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Chloroplast Function in Guard Cells of *Vicia faba* L.[1]

MEASUREMENT OF THE ELECTROCHROMIC ABSORBANCE CHANGE AT 518 NM

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DAVID A. GRANTZ[2], THOMAS GRAAN, AND JOHN S. BOYER[3]
United States Department of Agriculture/Agricultural Research Service, and Departments of Agronomy and Plant Biology, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Stomatal conductance is coupled to leaf photosynthetic rate over a broad range of environmental conditions. We have investigated the extent to which chloroplasts in guard cells may contribute to this coupling through their photosynthetic activity. Guard cells were isolated by sonication of abaxial epidermal peels of *Vicia faba*. The electrochromic band shift of isolated guard cells was probed *in vivo* as a means of studying the electric field that is generated across the thylakoid membranes by photosynthetic electron transport and dissipated by photophosphorylation. Both guard cells and mesophyll cells exhibited fast and slow components in the formation of the flash-induced electrochromic change. The spectrum of electrochromic absorbance changes in guard cells was the same as in the leaf mesophyll and was typical of that observed in isolated chloroplasts. This observation indicates that electron transport and photophosphorylation occur in guard cell chloroplasts. Neither the fast nor the slow component of the absorbance change was observed in the presence of the uncoupler carbonylcyanide *p*-trifluoromethoxyphenylhydrazone which confirms that the absorbance change was caused by the electric field across the thylakoid membranes. The magnitude of the fast rise was reduced by half in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Therefore, photosystem II is functional and roughly equal in concentration to photosystem I in guard cell chloroplasts. The slow rise was abolished by 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone indicating the involvement of the cytochrome b_6/f complex in electron transport between the two photosystems. Relaxation of the absorbance change was irreversibly retarded in cells treated with the energy transfer inhibitor, *N,N'*-dicyclohexylcarbodiimide. The slowing of the rapid decay kinetics by *N,N'*-dicyclohexylcarbodiimide confirms that the electrical potential across the thyalkoid membrane is dissipated by photophosphorylation. These results show that guard cell chloroplasts conduct photosynthetic electron transport in a manner similar to that in mesophyll cells and provide the first evidence that photophosphorylation occurs in guard cells *in vivo*.

Stomata control CO_2 uptake and water loss by plants (27). Steady state stomatal conductance is tightly coupled to photosynthetic rate in the underlying mesophyll cells (2, 26), but there is little information on the mechanism of this coordination. Independent responses by guard cells and mesophyll cells directly to the environment could result in the functional coupling that is observed under a wide range of conditions. Much is known about the properties of mesophyll cells which control photosynthetic responses to the environment, but little is known about similar properties of guard cells. An integrated view of the stomatal response to light, for example, requires information on the functional properties of chloroplasts from both cell types.

Stomatal conductance in intact leaves responds directly to light independently of associated changes in intercellular CO_2 (19). Isolated stomata open (6) and guard cell protoplasts swell (29) in response to light, establishing that the photoreceptors are located in the guard cells. Stomata respond independently to blue and red light (17, 29) although the blue light receptor remains unidentified. Action spectra (6, 11) and inhibitor studies (17) indicate that the red light response is mediated by guard cell chloroplasts.

Chl *a* fluorescence emission spectra (14, 21, 28) indicate the presence of both photosystems in guard cells. The kinetics of slow fluorescence quenching indicate the formation of the high energy state in guard cell chloroplasts (10, 27), but photophosphorylation remains to be demonstrated. An independent probe has been sought to confirm these conclusions regarding the functional capacity of guard cell chloroplasts.

Among the largest light-induced absorbance changes in chloroplasts and green cells is the carotenoid-Chl *b* absorbance band shift, with difference maximum at 518 nm and minimum at 480 nm (25). The magnitude of this electrochromic shift is a quasi-linear function of the thylakoid membrane potential generated by photosynthetic electron transport. The kinetic responses of the absorbance change to flash frequency and to photosynthetic inhibitors reflect properties of the electron transport components and the occurrence of energy conservation and photophosphorylation.

The electrochromic change in mesophyll thylakoid membranes (*e.g.* isolated from spinach or pea) rises biphasically. The fast phase occurs within 20 ns (25) and is due to primary charge separations performed by reaction centers of both photosystems. The slow phase occurs over several ms and reflects an additional electrogenic event during oxidation of plastoquinol (18). The action spectra and decay kinetics of the slow and fast rise components are indistinguishable, and both indicate the delocalized membrane potential (25).

The decay of the absorbance change reflects the dissipation of the membrane potential by proton movement across the thylakoid membrane (25). Relaxation is accelerated in isolated thylakoids by addition of ADP and Pi to create phosphorylating conditions (4). The rapid decay is abolished by energy transfer inhibitors (8) and is absent in algal mutants blocked at the terminal stage of photophosphorylation (7). The association of

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[2] This work represents part of the dissertation submitted by D. A. G. to the University of Illinois in partial fulfillment of the requirements for the Ph.D. Current address: Department of Biological Sciences, Stanford University, Stanford, CA 94305.

