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Potassium homeostasis in vacuolate plant cells

(cytosolic K^+/cytosolic pH/plant vacuole)

DAVID J. WALKER, ROGER A. LEIGH, AND ANTHONY J. MILLER*

Biochemistry and Physiology Department, Institute of Arable Crops Research (IACR)-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

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ABSTRACT **Plant cells contain two major pools of K^+, one in the vacuole and one in the cytosol. The behavior of K^+ concentrations in these pools is fundamental to understanding the way this nutrient affects plant growth. Triple-barreled microelectrodes have been used to obtain the first fully quantitative measurements of the changes in K^+ activity (a_K) in the vacuole and cytosol of barley (*Hordeum vulgare* L.) root cells grown in different K^+ concentrations. The electrodes incorporate a pH-selective barrel allowing each measurement to be assigned to either the cytosol or vacuole. The measurements revealed that vacuolar a_K declined linearly with decreases in tissue K^+ concentration, whereas cytosolic a_K initially remained constant in both epidermal and cortical cells but then declined at different rates in each cell type. An unexpected finding was that cytoplasmic pH declined in parallel with cytosolic a_K, but acidification of the cytosol with butyrate did not reveal any short-term link between these two parameters. These measurements show the very different responses of the vacuolar and cytosolic K^+ pools to changes in K^+ availability and also show that cytosolic K^+ homeostasis differs quantitatively in different cell types. The data have been used in thermodynamic calculations to predict the need for, and likely mechanisms of, active K^+ transport into the vacuole and cytosol. The direction of active K^+ transport at the vacuolar membrane changes with tissue K^+ status.**

Potassium is the major ionic osmoticum in plant cells and occurs in two major pools, one in the vacuole and one in the cytosol. The vacuolar pool is the largest and K^+ in this compartment has a purely biophysical function—the lowering of sap osmotic potential to generate turgor and drive cell expansion. In contrast, K^+ in the cytosol has both osmotic and biochemical roles (1, 2). When the external K^+ supply declines from sufficiency to deficiency the behavior of K^+ concentrations in each of these compartments is thought to differ; that in the cytosol remains relatively constant to maintain the rate of K^+-dependent processes, whereas that in the vacuole declines with other osmotica replacing it to maintain turgor (3). Cytosolic K^+ concentration is thought to decline only when the vacuolar K^+ concentration has been depleted to some minimum value below which it will not fall. The consequent changes in cytosolic K^+ are hypothesized to cause a decrease in the rate of biochemical processes and, thus, to a decline in growth (3).

The proposed behavior of K^+ concentrations in the cytosol and vacuole is broadly accepted and is supported by a variety of studies (e.g., refs. 4–8). However, it has never been quantitatively tested because the techniques used in the above studies measured compartmental K^+ concentrations either indirectly (e.g., ref. 4) or only semi-quantitatively (e.g., refs. 7 and 8). In this study we have used a new approach to measuring K^+ compartmentation in plant cells and its response to K^+ deficiency. Triple-barreled microelectrodes (9), able to measure K^+ activity (a_K), pH, and membrane potential (E_m), have been employed to determine these parameters in root cells of barley plants grown with different K^+ supplies. The incorporation of a pH-sensing barrel allows unequivocal assignment of a_K values to the vacuole or the cytosol, based on the pH differences between these compartments (10). The results obtained provide the first fully quantitative study of K^+ homeostasis in plants.

MATERIALS AND METHODS

Growth of Plants. Barley (*Hordeum vulgare* L. cv. Klaxon) seeds were germinated and grown in darkness over aerated 0.2 mM $CaSO_4$ for 5 days before transfer to either a full nutrient solution (FNS) containing 4.5 mM $CaCl_2$, 1.8 mM $Ca(NO_3)_2$, 0.2 mM FeNaEDTA, 2.2 mM $MgSO_4$, 1.1 mM NaH_2PO_4, and 0, 0.1, 0.5 or 5.0 mM KCl, plus micronutrients (pH adjusted to 5.8–6.0 with NaOH; contributing an additional 0.2 mM Na^+) (11) or to 0.5 mM $CaSO_4$ plus micronutrients, buffered with 1.0 mM 2-(*N*-morpholino)ethanesulfonic acid·Tris (pH 5.8) (designated $CaSO_4$). Growth for all treatments was at a constant 20°C, with a relative humidity of 75%, a photon fluence rate of 280–300 μmol photons m^{-2} sec^{-1}, and a day length of 16 h. Four plants were grown in a 0.6-liter pot until day 10 after sowing and then transferred to a 1.1-liter pot. Nutrient solution was replaced daily. The FNS and $CaSO_4$ solutions without added K^+ contained a background level of 2 μM K^+, and the $CaSO_4$ treatment contained 2.5 μM Na^+. Measurements were made 2, 9, and 16 days after transfer to the treatment solutions. In the experiment in Table 1, butyric acid (final concentration 10 mM) was added to FNS containing 0.5 mM KCl and the pH was adjusted to 5.8 with NaOH (final Na^+ concentration 11 mM) .

