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H^+ Extrusion and Potassium Uptake Associated with Potential Hyperpolarization in Maize and Wheat Root Segments Treated with Permeant Weak Acids[1]

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ABSTRACT

The rapid uptake of weak acids permeant in the uncharged form is accompanied in maize and wheat root segments by a hyperpolarization of the transmembrane electrical potential and an increase in K^+ uptake, suggesting a stimulation of the plasmalemma H^+ pump. The evaluation of weak acid-induced H^+ extrusion must take into account the alkalinization of the medium due to the rapid uptake of the uncharged form of the acid, partially masking the proton pump-mediated extrusion of H^+. The data corrected for this interference show that the lipophilic butyric acid and trimethyl acetic acid induce in maize and in wheat root segments a significant increase in 'real' H^+ extrusion, roughly matching the increase in net K^+ uptake. The presence of K^+ significantly increases the rate of uptake of the weak acid, possibly as a consequence of an alkalinization of the cytosol associated with K^+ absorption. In maize root segments, the effects of fusicoccin and those of butyric acid on both K^+ uptake and H^+ extrusion are clearly synergistic, thus suggesting distinct modes of action. These results support the view that the activity of the plasmalemma H^+ pump is regulated by the value of cytosolic pH.

We have previously reported that the treatment with permeant weak acids (such as butyric, isobutyric, trimethylacetic acids, and DMO[2]) induces in maize root segments a marked hyperpolarization of the PD (12, 14, 15; see also, for similar results in other materials, 1, 3, 6, 16, and 17). The finding that PD hyperpolarization is associated with an increase in the rate of K^+ uptake and is correlated with the rate of penetration of the weak acid and with a decrease in cell sap pH suggested that, in these experiments, the electrogenic proton pump at the plasmalemma is stimulated by the acidification of the cytoplasm consequent to the penetration and the dissociation of the weak acid in the cytosol (compare Sanders *et al.* [16] for the same conclusion from experiments in *Neurospora*).

If this interpretation is correct, the hyperpolarization associated with weak acid uptake should also be associated with an increase in proton secretion, which had not been demonstrated in the above-mentioned investigations. On the other hand, the demonstration of weak acid-induced changes in the rate of H^+ extrusion presents some experimental difficulties due in part to the strong buffering action of the weak acids, and also, even more seriously, to the fact that the rapid uptake of the uncharged form of the weak acid by the tissue results in an alkalinization of the medium, which may mask the acidification due to the secretion of H^+. Thus, for a correct evaluation of the 'true' H^+ movement across the plasmalemma, the titration values must be corrected for the changes in weak acid concentration.

Starting from these premises, in the present work we investigated the changes in 'real' H^+ extrusion induced in root segments by treatment with permeant weak acids. The proton extrusion values thus obtained were then compared with the results of experiments in which we investigated the effects of weak acids and of the H^+ pump-stimulating agent FC on K^+ uptake; the rationale of these experiments was the assumption that any changes in proton extrusion mediated by the proton pump should be associated with a corresponding change in the rate of net uptake of K^+ (or of another permeant cation, Marrè *et al.* (2, 12, 13). It was also thought that the comparison between weak acids and FC in their effects on H^+ extrusion, K^+ uptake, and transmembrane potential might be of interest in the study of the regulation of the activity of the proton pump.

The experiments reported in this paper were carried out by two different groups, working in two different laboratories and utilizing two different materials: wheat roots, by the Torino group, and maize roots, by the Milano group, We thought that the disadvantages arising from this heterogeneity of both materials and investigators would be compensated by a more general physiological significance of the results thus obtained and of their interpretations. A preliminary report of some of the results and conclusions of the present investigation was presented at the 16th FEBS meeting in Moscow, 1984 (11).

MATERIALS AND METHODS

As mentioned in the "Introduction," the results reported here were obtained in part in Milano, by a group working on the effects of BA on proton extrusion in maize roots, and in part in Torino, by a group working on the effects of TMA on wheat roots. The procedures utilized in the two laboratories for the two materials are thus separately described.

