Role of Potassium in Stomatal Opening in the Leaf of *Vicia faba*

R. A. FISCHER

Research School of Biological Sciences, Australian National University, Canberra, A. C. T. 2601, Australia

ABSTRACT

With isolated epidermal strips of *Vicia faba*, the intensity of potassium-staining in the guard cells of stomata was calibrated against the uptake of radioactively labeled potassium. By using this calibration, the quantity of potassium that had accumulated in the guard cells, as stomata of leaves of *Vicia* open in the light, was estimated. Results support the hypothesis that in leaves, as well as in isolated epidermal strips, potassium and an accompanying anion comprise the major, osmotically active solutes in the guard cells of open stomata.

Work with isolated epidermal strips from leaves has provided strong evidence that at least in some species, stomatal opening in the light requires external potassium and is associated with the accumulation of potassium by the guard cells. The species include *Zebrina pendula* (6), *Commelina communis* and *Allium cepa* (4), and *Vicia faba* (3). The role of potassium as the major osmotically active solute in the guard cells of open stomata can only be demonstrated with quantitative measurements. This was done using the uptake of rubidium-86 by isolated epidermal strips in the light (3).

Stomatal opening in leaves as distinct from isolated epidermal strips which also involves potassium accumulation in large amounts has been more difficult to demonstrate. Employing the cobalt-sodium nitrite stain for the presence of potassium, qualitative evidence that guard cell potassium increases markedly as stomata open has been obtained (4–6). Subsequently, Sawhney and Zelitch (10) have used the electron microprobe to estimate the amounts of potassium in guard cells of open and closed stomata of leaves of *Nicotiana tabacum*. Determinations were made on freeze-dried specimens which were compared to freeze-dried membrane filters with known amounts of potassium. Stomatal opening in light was associated with an increase of guard cell potassium concentration from 0.2 to about 0.5 M, stomatal aperture being linearly related to the potassium concentration. However, there was no mention made of how the concentration of potassium in freeze-dried guard cells is related to concentration in the same guard cells in their normal hydrated condition prior to freeze-drying.

Radioactive tracers cannot readily be used to obtain quantitative results for potassium accumulation by guard cells in intact leaves because of isotopic dilution in the leaf and because there are often intact epidermal cells in the epidermal strips which are taken in order to isolate the guard cells for tracer analysis. However, it has been possible to combine radioactive tracer measurements on appropriate isolated epidermal strips with potassium staining data for guard cells of both strips and intact leaves to obtain quantitative estimates of changes in guard cell potassium in the intact leaf of *Vicia faba*.

MATERIALS AND METHODS

Plants of *Vicia faba* (var. Early Long Pod) were grown individually in 2-liter containers of aerated nutrient solution (half-strength Hoagland’s solution, changed weekly) in a greenhouse during the late spring and summer months. Greenhouse air temperatures were usually within the range 20 to 30 C but extremes as low as 15 C and as high as 35 C were occasionally recorded. Expanded leaves two to six positions below the shoot apex of 4- to 8-week-old plants were used in all experiments.

Epidermal strips were taken and treated for radioactive tracer measurements of potassium uptake in exactly the manner described before (2, 3). Some experiments employed *Rb* with similar results to those obtained with *K* which was used in all other experiments. Results presented here were obtained after 5-hr exposure to labeled potassium, sufficient time to equalize specific radioactivity of potassium throughout. Results also contain no interference from intact epidermal cells, since the material was prepared and finally selected by microscopic observation of each strip so that the proportion of epidermal cells which were intact was always negligible (less than 1%). Under such conditions and with the 10-min period adopted for washing out free-space label, the amount of radioactivity remaining in the epidermal strips estimates guard cell potassium. This point is strongly supported by the fact that with all treatments of light, CO₂ concentration and KCl concentration, total potassium label in the strip always showed a very close linear relationship to aperture.