Electrophysiology. Triple-barreled microelectrodes for simultaneously measuring a_K, pH, and E_m were made and calibrated as in ref. 9, except that the K^+ sensor used contained 90% (wt/wt) Fluka K^+ ionophore I cocktail B (Fluka 60398). The reference barrel back-filling electrolyte was 200 mM NaCl. For intracellular measurements, a barley root, still attached to the plant, was perfused (10 ml min^{-1}) with aerated nutrient solution in a 1.5-ml Plexiglas chamber. Impalements were made in the epidermal and first two cortical cell layers, 10–20 mm from the tips of seminal roots. Intracellular measurements were only accepted if the E_m values were at least −40, −70, −90, or −100 mV for cells of plants grown with 5.0, 0.5, 0.1, or 0.002 mM K^+, respectively, as expected for barley root cells in these conditions (12, 13). Calibrations of the K^+ and pH barrels were performed before and after each root cell measurement. The K^+-selective barrel was calibrated against K^+ activity (a_K), not concentration (see ref. 9 for details).

Determination of Tissue K^+ Concentrations. Root apoplasmic solution was removed by a 10-min incubation in an iso-osmotic sorbitol solution (14), and after gentle blotting fresh weights of roots were recorded. No specific attempts

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Abbreviations: FNS, full nutrient solution; PPase, H^+-pumping pyrophosphatase.

*To whom reprint requests should be addressed.

were made to desorb K^+ from the cell wall as the high divalent cation concentrations in the nutrient solution meant that the cation exchange sites were filled by Ca^{2+} and Mg^{2+}. The tissue was oven-dried at 80°C for 72 h, reweighed, digested using a 85:15 (vol/vol) concentrated nitric acid:perchloric acid mixture and K^+ was determined by inductively coupled plasma atomic emission spectrometry.

Presentation of Results. Results are expressed as the mean ± SE minimum of six measurements for any single parameter, except for intracellular a_K values, which are expressed as the means and 95% confidence intervals (15). The number of measurements of vacuolar or cytosolic a_K, E_m, and pH for a given cell type in a given treatment sometimes differs because occasionally one of the ion-selective barrels failed to recalibrate after impalement and so the intracellular measurements made with that barrel were rejected.

Thermodynamic Calculations. The electrochemical potential gradients ($\Delta\mu/F$) for K^+ across the plasma membrane and tonoplast and the feasibility of symport and antiport mechanisms were determined using standard equations (16, 17). The free energy relationship of K^+ transport by the vacuolar H^+-pumping pyrophosphatase (PPase) was calculated using equation 18 from ref. 18 using a H^+:K^+ stoichiometry of 1.3:1.7 (19).

RESULTS

Measurement of Compartmental a_K. Shortly after impalement of a cell with an electrode, steady values of both a_K and pH were obtained and these remained constant until the electrode was withdrawn (Fig. 1). For the cell in Fig. 1 the a_K value of 66 mM was assigned to the cytosol because the pH-selective barrel reported a value of 7.4. Thus, the E_m-reporting barrel was measuring the membrane potential across the plasma membrane and this had a value of −129 mV. Similar recordings were used to obtain all of the data reported in this paper. The values of a_K separated into two populations when plotted as a function of pH (9). The more acidic population (pH 5.0–5.5) was assigned to the vacuole and the other to the cytosol. Tonoplast E_m was calculated by subtracting the mean of the E_m values for the cytosolic population from those of the vacuolar population and are reported using the sign convention of Bertl *et al.* (20).