[3] Current address: Department of Soil and Crop Science, Texas A & M University, College Station, TX 77843.

rapid decay kinetics with the proton fluxes of photophosphorylation has provided a technique for monitoring the kinetics of ATP synthesis in chloroplasts (25). Rapid decay kinetics in guard cells should reflect phosphorylating proton translocation.

We report for the first time the occurrence of electrochromic absorbance changes in guard cells. A DCMU-sensitive fast rise and a DBMIB[4]-sensitive slow rise establish that guard cell chloroplasts operate a noncyclic electron transport system and generate the thylakoid high energy state. A DCCD-sensitive, rapid decay indicates that this energy is conserved by photophosphorylation.

MATERIALS AND METHODS

Plants of *Vicia faba* L. cv Long Pod (Burpee Seed Co., Warminster, PA) were grown in a controlled environment for 4 weeks under 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR, from daylight fluorescent bulbs, at 28°C day (14 h)/18°C night (10 h). Pots were irrigated with tap water daily and fertilized twice weekly. Plants were adapted to darkness for 1 h before removal of epidermis from the abaxial surface of young, fully expanded leaflets. After peeling, the epidermal strips were rubbed to remove mesophyll debris and sonicated three times in fresh cold water (10). Peels were stored on ice in water or in 10 mM K-phosphate (pH 7) for all experiments.

Epidermal peels were periodically screened for contaminating mesophyll Chl using incident fluorescence microscopy. The sonicated peels were mounted on glass slides in water and viewed at 320×. For purposes of estimating contamination, each aggregate of mesophyll chloroplasts was assumed to have a Chl content equal to that of a whole mesophyll cell (14). Extracellular Chl was estimated from the projected areas of fluorescing subchloroplast particles relative to the area of a guard cell chloroplast in the same field of view. Fluorescence photomicrographs were taken using Kodak[5] technical pan film 2415.

For measurement of stomatal apertures from a dark-adapted plant, similar epidermal peels were removed, mounted on a glass slide in water and immediately measured with a calibrated ocular micrometer at 450×. For measurement of apertures following exposure to a range of conditions, similar epidermal peels were collected, half were sonicated to isolate guard cells, and the apertures were measured. Sonicated or unsonicated peels were placed in 10 mM K-phosphate (pH 7) at 28°C, in the dark or under an irradiance of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ (quartz iodide bulb, 500 w) measured after passing through an IR reflecting mirror (Optical Coatings Laboratory, Inc., Santa Rosa, CA) and 6 cm of water. Peels were incubated in glass Petri dishes with plastic lids through which humidified air or humidified CO_2-free air was passed. Dark treatments contained 0.1 mM ABA to ensure complete stomatal closure. After incubation for various periods, apertures were measured as above. Data are the means ± SE of 40 apertures, 10 on each of four peels.

For measurement of absorbance changes, separate preparations of peels were suspended on ice in 10 mM K-phosphate (pH 7) for 30 min prior to measurement. Pretreatment with inhibitors was in the same buffer for 15 min. Approximately 4 mg dry weight of sonicated epidermis, equivalent to about 1 μg guard cell Chl[6] was placed in a chamber consisting of an opaque rubber ring 7.5 mm × 5.0 mm i.d. held between two glass microscope slides. This sample chamber was placed in a clamp developed by D. R. Ort for measurement of flash-induced absorbance changes in attached leaves. A bifurcated quartz fiber light guide delivered actinic light to the sample and carried the measuring beam from the sample to the photomultiplier tube (Hamamatsu R 268). Another light guide carried the measuring beam from the monochromator to the sample. Both light guides were brought into contact with the sample chamber to minimize attenuation due to light scattering. For measurement of mesophyll cells, an attached leaf was placed between the two light guides, as described (12). All measurements were made at room temperature.

Actinic light for shutter flashes (pulse width = 10 ms) was provided by a 24 v/150 w tungsten-halogen bulb operated at 23 v from a regulated power supply. Single turnover flashes (pulse width = 6 μs) were delivered by a xenon lamp (FX 193 Flashtube; EG & G, Salem, MA) operated at 1500 v. Illumination was saturating, about 10^6 erg $cm^{-2} \cdot s^{-1}$, measured after passage through a heat filter and a red cut-off filter (Corning 2-64). The photomultiplier was protected by a Corning 4-96 filter and a green, wide band interference filter (66.1055; Rolyn, Arcadia, CA). The green filter was replaced by a blue, wide band interference filter (66.1050, Rolyn) below 500 nm, during measurement of the difference spectra. The measuring beam was provided by a diffracting monochromator (Instruments SA Inc., model AH 10) with spectral band width of 4 nm, and a tungsten bulb operated at 17 v from a regulated power supply. The time constant of the instrument was 300 μs (4).

Individual traces were recorded in digital form (Biomation 805 transient recorder) and accumulated as required by the signal to noise ratio, with a signal averaging oscilloscope (Nicolet 1172). When 10-ms shutter flashes were delivered, the absorbance change at 540 nm from an equal number of flashes was electronically subtracted from the change at 518 nm to minimize the contribution of nonelectrochromic changes. No such correction was performed for absorbance changes induced by single turnover flashes because the flashes were too short to be affected by the slower nonelectrochromic changes.

