

The role of sucrose in guard cell osmoregulation

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Abstract

Stomatal apertures are regulated by changes in the solute content of guard cells. The identity of the solutes involved in guard cell osmoregulation has been the subject of much study. Early consensus in the field held that carbohydrates derived from starch constitute the principal osmoticum. This starch–sugar hypothesis has been replaced by the present paradigm of guard cell osmoregulation by K^+ and its counterions. Recent studies, however, show that both K^+ and sucrose are primary guard cell osmotica, and that the use of these two solutes is separated into two distinct phases in which one or the other constitutes the dominant osmoticum. In the intact leaf, opening at the beginning of a daily cycle is supported by K^+ and its counterions, malate²⁻ and Cl^- . Malate²⁻ is the dominant counterion in growth chamber-grown leaves, whereas Cl^- predominates in a greenhouse environment. In the second half of the daily cycle, K^+ content in guard cells decreases drastically and sucrose becomes the dominant solute. Manipulation of stomatal apertures by altering ambient CO_2 concentration shows that either K^+ or sucrose accumulation can sustain rapid opening. The functional implications of two distinct osmoregulatory phases of stomatal movements remains to be elucidated. The guard cell content of K^+ , its counterions, and sucrose can be modulated by at least three osmoregulatory pathways in guard cells. Experimental conditions favouring three distinct pathways have been established, but major uncertainties remain about the control of guard cell solute content in the intact leaf.

Key words: Guard cells, stomatal apertures, sucrose, osmoregulation.

Introduction

This paper summarizes recent studies showing that sucrose plays a major role in guard cell osmoregulation. These findings were the unexpected outcome of research originally aimed at determining whether measurements of sucrose content in guard cells from intact leaves kept under well-controlled conditions could help solve the controversial issue of the relationship between guard cell photosynthesis and stomatal movements (Gotow *et al.*, 1988; Reckman *et al.*, 1990; Poffenroth *et al.*, 1992). Experiments run in a growth chamber under constant light showed that guard cell sucrose increased slowly early in the day, but accumulated rapidly in the afternoon. Subsequent studies showed that guard cell K^+ peaked at mid-morning and declined in the afternoon, that these osmoregulatory features were also seen in greenhouse conditions, and that they could also be found in a different species (Talbott and Zeiger, 1993, 1996; Amodeo *et al.*, 1996).

How could such novel and far reaching characteristics of guard cell osmoregulation have gone unnoticed after decades of detailed studies on solute content and fluxes in guard cells? Two major differences between the recent studies and extant literature might be pertinent to that question. Because of the initial interest in optimal photosynthetic rates in guard cells, intact leaves were used rather than detached epidermis or guard cell protoplasts, and the sucrose content was measured over the 12 h light phase of a daily cycle. In contrast, nearly all extant studies on guard cell osmoregulation have relied on experiments with isolated guard cells over short time-courses. As discussed below, these conditions favour K^+ -dependent osmoregulation and obscure the sucrose-dependent phase. The other novel factor was the use of high resolution, electrochemical detection of sugars in HPLC analysis of guard cell extracts.

The new findings unify the modern K^+ –malate²⁻ theory of guard cell osmoregulation with the basic tenets

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of the classical starch–sucrose hypothesis, and resolve two distinct osmoregulatory phases in which either K^+ or sucrose are the dominant solutes.

The K^+ and sucrose-dependent phases of guard cell osmoregulation

The nature of stomatal osmoregulation in guard cells of intact leaves has received little attention. In a pioneering study, Pearson (1973) analysed solutes in epidermal strips prepared from intact *Vicia faba* leaves at various times over the course of a daily cycle of opening. Although interpretation was complicated by the presence of other cells in the epidermal preparation and by insensitive, colorimetric analytical techniques, this study found that sucrose increased 6-fold more than malate²⁻ over a daily cycle of opening. Although Pearson was unable to obtain as good a correlation between sucrose increase and opening as he did between malate²⁻ accumulation and opening, this was primarily because the main period of sucrose accumulation occurred in the afternoon after the period of initial stomatal opening was completed.

Guard cell osmoregulation in the intact leaf during a daily cycle of stomatal movements was recently studied in *V. faba* (Talbott and Zeiger, 1996). At various times during the course of a light cycle, abaxial epidermal peels were sonicated and washed to isolate intact guard cells. Concurrent histochemical and HPLC measurements were used to estimate the contributions of K^+ , Cl^- , malate²⁻, sucrose and other monosaccharides to stomatal opening over the course of the light cycle. Use of electrochemical carbohydrate detection, which is both specific and highly sensitive (Johnson, 1986), permitted a much better quantification of individual carbohydrate species than was previously possible.

