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The Proton Electrochemical Transmembrane Gradients Generated by the Transfer Cells of the Haustorium of *Polytrichum formosum* and Their Use in the Uptake of Amino Acids

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ABSTRACT

The epidermal cells of the sporophyte haustorium of *Polytrichum formosum* are modified into transfer cells. These cells are located in a strategic place allowing them to control the exchanges between the two generations. Their plasmalemma creates proton gradients ($\Delta\psi$ and ΔpH) which increase during the development of the sporophyte. As the sporophyte grows from 2 to 4 cm long, the pH of the incubation medium of the haustoria decreases from 5.2 to 4.3, and the transmembrane potential difference (PD) hyperpolarizes form −140 to −210 millivolts. These gradients become rapidly larger than that generated by the plasmalemma of the basal cells of the sporophyte. They are used to energize the uptake of the solutes present in the apoplast of the gametophyte, particularly the amino acids. Below 20 micromolar α-aminoisobutyric acid uptake in the transfer cells is mediated by a saturable system and is optimal at acidic pH (4.0 and 4.5). It is strongly inhibited by compounds dissipating both $\Delta\psi$ and ΔpH (10 micromolar carbonylcyanide-*m*-chlorophenyl hydrazone) or only $\Delta\psi$ (0.1 molar KCl). The absorption of α-aminoisobutyric acid and of the other neutral amino acids tested induces an alkalinization of the medium and a depolarization of membrane potential difference which is concentration dependent. These data show that the uptake of amino acids by the transfer cells of the haustorium is a secondary translocation (proton-amino acid symport) energized by a primary translocation (proton efflux). More particularly, they show that transfer cells possess a membrane enzymic equipment particularly efficient to achieve the uptake of the solutes leaked in the apoplast from other cell types.

For 20 years, transfer cells, which are widely distributed in the plant kingdom, have held the attention of many researchers (12–14, 24, 26 and references therein). These cells, which result from the transformation of different cellular types, are characterized in particular by internal wall ingrowths that increase 5- to 10-fold the surface area of the plasmalemma and by numerous mitochondria. They are often found in contact with conducting cells (sieve-tubes or vessels), at the interface of the two generations (gametophyte-sporophyte), or at the plant-environment interface. Due to their structural particularities and their distribution, it has been suggested that they are involved in apoplast-symplast exchanges (13, 26).

Transfer cells that border conducting cells are not easily accessible for experimentation. However, the peripheral position of epidermal transfer cells allows a direct study of their properties, in particular by electrophysiological methods. In this respect, the epidermal transfer cells of the haustorium of bryophytes (14) deserve particular interest. Located at the interface of the two generations, they have a possible role in the loading of sporophyte symplast, in the same way as the phloem transfer cells of higher plants allow the loading of the conducting cells symplast. In the former case, the solutes originate from the gametophyte apoplast (6, 7, 14), and in the latter case, they originate from the leaf apoplast (9). Consequently, the study of their functioning should yield important information on the properties of exchanges between the gametophyte and the sporophyte. Another interest of the transfer cells of the haustorium of bryophytes lies in the absence of symplastic connections with the gametophyte (8, 14): only the apoplastic pathway is operating. Consequently, the properties of exchanges between the two generations are highly dependent on the properties of the plasmalemma of this cell type. The transfer cells which mediate the loading of the sieve tubes in higher plants do not exhibit this structural advantage since they communicate with the surrounding parenchyma cells by some plasmodesmata. Therefore, the possibility of a contribution of the symplastic pathway to phloem loading is presently being examined by some authors (23, 30).

Polytrichum formosum is one of the species of bryophytes in which the haustorium is bordered by epidermal transfer cells (8, 14, 24). Preliminary studies (7) have shown that the haustorium of this species acidifies the incubation medium and that this phenomenon is sensitive to ATPase inhibitors and metabolic uncouplers. Furthermore, the addition of glycine to the medium containing the haustorium induces a dramatic alkalinization of this medium. These results suggest that the uptake of glycine by the transfer cells is a secondary translocation (H^+-glycine symport) energized by a primary translocation process (H^+ extrusion) (7). In the present paper, the isolated sporophyte of Polytrichum was used as a model to study the electrochemical gradients created by transfer cells. Indeed, we were interested in determining whether this cell type not only increases the surface of exchange between the apoplast and the symplast but also generates electrochemical potential gradients different from those of the parenchyma

cells or from those of the cells from which its originates. As emphasized in the discussion, the data presently available (3, 16, 21, 27) do not provide a clear answer to this question. The other aim of this paper is to examine whether the electrochemical potential gradients generated by the transfer cells of the haustorium are used indeed to achieve active uptake of the organic solutes present in the apoplast of the gametophytes, as it was suggested by our previous results (6, 7), and then to bring information on the properties of exchanges between the two generations. The substrates selected for this study are α-AIB[1] and some other amino acids, since the gametophyte supplies several amino acids to the sporophyte during the development of this latter organ (29).