To ensure aerobic conditions the sample was removed from the measuring chamber every 10 min and swirled in aerated buffer for 5 min. For measurement of the slow absorbance rise, the sample was not aerated and was allowed to remain sealed in the measurement chamber for up to 1 h. To minimize any artifact due to sample geometry, the total signal for each treatment was obtained by adding several groups of flashes (at both 518 nm and 540 nm when necessary) separated by a resuspension in buffer, or buffer plus inhibitor.

DCCD and DCMU were obtained from Sigma Chemical Co. 4′-Deoxyphlorizin and FCCP were generous gifts to D. R. Ort from G. D. Winget, University of Cincinnati, and P. G. Heytler, E. I. du Pont de Nemours and Company, respectively. DBMIB was synthesized by D. R. Ort. DBMIB, DCMU, FCCP, DCCD, and ABA were dissolved in methanol and aliquots added to the buffer. This brought the methanol to 1% in treated preparations and so 1% methanol was added to all controls.

RESULTS

The sonication procedure described in "Materials and Methods" produced epidermal strips containing isolated guard cells with very little contamination by mesophyll cells or organelles (Fig. 1). It was estimated that following sonication greater than 99% of the Chl-containing cells remaining in these strips were guard cells and greater than 97% of the Chl content of these strips was accounted for by guard cells (Table 1). The isolated guard cells remained viable after sonication as determined by accumulation of the vital stain, neutral red (10), and remained fully functional in stomatal opening (Fig. 2). Both sonicated and

[4] Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCCD, *N,N′*-dicyclohexylcarbodiimide; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; CF_0/CF_1, proton channel/catalytic subunit of the chloroplast coupling factor (ATP synthetase).

[5] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

[6] Assuming 5 $cm^2 \cdot mg^{-1}$ dry weight of epidermis (11), 140 guard cells $\cdot mm^{-2}$ (10), and 4.5 pg Chl $\cdot$ guard $cell^{-1}$ (14).

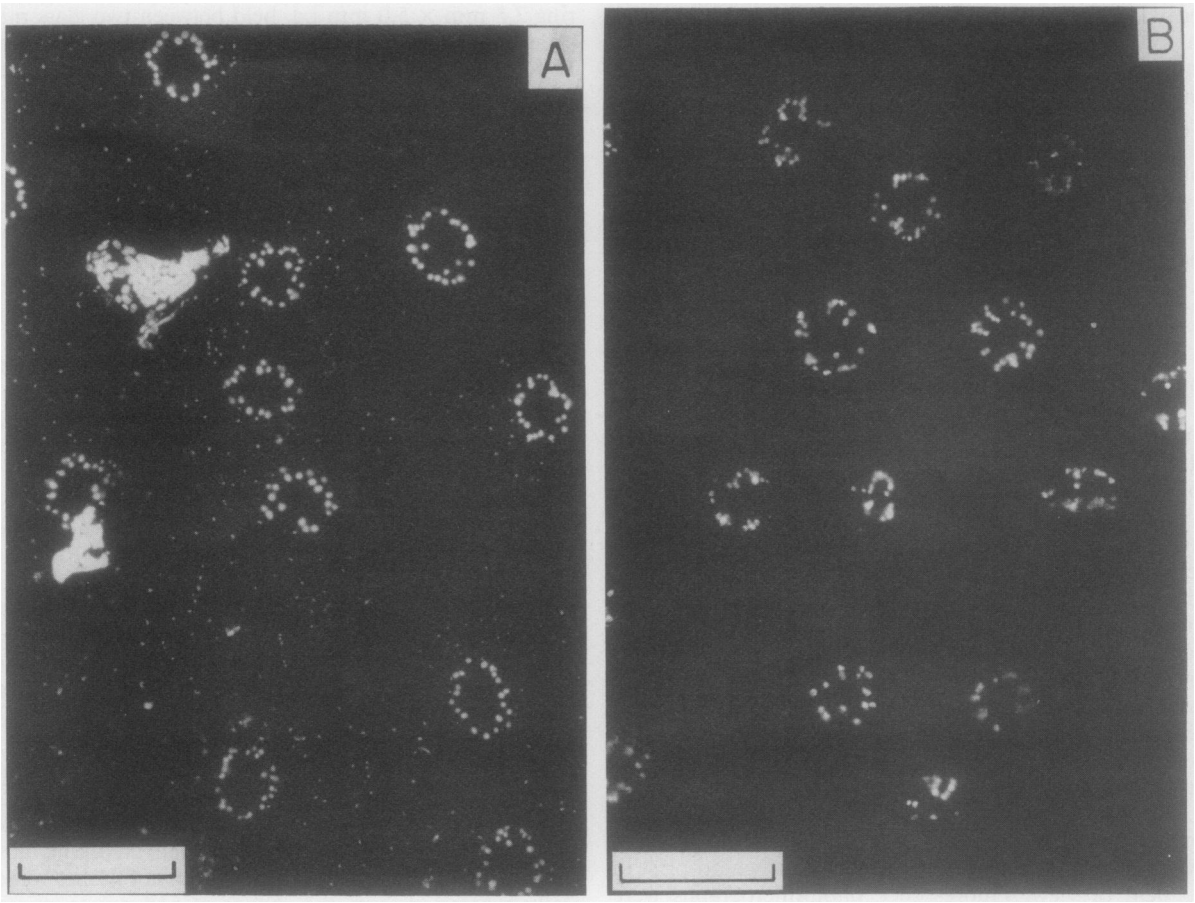

FIG. 1. Fluorescence photomicrographs of epidermal peels of *V. faba* before (A) and after (B) sonication. The small clustered fluorescing bodies are chloroplasts in guard cells. The irregular bodies in (A) are adhering mesophyll cells and the dispersed small bodies are subchloroplast fragments. The bar represents 100 μm.