The stain for potassium, employing cobalt sodium nitrite, was prepared as described by Macallum (8). Fresh reagent was prepared every 2 weeks and kept in a stoppered bottle. Isolated epidermal strips and strips taken directly from intact leaves were always floated for 1 min on distilled water before staining in order to remove most of the extracellular potassium. Strips were then floated 1 min on the staining solution at room temperature before transfer to ice-cold distilled water for at least 5 min to wash out excess stain. Strips were mounted between a slide and coverslip in a 1:1 mixture of 50% glycerine and concentrated ammonium sulfide solution in water, and observed at 400-fold magnification. The amount of black cobaltous sulfide precipitate in the guard cells (see Fig. 1) was scored as the percentage of the guard cell area covered by the precipitate. Scoring was aided by the preparation of photographic standards. During scoring the operator was unaware of the treatment of the strips observed. The ammonium sulfide-glycerin mixture alone gave no precipitate, nor did a concentrated solution of cobalt acetate.

---

1 Present address: International Maize and Wheat Improvement Center (CIMMYT), Londres 40, Mexico City, Mexico, 6, D. F.
Stomatal apertures were always measured microscopically in epidermal strips mounted in immersion oil on a glass slide. Where strips contained some intact epidermal cells, only stomata surrounded by broken epidermal cells, but otherwise selected at random, were measured.

RESULTS

Figure 1 shows typical results of staining for potassium. Potassium accumulation is evident in guard cells of stomata, opened in the light, of both intact leaves (A) and isolated epidermal strips floating on KCl solution (B). Several experiments with isolated epidermal strips having a negligible number of intact epidermal cells have established a linear relationship between the mean potassium staining of the guard cells and potassium content of the strips measured with radioactive tracers. For the material of Figure 2 an increase in staining score of 10% corresponds to an increase in guard cell potassium of $3.6 \times 10^{-5}$ moles cm$^{-2}$. Since the mean stomatal density was 5600 cm$^{-2}$ and the internal volume of an individual guard cell was $5 \times 10^{-8}$ cm$^3$ (determined by microscopic observations on the length, breadth, and depth of guard cells and assuming, as these measurements suggest, that guard cell volume does not alter greatly as stomata open), $3.6 \times 10^{-8}$ moles cm$^{-2}$ of potassium is equivalent to 65 mM potassium in the guard cell. Thus the degree of potassium staining can be related quantitatively to the concentration of potassium in the guard cell.

In studies with isolated epidermal strips, potassium staining (as well as uptake of potassium label) has always been linearly related to stomatal aperture. In order to show more convincingly than in Figure 1 that there is also a relationship in intact leaves between stomatal opening and potassium staining, an experiment was done in which both the aperture and the degree of staining were measured as stomata in leaves opened in the light (Fig. 3A), the source of experimental material being the same as that of Figure 2.

Stomatal aperture in Figure 3A refers to the mean aperture measured on epidermal strips taken from the leaf and floated 1 min on distilled water before mounting in oil. Again, aperture measurements were only made on stomata surrounded by broken epidermal cells (only approximately half the stomata are thus measurable with typical leaf material because the short floating time used with the strips permits many epidermal cells to remain intact). Under this condition, aperture is not influenced by back pressure from adjacent intact epidermal cells, and may therefore be expected to be a better indicator of guard cell solute concentration than mean aperture of all stomata in the strip or of stomata in situ in the leaf. It is because of the absence of this back pressure that stomata initially showed an aperture of 7.9 $\mu$m. In contrast, stomata in situ on the same leaf showed an aperture of 3.2 $\mu$m initially, and 11.8 $\mu$m after 5-hr illumination. The failure of stomata to close rapidly upon darkening at 5 hr is not unusual for *Vicia faba* (3).

Figure 3B shows that the observed changes in degree of
potassium staining in the guard cells are linearly related to the stomatal aperture movements of Figure 3A. The slope of this relationship was 10.0% per μm. From this and the data of Figure 2, it is possible to calculate that upon illumination of stomata in intact leaves the guard cell potassium concentration increases by 65 mM for each μm increase in aperture.

