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Comparative Studies of Fluorescence from Mesophyll and Guard Cell Chloroplasts in *Saxifraga cernua*[1]

ANALYSIS OF FLUORESCENCE KINETICS AS A FUNCTION OF EXCITATION INTENSITY

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ABSTRACT

The chlorophyll fluorescence induction curves from mesophyll and guard cell chloroplasts of *Saxifraga cernua*, including both the fast (O to P, the transients involved in the rise in variable fluorescence) and slow (P to steady state fluorescence due to quenching) components, were characterized over a range of excitation intensities using microspectrophotometry (with epi-lumination) equipped with apertures designed to eliminate cross contamination of the fluorescence signal between the two chloroplast types. At low excitation intensities, the fast fluorescence kinetics from guard cell plastids showed an extended I to D phase and a more rapid appearance of P while minimal quenching from P to steady state fluorescence was observed compared to the transients from mesophyll chloroplasts suggesting a lower activity of photochemical (electron movement via carriers between donor and acceptor sites) and nonphotochemical (such as membrane conformational changes) events which regulate the fluorescence induction curve kinetics. As the excitation intensity was increased, the quenching rates of guard cells were faster at initiating conditions for photophosphorylation and the fast and slow fluorescence kinetics from guard cells resembled those of the mesophyll cells.

Guard cell chloroplasts of *S. cernua* from intact epidermal peels showed a low temperature (77 K) fluorescence emission spectrum having three major peaks (at 685, 695, and 730 nanometers when excited at 440 nanometers) which were qualitatively similar to those in the spectrum obtained from mesophyll tissue.

These data suggest that *S. cernua* guard cell chloroplast photosystems I and II contribute to light-dependent stomatal activity only at high light intensities.

Two photoreceptor systems in stomatal guard cells have been identified according to their spectral responses as blue light and PAR light sensitive (5, 22). It has been suggested that the blue light receptor is a flavin, located on the plasma membrane, while the PAR receptor represents the two photosystems (PSI and PSII) found in guard cell chloroplasts (22). Important to stomatal function with respect to sensing changes in the quality of light is the differential response of the two photoreceptors to light intensity. The blue light receptor appears to reach saturation at much lower light intensities in initiating light-dependent stomatal activities such as ion uptake and malate synthesis, both of which are necessary to promote stomatal opening (4, 14). Thus, the current understanding of stomatal response with respect to light irradiances is that the PAR-dependent photosystem can function as a possible energy source only at moderate to high intensities (22).

Of equal interest with regard to the PAR-dependent system are reports describing a higher quantum yield efficiency for mesophyll cell photosystems as measured by CO_2 assimilation compared to the guard cell response indicated by an increase in stomatal conductance (12, 25). The underlying mechanisms which can account for these differences in the two chloroplast type photosystems are unknown. However, considering the biochemical differences which exist between guard and mesophyll cells (21), in particular the reported lack of key Calvin cycle enzymes in guard cells (17), one might speculate that some alterations of the guard cell photosystems exist in order to function solely as a light modulator for stomatal activity rather than participating in photosynthesis. Clearly then, further insight into the role of the PAR-dependent photosystem in guard cells may be possible by comparing the efficiencies of photosynthetic light conversion by both mesophyll and guard cell chloroplast photosystems.

As part of a larger experimental program designed to investigate the physiological mechanisms responsible for thermal acclimation of chloroplast function, we have examined the energetic conversion of absorbed light by guard cell chloroplast photosystems of *Saxifraga cernua*, a herbaceous perennial native to arctic regions, by analysis of the Chl *a* fluorescence induction curve (the Kautsky effect) for both mesophyll and guard cell chloroplasts over a physiological range of light or excitation intensities. Our experimental approach has been to measure the fluorescence induction response *in situ* using a microspectrophotometric system equipped with epi-lumination which was designed to permit an extremely high level of morphological resolution in order to eliminate possible cross contamination of the fluorescence signal between the two chloroplast types. Our analysis demonstrates that there exists a decreased capacity for electron transport by the guard cell PAR-dependent photosystem, compared to that observed for the mesophyll plastid of *S. cernua*, which could contribute to the whole leaf stomatal responses to red light only at high intensities.

MATERIALS AND METHODS

Plant Material. *Saxifraga cernua* was germinated from asexual bulblets on a soil mixture in environmental rooms at a constant temperature of 20°C and a continuous 24-h photoperiod at an intensity of 110 $\mu mol\ m^{-2}\ s^{-1}$ supplied by Sylvania Gro-lux

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fluorescent bulbs and 40-w incandescent bulbs. Plants were grown for 6 weeks to maturity, watered every other day, and fertilized every 10 d. Epidermal peels were prepared from leaf discs (1 cm in diameter) by removing the abaxial surface with a pair of broad edged forceps. The lower epidermal layer is removed easily from this species and shows minimal (<5%) damage to epidermal cells and no damage of guard cells as indicated by the uptake of neutral red stain into the cells. Epidermal peels showed considerable contamination with adhering mesophyll chloroplasts as judged by phase and epifluorescence microscopy. Approximately half of the mesophyll chloroplasts could be removed from the epidermal peel by brushing the tissue with a fine hair artist brush. Epidermal peels were floated on media at 0°C containing 20 mM Hepes buffer (pH 7.2) and 1 mM K_2HPO_4 until used for fluorescence measurements.

