Uptake and Release of Abscisic Acid by Runner Bean Root Tip Segments

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Uptake of abscisic acid by 5 mm long decapped root tips is a linear function of the external ABA concentration in the range of 2.9×10^{-8} M to 10^{-4} M and decreases dramatically with increasing pH. At pH 8.0 uptake rate is extremely low, even at high ABA concentrations. This indicated that nearly all of the ABA is taken up as the undissociated molecule ABAH. Uptake of ABA is influenced by agents modifying the pH gradients between the medium and the tissue such as salts of weak acids incubated at low external pH (inhibition of uptake and stimulation of ABA release by abolishing the pH gradients), protonophores such as CCCP (inhibition of uptake) and fusicoccin (stimulation of uptake by increasing the pH between medium and cytoplasm). It is concluded that ABA distributes between the compartments of the root cells according to the pH gradients with the undissociated molecule as the only penetrating species. Uptake and release occur without participation of a saturable component by diffusion. In contrast IAA permeates the plasmalemma as both IAAH and IAA^-.

Introduction

ABA of plant root tissue plays an important role in several physiological processes such as growth promotion and inhibition [1–5], geotropism [2, 5–8] and ion uptake [9, 10]. Since the primary site of ABA action seems to be the plasmalemma and since ABA transport is involved in the above mentioned processes, the uptake and release across the root cell plasmalemma is of special importance.

Astle and Rubery studied the uptake of radioactive ABA into bean root segments [11, 13] and into cells of bean root suspension cultures [12]. They detected beside a large diffusion component a relatively small saturable uptake component. The latter could be demonstrated only in apical 4 mm long root tip segments at external pH values in the range of 4.5–6.0. No ABA carrier could be detected at pH values above 6.0 and in the more basal parts of the roots.

In contrast experiments with mesophyll cells [14, 15] and own preliminary data with runner bean roots (cv. Weißer Riese) gave evidence that transmembrane transport of ABA does occur by diffusion. We have therefore reinvestigated the problem of ABA uptake and release by root cells of runner bean tissue.

Abbreviations: ABA, abscisic acid; ABAH, undissociated ABA; ABA^-, anion of ABA; CCCP, *m*-chlorocarbonylcyanidephenylhydrazone; FC, fusicoccin; IAA, indoleacetic acid; IAAH, undissociated IAA; IAA^-, anion of IAA.

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Materials and Methods

Runner bean seeds (*Phaseolus coccineus*, cv. Weißer Riese) were soaked for 3 h in tap water and germinated in moist vermiculite. After 3–4 days 5 mm long root segments were cut off 1 mm behind the root tip. Thus the biggest part of the root cap was removed to avoid abundant slime. Segments contained the non vacuolated parts of the roots and the growth zone. Preliminary experiments with lateral root tips showed identical ABA transport characteristics as the main root tips. Therefore part of the experiments was performed with lateral roots. Uptake experiments were performed at 20 °C in darkness in 1 ml of a medium containing 10 mM KCl, 0.5 mM KH_2PO_4 radioactive ABA or IAA of different concentrations buffered with 25 mM MES/NaOH or HEPES/NaOH. Segments were washed 3 times with 1 ml ice cold medium containing cold ABA or IAA of the same concentration, dried with filter paper, homogenised in liquid nitrogen, extracted over night in 1 ml 80% methanol and counted in a scintillation counter. The rinsing procedure lasted not longer than 10 s. Within that time efflux of incorporated radioactivity was found to be negligible. The first measurement of

tissue radioactivity was done one second after [^{14}C]ABA incubation. The obtained value, which was always very low, was considered to be due to the amount of radioactivity absorbed at the cell wall space and was therefore substracted from the subsequent uptake data. Uptake rates were calculated from the linear time course between 1 and 10 min. Within this time ABA metabolism was found to be negligible. [^{14}C]ABA, [^{3}H]ABA and [^{14}C]IAA were obtained from Amersham-Buchler, Braunschweig. The specific radioactivity was 348 MBq mmol^{-1}, 636 GBq mmol^{-1} and 2.04 GBq mmol^{-1} respectively. Experiments were performed 3–5 times with identical results.

Results

Kinetics of ABA uptake

At an external pH of 5.0 and an ABA concentration of 2×10^{-5} M uptake was nonlinear with time reaching equilibrium after about 60 min. At 0 °C uptake of ABA was extremely low, indicating that absorption to the cell wall material was negligible (Fig. 1). The rate of uptake was a linear function of the ABA concentration in the medium in the range of $10^{-6} - 6 \times 10^{-5}$ M at pH values between 5.0 and 8.0 (Fig. 2). At pH 8.0 uptake of ABA into root tissue was extremely low.