Guard cells accumulated K^+ rapidly during the initial phase of opening, in a pattern that generally matched the pattern of aperture increase (Fig. 1). Guard cell K^+ levels subsequently declined, sometimes reaching initial baseline levels by midday. This decrease of K^+ content occurred at times of steady or even increasing apertures and was seen in plants grown under both greenhouse and growth chamber conditions. A second peak of K^+ accumulation, usually about half the amount of morning accumulation, was observed in the afternoon. Guard cell K^+ content in the afternoon varied, ranging from 35–90% of morning levels. However, based upon a comparison of morning and afternoon K^+ -aperture ratios, afternoon K^+ levels were insufficient to account for the measured afternoon apertures. A similar pattern of transient morning K^+ accumulation was seen in stomata of both greenhouse and growth chamber-grown onion cotyledons (Amodeo *et al.*, 1996).

Measurements of sucrose content in guard cells of intact leaves from *V. faba* revealed the converse pattern

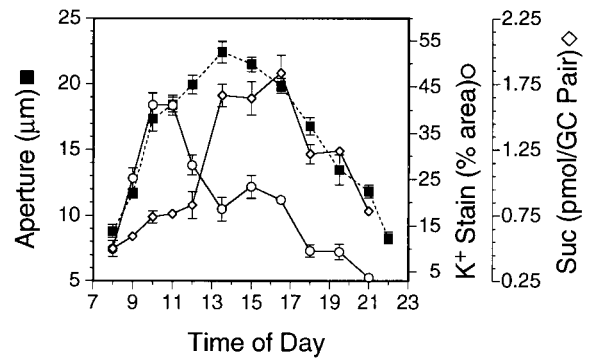


Fig. 1. Aperture, K^+ and sucrose content of *Vicia faba* guard cells over a daily light cycle of stomatal movements. Plants were grown in a growth chamber environment and leaves were sampled at intervals over the course of the light cycle for aperture measurement and solute analysis. Sucrose content was determined by HPLC and is expressed as pmoles per guard cell (GC) pair. Potassium was determined histochemically after Fischer (1971) and is expressed as the percentage of guard cell area covered by stain. Data after Talbott and Zeiger (1996).

of daily accumulation under both growth chamber and greenhouse conditions (Talbott and Zeiger, 1996). Sucrose levels in the morning rose slowly (Fig. 1) and sucrose content seemed insufficient to account for the observed aperture increases. There was, however, a rapid accumulation of sucrose around the mid-point of the light cycle, and afternoon patterns of sucrose content and apertures corresponded closely. In particular, the pattern of stomatal closure was closely correlated with a decrease in guard cell sucrose. A midday stomatal closure was frequently observed in greenhouse-grown *V. faba* stomata. In these cases, maximal K^+ accumulation corresponded to the first aperture peak and maximal sucrose accumulation corresponded to the second peak. Guard cell content of glucose and fructose did not show appreciable accumulation over the course of the light cycle. The same pattern of sucrose accumulation was seen in onion, although technical constraints prevented acquisition of a detailed time-course (Amodeo *et al.*, 1996).

In *V. faba*, use of the two known K^+ counterions, malate²⁻ and Cl^- , differed depending on the growth environment. In growth chamber conditions, malate²⁻ content of guard cells was generally correlated with K^+ content. Malate²⁻ content increased during a predawn phase of opening, peaked during the morning and declined at midday (Fig. 2). These growth chamber-grown guard cells showed only minor changes in Cl^- content over the light cycle. In greenhouse conditions, however, malate²⁻ levels showed little change over the course of the light cycle. Malate²⁻ accumulation was detected primarily during the predawn, presumably circadian rhythm-driven, phase of opening (Fig. 2). During the light cycle proper, substantial Cl^- accumulation was detected during the morning in a pattern correlated with that of K^+ accumulation.

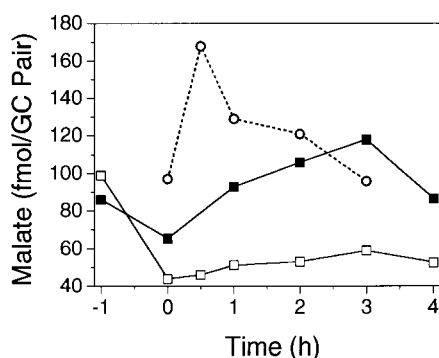


Fig. 2. Malate content of guard cells opening upon exposure to light. Stomata were exposed to light at time 0. Malate content of guard cells is shown for stomata in intact leaves of greenhouse-grown plants (open squares), stomata in intact leaves of growth chamber-grown plants (closed squares), and isolated stomata opening under $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ blue light (open circles). Malate content was determined by HPLC. Data after Talbott and Zeiger (1993, 1996).