Responses of Cytosolic and Vacuolar a_K to Changes in Tissue K^+ Status. After growth for 2–16 days in a solution containing 5.0 mM K^+, root K^+ concentration was about 150 mM, while the mean vacuolar a_K in both epidermal and cortical cells was over 100 mM and the cytosolic a_K was between 75 and 83 mM (Fig. 2). (The apparent discrepancy between tissue total K^+ concentration and the compartmental values arise because the microelectrodes were calibrated against K^+ activity, not concentration, whereas the tissue extraction procedure measures the total chemical amount.) When the external concentration of K^+ was decreased there was a decline in tissue K^+ concentration, which was matched by a proportionate and linear decline in vacuolar a_K in both epidermal and cortical cells. In contrast, cytosolic a_K remained relatively constant until the tissue K^+ concentration had declined to about 25 mM and then it behaved differently in epidermal and cortical cells. In epidermal cells, cytosolic a_K declined markedly with further decreases in tissue K^+ concentration and was only 39 mM in cells grown for 16 days in FNS containing 2 μM of K^+. In contrast, cortical cells grown under the same conditions still had a cytosolic a_K of 63 mM, but there was an indication that it was beginning to decline in response to the change in tissue K^+ concentration (Fig. 2). Growing the roots in $CaSO_4$ did not significantly affect the way vacuolar and cytosolic a_K behaved (Fig. 2).

Parallel Changes in Cytosolic a_K and pH. Vacuolar pH in both cell types was unaffected by K^+ supply (data not shown), but K^+ deficiency did affect cytosolic pH (Fig. 3). At cytosolic a_K values over 60–70 mM, the cytosolic pH was in the range 7.2–7.4, as expected (10). When cytosolic a_K fell below this range, cytosolic pH decreased by 0.2 pH units for every 10 mM change in cytosolic a_K, irrespective of whether the roots were grown on FNS or $CaSO_4$ and both epidermal and cortical cells followed the same relationship.

There is evidence from stomatal guard cells that cytosolic pH can act as a signal affecting the activity of K^+ channels in both the plasma membrane (21) and tonoplast (22). Thus, the decrease in cytosolic a_K may be linked to changes in K^+ channel activity caused by the acidification of the cytosol. To test this, cytosolic pH was perturbed by incubating K^+-replete