Wheat (Torino Group). *Material.* Wheat seeds (*Triticum aestivum*, cv Aquileia) sterilized for 10 min in 0.6% NaClO, and germinated for 2 d on filter paper imbibed with 0.5 mM $CaSO_4$ were placed in aerated 0.5 mM $CaSO_4$ and grown 10 to 13 d with

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[2] Abbreviations: DMO, 5,5-dimethyloxazolidine-2,4-dione; PD, transmembrane electrical potential difference; FC, fusicoccin; BA, butyric acid; TMA, trimethylacetic acid.

a photoperiod of 14 h light (800 lux)-10 h dark. The roots were thoroughly washed with 0.5 mM $CaSO_4$, cut in 20-mm-long segments, and randomized. Batches of segments of 0.6 g fresh weight (corresponding to 9–10 seedlings) were preincubated for 60 min in 10 ml of 2 mM Li tartrate and 0.5 mM $CaSO_4$ (pH 5) at 23°C. The segments were then washed and placed in the treatment incubation medium (final volume, 10 ml) containing either 2 mM tartaric acid brought to pH 5.03 with LiOH for the controls, or 2 mM TMA brought with LiOH to pH 5.03, for the treated samples, plus 0.5 mM $CaSO_4$ and K_2SO_4 as indicated. The media were vigorously aereated during both preincubation and incubation.

TMA and K^+ Uptake. TMA uptake was determined by measuring TMA contents in the medium at the beginning and at the end of the experiments. Aliquots of the medium adjusted to pH 5.03 (pH = pK) with NaOH were titrated to pH 8.2 (corresponding to complete neutralization of TMA at the 2 mM concentration used). The amount of OH^- equivalents required was taken as equal to one-half of the acid present. In the pH interval from 5 to 8, the shape of the titration curves of the TMA-containing medium collected at the end of incubation with the tissue exactly reproduced those of pure TMA/Li, giving the same pK value. This seems to rule out the possibility that TMA induces a secretion of other weak acids from the roots, which would interfere with the determination of TMA in the medium by titration.

K^+ net uptake was calculated from the difference between the initial and the final K^+ concentrations in the external medium measured by flame spectrometry.

Measurement of H^+ Extrusion. The changes in pH of the medium during our experiments result from two main processes acting in opposite directions: (a) the efflux of H^+ from the tissue, leading to acidification, and (b) the decrease of the weak acid (rapidly taken up in the uncharged form) leading to alkalinization. Thus, to calculate the true value of H^+ extrusion, one should first add at the end of the experiments an amount of weak acid equal to that which disappeared because of the uptake, and only then titrate from the new pH value thus obtained to the initial one. The value obtained by titration from the final to the initial pH would then reflect H^+ extrusion.

An evaluation of true H^+ extrusion (ΔH^+) in the presence of permeant weak acid can be effected in two ways: (a) by the empirical procedure described above; and (b) by the use of suitable equations dealing with the acid-base equilibria. Our buffer system is defined by C ($[AH] + [A^-]$), *i.e.* the concentration of the weak acid, and B, the concentration of the strong base added to the acid to obtain the actual pH value. The extrusion of H^+ from the tissue may be taken as equivalent to the addition of some strong acid which reduces from B_0 to B_t the concentration of the strong base acting in the buffer system, by converting it in a strong base-strong acid salt, inactive as far as the buffering action is concerned. Thus, if B_0 is the initial and B_t is the final concentration of the strong base active in the buffering system, we may write:

$$\Delta H^+ = (B_0 - B_t)\cdot 10^{-3}\cdot V\cdot g^{-1} \text{ fresh wt} \qquad (1)$$

where the amount of extruded protons ΔH^+ is expressed as eq·g^{-1} fresh weight and V is the incubation volume in ml. In other words, the buffer system at the end of incubation may be thought of as a mixture of the weak acid at the final C_t concentration and the final strong base concentration B_t as defined above.