In the next experiment (Fig. 3) we applied the technique of Astle and Rubery [11–13]. To each sample containing 18.5 KBq ml^{-1} [^{3}H]ABA (equivalent to 2.9×10^{-8} mol l^{-1}) increasing amounts of non labelled *cis-trans* ABA were added up to a final concentration of 10^{-4} mol l^{-1}. The external pH was 5.5. In the presence of a saturable component in the tissue radioactivity should decrease upon addition of non labelled ABA. However, no significant decrease of label at low ABA concentration compared to the label at high ABA concentration in the tissue could be detected. This indicates that ABA transmembrane transport occurs under acid conditions by simple diffusion (Fig. 3) and excludes a significant contribution of an ABA carrier.

At a given external ABA concentration (5×10^{-5} mol l^{-1}) uptake rates decreased dramatically with increasing pH. At pH 8.0 uptake is extremely low indicating that ABAH is the only ABA species penetrating the root cell plasmalemma (Table I).

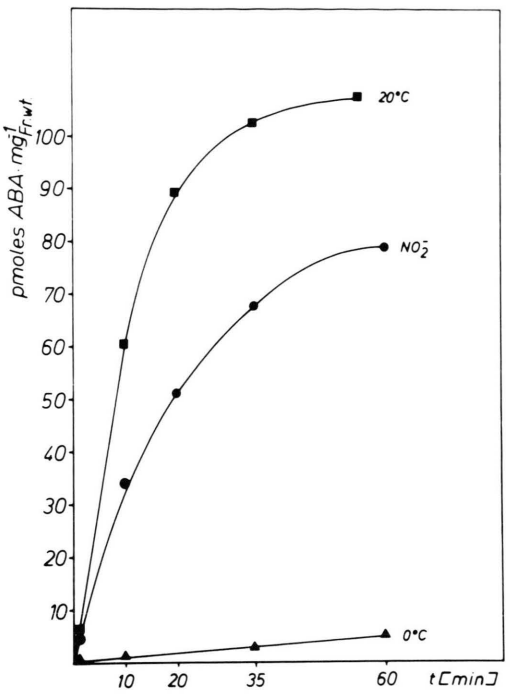

Fig. 1. Uptake of ABA into root tip segments of runner bean at room temperature and at 0 °C and treated with 10 mM KNO_2. pH of the medium was 5.0, external ABA concentration: 2×10^{-5} M. At the end of the experiment no significant effect of the nitrite treatment on elongation growth could be observed. It is therefore concluded that this treatment has no severe toxic effects on the roots.

Table I. Effect of external pH on uptake of radioactivity from [^{14}C]ABA in tissue of apical segments of runner bean roots. ABA concentration in the medium: 5×10^{-5} M.

pH	ABA uptake [pmol mg^{-1} fr. wt. h^{-1}]
5.0	185.7 ± 16.7
6.0	71.5 ± 6.5
7.0	22.7 ± 4.8
8.0	5.0 ± 4.4

pH-Gradients across the membranes of plant cells can be abolished when cells or tissues are incubated with salts of weak acids such as KNO_2 or Na-acetate at low external pH [14]. If ABA uptake is driven by pH gradients across the plasmalemma addition of KNO_2 at an external pH of 5.0 should inhibit ABA uptake. Fig. 1 demonstrates that 10 mM KNO_2 inhibits ABA uptake. pH gradients are diminished

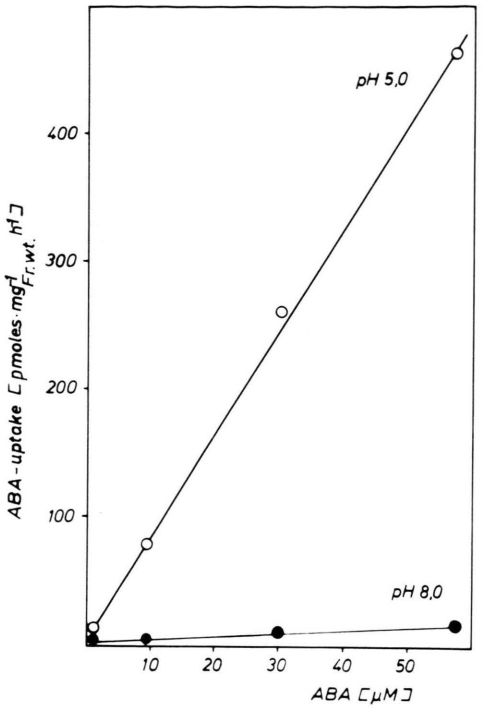

Fig. 2. Rates of ABA uptake depending on the ABA concentration in the medium.

Table II. Effect of 5 µM CCCP and 15 µM FC on uptake of radioactivity from [^{14}C]ABA into apical root segments of runner bean. ABA concentration in the medium: 5×10^{-5} M. After 60 min the pH of the unbuffered FC containing medium dropped to 5.53.