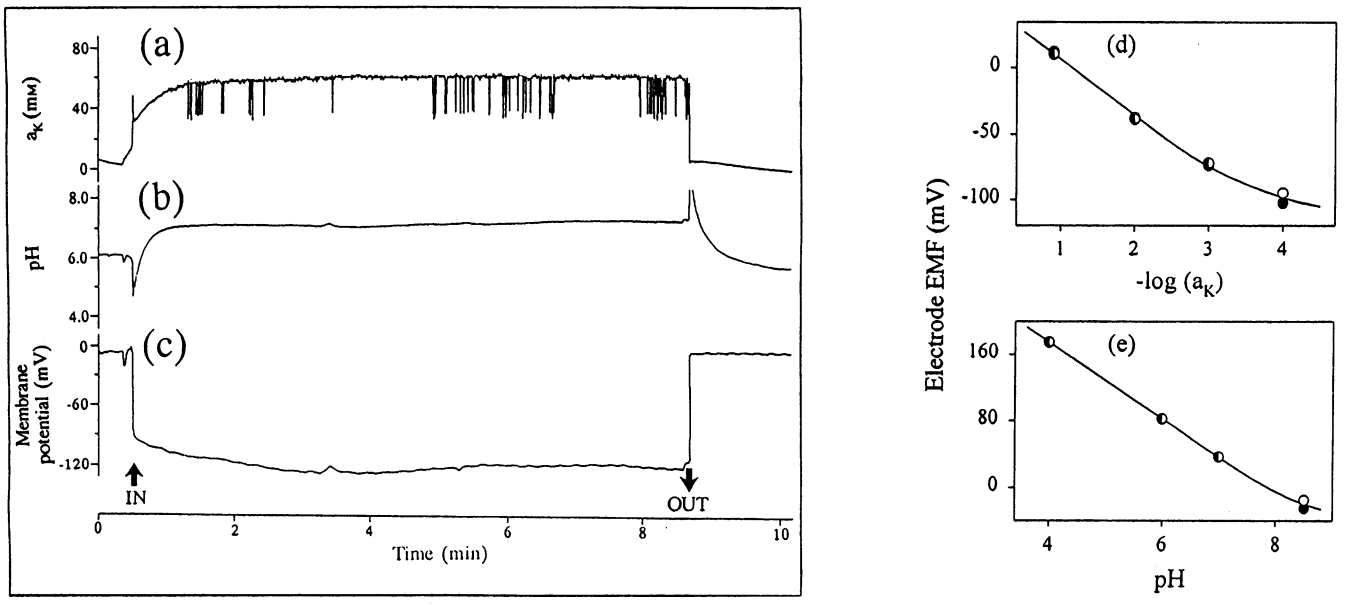

FIG. 1. Simultaneous recording of intracellular a_K (*a*), pH (*b*), and E_m (*c*) in the cytosol of a root epidermal cell of a 7-day old barley plant grown from day 5 in FNS containing 2 μM of K^+. Stable intracellular values were: a_K, 66 mM; pH 7.4; E_m, −129 mV. Potassium activities and pH were calculated using modified Nicolsky–Eisenman equations fitted to the combined data from calibrations for a_K (*d*) and pH (*e*) performed before (○) and after (●) the impalement.

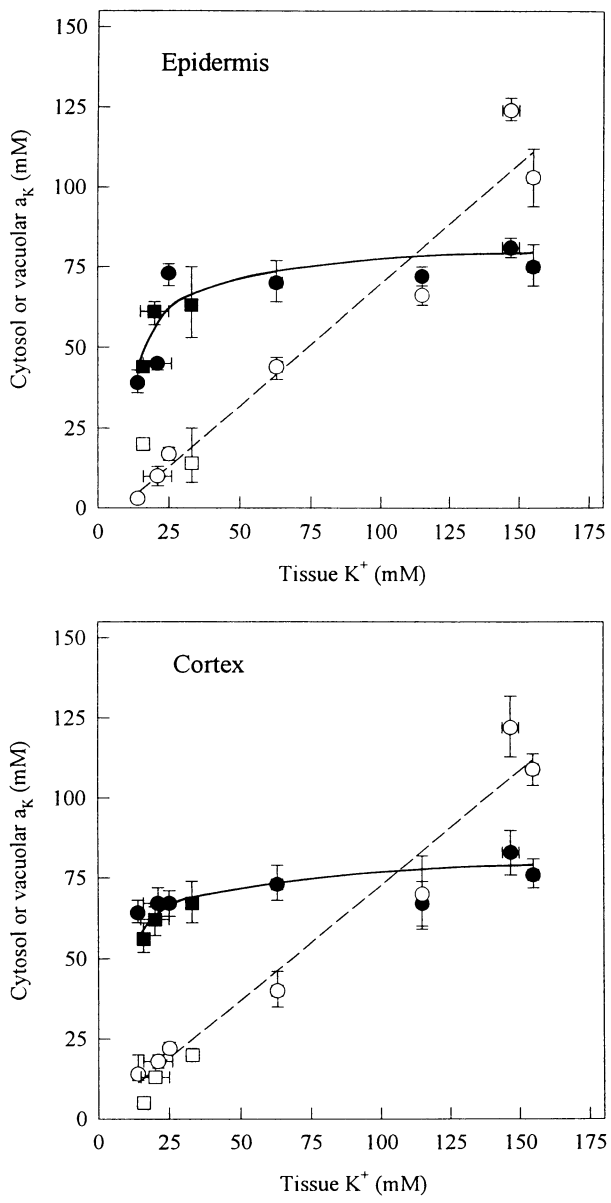

FIG. 2. The relationship between tissue K^+ concentration and vacuolar (open symbols) or cytosolic (solid symbols) a_K in epidermal and cortical cells of barley roots grown in either FNS with a range of K^+ concentrations (circles) or in $CaSO_4$ (squares). Linear regressions (dashed lines) are fitted to the vacuolar measurements and have the following parameters: epidermis, $y = 0.75x - 5.76$, $r^2 = 0.95$ ($P = 0.001$); cortex, $y = 0.72x + 1.2$, $r^2 = 0.96$ ($P = 0.001$). Solid lines through the measurements for the cytosol are drawn by eye. Bars show ± SE for the measurement of tissue K^+ concentration and 95% confidence limits for the a_K values.

roots for 2 h in FNS containing 10 mM butyric acid. This treatment acidified the cytosol but did not affect cytosolic a_K in either epidermal or cortical cells (Table 1). Thus, at least in the short term, the two parameters can vary independently.