The term B_t of eq. 1 can be calculated by the following:

$$B_t = \frac{K\cdot C_t - K\cdot[H^+]_t}{K + [H^+]_t} \qquad (2)$$

where C_t and $[H^+]_t$ are the values of C and $[H^+]$ measured at the end of incubation and K is the dissociation constant of the weak acid.

Eq. 2 is derived from the law of mass action. For the dissociation of a weak acid we can write:

$$\frac{[A^-]\cdot[H^+]}{[AH]} = K \qquad (3)$$

where K is the weak acid dissociation constant. In a buffer system, $[A^-]$ may be expressed as $B + [H^+] - [OH^-]$, where the term $[H^+]$ accounts for the contribution due to the self-dissociation of the unneutralized weak acid (each H^+ liberated corresponds to an A^- ion) and $-[OH^-]$ accounts for the decrease in A^- due to the hydrolysis of the weak acid-strong base buffer salt ($A^- + H_2O \rightarrow AH + OH^-$). Similarly, $[AH]$ may be taken as $C - B - [H^+] + [OH^-]$, where C is the concentration of the weak acid ($A^- + AH$). In our case, where $[OH^-]$ is equal to or less than 10^{-9}, the corresponding term may be omitted.

Substitution for $[A^-]$ and $[AH]$ in Eq. 3 gives:

$$\frac{B\cdot[H^+] + [H^+]^2}{C - B - [H^+]} = K \qquad (4)$$

which, solved for B and neglecting the $[H^+]^2$ term becomes:

$$B = \frac{K\cdot C - K\cdot[H^+]}{K + [H^+]} \qquad (5)$$

identical to Eq. 2 if C_t and $[H^+]_t$ are the values of C and $[H^+]$ measured at the end of the incubation time.

The procedure used in our experiments to determine $-\Delta H^+$ by Eq. 1 and 2 was as follows: B_0 was the amount in moles of the strong base (LiOH for wheat, NaOH for maize) initially used to bring 1 L of a 2 mM solution of the weak acid (TMA or BA) to the desired pH (5.03 for wheat, 5 for maize); $[H^+]_t$ was calculated from the pH of the medium determined at the end of the incubation time; C_t was calculated from the measurement of the decrease of total weak acid ($[A^-] + [AH]$) in the medium because of its uptake by the tissue; V was the volume of the incubation medium in ml; K was the dissociation constant of the weak acid ($K = 9.33\ 10^{-6}$, pK = 5.03 for TMA, $K = 1.54\ 10^{-5}$, pK = 4.81 for BA; values given for a temperature of 25°C in the Handbook of Physical Chemistry, CRC Press, Boca Raton, FL, 1980; no significant difference was found between these values and those experimentally found, by titration, in our system).

The validity of Eq. 1 and 2 in our experimental conditions was confirmed by experiments with pure TMA or BA solutions in which the amount of LiOH added was constant, the weak acid concentration was varied between 1 and 2 mM, and H^+ extrusion was simulated by the addition of known amounts of HCl, and the pH values thus obtained were measured.

A possible objection to our evaluation of $-\Delta H^+$ is that of the possibility of interferences due to the excretion of endogenous weak acids or, generally, of buffering substances from the tissue. This objection is also valid for any evaluation of ΔH^+ by back titration from the final to the initial pH, as routinely performed in the absence of permeant weak acids. In the present experiments, however, the titration curves in the 4 to 8 pH range did not reveal a significant appearance of buffering substance from the tissue.

Transmembrane Potential Measurement. PD measurements were performed by the conventional procedure (8) with standard glass microelectrodes filled with 1 M KCl under continuous flow of an aerated thermoregulated medium containing 2 mM tartaric acid or TMA brought to pH 5.03 with LiOH, 0.1 mM KCl, 0.5 mM $CaSO_4$, at 23°C. Cells of the second or third layer were measured at about 8 to 10 mm from the apex.