Cytofluorometric Measurements of Fluorescence Transients. A Zeiss photomicroscope II equipped with an epifluorescence illuminator (06 filter combination: band pass 436 nm, half bandwidth 8 nm excitation filter, T 460 beam splitter, and LP 470 barrier filter) were used for measurements of Chl fluorescence. The fluorescence signal was collected by a Zeiss MPMO1K microscope photometer and Zeiss power supply (model 477414). Excitation light from the illuminator was controlled by a Zeiss electronic shutter having a rise time of 3 ms. The analog signal from the power supply was routed to a Grass P16 DC preamplifier and displayed in parallel on a chart recorder (Perkin-Elmer) for determination of the slow fluorescence quenching transients. The signal from the preamplifier was increased by a factor of 10 and displayed on an oscilloscope (Tektronix 5111 storage oscilloscope) for measuring the faster components of the fluorescence induction curve. The oscilloscope recordings were triggered from the photometer signal and were photographed using a Polaroid camera. To assist in identifying the fast transients of the induction curve on the slower recording apparatus, a range of steady state fluorescence signals was used to calibrate both the oscilloscope and the chart recorder at all possible combinations of sensitivity settings.

In order to delimit both the area of excitation of the specimen and the fluorescing area striking the photometer, excitation and emission apertures having a diameter of 1.0 and 0.63 mm, respectively, were used. The area of the specimen illuminated was 2.46 mm^2 and the area of fluorescence measured by the photometer was 1.51 mm^2. The area of illumination very closely matched the area of guard cell pairs. If mesophyll chloroplasts, identified by their larger size, appeared above or below the guard cell pair, a new guard cell pair was selected.

Throughout all experiments, a 16× phase contrast Neofluar objective and a 1.6× diopter lens were used. Fluorescence measurements were taken over a range of excitation intensities (62.7–1110 $\mu E\ m^{-2}\ s^{-1}$). For low light intensities (62.7–407 $\mu E\ m^{-2}\ s^{-1}$), the excitation source was a 50-w, 12-v tungsten halogen lamp controlled by a Zeiss LPS-7.5 variable resistor power source. For high light intensities (greater than 407), a 50-w mercury vapor lamp was used and the intensity adjusted by using Zeiss neutral density filters (50%, 10%, and 3% transmission) in various combinations prior to passing the illumination through the Zeiss 06 filter combination. In order to eliminate possible photobleaching of the Chl due to high light intensities, the illumination periods were limited to less than 60 s which was sufficient time for fluorescence to reach steady state. Subsequent measurements of the induction curve showed complete recovery of the induction curve transients after a 20-min dark adaption period. Longer excitation periods up to 5 min at high intensity caused some damage to the photosystems which was evident by the lack of recovery of the induction curve after extensive dark adaption periods. The illumination intensities were calibrated by focusing the objective on to the surface of a quantum sensor (Li-Cor Quantum Meter, model LI-185) and correcting for differences in area of the excitation beam and the photodiode cover.

Fluorescence kinetics were obtained from preparations held at a constant temperature of 20°C by placing the slide on a water-cooled brass plate (7.5 × 3.0 × 1.0 cm) which was secured to the microscope stage. Temperature was held constant by circulating the coolant through a constant temperature bath (Tamson). A 3 × 1.5 cm section from the center of the brass plate was removed to allow focusing of the excitation beam onto the guard cells by transmitted light. Prior to each fluorescence measurement, a guard cell pair was first aligned and focused using dim green transmitted light. Since an extremely small section of the preparation was illuminated due to the delimitation of the excitation aperture, a dark adaption period of 2 to 3 min was required to ensure a maximum fluorescence yield between sampling. Each preparation was discarded after 15 min. All measurements were made in a darkened room in order to exclude any stray light contributing to the photometer signal.

The dark current signal from the photometer was used to establish a baseline voltage before and after each experiment. Background fluorescence was tested for by observing a signal from a blank portion of the microscope slide (minus sample) with and without the excitation and the internal trigger set at low sensitivity. Both signals were identical indicating the absence of filter luminescence.

Low Temperature Fluorescence Emission Spectra. Fluorescence emission spectra were measured at room temperature and 77 K with a fluorescence spectrofluorometer (SLM 4800, Urbana, IL) interfaced with a Tektronix 4051 desk-top computer. The spectrofluorometer cuvette was equipped with a home-built liquid N_2 cooled cryostat for obtaining low temperature emission spectra. Excitation at 440 nm (half bandwidth 4 nm) was provided by a 450-w xenon arc lamp (Osram). Fluorescence emission spectra between 640 and 770 nm (half bandwidth 2 nm) were measured at 1-nm increments. Reducing the emission bandwidth did not improve the resolution of the emission profile. For front face fluorescence of mesophyll and guard cell chloroplasts, whole leaf sections (4 × 1 cm) were positioned 45° to the excitation beam by adhering the leaf section to a cut microscope slide of similar dimensions with nontoxic silicone (Dow Corning) and inserting the section into a glass cuvette. Epidermal peels from leaf discs were prepared as described above except that the adhering mesophyll chloroplasts were removed by sonication according to Ogawa *et al.* (15) (Fig. 1D). Sections of peeled epidermis (4 × 1 cm) were floated on ice-cold distilled H_2O in a small (40 ml) beaker and sonicated three times for 15 s using a sonicator equipped with a 1.27-cm disruptor horn (model W200R, Heat Systems Ultrasonics, Inc., NY). This resulted in complete rupture of the epidermal cells, evident by the loss of cell turgor but did not damage stomata guard cells demonstrated by the concentration of neutral red dye in guard cell pairs. To test for possible alteration of the emission spectra due to sonication, whole leaf sections were also sonicated at the same settings. The fluorescence spectra were identical for both sonicated and control tissue. Individual epidermal strips were examined for remaining contaminating mesophyll chloroplasts by epifluorescence microscopy. 'Cleaned' epidermal strips were then layered on a glass section and held in place with another glass section cut from a microscope cover slip. Normally 20 to 30 layered strips were required in order to provide a fluorescence signal large enough to be detected by the emission photomultiplier tube. Tissue samples were frozen in a solution of 65% (v/v) glycerol.