Treatment	pH of the medium	Rate of ABA uptake [pmol mg^{-1} fr. wt. h^{-1}]
Control, unbuffered	6.0	19.0 ± 2.1
15 µM FC, unbuffered	6.0	109.6 ± 9.5
Control, buffered	6.0	13.8 ± 3.5
15 µM FC, buffered	6.0	30.2 ± 5.8
Control, buffered	5.5	116.3 ± 21.0
5 µM CCCP, buffered	5.5	25.0 ± 1.8

also by protonophores such as 5×10^{-6} M CCCP or enlarged by 1.5×10^{-5} M FC, which stimulates the electrogenic H^+ efflux from plant cells resulting in an external acidification.

The latter causes elongation growth and hyperpolarisation of the plasmalemma [16]. Cytoplasmic pH is not changed, whereas vacuolar pH is decreased in *Acer pseudoplatanus* cell [17]. In unbuffered medium ABA uptake should be stimulated by FC by an increase of the ΔpH between the medium and the cytoplasm, whereas CCCP should inhibit ABA uptake. Indeed ABA uptake is inhibited by the CCCP treatment and stimulated by FC (Table II). The FC effect is much larger in unbuffered medium.

ABA release

When root segments were preincubated with 5×10^{-5} M ABA for 1 h and then transferred to ABA free medium, a slow efflux of ABA was observed. When cellular pH gradients were abolished by the nitrite treatment, ABA efflux was stimulated rapidly (Fig. 4).

ABA uptake in root segments of different species

ABA uptake studies were performed too with root tips of *Phaseolus coccineus* cv. Preisgewinner, *Lepidium sativum* and *Zea mays* cv. LG 11, and with cell suspensions obtained from *Phaseolus coccineus* root cultures. In all cases no saturable uptake component could be detected.

IAA uptake

In Table III the pH dependency of IAA uptake into root tips of runner beans is shown. Similarly as described for ABA, IAA uptake decreases with in-

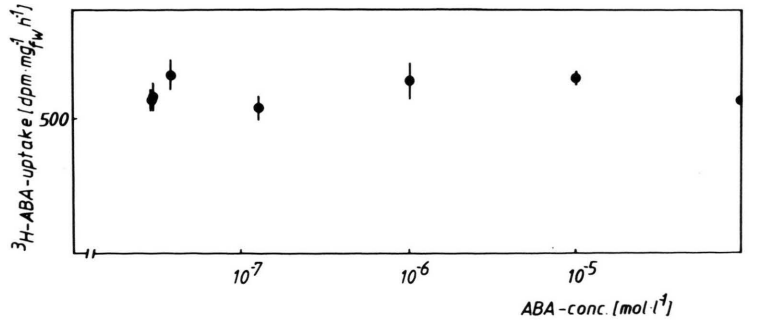

Fig. 3. Effect of increasing concentration of non radioactive cis-trans-ABA on uptake of radioactivity from (^{3}H)ABA (18.5 KBq ml^{-1}) into apical root segments of runner bean at an external pH of 5.5.

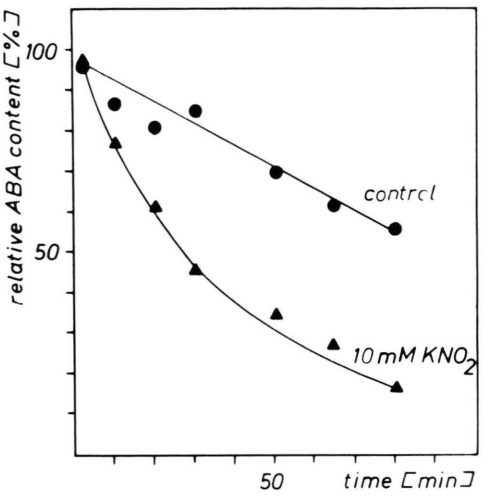

Fig. 4. Release of [^{14}C]ABA from preloaded segments. 16 segments were preincubated with 10^{-5} M ABA for 1 h at pH 5.0. After preincubation segments were washed with ABA free ice cold medium as described in materials and methods and transferred to 1 ml medium at pH 5.0 ± 10 mM KNO_2. Immediately after transfer one sample was removed and measured. This was taken as the 100% value at time zero. Further samples were taken at the time intervals as indicated in the figure. At zero time ABA content was 110 pmol mg^{-1} fr. wt.

Table III. Effect of external pH on uptake of radioactivity from [^{14}C]IAA into tissue of apical segments of gunner bean roots. IAA concentration in the medium: 10^{-5} M.

pH	IAA uptake [pmol mg^{-1} fr. wt. h^{-1}]
5.0	3814 ± 287
6.0	463 ± 29.3
7.0	226.8 ± 20.5
8.0	90.7 ± 4.2

creasing external pH. However, at pH 8.0 a much higher IAA amount (in the range of nmoles compared to a few pmoles in the case of ABA) is taken up by the root tissue, indicating that IAA^- is much more permeable for the root cell plasmalemma than ABA^-.

