J. Membrane Biol. 53, 199–205 (1980)

The Journal of
Membrane Biology

Ion Content and Aperture in "Isolated" Guard Cells of *Commelina communis* L.

E.A.C. MacRobbie and J. Lettau

Botany School, University of Cambridge, Downing Street, Cambridge CB2 3EA, England

Summary. "Isolated" guard cells of *Commelina communis* L., in epidermal strips in which all cells other than guard cells have been killed by treatment at low pH, will open to a degree dependent on the K(Rb)/Cl(Br) concentration in the bathing medium. Estimates of the changes with aperture of the ion concentrations in the guard cells were made by measurement of ^{86}Rb uptake from RbCl, of ^{82}Br uptake from $K^{82}Br$, and of potassium activity with a potassium-sensitive microelectrode. The osmotic effects of such changes were compared with the previous estimates of the osmotic changes required to change the aperture. The results suggest that a substantial fraction of the osmotic pressure of "isolated" guard cells is contributed by solutes other than KCl (or other potassium salts), and that, even in stomata opened by incubation on KCl solutions, a substantial fraction of the increase in osmotic pressure associated with opening is contributed by solutes other than KCl.

The accumulation of potassium salts in high concentrations in the guard cells of open stomata has been demonstrated by histochemical methods in some 50 species, including examples from both pteridophytes and gymnosperms and a wide range of angiosperms (Willmer & Pallas, 1973; Dayanandan & Kaufman, 1975). It is generally accepted that such accumulation of potassium salts is responsible for the turgor changes leading to changes in aperture, but the nature of the primary process responsible for the initiation of the set of changes in ion fluxes and ionic status has not been clearly established (*see* reviews by Raschke, 1975, 1977; Thomas, 1975; Hsiao, 1976; MacRobbie, 1977). Further, quantitative estimates of potassium concentrations in guard cells are available for only a few species, and there is a need for a detailed study of the relation between potassium content and aperture over the whole range of apertures, rather than simply in the open and the closed states.

The work reported in this and a following paper *(in preparation)* formed the starting point of a study of the stomata of *Commelina communis* L. in an attempt to establish the changes in the ionic state of the guard cell associated with change in aperture; such a description should include not only concentration figures but also flux and potential data. *Commelina* has been much used for stomatal work and was chosen because it allows the study of "isolated" guard cells, in epidermal strips in which all cells other than guard cells have been killed by treatment at low pH (Squire & Mansfield, 1972). In such tissue tracer measurements of ion concentration changes in guard cells are possible, and flux measurements may be made without the complications of contributions from subsidiary and other epidermal cells.

The present paper is concerned with measurements on such "isolated" guard cells, in particular with measurements of concentration changes with aperture. These figures may then be compared with the osmotic changes required to change the aperture, which were reported in the previous paper (MacRobbie, 1980), and the contribution of potassium salts to the total osmotic changes may be assessed. Estimates of concentration changes were made in two ways, by measuring potassium activity using double barrelled K-sensitive microelectrodes, with Corning potassium liquid ion exchanger (477317) in one barrel, and by measurements of tracer uptake of ^{86}Rb from RbCl or of ^{82}Br from KBr. The conclusion from these measurements is that for "isolated" guard cells, opening floating on KCl, the potassium changes are not adequate to account for the changes in aperture, and some other process must also be involved. A following paper *(in preparation)* will present results

0022-2631/80/0053-0199 $01.40

of potassium activity measurements in "intact" guard cells, in epidermal strips in which subsidiary and epidermal cells are still alive.

Materials and Methods

Preparation of Epidermal Strips

Plants of *Commelina communis* L., about 6 weeks old, were used. "Isolated" guard cells were prepared by floating strips of lower epidermis, about 20–80 mm^2 in size, at low pH for several hours, as described in the previous paper (MacRobbie, 1980). In general, a treatment for 2–4 hr in 10 mM MES (2-(N-morpholino) ethanesulphonic acid) at pH 3.9 was used. It was essential to check that epidermal cells had been killed by this treatment; the presence of even a small fraction of live epidermal cells, recognized by their microscopic appearance and the presence of crystals, led to much higher tracer uptakes and would invalidate the results. It was therefore important to scan the whole area of each individual strip and to discard any in which live epidermal cells were seen.