Thermodynamics of Transport at the Plasma Membrane and Tonoplast. The measurements of a_K, pH, and E_m can be used to determine both the electrochemical potential gradients for K^+ across the plasma membrane and tonoplast and the feasibility of achieving these gradients by different mechanisms of active K^+ transport. Although such calculations have been done for K^+ transport across the plasma membrane of root cells (16, 23), this is the first time it has been possible to extend them to different cell types and to the tonoplast. The values of the electrochemical potential gradient for K^+ at the

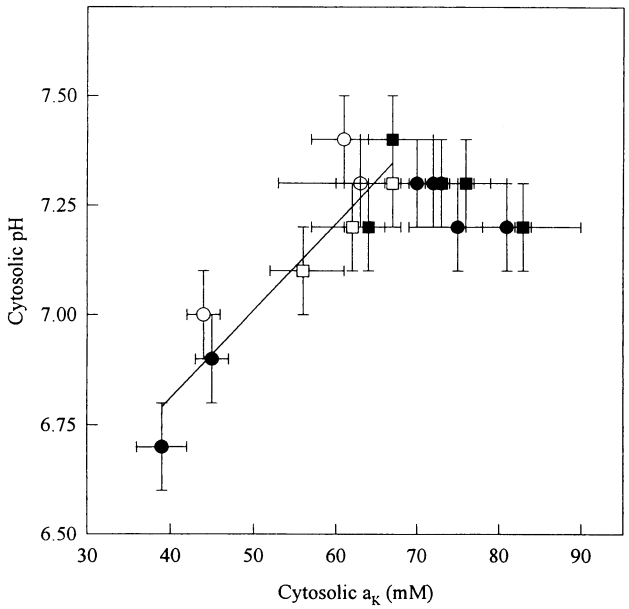

FIG. 3. The relationship between cytosolic pH and cytosolic a_K in epidermal (circles) and cortical (squares) cells of barley roots grown for 2–16 days in FNS containing a range of K^+ concentrations (solid symbols) or in $CaSO_4$ (open symbols). The regression line is fitted to all points with a cytosolic a_K of <70 mM and has the parameters: $y = 0.02x + 6.02$; $r^2 = 0.858$ ($P = 0.01$). Bars show 95% confidence limits for the measurements of a_K and ± SE for measurements of pH.

plasma membrane indicate that in roots grown in 5.0 mM K^+, inward transport of K^+ is passive in both epidermal and cortical cells (Table 2). In contrast, active uptake of K^+ across the plasma membrane must be invoked in both cell types in roots grown in 0.002, 0.1, or 0.5 mM K^+. In addition, a 1:1 H^+:K^+ symport is a feasible mechanism for achieving active K^+ uptake at the plasma membrane (Table 3), in agreement with the conclusions of others (16, 23) but now confirmed for both epidermal and cortical cells.

The tonoplast E_m varied from between −9 and −23 mV, was similar in both cell types, and did not change in any consistent way with the K^+ status of the roots (Table 2). With these values of tonoplast E_m, active transport of K^+ from cytosol to vacuole was necessary in both epidermal and cortical cells of roots grown in 0.5 or 5.0 mM K^+ (Table 2) in which the vacuolar a_K was greater than or equal to cytosolic a_K (Fig. 2). A passive mechanism for K^+ uptake into the vacuole could operate under these conditions only if the trans-tonoplast potential was between −30 and −40 mV, values much higher than those recorded (Table 2). In contrast, when roots were grown in 0.1 mM or 2 μM of K^+ and the vacuolar a_K was lower than that in the cytosol, the direction of K^+ transport across the tonoplast was changed with a requirement for active transport of K^+ from the vacuole to the cytosol (Table 2).

Two mechanisms have been proposed for active accumulation of K^+ in the vacuole: transport via the tonoplast PPase

Table 1. The effects of a 2-h pretreatment with 10 mM butyrate on the cytosolic a_K and cytosolic pH of epidermal and cortical cells of barley roots grown for 2 days in FNS containing 0.5 mM KCl

Cell type	Butyrate	a_K*	pH†
Epidermal	—	72 (66, 75) (8)	7.3 ± 0.1 (8)
	+	75 (66, 85) (5)	6.9 ± 0.1 (5)
Cortical	—	67 (63, 69) (7)	7.3 ± 0.1 (6)
	+	66 (76, 90) (16)	6.9 ± 0.1 (16)

*The values in the first set of parentheses indicate 95% confidence limits, those in the second the number of observations.
†Mean ± SE; values in parentheses indicate number of observations.