Table I. *Survey of Chl Contamination of Sonicated Epidermal Peels of Vicia faba*

Results of observations on three representative preparations of sonicated epidermal strips. Contamination was estimated from microscopic observations using incident fluorescence optics. The guard cells and contamination in many individual strips were pooled and converted to total Chl using published values for Chl content of guard and mesophyll cells (14). Contamination was present both as adhering mesophyll cells and as adhering subchloroplast particles which were derived from broken mesophyll cells by the sonication procedure. These particles were numerous but contained little Chl, as estimated from their projected areas under fluorescence optics.

	Guard Cells		Mesophyll Cells		Extracellular Chl[b]	Contamination	
	No.	Chl[a]	No.	Chl[a]		Cell	Chl[a]
		pg		*pg*	*pg*	%	
1	1054	4743	2	125	15.1	0.19	3.0
2	1086	4887	2	125	14.4	0.18	2.8
3	2082	9369	1	62.5	14.6	0.05	0.8

[a] Assuming that contaminating cells were spongy mesophyll cells containing 62.5 pg Chl and that each guard cell contained 4.5 pg Chl (14). [b] Estimated from projected areas of fluorescing subchloroplast particles relative to areas of guard cell chloroplasts containing 0.4 pg Chl (14).

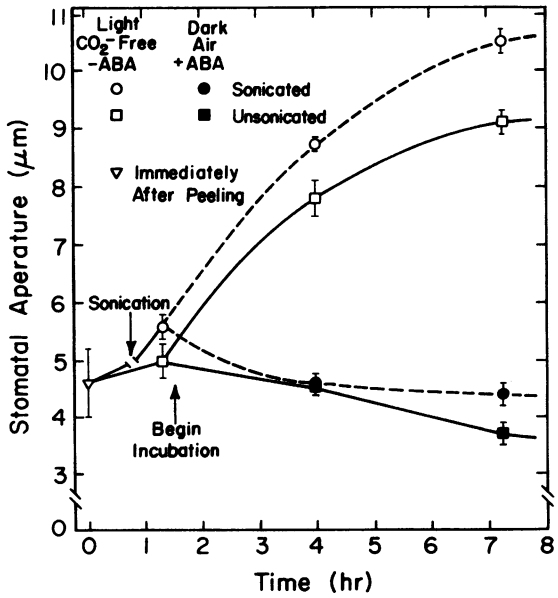

FIG. 2. Stomatal response in sonicated and unsonicated epidermal peels of *V. faba*. Peels were observed immediately after peeling or incubated in 10 mM K-phosphate (pH 7), in either light and CO_2-free air (▽, ○, □) or in darkness, normal air, and 10^{-4} M ABA (●, ■). Stomatal aperture was measured as described in "Materials and Methods."

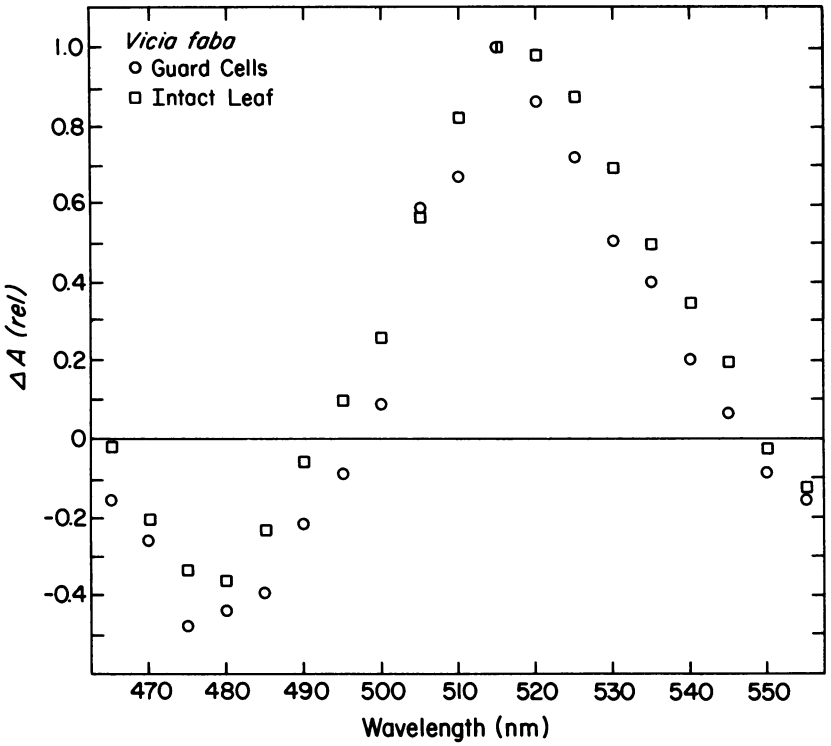

FIG. 3. Spectrum of flash-induced absorbance change in attached leaf (□) and isolated guard cells (O) of *V. faba*. The magnitude of the total absorbance change induced by one saturating, single-turnover flash is plotted as a function of measuring beam wavelength. Average of 32 flashes at each wavelength delivered at 1 Hz. Data are normalized to the absorbance change at 515 nm. At this wavelength, ΔA per flash was 2.28×10^{-3} for the leaf and 0.66×10^{-3} for the guard cells.