Historical background

Water movement between the guard cell and its surrounding tissue is driven by changes in guard cell osmotic potential. The resulting changes in turgor are translated, via a specialized cell wall structure, into changes in the size of the stomatal pore. The fundamental role of osmotic potential was demonstrated in the mid-nineteenth century by von Mohl (1856), who showed that open stomata could be made to close by placing them in solutions of increasing osmotic strength.

The starch–sucrose hypothesis

The nature of the osmoregulatory mechanisms in guard cells was the subject of much early debate. The most widely accepted hypothesis proposed that carbohydrates derived from starch hydrolysis provided the osmoticum necessary to drive stomatal opening (Meidner and Mansfield, 1968). This hypothesis was based on observations that stomata which had opened in response to light contained less starch than did closed stomata in the dark (Lloyd, 1908). Subsequent work confirmed that, in contrast with mesophyll tissue, guard cells accumulated starch at night and broke it down during the day (Pallas, 1964). Inverse relationships were shown between stomatal opening and guard cell starch content, both over the course of a natural light cycle (Willis and Jefferies, 1963) and after experimental treatments (Williams and Barratt, 1954). In some studies, however, stomatal aperture and guard cell starch content were found not to be correlated (Heath, 1949). In addition, guard cells of onion, which lack starch, were found to function normally (Heath, 1952).

Definite conclusions regarding the use of osmotically active sugars were hampered by the need to infer sugar accumulation from observations of starch content. Guard cells were known to import sugars from external sources (Pallas, 1964) and seemed to have photosynthetic capacity

(Scarath and Shaw, 1951), making inferences about sugar content based on measurements of starch content ambiguous. As Meidner and Mansfield (1968) stated, the starch–sugar hypothesis was questioned not because it was proved wrong, but because unambiguous evidence in its favour could not be obtained.

The potassium–malate theory

The demonstration of K^+ uptake by guard cells and its correlation with stomatal opening (Imamura, 1943; Fujino, 1967; Fischer and Hsiao, 1968), along with the identification of malate²⁻ as the counterion balancing this uptake (Allaway, 1973), presented an attractive, alternative hypothesis. There are numerous studies documenting K^+ uptake in isolated guard cell systems and correlating K^+ accumulation with stomatal opening (Fischer and Hsiao, 1968; Fischer, 1971, 1972; Humble and Raschke, 1971; Allaway and Hsiao, 1973; Pallaghy and Fischer, 1973; Outlaw and Lowry, 1977). Uptake of K^+ is driven by a H^+ gradient mediated by the activation of a vanadate-sensitive proton ATPase (Zeiger, 1983; Shimazaki and Kondo, 1987) and it is accompanied by malate²⁻ accumulation (Allaway, 1973) and Cl^- uptake (Schnabl, 1977; Schnabl and Raschke, 1980). Estimates based on osmotic potential requirements have shown that observed K^+ accumulation can account for a substantial portion of the solute requirements for opening (for a review see Outlaw, 1983). This body of research led to the formulation of the K^+ –malate²⁻ theory, which has replaced the starch–sucrose hypothesis of guard cell osmoregulation.

A role for carbohydrates within the K^+ –malate²⁻ theory

Potassium accumulation in the vacuole requires the accumulation of solutes in the cytosol to balance osmotic potentials between these two compartments, yet K^+ concentrations found in open stomata would be toxic to cytosolic metabolism. Carbohydrates are obvious candidates for cytosolic solutes (Raschke, 1975; Outlaw, 1983). In addition, there were indications that K^+ and its counterions cannot account for all the osmoticum required to support measured apertures of *Commelina benghalensis* stomata (MacRobbie and Lettau, 1980). Sucrose accumulation associated with stomatal opening under white light was detected spectrophotometrically in sonicated epidermal strips of *C. benghalensis* (Reddy and Das, 1986), and using amplified enzymatic assays of single dissected *V. faba* guard cell pairs (Outlaw and Manchester, 1979). Fusococcin treatment of stomata from *C. benghalensis* resulted in the accumulation of both sugars and malate²⁻ (Reddy *et al.*, 1983).