After the isolation procedure strips were floated on the experimental solutions, 10–90 mM KCl (or RbCl or KBr as appropriate), with 10 mM MES at pH 3.9 or with 10 mM MOPS at pH 6.7 (3-(N-morpholino) propanesulphonic acid). Guard cells will survive for days, at either pH, and will open widely if given KCl. In general, overnight treatment in a given solution, in a thermostatted cabinet at 30 °C in light, was used to open the stomata, to a degree dependent on the KCl concentration.

Tracer Measurements

Influx measurements, or measurements of total tracer content, were done by floating "isolated" epidermal strips, cuticle side up, on 3-ml portions of labeled solution in the wells of plastic culture dishes; solutions were $^{86}RbCl$ or $K^{82}Br$ of appropriate concentration, with either 10 mM MES at pH 3.9, or 10 mM MOPS at pH 6.7. After loading, strips were floated on a large volume of inactive solution, with changes, to remove free space activity before counting; the period of washing necessary was determined by efflux experiments (*see* Results). Apertures were measured, at 320× magnification with an eyepiece micrometer, on 10–20 complexes per strip; areas of strips were measured by observing at 12× magnification against mm graph paper. After measurement of area and aperture, strips were briefly submerged to rinse the top surface and digested in 0.5 ml of 100 mM HCl or NaOH, on the planchettes on which they were counted in a Tracerlab Omniguard (dried down with lens paper and 4 drops 2% sucrose), low background gas flow counting system.

Effluxes were measured by transferring strips to successive 0.7-ml portions of wash-out solution, again in the wells of plastic culture dishes, and counting both these wash-out solutions and the residue in the tissue after the wash-out.

Tracer experiments were done in a thermostatted cabinet, at 30 °C, under a bank of six 30 W warm-white fluorescent tubes; the light intensity at the level of the tissue was 36 Wm^{-2}, measured with a Kipp solarimeter.

Potassium Activity Measurements

Double barrelled K-sensitive microelectrodes, with Corning liquid ion exchanger (477317) in one barrel, were prepared as described by Zeuthen, Hiam and Silver (1974). The tips were about 1 μm. The use of double barrelled electrodes is likely to cause less damage to the cell than the insertion of two separate electrodes and avoids the problems of subtraction of a separately determined membrane potential from the potential between an internal ion-sensitive electrode and an external reference electrode, to allow calculation of K-activity; previous work on guard cells has used separate potential and K-sensitive electrodes (Penny & Bowling, 1974; Zlotnikova, Gunar & Panichkin, 1977). The electrodes were connected by means of chlorided silver wires to a solid state, unity gain voltage follower (input impedance $>10^{10}\ \Omega$), designed by Mr. P.L. Joyce of the Physiological Laboratory, Cambridge. The output from this was registered on a Tekman recorder. The preparation was earthed by means of an Ag/AgCl wire in the bath. A number of solutions were tried as filling solutions for the reference barrel – 3 and 0.1 M KCl, 1 and 0.1 M NH_4NO_3. Although there appeared to be more scatter in the values obtained with 3 rather than 0.1 M KCl, there was no evidence of large differences. For the measurements reported in this paper, the reference barrel contained 0.1 M KCl. Electrodes were calibrated in KCl solutions before and after insertion in cells, and had slopes of about 55 mV for a 10-fold change in K-activity over the concentration range 10 mM to 1 M KCl. Results were only used if the calibration after insertion in the cell was comparable with that done before insertion.

Epidermal strips were mounted, mesophyll-side down, with a piece of coarse plastic mesh for support, on a glass slide (5 × 15 × 1 mm); wax was used to attach the strip to the plastic to the glass. The slide was placed in a chamber on a microscope stage, with an overflow compartment and provision for flow of solution through the chamber. A Research Instruments Ltd. TVC 500 micromanipulator was used for the insertion, observed at 320× magnification with a Dyson long working distance objective.

Readings were taken immediately after insertion, and were steady for several minutes; recordings of up to 10–15 min after insertion were sometimes obtained, but the figures used were the initial steady values. Stimultaneous recordings of potential were not made in the work reported in this paper.