unsonicated guard cells were capable of wide stomatal opening during incubation in 10 mM K^+ salts, in light and CO_2-free air, though the initially larger aperture due to sonication persisted to quite wide apertures (Fig. 2).

The wavelength dependence of flash-induced absorbance changes in isolated guard cells was similar to that obtained from an attached leaf (Fig. 3). The characteristic maxima near 518 nm and minima near 480 nm (25) indicate that the flash-induced absorbance change in both guard cells and mesophyll cells was due to the electrochromic shift.

Mesophyll cells exhibited a biphasic absorbance increase at 518 nm when excited by saturating, single turnover flashes delivered at 0.1 Hz (Fig. 4, trace a). The kinetics of the fast rise,

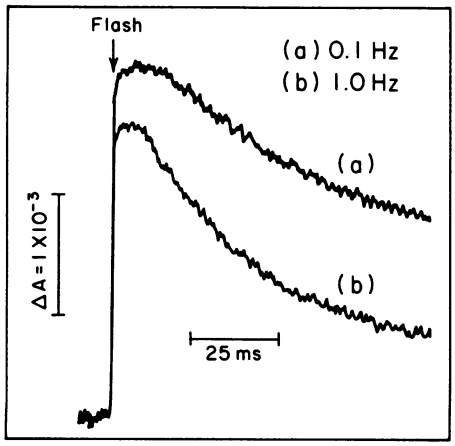

FIG. 4. Kinetics of absorbance increase and relaxation at 518 nm in attached leaf of *V. faba*. Absorbance change induced by one saturating, single-turnover flash. Average of 64 flashes delivered at 0.1 Hz (trace a) or 1 Hz (trace b). The fast component of the absorbance increase appears instantaneous on this time scale. The slow rise occurs within about 20 ms of the flash. Resolution of these components is obscured by the relaxation of the absorbance change which is occurring from the onset of the flash and continues into the subsequent dark period.

reflecting primary photochemical charge separations in the reaction centers, could not be resolved in this study ($t_{½}$ less than 20 ns; 25). The slow phase, completed within 10 ms in Figure 4, trace a, reflects an additional electrogenic event associated with noncyclic electron transport between the two photosystems (24). The $t_{½}$ of the slow rise was somewhat reduced and the relaxation kinetics were accelerated by increasing the flash frequency to 1 Hz (*cf.* trace a and b). The slow rise is not detectable under many experimental conditions, and the mesophyll cells in the well-aerated leaf of Figure 4 exhibited only a relatively small slow component.

Isolated guard cells in well-aerated buffer exhibited absorbance changes similar to those in mesophyll cells when excited with single turnover flashes (Fig. 5A, trace b) and with 10 ms flashes (Fig. 5B, trace b). The rise kinetics were distorted during the longer flash by the rapid decay occurring during the flash. These decay kinetics suggested that, in guard cell chloroplasts, as in mesophyll chloroplasts (*cf.* Figs. 4 and 5), the high energy state indicated by the electrochromic shift is associated with a pathway of low resistance to charge dissipation, through the otherwise impermeable lipid membrane.

To investigate whether the rapid decay represented movement of protons through the chloroplast coupling factor, the guard cells were treated with the energy transfer inhibitor, DCCD, which covalently binds to the CF_0 subunit, blocking the proton channel, and inhibiting phosphorylation at the terminal step (8). Following single turnover flashes (Fig. 5A) and 10-ms flashes (Fig. 5B), the decay kinetics were retarded substantially (*cf.* traces a and b) and to about the same rate in each case. The magnitude of the total absorbance change was larger in the presence of DCCD than in the control (Fig. 5B) since dissipation of the membrane potential by a proton current through CF_0 during the 10-ms flash was inhibited by DCCD. The effect of DCCD could not be reversed by extensive washing of the epidermal peels in fresh buffer.