Discussion

It was shown previously [11–13] that ABA uptake by basal parts of main and lateral bean roots occurs by diffusion without participation of a carrier. Additionally it was found that ABA uptake into the apical root segments was only carrier mediated at external pH values from 4.5 up to 6.0. At more alkaline conditions ABA was taken up just by diffusion. The saturable component at low external pH accounted for 10–15% of total uptake [13]. We can confirm those data with the non elongating basal segments and with apical segments at external pH higher that 6.0. In contrast to the above mentioned data, however, we are not able to find a saturable uptake component at low pH (Figs. 2, 3) in the range of $2.9 \times 10^{-8} - 10^{-4}$ M ABA. At low pH values between 4.5 and 5.0 about 50% of the ABA molecules are present as ABAH, which can pass biomembranes readily by diffusion [14, 15, 18].

A transport carrier which plays a significant role for transmembrane transport of ABA should work at pH values which we can expect in the compartments which are adjacent to the plasmalemma, *i.e.* the apoplast and the cytoplasm. It is well established fact that the cytoplasm is alkaline (pH 7.2–7.4). However, we have very poor information on the apoplastic pH of root tissue. Experiments with roots of several species (barley, buckwheat, maize, sunflowers etc.) placed in agar medium containing the pH indicator dye bromocresolpurple [15, 19] show the pH of the root surface of the different root zones, which reflects the approximate apoplastic pH of at least the outer cortical cell layers. Experiments of Evans *et al.* [5, 19] and unpublished experiments of Eggers show that the pH of the surface of the apical root parts is in the range of 6.0–6.5 or even higher. At this range of pH values, however, Rubery can not find a saturable component of ABA uptake. A higher H^+ concentration can be observed on the surface of the root hair zone, where, according to Rubery, no ABA carrier is present.

The physiological role of a carrier mediated ABA transport, which accounts for only approximately 15% of total ABA uptake and which works only at very low pH values which we cannot expect in the apoplast of the apical root segments, remains obscure. ABA passes the plasmalemma of runner bean root cells by diffusion only in the undissociated form ABAH, being trapped in the more alkaline compartment (cytoplasm) as the impermeable anion ABA^-, similarly as already demonstrated earlier with mesophyll cells [14, 18]. An efflux compartmental analysis for ABA with barley roots was performed by Behl *et al.* [20] and showed a

nine-fold accumulation of external ABA in the cytoplasm. This finding agrees well with our results.

Treatments which alter pH gradients, alter ABA uptake and release. Nitrite and CCCP-treatments which abolish pH gradients, inhibit ABA uptake. FC incubation which increases ΔpH, stimulates ABA uptake. The results of the CCCP- and FC-experiments agree with earlier findings [11–14, 21]. FC stimulates uptake not only in unbuffered but also in buffered medium. This could be explained by an increased cytoplasmic pH although Guern *et al.* [17] could not detect FC effects on cytoplasmic pH of *Acer* cells. The FC effect gives also a new explanation of its action on guard cells. When ABA uptake from the apoplast (which is equivalent to our medium) is stimulated by FC, ABA is withdrawn from the free space and thus from the only compartment which is directly connected with the guard cells. Additionally to its effects on K^+-uptake [16], FC could act via a withdrawal of ABA from the apoplast of mesophyll cells.

The pH dependency of IAA uptake was studied to comare IAA with ABA, which have identical pK values (4.75). There are two differences between transmembrane transport of ABA and IAA. At comparable external concentration IAA uptake rates are much higher (nmol mg^{-1} fr. wt. h^{-1}) as ABA uptake rates (pmol mg^{-1} fr. wt. h^{-1}). This observation agrees well with mesophyll cell data [14] and with the fact that the permeability coefficient of the plasmalemma for IAA is much higher as for ABA [15]. IAA is taken up, especially at high external pH, to a considerable amount not only as IAAH but also as IAA^-, whereas ABA^- seems to be impermeable as already demonstrated for mesophyll cells and guard cells [14, 22]. This can be due to the fact that IAA is more lipophilic and has a higher permeability coefficient than ABA [15].

A similar high permeability of the plasmalemma of crown gall cells for IAA^- was explained earlier [23] by the action of an IAA anion carrier.

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Note added in proof:

Since this paper was submitted, Daie and Wyse [1983] have published on ABA-uptake in sugarbeet root tissue. They have also concluded that ABA moves across the membrane as ABAH. However, they suggested that metabolic energy is necessary for the establishment of intracellular pH gradients. Their results are consistant with our findings.

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