MATERIALS AND METHODS

Plant material

Polytrichum formosum sporophytes were separated from the gametophyte by gentle pulling. This operation damaged neither the structural integrity of the transfer cells nor the uptake capacity of the haustorium (8). Sporophytes were then placed vertically with their haustoria set in a medium of particular composition according to the experiment, but which always contained 0.125 mM $CaCl_2$, 0.125 mM $MgCl_2$ and 0.100 mM KCl (standard medium). Isolation of the sporophytes transiently depolarized the PD, and the initial PD was restored after 1 h (data not shown). Therefore, experiments were started 1 h after sporophyte isolation. The temperature was 20 ± 1°C and light intensity was 12 Wm^{-2} (Sylvania fluorescent tubes). Unless otherwise stated, sporophytes 3.0 ± 0.5 cm long were used.

pH Measurements

One hundred sporophytes of *P. formosum* were isolated and affixed vertically to the wall of a 50 mL beaker with an inert paste (terostat), their haustoria dipping into 10 mL of standard medium (pH 6.0). Experiments were made with a PHM 62 pH meter (Radiometer, Copenhagen), using the K 4040 and G 2040 C electrodes. The pH meter was connected to a REC 61 Servograph recorder provided with a REA 160 Titrigraph module. Sporophytes 2 to 4 cm long were used.

Electrophysiological Measurements

Sporophytes were separated at different stages of development and were incubated in the standard solution buffered with 10 mM diNa-citrate and 20 mM diNa phosphate at the required pH (see "Results"). The sporophyte was fixed in a cuvette with terostat. Electrophysiological measurements were made in a Faraday cage, with the equipment previously described (25). Briefly, a glass micropipette (tip diameter < 1 μm, tip resistance 15 to 20 mΩ) and a reference micropipette (diameter 1 mm, filled with 3 M KCl in 1% agar) were connected to an electrometer-amplifier (model M707, WP Instruments, New Haven, CT). The output signal of the amplifier was monitored with an oscilloscope (DM 64, Telequipement, Tektronix UK Ltd, London, UK) and a chart recorder (Kipp and Zonen, Delft, The Netherlands). After 1 h preincubation the reference electrode were dipped into the bathing medium, and the glass micropipette was inserted into a transfer cell with a mechanical micromanipulator.

Intracellular pH Measurements

H^+-selective liquid membrane microelectrodes (1) were prepared with glass capillaries (GC 150-T 15, Clark Medical Instruments, Phymep, Paris). The microelectrodes were silanized by heating for 30 min at 200°C in the presence of *N*-trimethylsilyl dimethyl amine and filled with a few μl of resin (10.5 mg of Na-tetraphenylborate, 151.5 mg of tridodecyl amine and 1338.0 mg of nitrophenyloctylether) saturated with CO_2. The resine was backfilled into the tip with a long glass micropipette under microscopic control. The remainder of the electrode was then filled with a buffer (0.04 M KH_2PO_4, 0.023 M NaOH, and 0.015 M NaCl). The H^+-selective microelectrode and the reference micropipette (filled with 3 M KCl in 1% agar) were connected to an electrometer-amplifier (model FD 223, WP instruments, Inc.) and to a chart recorder (Kipp and Zonen, Delft, The Netherlands). The pH measurements were made in a Faraday cage. The sporophytes were prepared as previously for electrophysiological measurements. After calibrating the pH-selective microelectrode in a series of buffer solutions (pH 6.6 to pH 9.0) (Fig. 1), a transfer cell was impaled with the H^+-selective microelectrode. After measurement, the H^+-selective microelectrode was recalibrated (pH 9.0 to pH 6.6) (Fig. 1). Intracellular pH of transfer cells was measured by difference between the values obtained by H^+-selective microelectrodes and PD measurements (19). These intracellular pH values were statistical evaluations because the mean of PD measurements was subtracted from the values obtained by H^+-selective microelectrodes.

[α-^{14}C]AIB Uptake

For preincubation, incubation and rinsing, the sporophytes were fixed to a glass microscope slide, to allow easy and quick handling. Just the base (4 mm) of the sporophyte containing the transfer cells was in contact with the medium (Fig. 2). After 1 h preincubation in the medium, sporophytes were divided into groups of 12. Each group was incubated in 2 ml of standard medium containing 2.5 mM [α-^{14}C]AIB (16.5

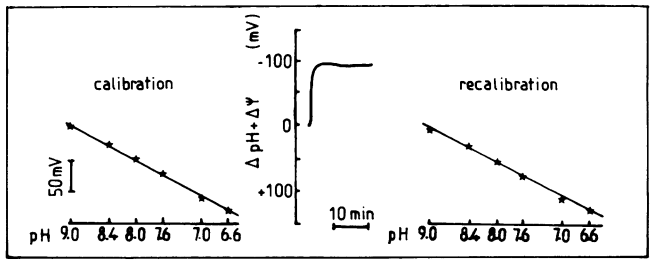

Figure 1. Measurement of cytoplasmic pH of transfer cells. After calibration the pH microelectrodes indicated ΔpH + Δψ. After impalement, the pH microelectrodes were recalibrated (pH 9.0 to 6.6).

[1] Abbreviations: α-AIB, α-aminoisobutyric acid; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; PD, transmembrane potential difference; $V_{max.}$ maximal velocity.