Table 2. Calculated values of the electrochemical potential ($\Delta\mu/F$) for K^+ at the plasma membrane and tonoplast of epidermal and cortical cells grown with different K^+ supplies, and comparison with the measured membrane potential (E_m) across each of these membranes

		Epidermal cells				Cortical cells			
		Plasma membrane		Tonoplast		Plasma membrane		Tonoplast	
$[K^+]_o$, mM	Time in K^+, d	$\Delta\mu/F$, mV	E_m, mV	$\Delta\mu/F$, mV	E_m, mV	$\Delta\mu/F$, mV	E_m, mV	$\Delta\mu/F$, mV	E_m, mV
5.0	16	−8	−83 ± 6 (7)	−27	−19 ± 7 (7)	−10	−85 ± 7 (7)	−20	−11 ± 9 (7)
0.5	2	+26	−105 ± 7 (8)	−12	−15 ± 8 (8)	+31	−99 ± 7 (6)	−10	−10 ± 6 (12)
0.1	2	+47	−124 ± 5 (6)	+1	−14 ± 6 (6)	+53	−119 ± 3 (6)	+7	−9 ± 10 (6)
0.002	9	+134	−128 ± 5 (9)	+17	−23 ± 11 (2)	+138	−134 ± 4 (20)	+19	−15 ± 6 (12)

Positive $\Delta\mu/F$ values indicate that movement of K^+ into the cytosol from either the external solution or the vacuole requires active transport. a_K values in nutrient solutions were calculated to be 0.0017, 0.083, 0.42, and 4.2 mM for solutions containing K^+ concentrations of 0.002, 0.1, 0.5, and 5.0 mM, respectively (24).

(19) or by a 1:1 H^+:K^+ antiport (25). Calculations of the free energy relationships for these mechanisms show that both are feasible (Table 3). For cells with low vacuolar a_K, active export of K^+ from the vacuole could be achieved by a 1:1 H^+:K^+ symport (Table 3).

DISCUSSION

We report the first fully quantitative study of changes in vacuolar and cytosolic a_K in plant cells in response to changes in K^+ availability. Previous approaches have either been unable to separate vacuole and cytosol (26) or were not truly quantitative (7, 8). The triple-barreled microelectrode measurements unequivocally identify compartmental K^+ activities and associated E_m values because the location of the tip is confirmed by the concomitant measurement of pH. The results show that there is no regulation of vacuolar K^+ content and vacuolar a_K declines proportionately with tissue K^+ concentration. The slope of the relationship between vacuolar a_K and tissue K^+ concentration was 0.75 in epidermal and 0.72 in cortical cells, consistent with the activity coefficient for K^+ in moderately concentrated solutions of KCl (24). Thus, K^+ in solution in the vacuole is freely ionized. In contrast to the behavior of K^+ in the vacuole, cytosolic a_K remained constant until the whole-root K^+ concentration had declined to below 25 mM and then it fell at different rates in epidermal and cortical cells. The behavior of vacuolar and cytosolic a_K is consistent with the model previously proposed (3) and directly defines, for the first time, the limits of cytosolic K^+ homeostasis in plant cells.

The trigger for the decline in cytosolic a_K, particularly in epidermal cells, at whole-root K^+ concentrations below about 25 mM remains unclear. It has been proposed (3) that decreases in cytosolic a_K are due to the vacuolar K^+ concentration reaching a minimum value of about 20 mM below which it will not decline and so further decreases in whole-tissue K content have to be at the expense of the cytosol. Although x-ray microanalysis studies have apparently confirmed this lower limit to vacuolar K^+ concentration in barley roots (8), the triple-barreled electrode measurements (Fig. 2) do not support it. Thus, some factor other than the ability to mobilize K^+ from the vacuole must be responsible for causing the decline in cytosolic a_K.

The observation that epidermal cells are less able to maintain cytosolic a_K than cortical cells may indicate that the difference in behavior is related to the distribution of the high-affinity K^+ transporter HKT1 (27). *In situ* localization studies in wheat roots have shown that this transporter is chiefly associated with root cortical cells (27). Thus, the maintenance of cytosolic a_K in cortical cells may result from their ability to use HKT1 to actively transport K^+ into the cell under conditions of extreme K^+ deficiency. However, the thermodynamic calculations (Table 2) indicate that K^+-deficient epidermal cells must also be actively transporting K^+ across the plasma membrane into the cytosol. Thus, the response of cytosolic a_K in each cell type may be related either to differences in the K^+ concentration gradients that the active K^+ transporters can maintain, or to differences in the activation properties of outward rectifying K^+ channels in the two cell types, with those in epidermal cells having a higher open probability resulting in the loss of K^+ through them.