Results

The aim is to examine the relationship between steady-state ion content and steady-state aperture in "isolated" guard cells. It is therefore necessary to establish conditions for steady aperture and, for tracer measurements, the washing regime for the determination of intracellular ion content after removal of free space activity.

Free Space Exchange

Initially influx and efflux were measured at pH 6.7 in 10–30 mM $^{86}RbCl$. Components in the efflux curve with half-times of 7–14 hr and 15–48 min could be distinguished, but it was difficult to separate any potentially cytoplasmic component, intermediate between free space exchange and the slow, presumably vacuolar, exchange. For example, in efflux experiments in 10 mM RbCl at pH 6.7, a component of 9.0 ± 1.1 (11) pmol mm^{-2}, with a half-time of 29 ± 4 (11) min could be distinguished. However, when the

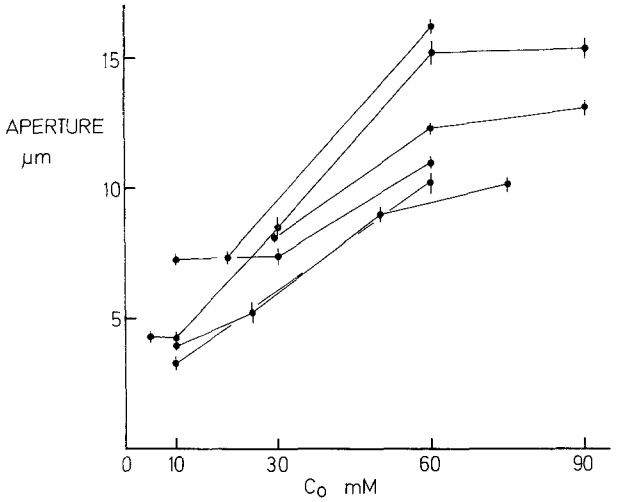

Fig. 1. Dependence of aperature on external concentration of K(Rb)/Cl(Br) in "isolated" guard cells, at pH 3.9; incubation for 16–24 hr

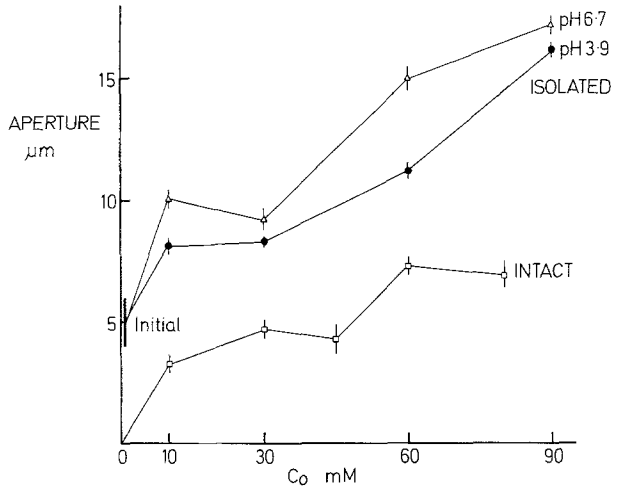

Fig. 2. Comparison of aperture of "isolated" guard cells in 10–90 mM KCl at pH 3.9 and 6.7; incubation for 24 hr after 17 hr pretreatment at pH 3.9 in the absence of KCl

efflux from frozen, thawed tissue was measured, a component of similar half-time also appeared with half-times of 20–40 min. It appears therefore that at pH 6.7 there is a component of the free space which exchanges slowly, presumably associated with fixed charge in the cell wall. Since this raises problems in removing free space activity without also losing significant intracellular activity, effluxes were also run at pH 3.9. At this pH the free space exchange was more rapid, and was effectively over in 10–15 min; if the fixed charges in the wall are protonated at this pH, this might be expected. At pH 3.9 the half-times for intracellular exchange were shorter, about 2 hr. Taken together, these figures mean that a wash of 10–15 min at pH 3.9 can be used to remove free space activity without losing a significant fraction of the intracellular activity.

With ^{82}Br there was no indication of a slow component of free space exchange, and the intracellular half-times were longer, so that a similar wash time could be used.