Sucrose and K^+ as alternative solutes

There is also solid evidence showing that carbohydrates are the major, if not the sole osmoticum supporting opening under certain experimental conditions. These

findings had their origin in experiments which determined that stomata of *V. faba* and *C. communis* had different opening kinetics when exposed to red or blue light. In addition, opening in red light was inhibited by DCMU, but resistant to KCN, while blue light-stimulated opening, which occurred under very low intensities, was DCMU-resistant but sensitive to KCN (Schwartz and Zeiger, 1984). These light quality studies have provided strong evidence in favour of the existence of multiple osmoregulatory mechanisms in the guard cell, despite a lack of understanding of the functional roles of the blue light and red (photosynthesis-dependent) light-sensing systems.

Under red light, histochemical studies of *V. faba* stomata in epidermal strips failed to detect either K^+ accumulation or starch degradation during opening (Tallman and Zeiger, 1988). In later HPLC studies, red light-treated stomata were shown to accumulate significant amounts of sucrose (Poffenroth *et al.*, 1992; Talbott and Zeiger, 1993). Measurements of other sugars and organic acids showed that opening under red light was not accompanied by significant increases in the guard cell content of glucose, fructose, malate²⁻, or the starch breakdown products maltose and maltotriose (Talbott and Zeiger, 1993).

Under low fluence rates of blue light ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$), an HPLC study of *V. faba* found that guard cells accumulated malate²⁻ during the first 30 min of irradiation, but malate²⁻ content declined thereafter even though apertures continued to increase (Talbott and Zeiger, 1993). The same transient time-course of accumulation was reported for K^+ in an earlier histochemical study of blue light-stimulated opening (Tallman and Zeiger, 1988). Sucrose, on the other hand, accumulated gradually under blue light, and became the dominant organic osmoticum after 60 min of irradiation. This two-phase pattern of guard cell osmoregulation, involving an early K^+ -malate²⁻ phase followed by sucrose-dominated phase, closely resembles that described for guard cells from intact leaves in the preceding section. Maltose and maltotriose increased rapidly at the onset of irradiation and remained high throughout the time-course, pointing to continuous starch hydrolysis.

Addition of low fluence rates of blue light to guard cells opening under red light stopped sucrose accumulation and stimulated K^+ uptake and malate²⁻ synthesis (Tallman and Zeiger, 1988; Talbott and Zeiger, 1993). The activation of proton pumping, K^+ uptake and starch hydrolysis by blue light, and the simultaneous inhibition of sucrose accumulation implies a modulation by light of key regulatory steps in the sucrose and starch metabolism of guard cells.

Guard cell osmoregulatory pathways and regulation

Guard cell content of the four solutes clearly implicated in guard cell osmoregulation; K^+ , Cl^- , malate²⁻ and

sucrose, can be regulated by at least three distinct osmoregulatory pathways (Fig. 3). The first pathway involves the uptake of K^+ and Cl^- from the apoplast, and the synthesis of malate²⁻ from carbon skeletons derived from starch (Fig. 3a). This pathway is associated with early morning stomatal opening in the intact leaf, and with the initial opening phase of isolated stomata stimulated with blue or white light.

A second pathway supplies sucrose from starch hydrolysis (Fig. 3b). This pathway is inferred from the high level of starch breakdown observed in the sucrose-dominated phase of isolated stomata opened under blue light (Tallman and Zeiger, 1988; Talbott and Zeiger, 1993), and the insensitivity of this sucrose accumulation to DCMU (Poffenroth *et al.*, 1992). Starch breakdown has also been implicated in measurements of sucrose accumulation in fusiccocin-treated *C. benghalensis* stomata (Reddy *et al.*, 1983). Both the K^+ -malate²⁻ and starch-sucrose pathways are activated by blue light (Ogawa *et al.*, 1978), and appear to require respiratory energy, as indicated by their sensitivity to cyanide (Schwartz and Zeiger, 1984).

A third pathway supplies sucrose from the products of photosynthetic carbon fixation in guard cells (Fig. 3c), and has been inferred from the DCMU-sensitive accumulation of sucrose in the absence of starch breakdown or external carbohydrate sources observed during red light-stimulated opening of isolated guard cells. A growing consensus that guard cell chloroplasts are capable of photosynthetic carbon fixation (Reckman *et al.*, 1990; Zeiger, 1990; Tallman, 1992; Assmann, 1993) has shifted controversy to the question of whether measured photosynthetic rates in guard cells suffice to support the observed rates of sucrose accumulation (Reckman *et al.*, 1990; Poffenroth *et al.*, 1992). The unusual properties of guard cell photosynthesis (Zeiger *et al.*, 1987; Mawson and Zeiger, 1991; Talbott and Zeiger, 1993) and the very limited set of conditions used to measure photosynthetic rates in guard cells, suggest that studies of guard cell photosynthesis in the intact leaf, particularly during the sucrose-dependent, afternoon phase, are essential for an accurate determination of the photosynthetic capacity of guard cells.