Maize (Milano Group). *Material.* Maize (*Zea mays*, Dekalb XL72A) seeds were sterilized for 30 min with 1% NaClO and

germinated in the dark for 2 d at 28°C on wet filter paper saturated with 0.5 mM $CaSO_4$. Approximately 24 h prior to the experiments, the seedlings were transferred to beakers containing about 2 L of aerated 0.5 mM $CaSO_4$ and maintained in the dark at 25° ± 1°C.

Butyric Acid Uptake. [1-^{14}C]Butyric acid was used as tracer. Twelve subapical root segments, 0.6 cm long (the 1-mm-long apical region removed), were washed in 5 ml of 0.5 mM $CaSO_4$ for 60 min; the medium was renewed after 30 min. The segments were then incubated with the labeled substance, with agitation (100 shakes/min) at 25°C, in 10 ml of a solution containing 0.5 mM $CaSO_4$, 2 mM BA brought with NaOH to pH 5, 5 mM Mes brought to pH 5 and 0.3, 1 or 5 mM K^+ (as sulfate), as indicated. At the end of the treatments, the segments were washed for 5 min with the corresponding nonlabeled solution (with this time of washing the uptake *versus* time curves passed through the origin). The tissue was then homogenized in ethanol and the radioactivity present was measured by liquid scintillation. All experiments were carried out in quadruplicate and repeated at least 3 times.

K^+ Uptake. ^{86}Rb was used as a tracer. Twelve subapical root segments, 0.6 cm long, were washed in 5 ml of 0.5 mM $CaSO_4$ as described above. FC, when present in the treatment, was added at the start of the second period of washing. The segments were then incubated with the labeled substance in 10 ml of a solution containing 0.5 mM $CaSO_4$, 2.5 mM K_2SO_4, 5 mM Mes brought to pH 5 with NaOH, and with or without 2 mM BA brought to pH 5 with NaOH and/or 10^{-5} M FC. Five mM Mes/Na buffer was added to the medium reduce pH changes during incubation, thus minimzing a pH effect on K^+ and weak acid uptake. At the end of the desired incubation time, the segments were washed twice at 4°C for 15 min with the corresponding nonradioactive solution and then heated to about 100°C for 10 min and homogenized in 0.1 M HNO_3.

Measurement of the pH Changes and Calculation of H^+ Extrusion. Thirty subapical root segments, 0.6 cm long, were washed in 5 ml of 0.5 mM $CaSO_4$ as described above. The segments were then transferred into a medium containing 0.5 mM $CaSO_4$, 2.5 mM K_2SO_4, 5 mM Mes, with or without 2 mM BA (both Mes and BA solution were brought to pH 5 with NaOH, see above), and/or 10^{-5} M FC, and incubated with agitation (100 shakes/min) at 25°C in the dark. The pH values were measured with a Radiometer PHM 84 pH meter.

Back titration of the media of the control and treated samples were undertaken as described by Lado *et al.* (5) with a Radiometer titrator.

True H^+ extrusion in the presence of BA was calculated by Eq. 1 and 2, as described for the experiments with TMA-treated wheat root segments. The known terms in the equation in this case were: (a) the pK of BA, taken as 4.81 at 25°C; (b) the amount of NaOH used to bring at zero time the 2 mM BA solution to pH 5; (c) the initial and final values of pH of the medium (measured); (d) the decrease in BA concentration in the medium during the experiment (calculated from the amount of [^{14}C]BA taken up by the tissue).

RESULTS

Wheat Roots. *Effects of TMA on PD.* Figure 1 shows that 1 or 2 mM TMA (brought with LiOH to pH 5.03) induced in the cortical cells of wheat root segments a rapid hyperpolarization similar to that already demonstrated in maize roots (12). In wheat, the induced hyperpolarization (about 30–40 mV) reached a maximum after 10 to 15 min of treatment, then slowly decreased. This decrease, which was not observed in maize, might be a consequence of a progressive metabolic damage due to the accumulation of TMA in the cells.