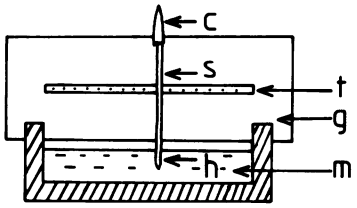

Figure 2. Device used for uptake experiments. For each condition tested, 12 sporophytes were fixed to a microscope slide. c, calyptra; s, seta; h, haustorium; t, terostat; g, glass microscope slide; m, incubation medium.

kBq) and buffered at pH 4.5 with 10 mM diNa citrate and 20 mM diNa phosphate. After uptake, the tissues were digested for 48 h in the darkness in the presence of 200 μL hyamine hydroxide and their radioactivity was counted by liquid scintillation spectrometry (Intertechnique SL 33) after addition of 10 ml scintillation cocktail (4 g 2,5-diphenyloxazole and 0.1 g 1,4-bis-2-[5-phenyloxazolyl]benzene in 1 L filtrated toluene). [α-^{14}C]AIB was supplied by Amersham France (Les Ulis).

RESULTS

pH Changes of the Incubation Medium

The rate of acidification of the medium which is sensitive to uncouplers, ATPase inhibitors and KCl (7) varied with stage of sporophyte development. After 1 h of incubation, the pH of the medium stabilized at pH 5.2, 4.6 and 4.3 ± 0.2 when sporophytes respectively 2, 3, and 4 cm high were used.

Measurements of Transmembrane PD of Transfer Cells

After a recovery phase (1 h), the PD measured was stable for at least 2.5 h (Fig. 3A). At pH 5.2, the measured PD ranged from −120 mV to −230 mV. This difference was mainly due to the hyperpolarization of PD during the development of the sporophytes (Fig. 3B): the values found were −142.3 ± 8.5 mV (mean ± SE, n = 9) for sporophytes 2 cm high, −180.5 ± 7.8 mV (mean ± SE, n = 9) for sporophytes 3 cm high and −213.6 ± 4.8 mV (mean ± SE, n = 9) for sporophytes 4 cm high. The PD then became less negative as the sporophyte completed its development. The PD was slightly sensitive to the external pH from pH 4.0 to 6.0 and did not vary from pH 4.5 to pH 5.5 (Fig. 3C).

In 3 cm-high sporophytes, the PD of the parenchyma cells at the basal extremity of the haustorium was only a third of the PD of the transfer cells. The PD of the epidermis capsula cells and the PD of the haustorium parenchyma cells were only half of the PD of transfer cells, and the PD of the cells of gametophytes leaves was also less negative than that of the transfer cells (Table I).

Effect of Various Effectors on PD

Addition of 10 μM CCCP induced an immediate depolarization, and the PD of the transfer cell reached about −100 mV (Fig. 4A). Another immediate depolarization followed a second addition of 10 μM CCCP. A third addition of CCCP

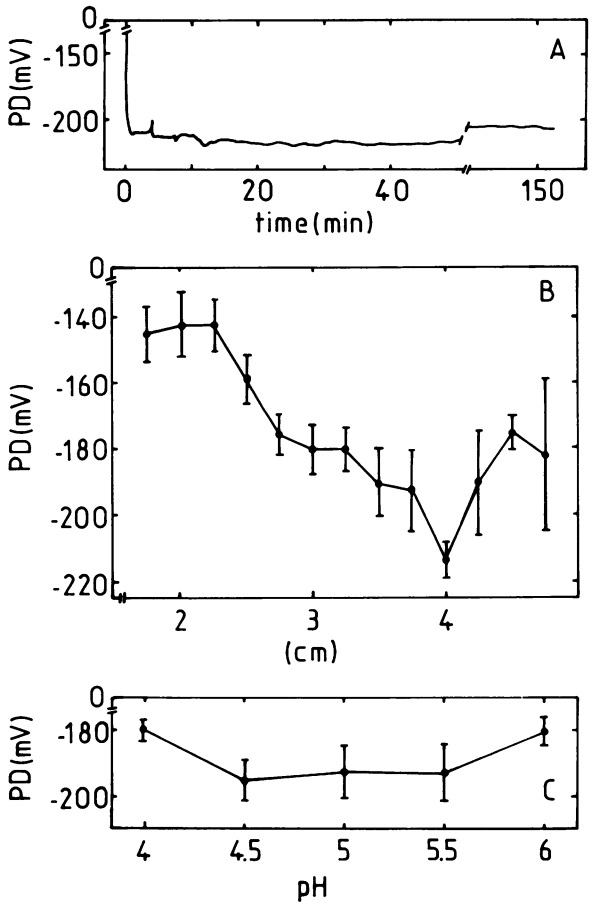

Figure 3. Various characteristics of the PD of *P. formosum* transfer cells. A, time course after impalement; B, changes during the development of the sporophyte (mean ± SE, $3 < n < 14$); C, pH dependence of the PD of *P. formosum* transfer cells (3.5 cm high sporophytes).

Table I. *PD of Different Cellular Types of P. formosum Sporophytes (Sporophytes 3 cm High Were Used, $n \geq 5$)*

Cellular Types	PD ± SE (mV)
Epidermis transfer cells of haustorium	−180 ± 8
Epidermis parenchyma cells of basal extremity of haustorium	−61 ± 5
Cortical parenchyma cells of haustorium	−105 ± 5
Epidermis capsula cells	−98 ± 4
Gametophyte leaf cells	−148 ± 8

induced no additional decrease of the PD which stabilized at values ranking from −60 to −50 mV, depending on the experiment and these values may represent diffusion potential. The protonophore was also tested on the epidermal capsula cells (Fig. 4B) and on the cortical parenchyma cells of the haustorium (Fig. 4C). Two successive additions of CCCP were followed by depolarizations and the final PD stabilized at about −50 mV. CCCP was also tested on the epidermal parenchyma cells of the basal extremity of the haustorium and did not affect the PD. This is not surprising since the differentiation of these cells is characterized by a strong protoplasmic regression (14). Dinitrophenol and ATPase inhibitors (dicyclohexylcarbodiimide, orthovanadate)