RESULTS

The important morphological features of *S. cernua* guard cells relative to our applicaton of epifluorescence for this study are

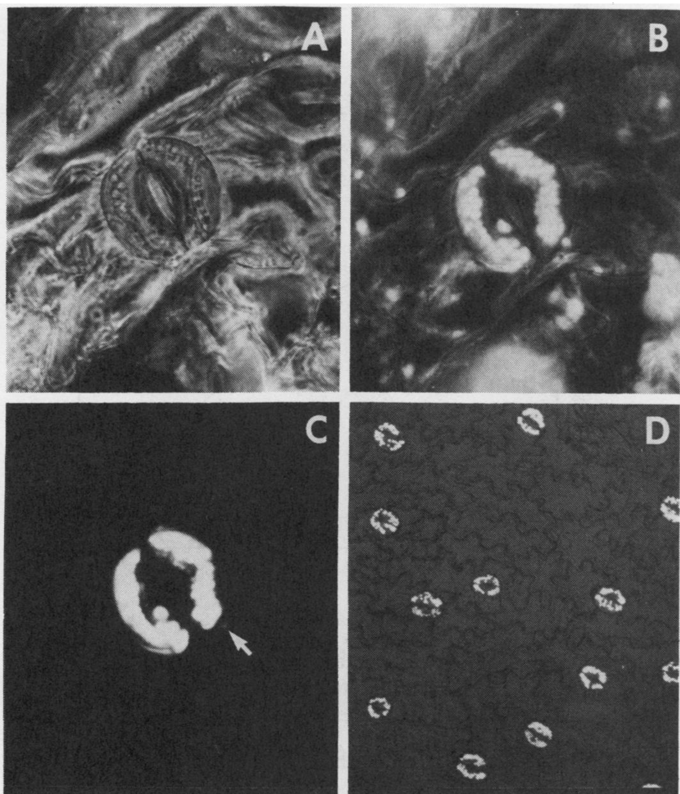

FIG. 1. Photomicrographs of *S. cernua* epidermal peels. These were made from color slides; therefore, the red fluorescence appears as either bright white spots or patches. A, Phase microscopy of a guard cell pair and surrounding epidermal cell. Mesophyll chloroplasts can be seen adhering to the epidermal tissue. Chloroplasts are also observed inside the guard cells. B, Fluorescence micrograph of the same sample as Figure 1A showing the fluorescing guard cell chloroplasts and the contaminating mesophyll chloroplasts. C, Same guard cell pair as in Figure 1A with the excitation aperture in place. Note the absence of contaminating fluorescence except for a single mesophyll chloroplast (arrow) adjacent to the guard cell wall. This would be removed from the working area of the photomultiplier by the addition of the emission aperture (see "Materials and Methods"). Magnification for A, B, and C: × 545. D, Combined low level transmission phase and fluorescence microscopy of sonicated epidermal tissue. The only source of fluorescence is from the guard cell chloroplasts. Magnification × 125.

shape, uniformity of size or area, and number of chloroplasts. *S. cernua* guard cell pairs approximate a circular shape (Fig. 1A) and are quite uniform in dimensions (width, 39.0 ± 0.56 μm; length, 44.0 ± 1.3 μm [mean ± SE]; $n = 12$). This facilitates the use of circular apertures for limiting the area under observation and eliminating fluorescing mesophyll chloroplasts adhering to the epidermal tissue (Fig. 1, B and C). Any mesophyll chloroplasts which could be detected by fluorescence adjacent to the guard cell walls were removed from the working field of the photomultiplier by either reducing the size of the emission aperture or moving the slide so the contaminant was no longer visible within the area of the emission aperture (Fig. 1C). *S. cernua* guard cells contain a large number of chloroplasts (21.7 ± 0.9 per guard cell [mean ± SE]; $n = 30$), although they are smaller than mesophyll chloroplasts. They thereby provide a readily detectable fluorescence signal. If apertures are constructed for the purposes described above and if epidermal peels having functional stomata can be obtained, fluorescence studies for any species should be possible.