**DISCUSSION**

The results are sufficiently quantitative to permit an estimation of the osmotic role of potassium in the opening of stomata in leaves upon illumination. An increase in concentration of 65 mM corresponds to a decrease in guard cell solute potential of about 3.0 bars if the anion accompanying potassium is assumed to be univalent. However, measurements with 32Cl suggest that chloride uptake is commonly only a small fraction of the potassium uptake by guard cells (Fischer and Pallaghy, unpublished data). Therefore, a major fraction of the accompanying anion may consist of divalent organic acid anions, in which case the osmotic equivalent of the potassium would be somewhat less than 3.0 bars.

Estimates of changes in guard cell solute potential suggest decreases of the order of 3 to 10 bars as stomata in leaves open (9). I have shown a decrease of 8 bars as stomata in *Vicia faba* leaves opened from 2 μm to 10 μm (2). Subsequent experiments suggest that the change in solute potential relative to aperture is linear but may have been underestimated to some extent and that a decrease of about 1.5 bars μm⁻¹ is more appropriate (Fischer, unpublished data). Nevertheless, it is apparent that the potassium accumulated by guard cells as the stomata in intact *Vicia faba* leaves open is very likely sufficient to account for the expected decrease in solute potential. A similar conclusion had been reached earlier for the case of stomata in isolated epidermal strips (3). These quantitative data rule out the possibility that the changes in guard cell potassium simply involve catalytic amounts of potassium. Also, in *Vicia* stomata, a significant role for sucrose, proposed as the major osmotic solute in the classical theory of stomatal opening (7), appears unlikely.

Because of the limitations of technique, it has been necessary in this study to measure stomatal opening in terms of changes in the aperture of stomata surrounded by broken epidermal cells. There is, however, no evidence to suggest that the effect of intact epidermal cells on aperture is not simply the passive effect of back pressure (9), as governed by relative turgor pressures and the geometry of the stomatal apparatus.

It has been suggested that the potassium accumulated during stomatal opening in leaves may come from adjacent epidermal cells (10). This is difficult to test, because the concentration changes in epidermal cell potassium need only be small. Guard cells occupy about 7% of the surface area of the epidermis in *Vicia faba* and if it is assumed therefore that the ratio of guard cell to epidermal cell volume in the epidermis is 1:13, stomatal opening with an increase in guard cell potassium concentration of 300 mM would be accompanied by a fall in the epidermal cell concentration of only 23 mM. It is not surprising that potassium staining (Fischer, unpublished results) or electron probe measurements (10) of epidermal cells provide no clear evidence about this. Since epidermal cells appear to lack any special protoplasmic connections with the guard cells (9), they would probably have to lose potassium to the free space in order for the guard cells to obtain it. Actually, most healthy plant systems show very little loss of potassium to their free space even when placed in distilled water (1). Perhaps epidermal cells are specially adapted in this respect: indeed, guard cells must be in order to dispose of their potassium on closing.
A separate possibility is that the potassium of the transpiration stream could reach the immediate guard cell environment by both mass flow and ionic diffusion in the free space of the epidermis. Guard cells appear to have a great affinity for potassium (3) and therefore they may be able to compete very effectively for free-space potassium.

It has been demonstrated that, with respect to quantitative potassium accumulation in the guard cells during stomatal opening in light, as well as with respect to other important parameters (2), stomata in leaves of *Vicia faba* behave similarly to those in isolated epidermal strips floating on dilute potassium solutions. Thus the use of suitable isolated epidermal strips remains probably the most satisfactory technique for examining the much disputed and little understood area of metabolic control of stomatal behavior.

Acknowledgment—This work was carried out while the author held a Queen Elizabeth II Fellowship in the Department of Environmental Biology at the Research School of Biological Sciences, A.N.U. I am very grateful to Drs. C. K. Pallaghy and C. B. Osmond for their advice and assistance.

LITERATURE CITED