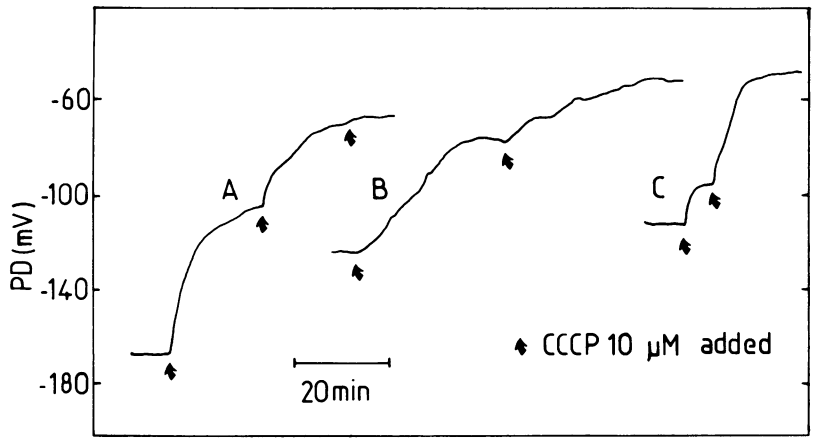

Figure 4. Effect of CCCP on the transmembrane PD of transfer cells (A), epidermis capsula cells (B) and cortical parenchyma cells of the haustorium (C). These experiments were repeated three times with similar results.

also induced a large decrease in PD of transfer cells (data not shown).

A low KCl concentration (1 mM) induced little or no decrease of the PD (Fig. 5). The addition of higher KCl concentrations (Fig. 5), was followed by an immediate decrease in PD, whose amplitude was dependent on the final concentration of KCl used: 32.9 ± 1.3 mV (mean ± SE, n = 8) with 10 mM KCl and 117.0 ± 3.1 mV (mean ± SE, n = 9) with 100 mM KCl. In some cases, a slight hyperpolarization consecutive to the initial depolarization could occur. However, no hyperpolarization occurred during the first 2 h following addition of 100 mM KCl.

Measurements of Intracellular pH of Transfer Cells

These experiments were carried out at pH 4.6 with 3 cm high sporophytes. The pH microelectrode recorded $\Delta pH + \Delta\psi$ (Fig. 1); the $\Delta\psi$ measured in Figure 3B (−180 ± 7.8 mV) was substracted from these overall recordings, and the average pH value estimated in this way (data from Figs. 1 and 3B) was 7.6 ± 0.2 (mean ± SE, n = 5).

Characterization of α-AIB Uptake

Uptake of α-AIB was linear with time for the first 2 h of incubation. The rate of α-AIB uptake changed as the sporophytes matured; it reached 7.5 nmol h^{-1} $sporophyte^{-1}$ when sporophytes 2.5 cm high were used and 12.5 nmol h^{-1} $sporophyte^{-1}$ with sporophytes 3.5 cm high (data not shown).

Uptake of α-AIB was strongly stimulated by acidic pH values, more particularly at pH 4.0 and 4.5 (Fig. 6). From pH 6.0 to pH 9.0 uptake was strongly inhibited. These differences cannot be ascribed to the composition of the buffers used (Fig. 6).

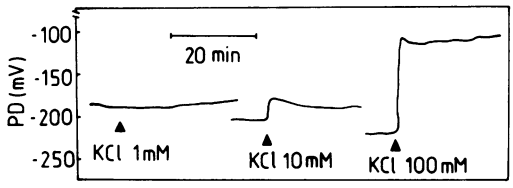

Figure 5. Effect of various KCl concentrations on the transmembrane PD of transfer cells. These experiments were repeated five times with similar results.

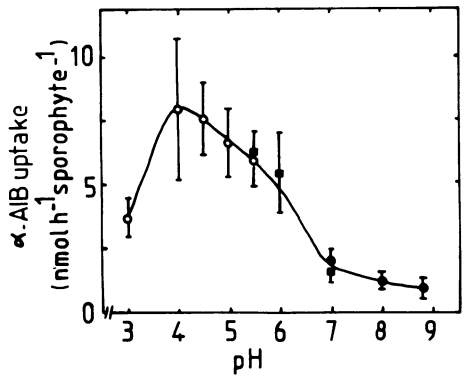

Figure 6. pH-dependence of [α-^{14}C]AIB uptake by *P. formosum* sporophytes. Three differents buffers were used: (○) 10 mM citrate, 20 mM diNa phosphate for pH 3 to 5,5; (■), 20 mM Mes/NaOH for pH 5.5 to 7.0; (●), 20 mM Tricine/NaOH for pH 7.0 to 8.8. After incubation (1 h), the sporophytes were rinsed in one bath (1 min) of unlabeled medium, then rapidly frozen and lyophilized. Each point is the mean of 12 sporophytes ± SE.