Effect of K^+ on TMA Uptake. Similarly to what is known for this and other weak acids when fed to maize roots, TMA rapidly penetrated the cells. Figure 2 shows that TMA accumulation in wheat roots in 60 min was markedly enhanced by the presence of K^+ in the medium.

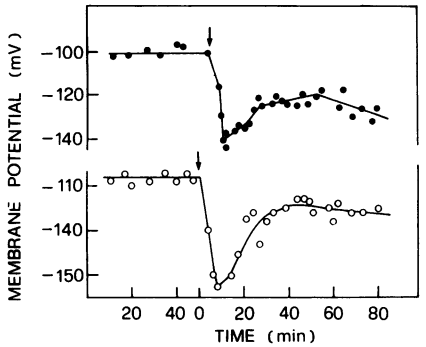

FIG. 1. Effect of TMA on the transmembrane potential of wheat root cortical cells. Control: 2 mM Li tartrate, 0.5 mM $CaSO_4$, 0.05 mM K_2SO_4, pH 5.03, 1 mM (●) or 2 mM (○) TMA brought to pH 5.03 with LiOH, was added where indicated by the arrows.

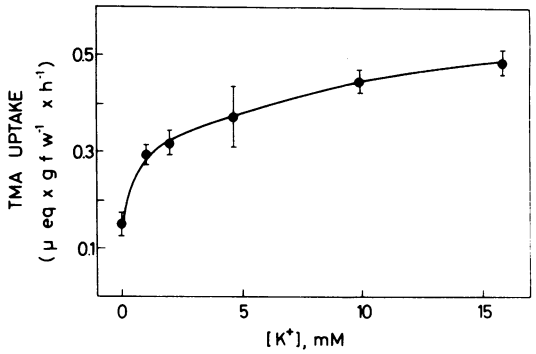

FIG. 2. Effect of K^+ in the medium on TMA uptake in 60 min in wheat root segments. TMA (2 mM) brought to pH 5.03 with LiOH; 0.5 mM $CaSO_4$; K_2SO_4 as indicated.

Effects of TMA on H^+ Extrusion and K^+ Uptake. The data of Table I summarize the results of several experiments in which proton extrusion and net K^+ uptake were measured in the presence of equivalent concentrations of either tartaric acid (controls) or TMA. The rationale for choosing tartaric acid for the controls was that this hydrophilic acid penetrates the wheat root cells only very slowly and that it does not significantly influence H^+ extrusion and K^+ uptake in this material (data not shown). K^+ (1 mM) was also present in the control as well as in the TMA-containing media, thus ensuring high values of both proton extrusion and TMA (when present) uptake (Table I). The data of Table I show that both K^+ uptake and proton extrusion (corrected for the weak acid uptake, see "Materials and Methods") are much higher in the presence of the easily permeating TMA than in that of tartaric acid, and the ratio between the two effects of TMA on K^+ uptake and on H^+ extrusion is about 1.4. Thus, most of K^+ uptake seems accounted for by an exchange with H^+. This suggests that the acidification induced by the permeant weak acid has indeed stimulated an electrogenic mechanism mediating H^+/K^+ exchange, presumably identifiable with the proton pump.

Maize Roots. *BA Uptake.* The rate of BA uptake was practically constant for the first 15 min, then progressively decreased, parallel with the increased accumulation within the tissue (Fig. 3).