Table 1. Steady apertures over uptake periods of 13–45 hr at pH 3.9

C_o (mM)	Time (hr)	Aperture (μm)		
		Range of strip means		Overall mean
		Minimum	Maximum	
10, RbCl	16–17	3.0±0.3(28)	6.3±0.3(17)	4.8±0.15(128,7)
	44–45	4.2±0.2(18)	5.8±0.3(18)	4.9±0.1 (107,6)
20, KBr	15	6.9±0.5(16)	7.7±0.5(15)	7.2±0.2 (59,4)
	22	5.4±0.4(21)	9.5±0.4(16)	7.5±0.2 (119,7)
	42–43	5.4±0.3(15)	8.6±0.2(16)	7.3±0.2 (92,6)
60, KBr	13–14	14.2±0.8(12)	16.5±0.7(12)	15.0±0.4 (51,4)
	21	14.4±0.8(16)	20.0±0.6(16)	16.9±0.3 (96,6)
	42–43	16.3±1.7(16)	20.5±0.6(11)	17.4±0.5 (64,5)
60, RbCl	20–24	10.4±0.4(17)	13.9±0.9(19)	12.3±0.2 (130,7)
	41–42	10.0±0.6(23)	12.1±0.6(14)	11.0±0.3 (113,6)
90, RbCl	20–24	10.9±0.6(18)	16.1±0.7(19)	13.1±0.3 (101,6)
	44	16.7±0.8(20)	17.5±1.0(14)	17.0±0.6 (34,2)

Strip means give mean, SEM (number of pores measured). Overall means give mean, SEM (number of pores measured, number of strips). Uptake times quoted refer to a period of about an hour around that mean time, during which the apertures in individual strips were measured.

Table 2. Relation between ^{86}Rb content and aperture

C_o (RbCl) (mM)	Regression: a (pmol·mm^{-2})	$Q^* = a + bA$ b (pmol·mm^{-2} μm^{-1})	r (df)
30, 60, 90	− 8.5	6.6	0.95 (8)
30, 60	−20.4	9.7	0.93 (4)
10, 25, 50, 75	−19.1	10.6	0.96 (8)
30, 60, 90	−55.0	13.4	0.903(17)
10, 60	−56.7	14.8	0.818(12)

Strips were loaded for 16–24 hr, in RbCl at pH 3.9.

Behavior of Isolated Guard Cells

In view of the advantages of pH 3.9 for the tracer experiments, the behavior of "isolated" guard cells was studied both at pH 3.9 and at pH 6.7.

Table 1 shows that at external concentrations between 10 and 90 mM apertures at pH 3.9 are steady over the period of 15–45 hr of salt loading. At pH 6.7 there was little further change up to 80 hr of incubation in guard cells which still appeared healthy after this treatment, but there was evidence of deterioration in some guard cells in the strip after the prolonged incubation of 80 hr. At pH 3.9 there was significantly wider opening with such very long incubations, e.g., increases from 8 to 11 μm at 30 mM, and 11 to 18 μm at 60 mM. This seems likely to be the result of cell wall changes, and the measurements of content are therefore restricted to times less than 45 hr; in general, an incubation time of 16–24 hr was used in such experiments.

Figure 1 shows the relation between aperture and external concentration in a number of experiments at pH 3.9. Figure 2 compares the apertures after 24 hr incubation in 10–90 mM KCl at pH 3.9 and 6.7 (following a 17-hr pretreatment at pH 3.9 in the absence of KCl) and shows that, although the apertures at a given concentration are higher at pH 6.7 than at 3.9, the differences are not large. Figure 2 also shows, for comparison, the apertures attained by guard cells in intact strips, with subsidiary and epidermal cells alive, after incubation in 10–80 mM KCl for 17–22 hr. It is clear that much lower apertures are reached in guard cells required to expand against the turgor of intact subsidiary cells.