Uptake of α-AIB exhibited saturable kinetics. Lineweaver-Burk (Fig. 7) or Eadie-Hofstee plots showed the existence of two lines of different slope. The uptake data could be best fitted by the superimposition of a diffusive component to a single saturable component possessing an apparent K_m of 0.85 mM and an apparent V_{max} of 0.25 nmol min^{-1} $sporophyte^{-1}$.

Effect of Various Effectors on Uptake

The protonophore CCCP, strongly inhibited α-AIB uptake (Fig. 8). Inhibition exerted by CCCP varied from 60% with 0.5 µM CCCP to 95.6% with 50 µM CCCP. In the presence of 0.1 mM dinitrophenol, inhibition was 93%. A slight stimulation of α-AIB uptake was observed in the presence of 1 mM KCl in the medium, while high KCl concentrations inhibited the absorption of the amino acid (Fig. 9). At pH 4.5, inhibition was 21.5% with 10 mM KCl and 45.7% with 100 mM KCl. In addition, α-AIB uptake was lower and the inhibition exerted by KCl was more marked when the incubation solution pH was closer to neutrality. At pH 6.0, inhibition of α-AIB uptake was 32.2% with 10 mM KCl and 74.1 with 100 mM KCl.

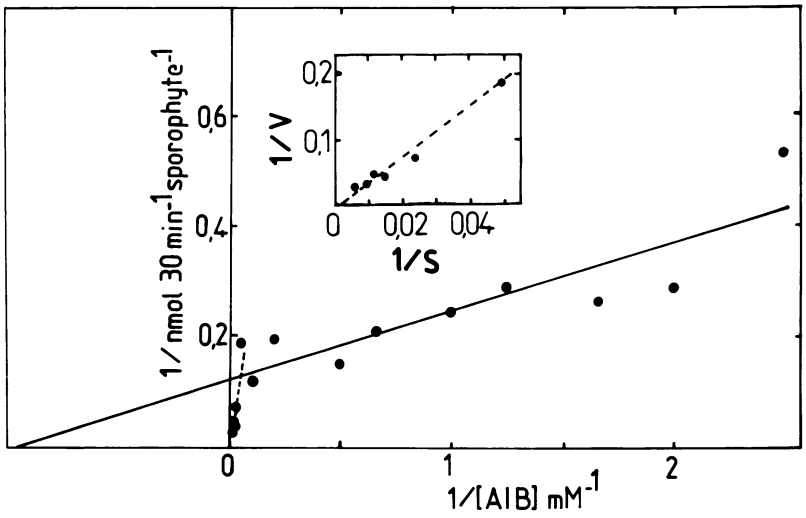

Figure 7. Lineweaver-Burk plots of [α-^{14}C]AIB uptake. The inset details the highest α-AIB concentrations used. After preincubation (1 h), each group of 12 sporophytes was transferred to 2 mL of incubation medium buffered at pH 4.5 (10 mM citrate, 20 mM diNa-phosphate) containing [α-^{14}C]AIB (16.5 kBq) in concentrations ranging from 0.05 to 150 mM. After 30 min of uptake, the sporophytes were prepared as described above for radioactivity measurements. Each point was obtained from 12 sporophytes.

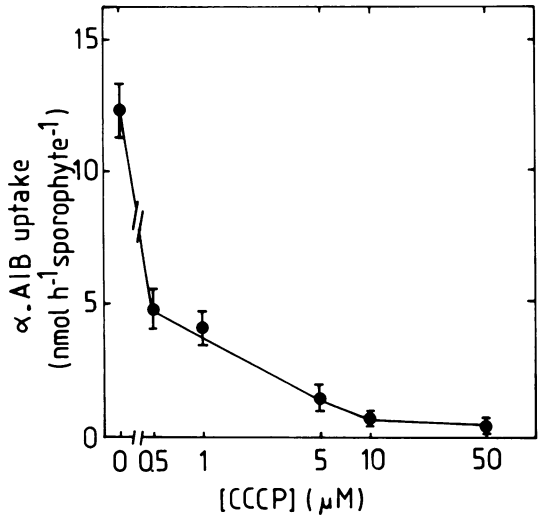

Figure 8. Effect of CCCP on the rate of [α-^{14}C]AIB (2.5 mM) uptake. After preincubation (2 h) in a solution buffered at pH 4.5 with 10 mM citrate and 20 mM diNa phosphate with or without uncouplers (CCCP) at different concentrations, the sporophytes were incubated (1 h) on a similar solution containing [α-^{14}C]AIB 2.5 mM (16.5 kBq) and they were processed as previously described. Each point was the mean of 12 sporophytes ± SE.

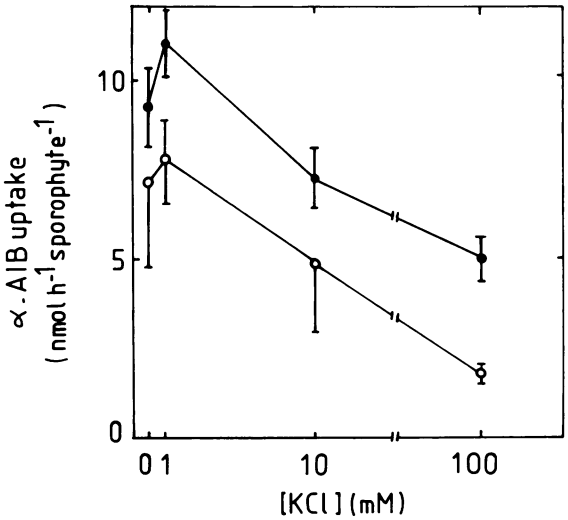

Figure 9. Effect of increasing KCl concentrations on the rate of [α-^{14}C]AIB (2.5 mM) uptake. The sporophytes were incubated (1 h) in a solution buffered at pH 4.5 (●) or pH 6 (○) with 10 mM citrate and 20 mM diNa phosphate containing KCl at various concentrations and 2.5 mM [α-^{14}C]AIB (16.5 kBq). Each point was the mean of 12 sporophytes ± SE.