To probe the electrochromic nature of the absorbance change, the guard cells were treated with FCCP, a weak acid uncoupler that facilitates proton transport through the membrane without

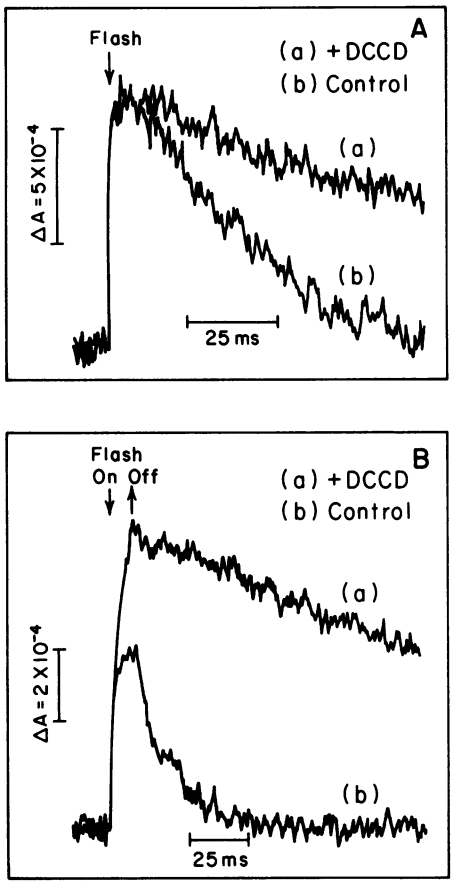

FIG. 5. Effect of 10^{-4} M DCCD on the relaxation kinetics of the absorbance change in isolated guard cells. A, Absorbance change at 518 nm induced by one saturating, single-turnover flash. Average of 512 flashes delivered at 1 Hz and collected in groups of 256. B, Absorbance change at 518 nm induced by one 10-ms flash was corrected by subtraction of change at 540 nm. Average of 576 flashes delivered at 1 Hz. Note the longer time scale in this figure than in A. The two traces were obtained from different guard cell preparations with different Chl contents, therefore the absolute magnitudes are not directly comparable.

passage through the coupling factor. FCCP accelerated the decay of the absorbance change (Fig. 6) as expected for the electrochromic shift (8). In the presence of 0.1 μM FCCP the decay was substantially enhanced (Fig. 6, *cf.* curve a and b). The apparent magnitude of the absorbance change was slightly reduced by this concentration of FCCP, but this probably resulted from accelerated decay of the absorbance increase during the response time of the instrument. Indeed, the decay was accelerated to such an extent by 10 μM FCCP (curve c) that no absorbance increase was detected, although the primary charge separations would not be affected by the uncoupler.

For measurement of the rapid decay kinetics described above, the guard cell sample was removed from the measurement chamber every 10 min for a 5-min resuspension in aerated buffer. If the guard cells were not resuspended in this manner, a reversible retardation of the decay kinetics occurred (data not shown) preceded by a reversible increase in the slow component. Both changes were reversible even after more than 1 h of enclosure in the chamber, by a 5-min resuspension. For measurement of the slow rise in guard cells, the samples were sealed in the chamber for 20 min prior to measurement, at which time stable kinetics were achieved. The slow rise was also most apparent at low flash frequencies, as observed in mesophyll cells (Fig. 4), so that flash groups were routinely delivered at 0.1 Hz.

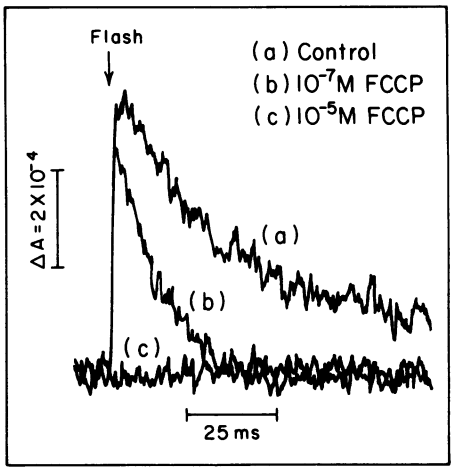

FIG. 6. Effect of FCCP on the relaxation kinetics of the absorbance change at 518 nm in isolated guard cells. The control sample (trace a) and sample treated with 10^{-7} M FCCP (trace b) represent the average of 512 flashes delivered at 1 Hz and collected in groups of 128. The sample treated with 10^{-5} M FCCP (trace c) is the average of 256 flashes delivered at 1 Hz.

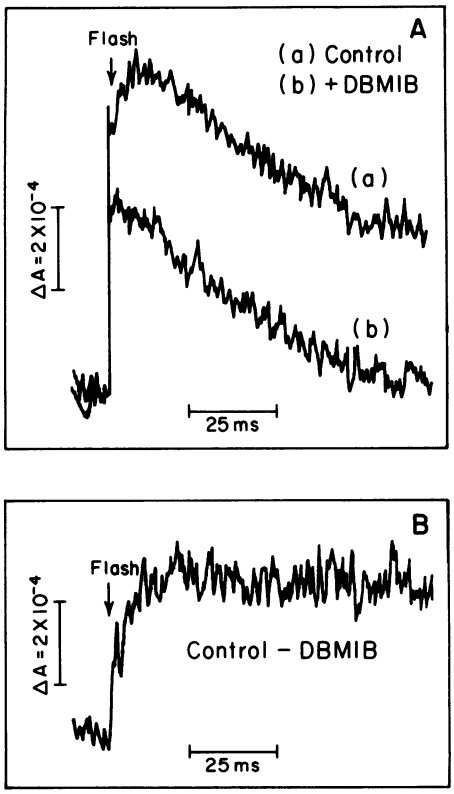

FIG. 7. A, Effect of 10^{-5} M DBMIB on the slow phase of the absorbance increase at 518 nm in isolated guard cells. Average of 512 single turnover flashes delivered at 0.1 Hz. Flashes were collected in groups of 256 with the sample resuspended for 5 min in aerated buffer between groups. The sample was sealed in the measurement chamber for 20 min prior to measurement to enhance the slow rise. B, The difference between treated and control traces showing predominately the slow phase of the absorbance increase.