It is also important to establish whether "isolated" guard cells are still responsive to the same factors as are important in controlling aperture in the intact leaf. These effects have not been studied in detail but the evidence suggests that the same factors are still important. The effects of abscisic acid on fluxes will be discussed in another paper, and it is clear that such "isolated" guard cells are still sensitive to ABA. Itai and Meidner (1978) reported that only guard cells surrounded by live subsidiary cells closed in response to ABA. However, "isolated" guard cells lose a significant fraction of their tracer content in the period immediately after the addition of ABA, and their result reflects the importance of subsidiary cell turgor in producing very small apertures, rather than an insensitivity of "isolated" guard cells to ABA. "Isolated" guard cells change their aperture in response to changes in condition, but in the absence of turgid subsidiary cells, they do not close completely.

The same is true of CO_2. Figure 3 shows the effects of adding $NaHCO_3$ to the bathing solution (30 mM RbCl, 10 mM MOPS at pH 6.6), for four "isolated" epidermal strips; the aperture diminishes, and the effect is reversible on removal of the bicarbonate. Thus "isolated" guard cells are still sensitive to CO_2.

Tracer Measurements of Content

The relation between ^{86}Rb content (Q^*, pmol mm^{-2}) and aperture (A, μm) was established by loading "isolated" strips overnight in varying concentrations of RbCl, then measuring aperture and total tracer content after wash-out of free space activity. There was a linear relation between Q^* and A, and Fig. 4 shows one such regression. Table 2 shows the values obtained for 5 such regressions.

So far, only one similar full experiment with $K^{82}Br$ has been done which yielded the regression Q^* (Br) $= -63 + 13.6\ A$ ($r = 0.971$,17 df). Other measurements of ^{82}Br uptake and aperture are consistent with this. This suggests that, as might be expected, "isolated" guard cells opening by exposure to K(Rb)/Cl(Br) do so by uptake of halide ions from the solution, rather than by synthesis of malate to balance the cation uptake.

For the purposes of calculation, two regressions were used, that shown in Fig. 4 ($Q^* = -55 + 13.4\ A$) and a less steep slope ($Q^* = -19.1 + 10.6\ A$). From these relations, concentration changes with aperture were estimated, and the osmotic values of such concentrations of KCl (π_{KCl}, taken from the Handbook of Chemistry and Physics, Weast, CRC Press). This required estimates of stomatal frequencies and guard cell volumes. Frequencies were calculated from counts on photographs of epidermal strips or from estimates of the fractional areas of complexes in such photographs. Volumes were estimated from measurements on photographs of guard cells taken with Nomarski optics (640×) at various levels of focus. The figures used were: frequency of complexes 60 mm^{-2}, (single) guard cell volume 5 pl at 5 μm open, 6 pl at 10 μm, and 7 pl at 15 μm.

Using these figures, the results showed Rb concentrations increasing from 20–56 mM at 5 μm aperture to 174–167 mM at 15 μm. Figure 6 shows the calculated changes in concentration, and in π_{KCl}, with aperture.

Potassium Activity Measurements

Potassium activity measurements were also made on "isolated" guard cells. After the low pH treatment

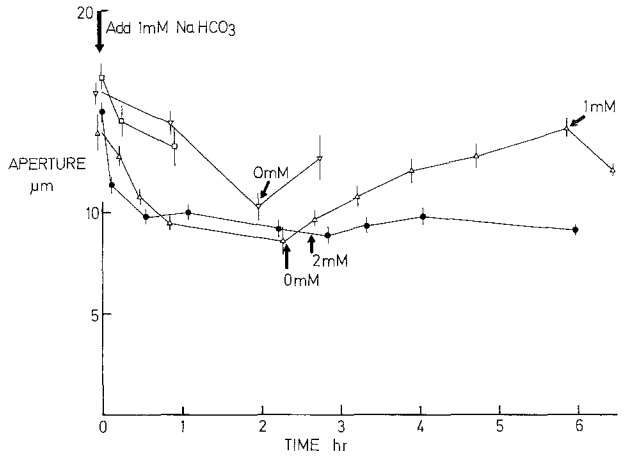

Fig. 3. Effects on the aperture of "isolated" guard cells of adding and removing $NaHCO_3$ (1 or 2 mM) in the bathing solution of 30 mM RbCl, 10 mM MOPS at pH 6.6