Effect of Addition of α-AIB and of Other Amino Acids on the PD and on the pH of Incubation Medium

The addition of α-AIB to the incubation medium changed the acidification response of the sporophytes and the extent of this effect depended on the concentrations used. The rate of acidification was simply slowed down after addition of 1 mM α-AIB, while a strong alkalinization was observed after addition of 20 mM α-AIB (Fig. 10A). In the same way, addition of 5 mM threonine (7), leucine or methionine (Fig. 10B) induced an alkalinization of pH.

The addition of α-AIB (1–20 mM) induced, after a lag time of a few minutes, a depolarization which exhibited a simple saturable phase as a function of α-AIB concentration (Figs. 11 and 12A). Eadie-Hofstee and Lineweaver-Burk plots (Fig. 12B) yielded an apparent K_m of about 2 to 3 mM and V_{max} of 57 mV for this depolarization. Threonine, leucine and methionine also induced reversible depolarization of the PD (Fig. 11). These decreases occurred in general, after a lag time of 1 or 2 min. After addition of 5 mM amino acid, this depolarization reached: 55.7 ± 13.0 mV with threonine, 57.0 ± 3.1 mV with methionine and 45.0 ± 2.8 mV with leucine (mean ± SE, n = 5).

DISCUSSION

The sporophyte of bryophytes is very dependent on the gametophyte for its nutrition (14, 29). Its haustorium, which

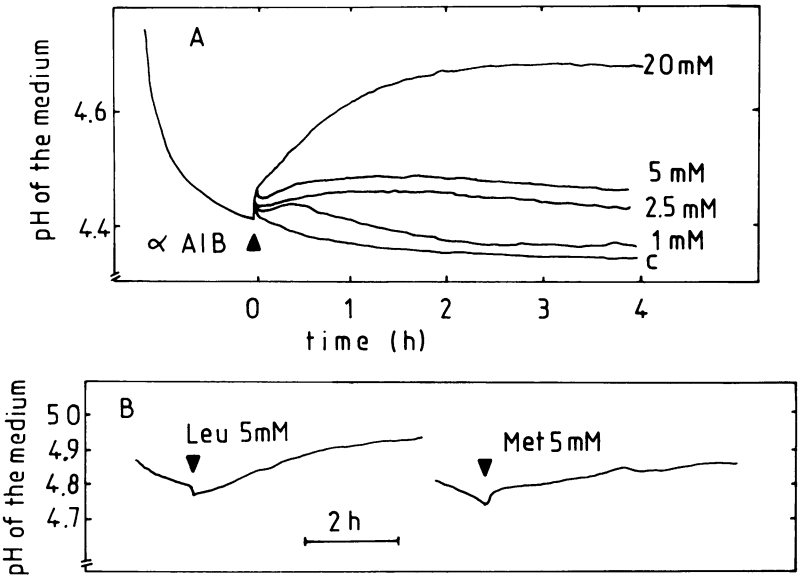

Figure 10. Effect of the addition of neutral amino acids on the pH of a medium containing 100 sporophytes (3–4 cm long). A, 1 to 20 mM α-AIB, although the pH of the medium at the time of amino acid addition varied somewhat (4.3–4.5), all curves were drawn with an initial pH of 4.4, (c: control); B, 5 mM leucine and 5 mM methionine. These experiments were repeated four times with similar results.

is embedded in the vaginula of the gametophyte, is bordered externally by transfer cells in several species of mosses (14, 24, 26). The epidermal transfer cells present numerous advantages, as experimental material. From a thematic point of view, the interest of their study is double since it provides information both on the functioning of the transfer cells *per se* and on the properties of the exchanges between the two generations. From a methodological point of view, they also offer unique features: (a) their location allows an easy electrophysiological study; (b) the small size of the vacuome (10% of the total cell volume) compared to that of most plant cells (about 90%) allows the interpretation of the electrophysiological data in terms of transplasmalemma potential difference; (c) the lack of symplastic connections with the gametophyte (see Introduction) allows the exclusion of any symplastic event in the interpretation of the data; (d) the data are reliable because the excision of the sporophyte does not affect its uptake capacity (8) and affects only briefly the gradients generated by the plasmalemma. In particular, after a short recovery period, the transmembrane PD remains stable for at least 150 min (Fig. 3A). This stability, which is not observed in some tissues excised after dissection (16), allowed to obtain results, comparable from one recording series to another one (Fig. 3B for example). All these peculiarities show that the excised sporophyte provides a simple and convenient model for studying transfer cell physiology and the properties of nutrient exchange between the two generations of a bryophyte.