With these three osmoregulatory pathways in mind, the question of solute content regulation during stomatal movements is now addressed.

Potassium and its counterions

The mechanisms regulating K^+ and anion fluxes in guard cells have been extensively studied and recently reviewed (Assmann, 1993; Schroeder, 1995; MacRobbie, 1997). The most important implication of the intact leaf studies is that guard cell K^+ content in the early afternoon declines to as little as 13% of maximum

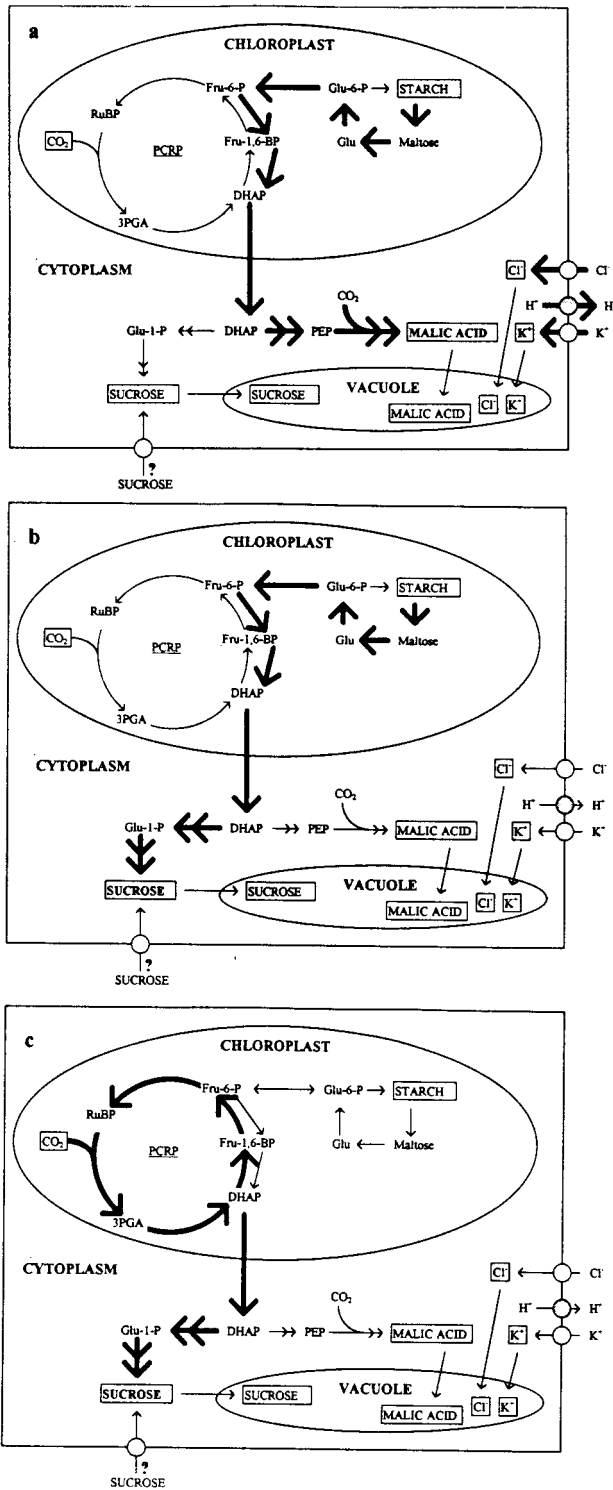


Fig. 3. Osmoregulatory pathways in guard cells controlling solute accumulation. (a) Pathway operating during the early phase of blue light-stimulated opening. Potassium and Cl⁻ are taken up from the apoplast in conjunction with H⁺ extrusion while malate²⁻ is formed from the products of starch breakdown. (b) Pathway operating during later times of blue light-stimulated opening, involving sucrose synthesis from starch breakdown. (c) Pathway seen under red light illumination, involving the formation of sucrose from the products of the photosynthetic carbon reduction pathway (PCRCP).

morning levels, at a time at which stomatal apertures are maximal or still increasing (Fig. 1; Talbott and Zeiger, 1996). Thus, to the extent that it is possible to generalize from findings in two divergent species such as *V. faba* and onion, these results indicate that the regulation of stomatal closing is primarily associated with a loss of sucrose, and not K⁺.