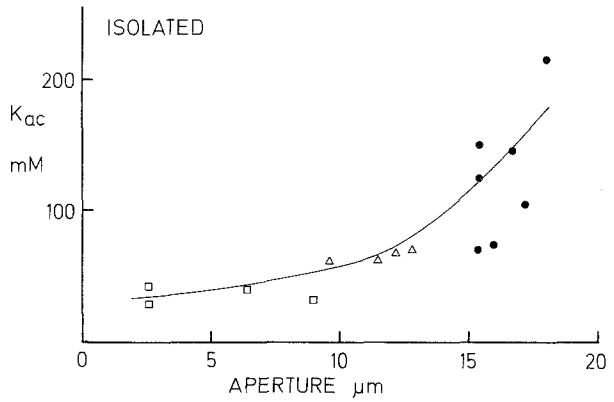

Fig. 5. Measurement of potassium activity (K_{ac}) in "isolated" guard cells, after incubation for not more than 24 hr in 10–50 mM KCl, 10 mM MES pH 3.9. Each point is a single measurement. □ 10 mM △ 30 mM ○ 50 mM

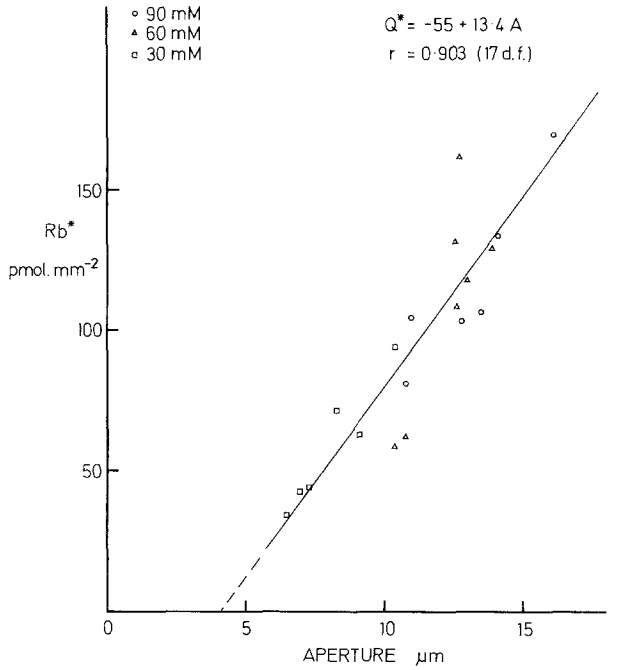

Fig. 4. Relation between ^{86}Rb content (Q^*, pmol mm^{-2}) and aperture (A, μm) after loading "isolated" strips overnight in 30–90 mM RbCl, 10 mM MES at pH 3.9. □, 30 mM; △, 60 mM; ○, 90 mM

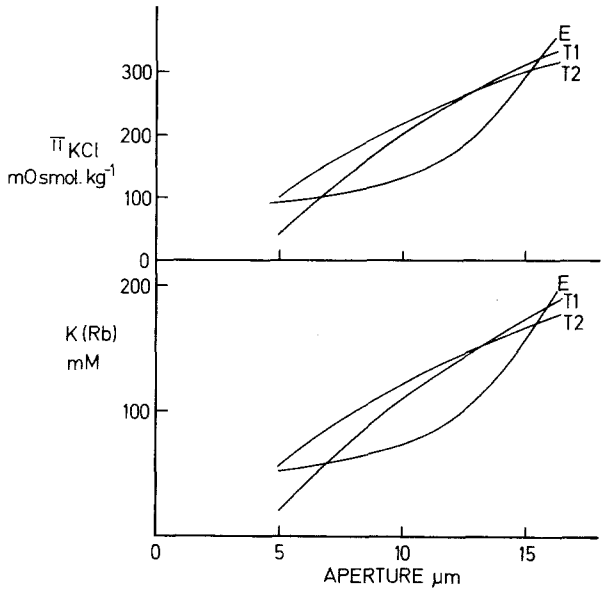

Fig. 6. Calculated values of ^{86}Rb concentration estimated from tracer experiments ($T1$ and $T2$), and of K concentration estimated from potassium activity (E), plotted against aperture; osmotic values of these concentrations of KCl (π_{KCl}). $T1$ is calculated from the regression $Q^* = -55 + 13.4A$, and $T2$ from $Q^* = -19.1 + 10.6A$

