of *Catharanthus roseus,* Guern *et al.* (11) showed by a ^{31}P-NMR technique that pyrophosphate-induced vacuolar acidification was reversed by the addition of CCCP. In our experimental system, an increase of pH_v (from 5.18–5.65) could also be observed after 20 min preincubation with CCCP; a nonnegligible increase of the accumulation capacity in the vacuole could thus be induced since the percentage of dissociated molecule increased from 73 to 89%. Studies with membrane

vesicles or with vacuole preparations are now necessary to manipulate the hormone transport more easily.

ACKNOWLEDGMENTS

We thank Dr. W. Rademacher (BASF, Limburgerhof) for his generous gift of LAB 173711. The authors are very grateful to G. Garello and H. Le Bris for their excellent technical assistance.

LITERATURE CITED

1. **Astle MC, Rubery PH** (1980) A study of abscisic acid uptake by apical and proximal root segments of *Phaseolus coccineus* L. Planta **150:** 312–320
2. **Astle MC, Rubery PH** (1983) Carriers for abscisic acid and indole-3-acetic acid in primary roots: their regional localisation and thermodynamic driving forces. Planta **157:** 53–63
3. **Astle MC, Rubery PH** (1985) Uptake of abscisic acid by suspension-cultured *Phaseolus coccineus* L. cells: evidence for carrier participation. J Exp Bot **36:** 469–484
4. **Astle MC, Rubery PH** (1987) Carrier-mediated ABA uptake by suspension-cultured *Phaseolus coccineus* L. cells: stereospecificity and inhibition by ionones and ABA esters. J Exp Bot **38:** 150–163
5. **Barthe Ph, Bulard C** (1983) Anaerobiosis and release from dormancy in apple embryos. Plant Physiol **72:** 1005–1010
6. **Bianco-Colomas J, Hugues M** (1990) Establishment and characterization of a betacyanin producing cell line of *Amaranthus tricolor:* inductive effects of light and cytokinin. J Plant Physiol **136:** 734–739
7. **Clark KA, Goldsmith MHM** (1986) Roles of transport and binding in the specific ΔpH-dependent accumulation of auxin by zucchini membrane vesicles. *In* M Bopp, ed, Plant Growth Substances. Springer-Verlag, Berlin, pp 203–208
8. **Daie J, Wyse R** (1983) ABA uptake in source and sink tissues of sugar beet. Plant Physiol **72:** 430–433
9. **Drake GA, Carr DJ** (1981) Flux studies and compartmentation analysis of gibberellin A_1 in oat coleoptiles. J Exp Bot **32:** 103–119
10. **Felle H** (1989) pH as a second messenger in plants. *In* WE Boss, DJ Morre, eds, Second Messengers in Plant Growth and Development. Alan R Liss, New York, pp 145–166
11. **Guern J, Mathieu Y, Kurkdjian A, Manigault P, Manigault J, Gillet B, Beloeil JC, Lallemand JY** (1989) Regulation of vacuolar pH of plant cells. II. A ^{31}P NMR study of the modifications of vacuolar pH in isolated vacuoles induced by proton pumping and cation/H^+ exchanges. Plant Physiol **89:** 27–36
12. **Heilmann B, Hartung W, Gimmler H** (1980) The distribution of abscisic acid between chloroplasts and cytoplasm of leaf cells and the permeability of the chloroplast envelope for abscisic acid. Z Pflanzenphysiol **97:** 67–68
13. **Kaiser WM, Hartung W** (1981) Uptake and release of abscisic acid by isolated photoautotrophic mesophyll cells, depending on pH gradients. Plant Physiol **68:** 202–206
14. **Kurkdjian A, Guern J** (1978) Intracellular pH in higher plant cells. I. Improvements in the use of the 5,5-dimethyloxazolidine-2[^{14}C], 4-dione distribution technique. Plant Sci Lett **11:** 337–344
15. **Kurkdjian A, Guern J** (1981) Vacuolar pH measurement in higher plant cells. I. Evaluation of the methylamine method. Plant Physiol **67:** 953–957
16. **Kurkdjian A, Guern J** (1989) Intracellular pH: measurement and importance in cell activity. Annu Rev Plant Physiol Plant Mol Biol **40:** 271–303
17. **Le Page-Degivry M-Th, Barthe Ph, Prévost I, Boulon B** (1989) Regulation of abscisic acid translocation during embryo maturation of *Phaseolus vulgaris*. Physiol Plant **77:** 81–86
18. **Le Page-Degivry M-Th, Duval D, Bulard C, Delaage M** (1984) A radioimmunoassay for abscisic acid. J Immunol Methods **67:** 119–128
19. **Martin HV, Pilet PE** (1987) Effect of pH on IAA uptake by maize root segments. Plant Physiol **83:** 262–264
20. **Milborrow BW, Rubery PH** (1985) The specificity of the carrier-mediated uptake of ABA by root segments of *Phaseolus coccineus* L. J Exp Bot **36:** 807–822
21. **Murashige T, Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497
22. **Nour JM, Rubery PH** (1984) The uptake of gibberellin A_1 by suspension-cultured *Spinacia oleracea* cells has a carrier-mediated component. Planta **160:** 436–443
23. **Ohlrogge JB, Garcia-Martinez JL, Adams D, Rappaport L** (1980) Uptake and subcellular compartmentation of gibberellin A_1 applied to leaves of barley and cowpea. Plant Physiol **66:** 422–427
24. **Rademacher W, Maisch R, Liessegang J, Jung J** (1989) New synthetic analogues of abscisic acid: their influence on water consumption and yield formation in crop plants. *In* KH Kreeb, H Richter, TM Hinckley, eds, Structural and Functional Responses to Environmental Stresses. SPB Academic Publishing, The Hague, pp 147–154
25. **Waddel WJ, Butler TC** (1959) Calculation of intracellular pH from the distribution of DMO. Application to skeletal muscle of the dog. J Clin Invest **38:** 720–729
26. **Zeevaart JAD, Creelman RA** (1988) Metabolism and physiology of abscisic acid. Annu Rev Plant Physiol Plant Mol Biol **39:** 439–473
27. **Zeevaart JAD, Milborrow BV** (1976) Metabolism of abscisic acid and the occurrence of epi-dihydrophaseic acid in *Phaseolus vulgaris*. Phytochemistry **15:** 493–500