Fast Kinetics of Variable Fluorescence (O to P). The red fluorescence observed in guard cell chloroplasts originates from the Chl *a* of PSII. Low temperature (77 K) fluorescence emission spectra for intact sonicated leaf tissue and epidermal layers excited by light of 440 nm revealed not only the presence of the PSII reaction center Chl *a* protein complex at 695 nm in both tissue types as shown in Figure 2, but also the light-harvesting Chl *a*/*b* protein complex and the PSI Chl complex at 685 and 730 nm, respectively (13). Due to the differences in Chl concentration between the two samples, further quantification such as the relative amounts of Chl-protein complexes present in the two chloroplast types was not possible. The fast fluorescence transients from both chloroplast types show a number of distinct differences in the appearance and time course of the transients during changes in excitation intensity. Figure 3 (A–D) shows typical fast fluorescence induction kinetics for both guard cell and mesophyll chloroplasts recorded at two excitation intensities. At the lowest excitation level used in this study (62.7 $\mu E\ m^{-2}\ s^{-1}$), mesophyll chloroplasts show a typically small residual fluorescence level (O) (18) followed by a slow rise in variable fluorescence progressing through the I to D and D to P phases (Fig. 3A) within 350 ± 10 ms (Fig. 5). Increasing the excitation intensity (Fig. 3B) results in a relative increase in the amount of variable fluorescence, shown by an increase in the ratio of P/O (Fig. 4), accompanied by a faster rise to maximum fluorescence of approximately 100 ms (Fig. 5). Similar responses of the induction curve to increased excitation have been reported for isolated spinach mesophyll chloroplasts (19).

Guard cell chloroplasts show an extended I to D phase, referred to as Fpl by Zeiger *et al.* (23) at the low excitation intensity (Fig.

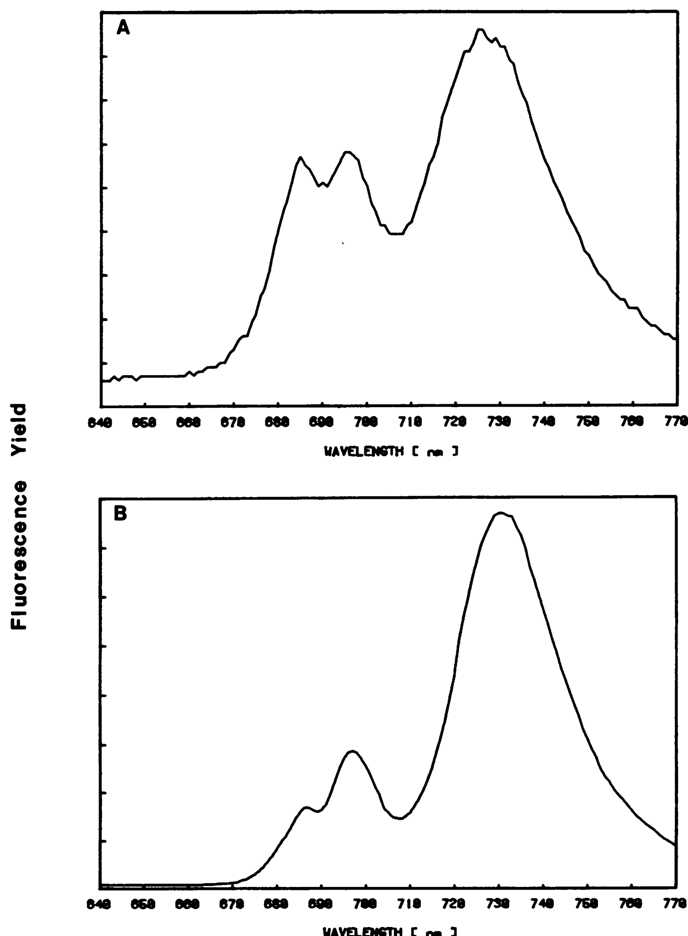

FIG. 2. Low temperature (77 K) fluorescence emission spectra of sonicated epidermal peels (A) and sonicated whole leaf sections (B) from *S. cernua*.

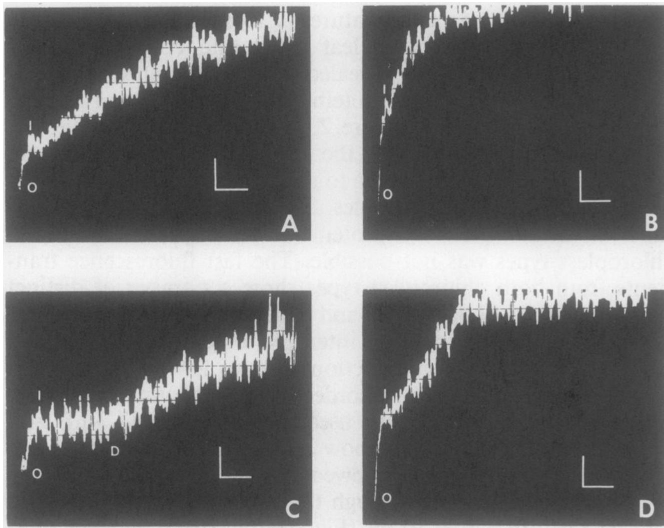

FIG. 3. Fast changes in the Chl *a* induction curve for mesophyll (A and B) and guard cells (C and D). The excitation intensity for A and C was 62.7 μE m^{-2} s^{-1}; for B and D, the intensity was 407 μE m^{-2} s^{-1}. The slow changes for each trace are shown in Figure 5. The time bar equals 50 ms for each recording; the vertical bar equals 20 mv for A and C and 50 mv for B and D.