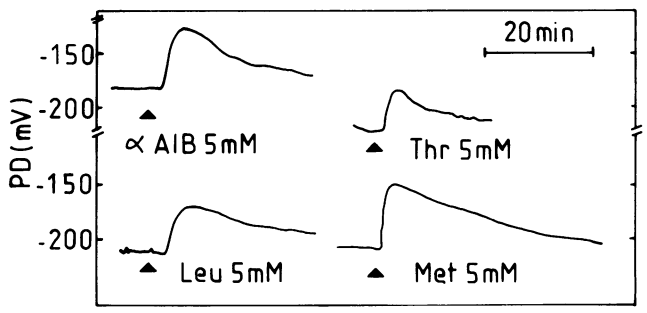

Figure 11. Effect of the addition of neutral amino acids on the PD of *P. formosum* transfer cells. All concentrations are final concentrations. These experiments were repeated five times with similar results.

Although the characteristics of the transfer cells suggest that these cells play a major physiological role (8), the data concerning the properties of their plasmalemma are still scarce and sometimes not very conclusive. The transmembrane PD of the giant transfer cells induced by nematodes in the roots of *Impatiens balsamina*, are about −125 mV just after gall excision, reaches −160 mV 3 h later, and still changes during the aging process to reach −207 mV 23 h after the excision (16). The PD of the cells from which they derive is not known but the PD of the parenchyma cells of the gall is almost identical to that of the transfer cells and exhibits the same evolution during aging (−118 mV just after excision, −207 mV 23 h later) (16). In the leaf of *Pisum sativum*, the plasmalemma of the transfer cells exhibits a higher ATPase activity than the sieve tubes (3). Yet, the plasmalemma of the phloem parenchyma cells seems to exhibit an ATPase activity similar to that of the transfer cells (Figs. 2 and 3 of reference 3). In *Vallisneria spiralis*, an aquatic angiosperm, the PD of the epidermal transfer cells is similar to that of the leaf parenchyma cells (27). In various species, and more particularly in *Capsicum annuum*, Fe deficiency leads to a stimulation of H^+ excretion by the roots and to a concomittant increase of malate and citrate synthesis. These responses are accompanied by a transformation of the rhizodermis cells into transfer cells (21). Our results show that the transfer cells of the haustorium of *Polytrichum formosum* generate pH and electrical gradients that may be very strong. The gradients increase as the sporophyte develops (Fig. 3). A similar trend was noted during the development of angiosperm embryos, at least for the embryo of *Vicia faba*. In this organ, the PD of the cells of the superficial layer changes from −32 mV at the eighteenth day after flowering to −140 mV at the 45th DAF; in the same time interval, the pH to which the tissues equilibrates their incubation medium changes from 6.5 to 5.7 (22) (R El Ayadi, in preparation). However, the gradients gener-

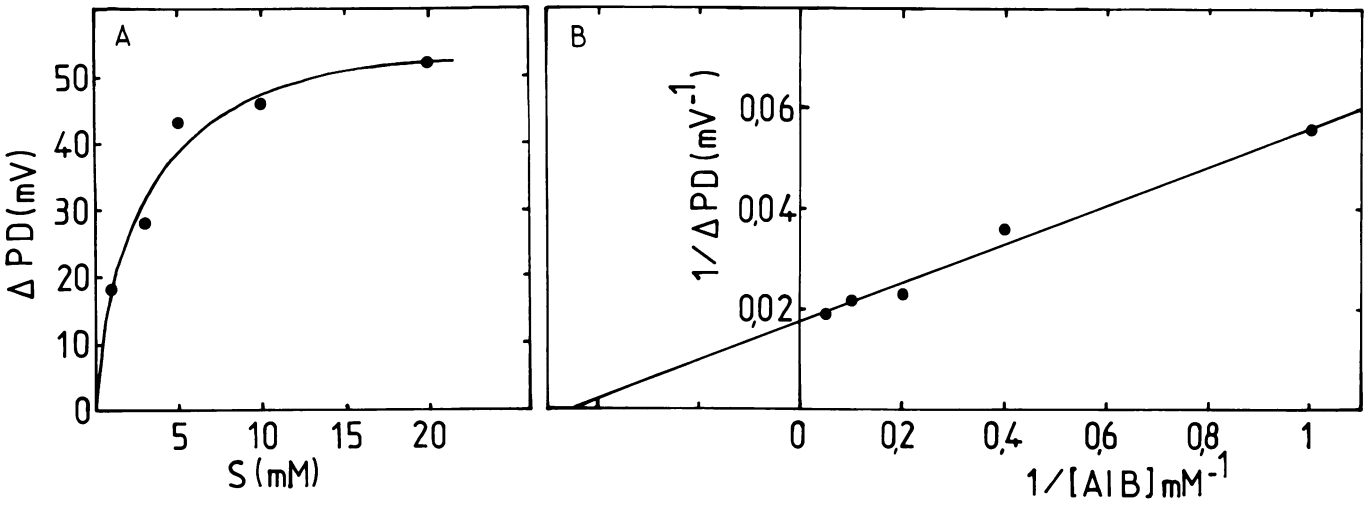

Figure 12. Concentration-dependence of α-AIB induced depolarizations (ΔPD). Michaelis-Menten (A) and Lineweaver-Burk (B) plots. Each point is the mean of five experiments.

ated by the transfer cells of the haustorium of *Polytrichum* are much more dramatic than those noted for the superficial cells of the angiosperm embryos. The PD of the transfer cells of *Polytrichum* hyperpolarizes from −142 to −213 mV and the pH of equilibration of their incubation medium decreases from 5.2 to 4.3 when the sporophytes grow from 2 to 4 cm long.