The intact leaf studies also provide new information regarding the relative abundance of Cl⁻ and malate²⁻ in opening stomata. Aside from the special cases of Liliaceae such as onion, which seem to use Cl⁻ exclusively (Schnabl and Ziegler, 1977; Schnabl and Raschke, 1980), the prevailing view in the stomatal literature is that malate²⁻ is the primary counterion for K⁺. In isolated stomata from *V. faba*, the proportion of Cl⁻ used was found to range from zero to 45%, depending on the external concentration of Cl⁻ in the incubation solution (Raschke and Schnabl, 1978). The observed variation was proposed to depend on Cl⁻ supply (Van Kirk and Raschke, 1978).

Malate²⁻ content of growth chamber-grown guard cells from intact leaves tracked changes in K⁺ content in a pattern that was consistent with that reported for isolated stomata (Talbott and Zeiger, 1996). In contrast, little change in malate²⁻ content was detected under greenhouse conditions over the course of the light period (Fig. 2) and K⁺ content of the guard cells was correlated with Cl⁻ fluxes. It is unlikely that Cl⁻ availability differed under the two growth conditions. There were, however, other important differences between the growth chamber and greenhouse environments, including prevailing relative humidity and spectral quality (Lu *et al.*, 1993; Talbott *et al.*, 1996). Guard cells from the two environments also have contrasting sensitivities to light and CO₂ (Talbott *et al.*, 1996). Any of these factors could have influenced the osmoregulatory pathways controlling Cl⁻ uptake and malate²⁻ synthesis. For instance, blue light stimulates malate²⁻ formation (Ogawa *et al.*, 1978), and the growth chamber was enriched in blue light, as compared to the greenhouse.

The levels of malate²⁻ found in stomata from growth chamber-grown plants were substantially less than those found in isolated stomata of epidermal peels (Fig. 2). Maximum malate²⁻ accumulation in intact guard cells was approximately 6.9 fmol μm⁻¹ of opening, as opposed to the 43 fmol μm⁻¹ found in isolated guard cells. These differences argue further for effects of environment and experimental conditions on the malate²⁻ content of guard cells. The most interesting new result, however, is the observation that stomata of greenhouse-grown leaves, which most closely approximate those from a natural environment, show little malate²⁻ accumulation over the light cycle. This result indicates that Cl⁻ may have a greater physiological role in balancing K⁺ than currently thought.

Sucrose

The characterization of a sucrose-dependent phase of guard cell osmoregulation in the intact leaf provides an additional incentive to seek understanding of the pathway(s) that control sucrose accumulation and loss in guard cells. In addition to the two sources for sucrose available in isolated guard cells, starch breakdown and photosynthetic carbon fixation (Fig. 3), stomata in intact leaves have a third possible source, apoplastic sucrose. Each source implicates significantly different control mechanisms, which will require resolution for a thorough understanding of guard cell osmoregulation.

Evidence for sucrose supply from starch breakdown and photosynthetic carbon fixation in isolated guard cells was mentioned above; little is known about the regulation of sucrose content in guard cells from intact leaves. Measurements of acid-soluble polysaccharides (including starch) over a daily cycle of opening showed that in *Commelina cyanea*, polysaccharide levels during most of the light cycle remained roughly similar to predawn levels (Pearson, 1973). In *V. faba*, there was a large decline in polysaccharides upon stomatal opening, but polysaccharide levels quickly rose to exceed predawn values. In neither species were the changes well correlated with changes in malate²⁻ or sucrose. In a later study with excised *V. faba* guard cells, starch content was found to decrease in conjunction with malate²⁻ and sucrose accumulation, in response to white light and CO₂-free air (Outlaw and Manchester, 1979). In stomata of intact *V. faba* leaves, pilot HPLC measurements of maltose and maltotriose suggested that starch breakdown could occur at peak periods of both malate²⁻ and sucrose production (L. Talbott and E. Zeiger, unpublished results).

The absence of starch reserves in guard cells of onion has been an historical argument against a role of guard cell carbohydrate in osmoregulation (Heath, 1952). The finding of a sucrose-dependent osmoregulatory phase in onion similar to that observed in *V. faba* (Amodeo *et al.*, 1996), would indicate that, in the absence of starch, sucrose accumulation would depend on guard cell photosynthesis and/or apoplastic uptake. However, onion guard cells have been shown to contain soluble fructans which may serve as a source of carbohydrate during opening (Darbyshire and Allaway, 1981). Sugars generated by fructan hydrolysis have been suggested to play a role in osmotic adjustments of leaf tissue during bulbing (Darbyshire and Henry, 1978). Soluble fructans could serve as a source of carbohydrates supporting the sucrose accumulation seen in onion stomata.