Figure 3 shows the results of an experiment in which we compared BA uptake at two different pH values, 5 and 5.5, and the initial BA concentration was calculated in such a way as to

Table I. *Effects of TMA and K^+ on H^+ Extrusion and on K^+ Net Uptake in Wheat Root Segments*

	H^+ Extrusion			K^+ Uptake	H^+ Extrusion/ K^+ Uptake
	No K^+ (1)	1 mM K^+ (2)	Δ by K^+ (2) − (1) (3)	1 mM K^+ (4)	(3)/(4)
			μeq/g fresh wt·h		
2 mM tartrate	−0.26 ± 0.07[a]	2.11 ± 0.16	2.37	3.20 ± 0.27	0.74
2 mM TMA	0.40 ± 0.08	4.90 ± 0.19	4.50	6.14 ± 0.3	0.73
By TMA	0.66	2.79	2.13	2.96	0.72

[a] Values are the means ± SE of four separate experiments.

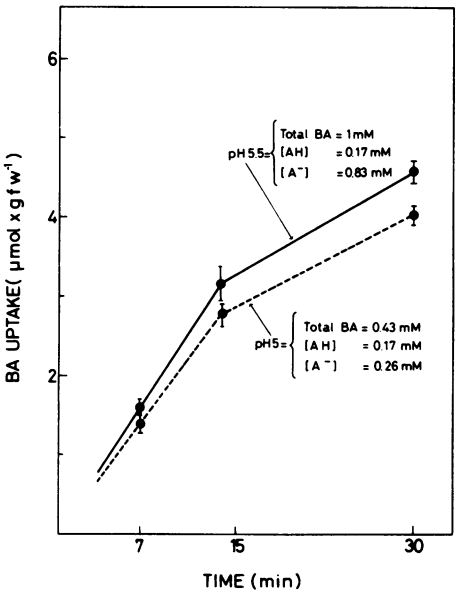

FIG. 3. Uptake of BA at two different pH values. Total BA concentrations were such that only the charged form concentration were different at the two pH values.

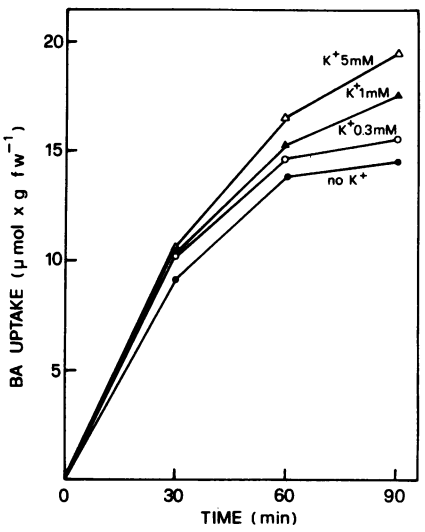

FIG. 4. Effect of K^+ in the medium on BA uptake in maize root segments. (●), Control: 2 mM BA, brought to pH 5 with NaOH; 0.5 mM $CaSO_4$; (○), control + 0.15 mM K_2SO_4; (▲) control + 0.5 mM K_2SO_4; (△), control + 2.5 mM K_2SO_4.

obtain the same concentration of the uncharged form (0.17 mM at both pH values), whereas the concentration of the dissociated form was 0.26 mM at pH 5, and 0.83 mM at pH 5.5. It is seen that BA uptake was very little influenced (less than 10%) by the difference in concentration of the dissociated acid (more than 3-fold higher at the higher pH). This confirms that this weak acid penetrates into the cells almost exclusively in the uncharged form. (See also, for the effect of pH on BA uptake, references 14 and 16)

Effects of K^+ on Butyric Acid Uptake. Previous work had shown that undissociated BA rapidly penetrates maize root segments (14). Figure 4 shows that the presence of K^+ in the medium significantly stimulated its uptake. This effect was already detectable after 30 min, and became more evident, for the higher K^+ concentration, at 60 and 90 min of incubation. The K^+-induced stimulation of weak acid uptake in maize roots was similar (although quantitatively smaller) to that described for wheat root segments, thus suggesting the same interpretation.