The slow rise phase was isolated by delivering single turnover flashes at 0.1 Hz to samples which had been sealed in the measurement chamber for greater than 20 min, in the presence (Fig. 7A, trace b) or absence (trace a) of the quinol oxidation

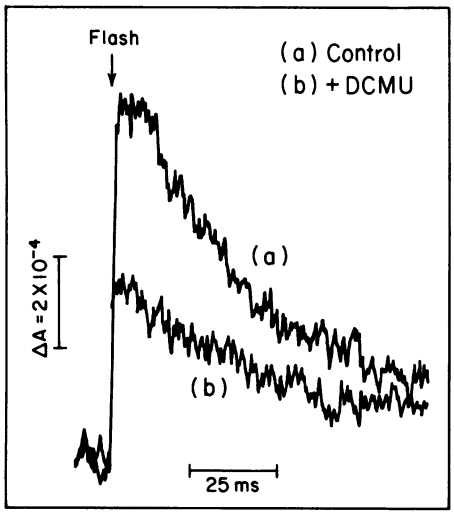

FIG. 8. The effect of 10^{-5} M DCMU on the fast phase of the absorbance increase at 518 nm in isolated guard cells. The sample was measured immediately following enclosure in the measurement chamber. Average of 512 flashes delivered at 1 Hz and collected in groups of 128.

antagonist, DBMIB. The computer subtracted difference between the two traces (Fig. 7B) represents the slow rise, which is abolished by DBMIB (18).

The electrochromic shift reflects the delocalized membrane potential contributed by both PSI and PSII (25). To determine whether PSII is functional in guard cell chloroplasts, the effect of DCMU on the fast rise component was investigated. In guard cells measured immediately after sealing into the measurement chamber and excited by flashes delivered at 1 Hz, the effect of DCMU was to decrease the amplitude of the fast rise (Fig. 8; *cf.* trace a and b) by about 50%, as expected if PSI and PSII contribute approximately equally to the thylakoid membrane potential. The fast rise was reduced by DCMU independently of the slow rise or of the decay kinetics when these were manipulated as above, with prolonged enclosure or with DCCD (not shown).

DISCUSSION

The sonicated epidermal peels used in these studies are sufficiently free of epidermal cells (10) and of mesophyll cells and organelles to allow results to be interpreted unambiguously as guard cell functions. The purity of the sonicated guard cells of *Vicia* (greater than 99% on a Chl-containing cell basis) compares favorably with the purity of guard cell protoplast preparations (95–98% on a total cell basis; 3, 5, 14). Sonication also avoids lengthy enzymic digestion and maintains cell wall attachments.

The sonicated guard cells were functional in stomatal opening. Substantial base line opening is always found in epidermal peels of *Vicia* as a result of the loss of back pressure from epidermal cells ruptured during peeling. The further increase in baseline aperture due to sonication is apparently due to disruption of the remaining epidermal cells. This seems to be a mechanical effect since only slight closure from the dark-adapted state was observed in both sonicated and unsonicated peels under conditions known to cause stomatal closure in this species. The continued ability of sonicated guard cells to function in stomatal opening (20; Fig. 2), synthesis of malate (11) and accumulation of neutral red (10) and K^+ (20) shows that measurements on these peels represent physiological processes occurring *in vivo*.

The magnitude of the electrochromic absorbance change measured at 518 nm is compatible with the limited sample size of isolated guard cells and allowed the spectrum as well as the induction and decay kinetics to be observed. The difference spectrum of the light-induced absorbance change for guard cells was similar to that for mesophyll cells in the intact leaf. The characteristic maxima and minima in the difference spectra and the sensitivity of the absorbance decay to uncoupling by FCCP demonstrate that the phenomenon observed in both cell types was the electrochromic shift. Observation of the electrochromic shift in guard cells confirms that charge separation and accumulation of the high energy state occur in guard cell chloroplasts as previously inferred from analyses of fluorescence quenching (10, 28). Fluorescence intensity in intact cells is a complex function of the redox status of the electron transport chain and of membrane conformation (15), as well as of membrane energization, while the electrochromic shift reflects directly the electrical component of the high energy state (25). Observation of both phenomena in intact guard cells establishes that guard cell chloroplasts are functional in photosynthetic energy conversion *in vivo*, and thus are likely to be of physiological significance in stomatal function.