Alternatively, the decline in cytosolic a_K may be related to changes in cell turgor. Potassium deficiency causes a decline in cell turgor of about 0.2–0.4 MPa in mature wheat root cells (28, 29). This is equivalent to a decrease in cell osmotic pressure of about 80–160 mosmol·kg^{-1} and both vacuolar and cytosolic osmotic potential must change in parallel by this amount to maintain osmotic equilibrium across the tonoplast. Potassium may be one of the solutes contributing to the decrease in cytosolic osmotic potential during this change in turgor. The difference of 40 mM in cytosolic a_K between epidermal cells grown in K^+-replete and K^+-deficient conditions (Fig. 2), if matched by a parallel decline in a monovalent anion, would be equivalent to an osmotic pressure decrease of about 0.2 MPa

Table 3. Thermodynamics of various active K^+ transport mechanisms at the plasma membrane and tonoplast

				$\Delta G'/F$, mV			
				Plasma membrane*	Tonoplast*		
		Mean a_K, mM		H^+:K^+		H^+:K^+	H^+:K^+
K^+ status	Cell type	Cytosol	Vacuole	symport	PPase	antiport	symport
Replete	Epidermis	81	124	—	−57	−107	—
Starved	Epidermis	45	10	−59	—	—	−96
Replete	Cortex	83	122	—	−75	−102	—
Starved	Cortex	67	18	−81	—	—	−120

Potassium-replete seedlings were grown for 2 days in FNS containing 5 mM K^+, while starved seedlings were grown for 9 days in FNS with 0.002 mM K^+. A negative value of $\Delta G'/F$ shows the feasibility of the mechanism to maintain the measured K^+ gradients. Values of a_K in the external solution were 4.2 mM (replete) and 1.7 μM (starved).
*H^+:K^+ stoichiometry is 1:1 for all mechanisms except PPase where 1.3H^+:1.7K^+ is used (19).

and thus within the range of measured changes in turgor (28, 29). However, there is little difference in the responses of turgor to K^+ deficiency in epidermal and cortical cells (29) yet the cytosolic a_K declines more in epidermal cells than cortical cells (Fig. 2). A detailed study of the interrelationships between cytosolic a_K and turgor in responses to K^+ deficiency is needed to clarify the precise role of turgor in determining the decline in cytosolic a_K in these two cell types.

The parallel decline in cytosolic pH and cytosolic a_K in K^+-deficient cells (Fig. 3) is the first time such a relationship has been reported. In *Neurospora*, cytosolic pH was decreased by K^+ starvation, but changes in cytosolic a_K were not determined (30). The decrease in pH raises the possibility that lower growth rates caused by K^+ deficiency are not due solely to changes in cytosolic a_K but are also caused by the effects of lowered pH on the activity of metabolic processes in the cytosol. The reason for the parallel decreases in cytosolic a_K and pH is unclear, but it is not due to direct effects of short-term changes in cytosolic pH on K^+ transport (Table 1). Therefore, the relationship must indicate a long-term effect of K^+ status on cytosolic pH. Three mechanisms contribute to the maintenance of cytosolic pH in plant cells (31): physicochemical buffering (32), the biochemical pH-stat (33), and H^+ export from the cytosol to the external medium or the vacuole (31). Presumably, effects on one or several of these contribute to the change in cytosolic pH in K^+-starved cells.

The direction of the electrochemical gradient for K^+ at the tonoplast depended upon the external K^+ supply (Table 3). The measurements we have made indicate that both a 1:1 H^+:K^+ antiporter (26) and the tonoplast PPase (19) are feasible mechanisms for active K^+ transport into the vacuole and thus the measurements cannot contribute to resolving whether the PPase really does operate as a K^+ transporter (19, 34, 35). The argument that in severely K^+-depleted cells there is a need for active transport of K^+ out of the vacuole is new. Our calculations suggest that this could be achieved by a 1:1 H^+:K^+ symport. This transporter will be electrogenic and so its activity may be detectable by patch clamping.

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