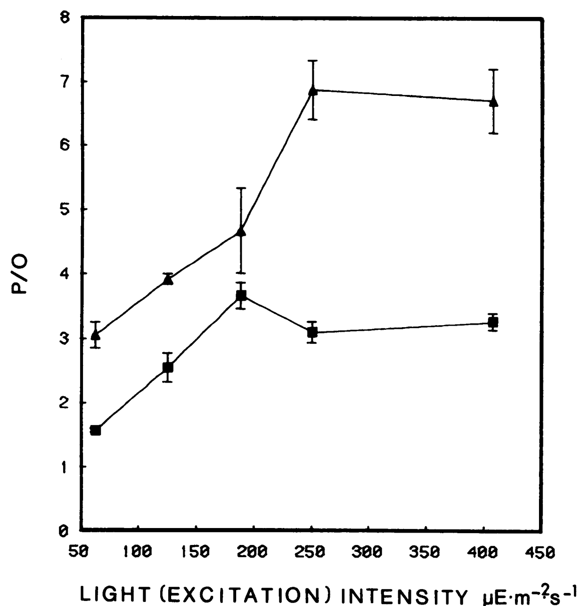

FIG. 4. Effect of excitation illumination on P/O ratios for guard (■) and mesophyll cells (▲). Each point is the average ± SE (bars), where n = 5; where bars are absent, SE is smaller than symbols.

3C). The duration of this phase decreases as excitation intensity increases (Fig. 3D). While the I-D-P induction kinetics from mesophyll cells can not be resolved at the higher excitation intensities (Fig. 3B), these features persist in guard cells (Fig. 3D). At even higher excitation intensities (1100 μE m^{-2} s^{-1}), the I-D-P phases shown for guard cells shown in Figure 3D appear identical to the simple biphasic rise kinetics found in mesophyll chloroplast fluorescence. In contrast to the rate of electron flow from water to Q[2] in mesophyll chloroplasts, guard cell chloroplasts show a reduced amount of time required to reach maximum fluorescence, particularly at the low intensities (Fig. 5). As

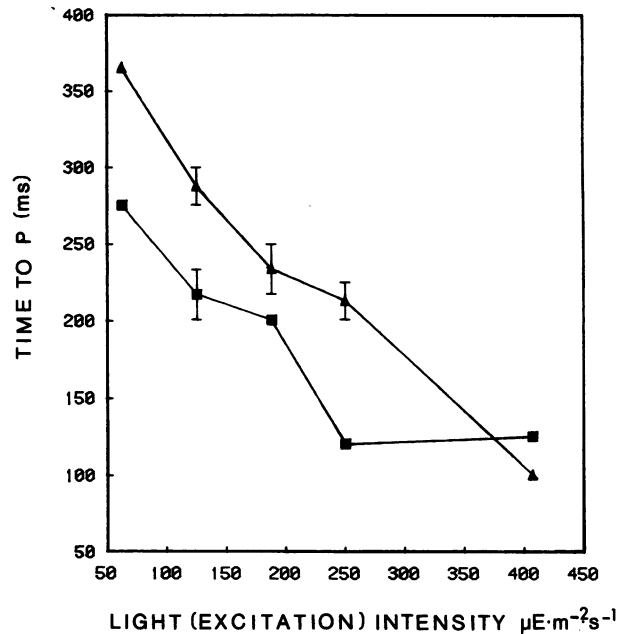

FIG. 5. The time required for variable fluorescence to reach a maximum as a function of excitation intensity for guard (■) and mesophyll (▲) cells. Each point is the average ± SE, where n = 5.

intensity increases however, both chloroplast types show similar times required to reach P due to a more rapid increase in fluorescence observed for mesophyll chloroplasts. Guard cell chloroplasts also show a lower P/O ratio at the low to moderate excitation intensities compared to the mesophyll chloroplast fluorescence due to the reduced relative height of P (Fig. 4). The reduced ratios appear to be a result of a lower D to P (or f_{max}) height compared to the mesophyll response. These observations are consistent with the reduced time taken to reach P mentioned above. This response by the guard cell chloroplasts may be due to either a more rapid reoxidation by oxidants following Q or dissipation of excitation by a nonradiative process.

Slow Kinetics of Variable Fluorescence (P to T). Ogawa *et al.* (15) have reported that fluorescence quenching by guard cell chloroplasts from P to steady state is much slower than that in mesophyll chloroplasts and lacks the secondary fluorescence peak or wave M which manifests itself in mesophyll cells. Examples of the slow quenching kinetics are shown in Figure 6 for both mesophyll and guard cell chloroplasts at the same two excitation intensities as Figure 3. At the lowest intensity (Fig. 6A), mesophyll chloroplasts show considerable fast quenching (P to T) which increased in rate with increasing intensity (Fig. 7). Guard cell chloroplasts show minimal quenching at the low light intensities (Fig. 6C) which increases in rate at higher excitation intensities (Fig. 6D) and approaches the quenching rates shown by mesophyll chloroplasts (Fig. 7). A secondary fluorescence rise (M) from the guard cell induction curve was never observed at any intensity.