The $t_{1/2}$ of the rapid absorbance relaxation observed in guard cells is of the same order of magnitude (few tens of ms) as the decay in isolated chloroplasts under phosphorylating conditions (25). The sensitivity of the decay to DCCD demonstrates that the charge dissipation is likely due to protons crossing the membrane through the coupling factor during photophosphorylation. It should be noted that DCCD would not distinguish between CF_1- and non CF_1-mediated proton transport through CF_0. Removal of CF_1 from thylakoids results in preparations with high proton permeability and little phosphorylating activity, but DCCD decreases the conductivity without reestablishing photophosphorylation (8). However, the electrochromic shift in intact leaves and in guard cells (*cf.* Figs. 4b, 6a, and 8a) show similar decay kinetics. Both are *in vivo* conditions and, since the intact leaf clearly was photophosphorylating, the similarity of the decay in guard cells indicates no apparent damage (which would be manifested as an accelerated decay) in these preparations. Had damage to the proton translocating CF_0/CF_1 channels been present, accelerated decay would have been evident, as was the case when 0.1 μM FCCP was present (known to increase proton permeability of thylakoids) and an accelerated decay was clearly observed.

The acceleration by the uncoupler, FCCP, of the decay of the absorbance change is diagnostic for the electric field-indicating electrochromic shift. Because FCCP increases the membrane permeability to protons, it dissipates both the pH gradient and the membrane potential of the chloroplast high energy state.

The slow rise of the electrochromic shift has been difficult to document in a variety of organisms. The frequency and duration of the flash excitation may determine whether it is observed (7, 18), since an intermediate redox poise of the electron transport chain and a low level of membrane energization are thought to enhance the extent of the slow rise. The enhancement of the slow rise in guard cells during prolonged enclosure probably resulted from mildly reducing conditions due to O_2 depletion. Observation of a DBMIB-sensitive slow phase of the electrochromic shift in guard cells indicates that intersystem electron transport in guard cell chloroplasts functions as it does in mesophyll chloroplasts. DBMIB interacts with the Cyt b_6/f complex to inhibit plastoquinol oxidation (22). The difference between the absorbance changes in the presence and absence of low concentrations (0.5 μM) of DBMIB has been suggested (18) as a means of isolating the slow component of the absorbance increase in isolated chloroplasts. In the present study, using intact cells in the presence of considerable lipophilic cuticle, a somewhat higher concentration (10 μM) was required to achieve this effect. In isolated chloroplasts, DBMIB at this concentration may bind to the DCMU site of PSII (22). This inhibition of PSII may explain the decrease in the fast rise in the presence of DBMIB

(Fig. 7A, trace b).

The possibility that PSII may be absent or deficient in guard cell chloroplasts is suggested by ultrastructural studies (1) showing a deficiency of granal lamellae, in which PSII is known to be concentrated. However, inhibition of half of the rapid electrochromic rise by DCMU in the present study confirms earlier indications of PSII activity in guard cells based on Chl *a* fluorescence (10, 14, 21, 28), cytochemistry (23), and photosynthetic O_2 evolution from guard cell protoplasts (3, 21).

The remaining half of the fast rise, not inhibited by DCMU, reflects charge separations of the PSI reaction centers. This is consistent with earlier suggestions of PSI activity in guard cells based on fluorescence emission spectra, light-induced absorbance changes at 700 nm (14), and cytochemical observations (23). Far-red light, which is absorbed preferentially by PSI, was found to alter the restoration kinetics of PSII fluorescence in guard cells of *Chlorophytum* (9), also suggesting functional PSI as well as intersystem electron transport in guard cell chloroplasts.

Whether guard cell chloroplasts contribute substantially to cellular pools of ATP (27) and reducing equivalents (10) or rather provide information regarding the presence of PAR as a trigger for stomatal opening (14), is not yet clear. However, the two postulated roles may differ quantitatively rather than qualitatively. Light information is likely to be transduced in the usual currency of photosynthetic high energy intermediates, ATP and reduced ferredoxin or NADPH. While apparently lacking key enzymes of the 'dark reactions' of the Calvin-Benson Cycle (13), with respect to the 'light reactions', guard cell chloroplasts seem to contain the full photosynthetic machinery described for mesophyll chloroplasts. The presence of both PSI and PSII and the intersystem electron transport system, including the Cyt b_6/f complex, imply that reducing equivalents are produced in guard cell chloroplasts. The decay kinetics of the light-induced thylakoid membrane potential, under a variety of experimental conditions, suggest that photophosphorylation also occurs in guard cells. Light-generated ATP and reducing equivalents would certainly be used in routine synthesis and maintenance of cellular components. More relevant to stomatal regulation are mechanisms through which chloroplast function might regulate cation uptake, proton efflux, synthesis of organic anions, or whatever process is ultimately found to be the initial event in stomatal movement. The physiological role of guard cell chloroplasts, involving energy or some other form of information, such as reductive light activation of chloroplast enzymes (16), is not yet clear. However, the functional competence of guard cell chloroplasts seems well established.

Independent responses to environmental stimuli by guard and mesophyll cells are consistent with the coupling of photosynthesis and stomatal conductance which is observed in intact plants and might also explain the striking limitations to this coupling that can be induced by manipulating ambient CO_2, light quality, or exogenous ABA. Direct communication of carbon assimilation capacity to the stomatal apparatus should be capable of coordinating the two processes during such manipulations, while subtle differences in receptors of the two cell types could easily lead to loss of coordination. Given that guard cells do respond directly to the environment, it will be important in the future to elucidate these subtle differences and to characterize their role in the overall integration of stomatal function in the activity of intact plants.

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