to kill subsidiary/epidermal cells, strips were incubated for not more than 24 hr in various concentrations of KCl, pH 3.9, to open the stomata. Figure 5 shows the results of incubation in 10, 30 and 50 mM KCl, and the smooth curve drawn through these points was used for further calculation. The K-activity was converted to concentration by assuming the activity coefficient of KCl, a reasonable assumption given the conditions of incubation and one supported by comparison of ^{86}Rb and ^{82}Br tracer experiments. The results suggest that the potassium concentration rises from 51 mM at 5 μm open, to 157 mM at 15 μm open. While this agrees well with the tracer results, the shapes of the two curves are different; the tracer work

Table 3. Comparison of π_{KCl} with osmotic changes required

Aperture	7–9 μm	11–14 μm	16 μm
Minimum π (mosmol kg^{-1})	330	620	1600
π_{KCl} (mosmol kg^{-1})	150–200	240–300	350
$d\pi/dA$ (mosmol kg^{-1} μm^{-1})	74	121	188
$d\pi_{KCl}/dA$ (mosmol kg^{-1} μm^{-1})(E)	5–10	18–45	55
$d\pi_{KCl}/dA$ (mosmol kg^{-1} μm^{-1})(*T*)	20–32	15–25	10–20

gave a linear relation between Q^* and A, which produces a curve of concentration against aperture which is concave downwards, since the cell volume increases with aperture; by contrast, the potassium activity curve as measured by the electrode is concave upwards. Thus the agreement between the two methods is fair rather than good.

The osmotic values of KCl solutions having the potassium activities measured with the electrode were also calculated. Figure 6 shows the changes in concentration, and in π_{KCl}, with aperture, compared with the values estimated from the tracer work.

Discussion

The results show that "isolated" guard cells open to an extent dependent on the K(Rb)/Cl(Br) concentration in the solution, and that the K(Rb) content increases markedly with aperture. The opening is not very sensitive to pH. However, at pH 3.9 the concentrations in the guard cell and the rate of increase of concentration with aperture are both much less than would be expected from the osmotic measurements in the previous paper.

Accurate measurements of the plasmolytic point were not made in the previous paper since closely spaced sucrose concentrations were not used, and careful detection of incipient plasmolysis was not attempted. Nevertheless, we may consider the highest external osmotic concentrations at which guard cells were clearly not plasmolysed as providing an underestimate of the osmotic pressure of the guard cells. On this basis (from Table 2 of the previous paper) we may compare the minimum osmotic concentrations of "isolated" guard cells with the figures for π_{KCl} from Fig. 6. This is shown in Table 3, and it is clear that the amounts of K(Rb)/Cl(Br), estimated either from tracer measurements or from K-electrode measurements, are not adequate to account for the osmotic pressures estimated for guard cells.

The slopes of the π_{KCl} curves are also quite inadequate to account for the measured osmotic changes required to change the aperture, estimated in the previous paper. This comparison is also shown in Table 3.

Thus the conclusion is clear, that a substantial fraction of the osmotic pressure of "isolated" guard cells is contributed by solutes other than KCl, even when the strips are opened by incubation on KCl solutions. Further, a substantial fraction of the increase in osmotic pressure associated with stomatal opening in these conditions is contributed by solutes other than KCl. The measurements of cell volume are probably the biggest uncertainty in the conversion of tracer content to concentration, but the volumes cannot be overestimated to an extent that would reconcile the figures for π and π_{KCl} and this uncertainty is not present in the measurements of K_{ac}.

There is no evidence from the osmotic measurements that the elastic properties of "isolated" guard cells differ from those of intact guard cells, or are sensitive to pH. In this paper it is also argued that up to 45 hr at pH 3.9, or longer at pH 6.7, there are no marked changes in the wall properties. It is therefore argued that the discrepancy must arise from the contribution of some other solute, and not from cell wall loosening. The accumulation of K(Rb)/Cl(Br) contributes to the generation of turgor leading to opening, but some other process must also be involved.