Effects of Butyric Acid on H^+ Extrusion and K^+ Uptake in the Absence and in the Presence of Fusicoccin. Table II summarizes the results of several experiments on the effects of 2 mM BA on proton extrusion and K^+ (^{86}Rb) uptake in maize root segments. In these experiments, both the controls and the BA-treated samples contained as a buffer 5 mM Mes, which is not taken up by this material and which does not influence proton extrusion when its concentration is varied in the 1 to 50 mM range. In these experiments, the effects of BA were determined both in the absence and in the presence of FC, which is thought to stimulate the activity of the H^+ pump (7). Table II shows that in maize root segments (similarly to that described for TMA in wheat roots) BA markedly stimulated both proton extrusion and K^+ uptake. Here again, the BA-induced increase in proton extrusion was roughly accounted for by the increase in K^+ uptake, which suggests the same conclusion, namely that the acidification of the cytosol by the weak acid leads to an activation of a mechanism mediating H^+/K^+ exchange.

Interaction between Butyric Acid and Fusicoccin in Stimulating H^+ Extrusion and K^+ Uptake. The data of Table III (re-elaborated from those of Table II) show that in maize root segments the stimulation of both proton extrusion and K^+ uptake by BA is clearly more than additive with the similar (although much larger) effects of FC. The parallelism of the effects of FC and BA on H^+/K^+ exchange strongly suggests that both types of treatments act by activating the same terminal system, presumably the plasmalemma proton pump. On the other hand, the finding that the responses of electrogenic H^+/K^+ exchange to FC and to BA are more than additive suggests different mechanisms of action of the two compounds.

DISCUSSION

The discussion of the present results is based on the following assumptions: (a) TMA and BA penetrate only or mainly in the

Table II. *Effects of BA on H^+ Extrusion and K^+ Uptake in Maize Root Segments, in the Presence and in the Absence of Fusicoccin*

Final pH were measured after 2 h of treatment. The incubation mixture contained, in a final volume of 10 ml, 0.5 mM $CaSO_4$, 5 mM Mes plus or minus 2 mM BA and/or 10^{-5} M FC. The pH of the Mes and BA solutions adjusted to 5 with NaOH. Data are the means ± SE of four separate experiments.

	BA Uptake	Final pH	H^+ Extrusion (1)	K^+ Uptake (2)	H^+ Extrusion/K^+ Uptake (1)/(2)
			μmol·g⁻¹ fresh wt·2 h⁻¹		
		4.70 ± 0.02	3.8 ± 0.14	6.5 ± 0.09	0.60
BA	19.65	5.31 ± 0.03	5.7 ± 0.35	7.9 ± 0.28	0.72
FC		3.65 ± 0.019	16.3 ± 0.22	19.2 ± 0.40	0.85
BA + FC	22.7	4.54 ± 0.012	23.5 ± 0.50	24.5 ± 0.47	0.96

Table III. *Comparison between the Effects of BA, FC, and BA plus FC on H^+ Extrusion and K^+ Uptake*

Treatment	H^+ Extrusion	K^+ Uptake	H^+ Extrusion/ K^+ Uptake
	μmol·g⁻¹ fresh wt·2 h⁻¹		
BA	1.9 ± 0.37[a]	1.4 ± 0.29	1.3
FC	12.5 ± 0.25	12.7 ± 0.40	0.9
BA + FC	19.8 ± 0.50	18.0 ± 0.47	1.1

[a] Data are ± SE of the differences obtained from Table II by subtracting the control values.

undissociated form; (b) they induce an acidification of the cytosol; (c) some metabolism of the weak acids does not significantly interfere with their effects on H^+ extrusion.

The assumption that the weak acids are taken up in the undissociated form is based on the finding that their uptake is strongly inhibited by a rise of pH from 5 to neutrality (for BA, see Refs. 14 and 16; for TMA, M. Bellando, unpublished data). Further evidence in this direction is given by the data of Figure 3, showing that the BA uptake rate is almost insensitive to large changes in the concentration of the dissociated acid form.