Although guard cell quenching rates increase with higher excitation intensities, total quenching, defined as (P–T)/T (6), remained relatively constant at low to moderate intensities and did not approach that shown by mesophyll cells (Fig. 8). This lack of total quenching is reported to reflect a lower energetic state of the thylakoid membranes, thereby reducing photophosphorylation and limiting ATP production.

DISCUSSION

Guard cell chloroplasts of *S. cernua* possess both PSI and PSII based on the spectrophotometric criteria used for this study.

[2] Abbreviation: Q, primary electron acceptor of PSII.

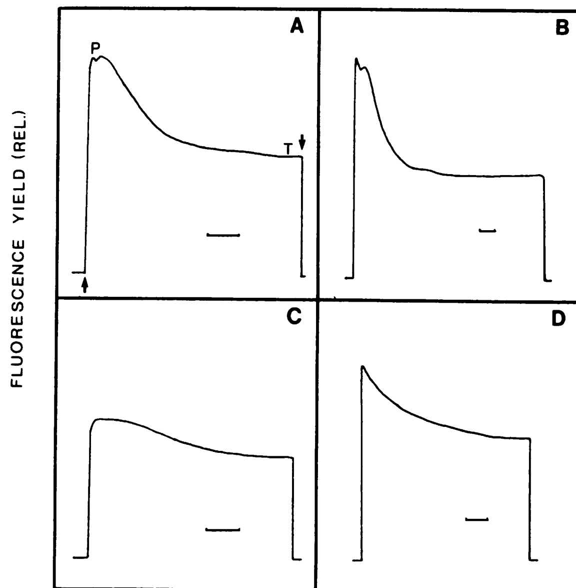

FIG. 6. Traces of the slow quenching changes of Chl fluorescence from dark-adapted mesophyll (A and B) and guard cells (C and D). The excitation intensity for A and C was 62.7 μE m^{-2} s^{-1}; for B and D, the intensity was 407 μE m^{-2} s^{-1}. The time bar equals 10 s.

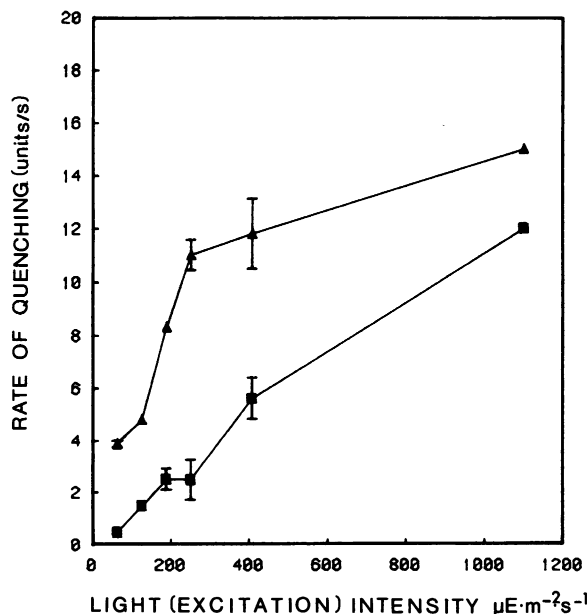

FIG. 7. Changes in the maximum rate of fluorescence quenching ($-dF/dt$, relative units s^{-1}) from peak P for guard (■) and mesophyll (▲) cells due to increasing excitation intensity. Each point is the average ± SE, where $n = 5$.

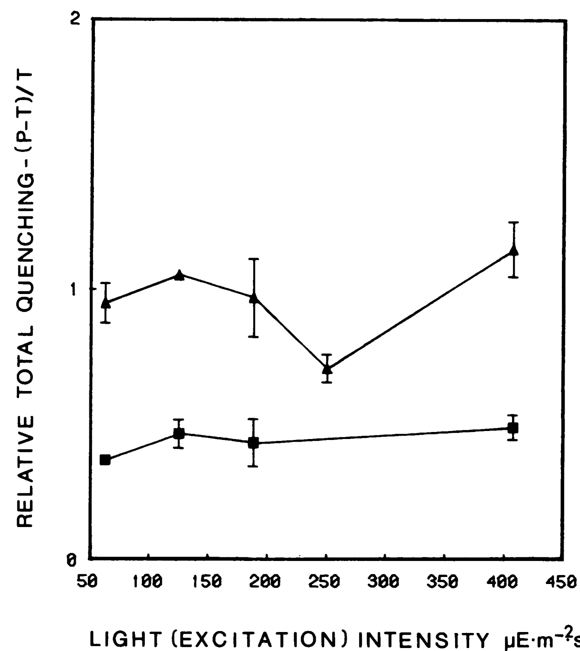

FIG. 8. Changes in total quenching [(P–T)/T] for guard (■) and mesophyll (▲) cells as a function of excitation intensity. Each point is the average ± SE, where $n = 5$.