Rates of sucrose accumulation in *V. faba* guard cells range from 43 fmol h⁻¹ in peels to 150 fmol h⁻¹ in guard cells of intact leaves (Talbott and Zeiger, 1993, 1996). Could guard cell photosynthesis be a source of the accumulating sucrose? Rubisco activity in guard cells of

V. faba, was 27 μmol CO₂ mg⁻¹ chlorophyll h⁻¹ (Gotow *et al.*, 1988). Taking each guard cell to contain 2 pg of chlorophyll, this carboxylation rate would account for 3–10.5% of the observed sucrose accumulation. This estimate agrees with one for pea guard cells, in which guard cell photosynthesis could account for about 2% of the sugar requirement for opening (Reckman *et al.*, 1990). Yet, can estimates based on photosynthetic rates of guard cell protoplasts, which require drastic manipulation for their isolation, be taken to represent intact leaf conditions? Estimates of guard cell Rubisco content which were 8–12 times higher by immunological assay than by assay of active Rubisco sites (Reckman *et al.*, 1990) might underscore yet to be resolved regulatory features of guard cell Rubisco. Measurements of daily courses of Kautsky fluorescence transients from guard chloroplasts of green house grown *V. faba* leaves showed that the kinetic features of the transients associated with photosynthetic carbon fixation were readily observed in the middle of the day, but could not be resolved in the early morning and late afternoon (A. Srivastava and E. Zeiger, unpublished results). Inhibition of photosynthetic carbon fixation in the early morning and late afternoon has important implications for the functioning of the xanthophyll cycle of guard cells in light and CO₂ sensing (Zeiger and Zhu, 1998). Much remains to be learnt about guard cell photosynthesis and its role in carbon fixation and sensory transduction.

A few studies have documented sucrose uptake into guard cells. Measurements with illuminated guard cell protoplast of *C. benghalensis* found uptake rates of 1251 fmol cell⁻¹ h⁻¹ (Reddy and Das, 1986). Addition of fusicoccin to the medium almost doubled that rate. This uptake capacity would be more than sufficient to support observed rates of sucrose accumulation in guard cells. One study of sucrose in the guard cell apoplast of *V. faba* suggests that external sucrose supply is sufficient to satisfy the observed accumulation rates (Lu *et al.*, 1995). However, a competence for sucrose uptake is common to most plant cells, so proof of an osmoregulatory pathway supplying apoplastic sucrose must await direct demonstration that apoplastic sugar contributes to guard cell sucrose content in opening stomata. In *C. benghalensis* epidermal peels, increasing external sucrose concentrations did enhance stomatal opening in white light (Reddy and Das, 1986), but external sucrose had no effect on red light-stimulated opening in epidermal peels of *V. faba* (L. Talbott and E. Zeiger, unpublished data).

Functional implications of multiple osmoregulatory pathways

The identification of two osmoregulatory phases in guard cells, an initial K⁺ phase and a subsequent sucrose phase,

implies a functional distinction between the two solutes. The close relation between morning opening and K^+ uptake could indicate that K^+ accumulation is used primarily for rapid opening, whereas afternoon sucrose is used primarily for turgor maintenance. This possible relationship was tested with growth chamber-grown *V. faba* guard cells, which were shown to have a very high sensitivity to CO_2 (Talbot *et al.*, 1996). Experiments in which stomata were closed by elevated CO_2 during the morning, K^+ -dominated phase and then rapidly reopened by lowering ambient CO_2 showed that reopening was accompanied by rapid K^+ accumulation (Fig. 4; Talbot and Zeiger, 1996). However, reopening after a similar manipulation during the afternoon, sucrose-dominated phase was accompanied by rapid sucrose accumulation, but very little K^+ increase (Fig. 4). There were no obvious kinetic differences in the reopening during the morning and afternoon phases arguing against a distinction between K^+ and sucrose based on the capacity of the two osmoregulatory pathways to sustain rapid opening.

It was also investigated whether the separation between morning and afternoon phases was of a circadian nature (Talbot and Zeiger, 1996). Ambient CO_2 was elevated throughout the morning hours of the light cycle to suppress stomatal opening in growth chamber-grown *Vicia* leaves, and then lowered to normal levels in the afternoon. This manipulation displaced the first opening

of the day into the sucrose-dominated phase of the normal daily cycle. Changes in guard cell K^+ and sucrose during the delayed opening resembled those typical of the morning, K^+ -dependent phase and not those of the afternoon, arguing against a circadian dependency of the two osmoregulatory phases (Talbot and Zeiger, 1996). It is of further interest that, because of incomplete stomatal closure and high ambient $[CO_2]$, photosynthetic rates remained stable despite the delay of stomatal opening until the afternoon (Talbot and Zeiger, 1996). Since the supply of sucrose from mesophyll photosynthesis had not decreased, as compared to a normal daily cycle, the use of K^+ in the delayed opening phase appears to rule out the explanation that K^+ is normally used in the morning because of insufficient sucrose supply from the mesophyll.