The assumption that BA and TMA do indeed acidify the cytosol is accepted here by extrapolation of the data of other authors (15, 16) and is also supported by the finding that in wheat and maize roots, both BA and TMA induce a significant decrease of cell sap pH (for maize roots, see Ref. 15; for wheat roots, M. Bellando, unpublished data). Actually, cell sap pH essentially reflects the vacuolar pH; however, it seems reasonable to assume that in a weak acid-treated tissue, cytosolic and vacuolar pH would change in the same direction.

The possibility of metabolism of the weak acid has not been thoroughly investigated in this paper. In roots, we found that less than 2% of the [^{14}C]BA taken up in 90 min (when fed at the 2 mM concentration) is metabolized to CO_2, and even less to lipids or other acid-soluble materials. The chances of metabolism of TMA appear even less likely. On the other hand, it seems unlikely that some metabolism of the weak acid would significantly interfere with the results and the conclusion of the present paper.

With these assumptions the results presented in this paper suggest the following conclusions:

Cytoplasm acidification by weak acids permeating in the uncharged form is accompanied in maize and in wheat root segments by an increase in the rate of H^+ extrusion, roughly corresponding to an increase in K^+ uptake. This result, when integrated with the finding that in both materials weak acids also induce a hyperpolarization of PD, confirms the hypothesis that the acidification of the cytoplasm indeed increases the activity of the electrogenic proton extrusion mechanism. This conclusion is in full agreement with the view, supported by *in vitro* evidence, that electrogenic proton extrusion is mediated by a vanadate-sensitive plasmalemma ATPase with a pH optimum close to 6.5 (9, 10, 16, 18).

The presence of K^+ in the medium significantly increases the uptake of weak acids from the medium (BA in the case of maize, TMA in that of wheat). Of the two main and more direct effects of K^+ influx, namely PD depolarization and stimulation of H^+ extrusion, the latter seems more easily interpretable as a cause of the increased weak acid uptake. In fact, H^+ extrusion is strictly dependent on the parallel uptake of K^+ (see 2, 4, 7, 11 for a discussion of the coupling between H^+ extrusion and cation uptake). Thus, it seems reasonable to assume that the activation of the pump by K^+ should be associated with an increase in cytosolic pH. This would increase the rate of dissociation of the weak acid in the cytoplasm, thus keeping low the cytosolic concentration of the permeant undissociated form, making steeper its concentration gradient across the plasmalemma, and accelerating its net inward diffusion. The conclusion that an alkalinization of the cytosol would accelerate weak acid uptake is supported by the finding that the opposite condition, namely the acidification of the cytosol by mM BA or TMA does indeed strongly inhibit the uptake of a different (^{14}C-labeled) weak acid (such as DMO or BA) fed at μM concentration (G. Romani, M. T. Marrè, M. Bellando, G. Alloatti, E. Marrè, unpublished data).

Proton extrusion and K^+ uptake are stimulated, and PD is hyperpolarized by treatment with either permeant weak acids or with the toxin FC (7, 14). When both FC and a weak acid are present together, the effects of the two agents on H^+ extrusion and K^+ uptake are clearly synergistic. This strongly suggests that the two agents influence the proton pump through different mechanisms. In a simple interpretation or model, H^+ pump activation by weak acids would depend on increased cytosolic H^+ concentration, with cytosolic H^+ acting as a substrate (thermodynamic factor) and/or as an activator of the plasmalemma ATPase (kinetic factor), whereas the effect of FC would depend only on the activation of the enzymic machinery of the pump (possibly by antagonizing some endogenous, unidentified, inhibitor).

In conclusion, the present results clearly indicate the importance of cytosolic pH for the regulation of the activity of the H^+ pump. This conclusion is not in contrast with the view that also other factors, such as the PD value and the concentrations of specific natural chemical effectors, among which endogenous hormones of products of hormone action, may be equally important for the modulation of electrogenic H^+ transport.

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