Similar findings have been reported for guard cell chloroplasts of *Vicia faba* (15, 20) and *Chlorophytum comosum* (23). The possibility of contamination from mesophyll chloroplasts in related studies has resulted in some concern (16, 24). The high resolution afforded by the microepifluorescence system described in this report eliminates all possible contamination of the fluorescence induction signal by the mesophyll cell population. An important feature of our experimental apparatus is the flexibility of the aperture system for delimiting the area of tissue which is excited by the light source thereby permitting examination of the fluorescence kinetics for any species provided epidermal peels can be obtained. An additional criterion which is necessary in order to obtain acceptable resolution of the initial fluorescence transients is a relatively large guard cell chloroplast population to provide a fluorescence signal which requires minimal amplification. Otherwise, electrical noise inherent in the system can obscure the fluorescence induction kinetics. Clearly in this case guard cell chloroplast populations are sufficiently large to give reliable fluorescence induction curves.

A major conclusion in this work is that, although both chloroplast types show similar general features in the fluorescence rise and quenching kinetics, guard cell chloroplasts require higher excitation intensities compared to their mesophyll counterparts in order to show induction curves which reflect similar rates of electron flow and photophosphorylation activity. This difference could come about in two very different ways. Are there differences in fluorescence between the two chloroplast types or are they merely caused by differences in the tissue preparations which are examined? One potentially significant difference in the tissues has to do with the concentrations of chloroplasts which could lead to shading or back scattering. The guard cell preparations contain single layers of cells, whereas the mesophyll cell preparations contain multiple cell layers (as is the case *in vivo*) and hence more layers of chloroplasts. This layering may increase the attenuation of light received by lower level chloroplasts but could also increase the effectiveness of low light intensities and increase fluorescence from the mesophyll cell preparations as more of the excitation beam is captured to be re-emitted.

This argument can, we believe, be overcome by considering the aperture size and therefore the area of excitation which is very small and covers only one guard cell pair. The guard cells themselves contain a high concentration of chloroplasts and hence exhibit intracellular layering of chloroplasts. Most of the fluorescence emission can be expected to arise from the top most layers of chloroplasts of both tissues since epifluorescence was measured. Similarly, as shown in Figure 7, the magnitude of the differences in the fluorescence kinetics of the two cell types is too large to be accounted for by a simple layering effect. Finally, the difference in the chloroplast types persists even when relative measures of fluorescence such as P/O and (P-T)/T are made.

Analysis of the fast fluorescence rise permits an examination

of the general characteristics of altered electron excitation of PSII during the development of the light-adapted state in photosynthetic tissue and reflects Q reduction due to electron flow from the water splitting site via intersystem electron carriers (18). Differences in the kinetics of the variable fluorescence yield between mesophyll and guard cell chloroplasts have been reported for *C. comosom* (23). For *S. cernua*, the major difference observed in the I-D-P transients of the induction curve between the two chloroplast types is an extended I to D phase followed by a more rapid occurrence of P in the guard cell chloroplasts at the lower excitation intensities. Zeiger *et al.* (23) have proposed that the more rapid appearance of P in guard cells may be due to greater reducing conditions present in the chloroplasts. However, intrinsic differences in the population of PSII electron carriers may exist. The presence of two subpools of intersystem oxidants in mesophyll chloroplasts which differ according to their rate of reduction has been proposed earlier (8), based on fluorescence induction kinetics. The extended fluorescence deflection point we observe in the fast kinetics in guard cell chloroplasts suggests that there may exist a different proportion of oxidants associated with PSII electron carriers compared to that found in mesophyll chloroplasts. Second, the more rapid appearance of P (Fig. 5) suggests a decreased electron transport capacity in guard cell chloroplasts relative to mesophyll chloroplasts (10). Thus, at low light intensities the reduced electron transport capacity would not favor a significant energetic contribution to light-dependent stomatal activity. Under these low light conditions, light-dependent stomatal opening would have to rely primarily on energy derived from the blue light photoreceptor which is observed in whole leaf studies (9).

Our proposal that the energy requirements for stomatal opening under low light conditions cannot be supported by the guard cell chloroplast photosystem is further demonstrated by the lower fluorescence quenching rates observed at low excitation intensities in guard cell chloroplasts. The rate of quenching is related to several factors such as changes in the proton concentration of the intrathylakoid spaces coupled to Mg^{2+} efflux into the stromal region, development of the prephosphorylation state of the thylakoid membrane and a change in the distribution of the excitation energy between the two photosystems (3, 7, 13). The quenching rates for both mesophyll and guard cell chloroplasts can also be reduced by high cellular CO_2 presumably due to a dissipation of the high energy state of the photosynthetic membranes, the concentration of which may be lower in mesophyll tissue as a result of photosynthesis (11). Thus, the requirement for high light intensities suggests that, in guard cells, conditions prerequisite for rapid fluorescence quenching may not be as readily established compared to mesophyll cells. The reduction in grana stacking observed in guard cell chloroplast ultrastructure (1) may play an important role in regulating fluorescence quenching. Reduced stacking may result in a lower light-harvesting PSII pigment-protein complex/PSI complex ratio in guard cells relative to that found in mesophyll chloroplasts thereby limiting the efficient transfer of excitation energy to PSI (2). Confirmation of this must await further studies.

In summary, our results demonstrate that guard cell chloroplast photosystems in *S. cernua* require higher excitation intensities compared to their mesophyll counterparts in order to show several features of the fluorescence induction curve which indicate rapid electron movement through PSII and PSI. This minimal response of the induction curve at low light intensities can account for the lack of a red light response in stomatal activity observed in whole leaf studies (12, 25). The presence of the more light sensitive blue photoreceptor can compensate for the lack of involvement of the guard cell chloroplast photosystems at low intensities (22).