Taken together, the daily cycle and CO_2 experiments suggest an osmoregulatory sequence that requires K^+ to be used first. Potassium accumulation to some critical level would turn on the osmoregulatory pathways involved in sucrose supply and shut off the pathway regulating K^+ accumulation until the sequence is reset by a period of closure in the dark. Functionally, the sucrose phase could be more tightly coupled to photosynthetic rate, and thus represent a mechanism whereby stomatal apertures are more finely tuned to continuous changes in mesophyll photosynthetic rates during midday and the early afternoon. Fine tuning between stomatal conductance and mesophyll photosynthesis is likely to dominate the control of stomatal movement at these times. The morning K^+ phase, on the other hand, would be associated with a consistent opening stimulus at sunrise, which in the absence of water stress, supports high stomatal apertures before maximal photosynthetic rates (Tenhunen *et al.*, 1987).

Environmental sensing

Much of the current thinking on the sensory transduction of environmental signals in guard cells hypothesizes that signal sensing is coupled to the regulation of stomatal apertures via the activation of a specific osmoregulatory pathway. For instance, malate²⁻ supply from CO_2 -stimulated PEP carboxylase activity would modulate stomatal responses to CO_2 (Raschke, 1975), or K^+ uptake driven by a light-activated proton ATPase would modulate stomatal responses to light (Zeiger, 1983). However, the finding that CO_2 pulses applied in the morning or the afternoon elicit very similar stomatal responses supported by two different osmoregulatory pathways (Fig. 4) pose important questions about the relation between signal sensing and guard cell osmoregulation. Pathway-specific sensing would require that both the K^+ and the sucrose pathways have a capacity for CO_2 sensing, and implies that each osmoregulatory pathway has multiple environmental sensors. Although certainly possible, such design

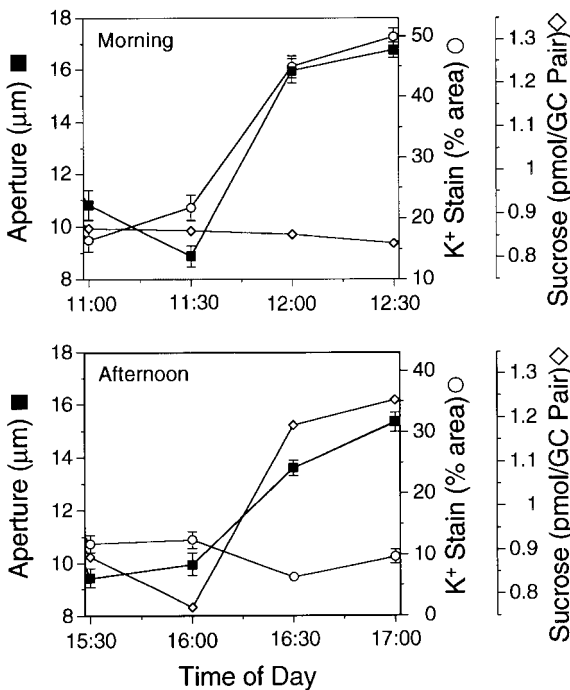


Fig. 4. Solute content of guard cells in intact growth chamber-grown leaves during reopening after closure in response to elevated CO_2 . Aperture, K^+ content, and sucrose content are shown for reopening in the morning (K^+ -dominated) and afternoon (sucrose-dominated) phases. Measurements are as in Fig. 1. Data after Talbot and Zeiger (1996).

could be overly complex. An alternative mechanism would involve a design in which environmental signals are transduced into apertures through a solute-independent parameter such as guard cell turgor. Under this scheme, signals would be sensed by a turgor-sensitive system, such as stretch activated channels sensing plasma membrane tension (Cosgrove and Hedrich, 1991). Prevailing cellular conditions would then determine which osmoregulatory pathway(s) is activated to generate the required solutes supporting the apertures specified by the solute-independent system. Such a scheme does not necessarily rule out environmental interaction with a specific osmoregulatory pathway, such as the sensing of mesophyll photosynthetic rates by the sucrose-dependent phase discussed above, but a solute-independent sensing mechanism could add much flexibility to the regulation of stomatal apertures by multiple environmental signals.

The characterization of a major role of sucrose in guard cell and the two phases of osmoregulation in the intact leaf opens new research opportunities for the understanding of the regulation of stomatal movements and the control of leaf gas exchange.

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