At the intermediary stage (3 cm long sporophytes) used for the study of amino acid uptake by the transfer cells of the haustorium, the PD of these cells is −180 mV. This value is much more negative than that of the surrounding cells (Table I). However, these values cannot be compared directly because in most cases the PD measured in the transfer cells is a transplasmalemma PD while the PD measured in the other cells is, in most cases, the sum of the transplasmalemma plus transtonoplast PDs. Indeed, the differentiation of the transfer cell of the haustorium is accompanied by a dramatic regression of the vacuome, this organelle occupying less than 10% of the volume limited externally by the plasmalemma (8). However, since the electrical gradient established by the tonoplast is small (+10 to +30 mV), at least in angiosperms (28), and since the epidermis of the seta and of the capsula stabilize their incubation medium at values close to pH 5.7 (7) instead of 4.6 for the haustorium, it is possible to conclude that the plasma membrane of the transfer cell of the haustorium establishes and maintains a protonmotive force much larger than that of the surrounding cells and that of the cells from which they come. The cytoplasmic pH of the transfer cells is close to 7.6. This value is one of the most alkaline among those recorded in various cells (internodal cells of *Chara*, thallus cells and rhizoid cells of *Riccia*, root corpus, root hairs and leaf cells of angiosperms, coleoptiles, suspension cultures of angiosperms) with H^+-selective microelectrodes (pH 7.1 to 7.6) (11) or by ^{31}PNMR (pH 6.7–7.7) (20). Consequently, if the pH of equilibrium of the incubation medium gives a good account of the apoplastic pH of the transfer cells, the proton motive force developed by the plasmalemma of these cells reaches 33.1 kJ under the experimental conditions used in the present study. This very high value is similar to that noted for the epidermal cells of *Riccia fluitans*, an hepatic liverwort (15). However, it is not yet the maximum value, since $\Delta\psi$ (Fig. 3) and ΔpH still increase when the sporophytes get older. The data indicate that the important protonmotive force developed by the plasmalemma in the transfer cells energizes the uptake of the solutes leaked in the gametophyte apoplast.

Since the growth of the sporophyte is accompanied by a decrease of the amino acid content of the gametophyte (29), a study of the ability of the transfer cells of the sporophyte to absorb this kind of substrate was pertinent. In higher plants, the uptake of amino acids has been studied extensively in source and in sink tissues. The H^+-amino acid symport occurring for neutral amino acids has been demonstrated repeatedly (10, 17, 18, 31, 32, and references therein) and the recent papers concern the number of different systems mediating the transport of the various amino acids, neutral, basic and acidic (4, 18, 32). By contrast, the exchanges of amino acids between the two generations in higher plants are still poorly known. The uptake of α-AIB by freshly isolated soybean embryos is a passive-like, nonsaturable process, which has no electrophysiological response (2). Incubation of these embryos in a nitrogen-free medium allows the derepression of a high affinity, saturable system mediating the uptake of amino acids with proton symport (2).

In mosses, the competition for the nutrients between the sporophyte and the gametophyte is extremely strong since both these organisms are of slightly different size. In our material, the transfer cells of the haustorium likely play a major role in the uptake of the solutes present in the apoplast of the sporophyte vaginula. Indeed, removal of the haustorium decreases three-fold the ability of the sporophyte to absorb the ^{86}Rb present in the incubation medium (5), and about four- to sixfold the ability of absorb the amino acids (8). Besides, the addition of amino acids to the incubation medium of sporophytes without haustorium (excised) induces a much weaker alkalinization than that obtained in a medium only containing the haustorium (7). In principle, the tough

competition between the two generations must result in vivo in the presence of high-affinity uptake systems able to absorb the organic nutrients leaked in the gametophyte apoplast. Our previous data concerning glycine (6) and the present work show the existence of such a system for neutral amino acids. The apparent kinetic parameters of glycine uptake (K_m = 1 mM; V_{max} = 0.5 nmol min^{-1} $sporophyte^{-1}$) are very similar to that measured for α-AIB, but the uptake of the natural amino acid exhibits a single phase up to 100 mM (no diffusion-like component), unlike that of α-AIB. The optimum of uptake at strongly acidic pH (Fig. 6), the sensitivity of absorption to chemicals dissipating either ΔpH and $\Delta\psi$ (Fig. 8) or only $\Delta\psi$ (Fig. 9), the pH and PD transients induced by the addition of amino acids to the medium (Figs. 10 and 11) demonstrate that the transfer cells of the haustorium take up these amino acids with proton symport. This transport is energized by the gradients described above, and particularly by $\Delta\psi$, as shown by the extreme sensitivity of the uptake to the compounds collapsing this component of the proton motive force (Figs. 4 and 9). A similar mechanism has been demonstrated in the rhizoids of *R. fluitans* (15 and references therein).

In conclusion, the transfer cells characterized in particular by the extension of the exchange surface between the apoplast and the symplast and by numerous mitochondria fulfill well the function expected from their structural peculiarities. The example studied shows that their plasmalemma may generate pH and electrical gradients stronger than that of the nondifferentiated surrounding cells. In the case of Bryophytes, the very strong protonmotive force established by the transfer cell of the haustorium is used to energize an efficient absorption of the amino acids leaked in the apoplast of the gametophyte.

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