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LITERATURE CITED

1. ALLAWAY WG, G SETTERFIELD 1972 Ultrastructural observations on guard cells of *Vicia faba* and *Allium porrum*. Can J Bot 50: 1405-1413
2. BARBER J 1983 Membrane conformational changes due to phosphorylation and the control of energy transfer in photosynthesis. Photobiochem Photobiophys 5: 181-190
3. BRIANTUS JM, C VERNOTTE, M PICAUD, GH KRAUSE 1979 A quantitative study of the slow decline of chlorophyll a fluorescence in isolated chloroplasts. Biochim Biophys Acta 548: 128-138
4. HSIAO TC, WG ALLAWAY, LT EVANS 1973 Action spectra for guard cell Rb^+ uptake and stomatal opening in *Vicia faba*. Plant Physiol 51: 82-88
5. JARVIS PG, JIL MORISON 1981 The control of transpiration and photosynthesis by the stomata. *In* PG Jarvis, TA Mansfield, eds, Stomatal Physiology. Cambridge University Press, Cambridge, pp 247-279
6. KLOSSON RJ, GH KRAUSE 1981 Freezing injury in cold-acclimated and unhardened spinach leaves: effects of freezing on chlorophyll fluorescence and light scattering reactions. Planta 151: 347-352
7. KRAUSE GH 1974 Changes in chlorophyll fluorescence in relation to light dependent cation transfer across thylakoid membranes. Biochim Biophys Acta 333: 301-313
8. MALKIN S, B KOK 1966 Fluorescence induction studies in isolated chloroplasts. 1. Number of components involved in the reaction and quantum yields. Biochim Biophys Acta 126: 413-432
9. MANSFIELD TA, AJ TRAVIS, RG JARVIS 1981 Responses to light and carbon dioxide. *In* PG Jarvis, TA Mansfield, eds, Stomatal Physiology. Cambridge University Press, Cambridge pp 119-135
10. MELIS A, GW HARVEY 1981 Regulation of photosystem stoichiometry, chlorophyll *a* and chlorophyll *b* content and relation to chloroplast ultrastructure. Biochim Biophys Acta 637: 138-145
11. MELIS A, E ZEIGER 1982 Chlorophyll a fluorescence transients in mesophyll and guard cells: modulation of guard cell photophosphorylation by CO_2. Plant Physiol 69: 642-647
12. MORISON JIL, PG JARVIS 1983 Direct and indirect effects of light on stomata in *Commelina communis* L. Plant Cell Environ 6: 103-109
13. MURATA N, K SUGAHARA 1969 Control of excitation transfer in photosynthesis. III. Light-induced decrease of chlorophyll a fluorescence related to photophosphorylation system in spinach chloroplasts. Biochim Biophys Acta 189: 182-192
14. OGAWA T, H ISHIKAWA, K SHIMADA, K SIBATA 1978 Synergistic action of red and blue light and action spectra for malate formaton in guard cells of *Vicia faba*. Planta 142: 61-65
15. OGAWA T, D GANTZ, J BOYER, GOVINDJEE 1982 Effects of cations and abscisic acid on chlorophyll *a* fluorescence in guard cells of *Vicia faba*. Plant Physiol 69: 1140-1144
16. OUTLAW WH JR, BC MAYNE, VE ZENGER, J MANCHESTER 1981 Presence of both photosystems in guard cells of *Vicia faba* L: Implications for environmental signal processing. Plant Physiol 67: 12-16
17. OUTLAW WH JR 1982 Carbon metabolism in guard cells. Recent Adv Phytochem 16: 185-222.
18. PAPAGEORGIOU G 1975 Fluorescence: an intrinsic probe of photosynthesis. *In* Govindjee, ed, Bioenergetics of Photosynthesis. Academic Press, New York, pp 320-371
19. THORNE SW, NK BOARDMAN 1971 The effect of temperature on the fluorescence kinetics of spinach chloroplasts. Biochim Biophys Acta 234: 113-125
20. VAUGHN KC, WH OUTLAW JR 1983 Cytochemical and cytofluorometric evidence for guard cell photosystems. Plant Physiol 71: 420-424
21. WILLMER CM 1981 Guard cell metabolism. *In* PG Jarvis, TA Mansfield, eds, Stomatal Physiology. Cambridge University Press, Cambridge pp 87-102
22. ZEIGER E 1983 The biology of stomatal guard cells. Annu Rev Plant Physiol 34: 441-475
23. ZEIGER E, P ARMOND, A MELIS 1980 Fluorescence properties of guard cell chloroplasts: evidence for linear electron tranport and light-harvesting pigments of photosystem 1 and 11. Plant Physiol 67: 17-20
24. ZEIGER E 1981 Novel approaches to the biology of stomatal guard cells: protoplast and fluorescence studies. *In* PG Jarvis, TA Mansfield, eds, Stomatal Physiology. Cambridge University Press, Cambridge pp 103-117
25. ZEIGER E, C FIELD 1982 Photocontrol of the functional coupling between photosynthesis and stomatal conductance in the intact leaf: blue light and PAR-dependent photosystems in guard cells. Plant Physiol 70: 370-375