The question arises of how the mechanism of opening under these experimental conditions is related to the normal processes of opening. Evidence to be presented in a following paper suggests that in intact strips, while potassium accumulation is responsible for wide opening, and determines the final aperture reached above about 10 μm, there are discrepancies at low apertures. In "intact" guard cells below about 10 μm, as in "isolated" guard cells, the amounts of potassium and the rates of increase in K with aperture do not seem to be adequate to explain the osmotic changes required. Again some other process must also be involved. Hence the discrepancy between potassium changes and osmotic changes established for "isolated" guard cells in this paper may be also of relevance in intact stomata. Rogers, Powell and Sharpe (1979) also found discrepancies in the accepted relation between potassium accumulation and aperture in "isolated" guard cells of *Vicia faba* (surrounded by ruptured epidermal cells); in the period between 3 and 5 hr of incubation on 10 mM KCl, at 10–30 °C, potassium content as determined by potassium cobaltinitrite staining decreased markedly, yet the apertures increased by two- to threefold. The staining method has the disadvantage that it is not quantitative, since there appears to be a threshold K-content for visible staining, but the qualitative indications are clear.

Thus while potassium salt accumulation is undoubtedly important in turgor generation in guard cells and may be responsible for wide opening in intact stomata, it does not appear to be the whole story. It is important to establish the nature of the other process involved, and the opening of "isolated" guard cells provides a convenient system for further study.

The support of the Science Research Council for this work is gratefully acknowledged. Thanks are also due to Mr. J. Banfield for skilled technical assistance.

References

Dayanandan, P., Kaufman, P.B. 1975. Stomatal movements associated with potassium fluxes. *Am. J. Bot.* **62**:221

Hsiao, T.C. 1976. Stomatal ion transport. *In:* Encyclopedia Plant Physiol. N.S. U. Lüttge and M.G. Pitman, editors. Vol. IIB; p. 195. Springer Verlag, Berlin

Itai, C., Meidner, H. 1978. Effect of abscisic acid on solute transport in epidermal tissue. *Nature (London)* **271**:653

MacRobbie, E.A.C. 1977. Functions of ion transport in plant cells and tissues. *Int. Rev. Biochem. Plant Biochem. II* **13**:211

MacRobbie, E.A.C. 1980. Osmotic measurements on stomatal cells of *Commelina communis* L. *J. Membrane Biol.* **53**:189

Penny, M.G., Bowling, D.J.F. 1974. A study of potassium gradients in the epidermis of intact leaves of *Commelina communis* L. in relation to stomatal opening. *Planta* **119**:17

Raschke, K. 1975. Stomatal action. *Annu. Rev. Plant Physiol.* **26**:309

Raschke, K. 1977. The stomatal turgor mechanism and its response to CO_2 and abscisic acid: Observations and a hypothesis. *In:* Regulation of Cell Membrane Activities in Plants. E. Marré and O. Ciferri, editors. p. 173. Elsevier/North Holland, Amsterdam

Rogers, C.A., Powell, R.D., Sharpe, P.J.H. 1979. Relation of temperature to stomatal aperture and potassium accumulation in guard cells of *Vicia faba*. *Plant Physiol.* **63**:388

Squire, G.R., Mansfield, T.M. 1972. A simple method of isolating stomata on detached epidermis by low pH treatment: Observations of the importance of subsidiary cells. *New Phytol.* **71**:1033

Thomas, D.A. 1975. Stomata. *In:* Ion Transport in Plant Cells and Tissues. D.A. Baker and J.L. Hall, editors. p. 377. Elsevier/North Holland, Amsterdam

Willmer, C.M., Pallas, J.E. 1973. A survey of stomatal movements and associated potassium fluxes in the plant kingdom. *Can. J. Bot.* **51**:37

Zeuthen, T., Hiam, R.C., Silver, I.A. 1974. Microelectrode registration of ion activity in brain. *In:* Ion-selective Microelectrodes. H.J. Berman and N.C. Hebert, editors. p. 145. Plenum Press, New York

Zlotnikova, I.F., Gunnar, I.I., Panichkin, L.A. 1977. Measurement of intracellular potassium activity in *Tradescantia* leaf epidermal cells. *Izv. Timiryazev S-Kh. Akad.* **2**:10 (*in Russian*)