Structural Flexibility of Chiral Macroaggregates of Light-Harvesting Chlorophyll *a/b* Pigment−Protein Complexes. Light-Induced Reversible Structural Changes Associated with Energy Dissipation[†]

Virginijus Barzda, Anita Istokovics, Ilian Simidjiev, and Gyözö Garab*

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, P.O. Box 521, H-6701 Hungary

Received January 17, 1996; Revised Manuscript Received April 16, 1996[⊗]

ABSTRACT: In this paper, we show that stacked lamellar aggregates of the purified chlorophyll *a/b* light-harvesting antenna complexes (LHCII) and granal thylakoid membranes are capable of undergoing light-induced reversible changes in the chiral macroorganization of the chromophores as well as in the photophysical pathways. In granal thylakoids, the light-induced reversible structural changes, detected by circular dichroism (CD) measurements, are accompanied by reversible changes in the fluorescence yield that indicate an increased dissipation of the excitation energy. These changes become gradually more significant in excess light compared to nonsaturating light intensities, and can be eliminated by suspending the membranes in hypotonic, low-salt medium in which the chiral macroaggregates are absent. In lamellar aggregates of LHCII, the light-induced reversible changes of the main, nonexcitonic CD bands are also accompanied by reversible changes in the fluorescence yield. In small aggregates and trimers, no light-induced ΔCD occurs, and the fluorescence changes are largely irreversible. It is proposed that the structural changes are induced by thermal effects due to the excess light energy absorbed by the pigments. Our data strongly suggest that the structure and function of the antenna system of chloroplasts can be regulated by the absorption of excess light energy with a mechanism independent of the operation of the photochemical apparatus.

In photosynthesis, the structure of the antenna and the reaction centers appears to be optimized for an operation with a high quantum efficiency. On the other hand, it has been well established that plants are capable of regulating the dissipation of the excess light energy absorbed by photosystem II (PSII)[1] and protect themselves against damage by excess radiation [for a review, see Horton et al. (1994)].

Regulation of energy utilization and dissipation in PSII has been studied mainly by Chl fluorescence induction kinetic measurements, which revealed several forms of nonphotochemical quenching mechanisms. When exposed to excess light, energy-dependent quenching (qE) which is associated with high transmembrane ΔpH (Briantais et al., 1979) has been shown to play a protective role against photoinhibitory damages.

The fact that energy dissipation is not arrested upon the interruption of the illumination with excess light and the high quantum efficiency is restored only gradually indicates that intense light induces slowly reversible structural changes which alter the photophysical and/or photochemical reaction pathways. The nature of these reversible changes and the underlying physical mechanism(s) have not been clarified. Some authors have suggested the involvement of the reaction center (Weis & Berry, 1987), whereas others proposed that the regulation takes place in the antenna via the xanthophyll cycle (Gilmore & Yamamoto, 1991) or through changes in the macroaggregation of the chlorophyll *a/b* light-harvesting complex of PSII (LHCII) (Horton et al., 1991, 1994).

In chloroplasts, the chlorophyll (Chl) molecules are bound to different pigment−protein complexes, in which the distances between the pigment molecules and their mutual orientation are well-defined (Breton & Vermeglio, 1982). The antenna and reaction center complexes are clustered into particle-aggregates of 150−180 Å in diameter. For PSII, these probably contain a single reaction center surrounded by a variable quantity of LHCIIs (Staehelin, 1976). PSII units have been shown to be interconnected by energy transfer interactions (Joliot et al., 1973; Lavergne & Trissl, 1995).

By means of CD spectroscopic techniques, it has been shown that in granal thylakoid membranes PSII particles are assembled into psi-type macroaggregates, densely packed chirally organized macroarrays with an estimated diameter of 200−400 nm (Garab et al., 1988c; Finzi et al., 1989; Barzda et al., 1994), the stability and size of which are controlled by the ionic strength and the osmotic pressure of the medium and the peripheral LHCII content of the membranes (Garab et al., 1991; Busheva et al., 1991; Liker & Garab, 1995) (psi, polymer or salt-induced). Purified LHCII also readily forms lamellar aggregates (Kühlbrandt, 1994) which exhibit psi-type CD features (Barzda et al., 1994).

[†] This work was supported by grants from the Hungarian Research Fund (OTKA T019226 and T017827).

* Correspondence should be addressed to this author. Phone: +(36-62)-433131. Fax: +(36-62)-433434. E-mail: gyozo@everx.szbk.u-szeged.hu.

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1996.

[1] Abbreviations: Chl, chlorophyll; CD, circular dichroism; ΔCD, light-induced reversible changes in circular dichroism; LHCII, chlorophyll *a/b* light-harvesting complex of photosystem II; PSII, photosystem II; psi, polymer or salt-induced; qE, energy-dependent nonphotochemical quenching of chlorophyll fluorescence.

S0006-2960(96)00114-6 CCC: $12.00

By measuring light-induced CD changes (ΔCD) in thylakoid membranes, earlier we have shown that the chiral macroorganization of particles in the membranes can undergo reversible rearrangements, which are sensitive to nigericin and NH_4Cl but largely insensitive to gramicidin (Garab et al., 1988b). However, the mechanism of these structural changes and their significance in the regulation of the photophysical pathways have not been investigated.

In this work, by studying ΔCD and Chl fluorescence induction kinetics, we show that thylakoid membranes and surprisingly also lamellar aggregates of purified LHCII are capable of undergoing light-induced reversible structural changes, which bring about alterations in the photophysical pathways and thus are likely to be involved in the regulation of the energy dissipative pathways in the antenna.

EXPERIMENTAL PROCEDURES

Pea chloroplasts and thylakoid membranes were isolated as described earlier (Garab et al., 1988a). LHCII was isolated from 2-week-old pea leaves according to the method of Krupa et al. (1987) with minor modifications. The solubilization step with Triton X-100 was critical in obtaining LHCII macroaggregates exhibiting ΔCD: it was performed with different detergent concentrations between 0.6 and 0.8% (v/v). The Chl *a/b* ratio varied between 1.0 and 1.15. In these preparations, SDS−PAGE revealed the presence of the 25 and 27 kDa apoproteins. Minor complexes were present at very low concentrations and could only be detected by silver staining. As judged from Western blots for the D1 protein, the samples contained no contamination from PSII reaction centers.

CD spectra were recorded in a Jobin Yvon CD6 dichrograph; the Chl concentration was adjusted to 10 μg/mL with thylakoid membranes and to 20 μg/mL with LHCII. The dichrograph was equipped with a side-illumination attachment; the photomultiplier was protected with crossing filters (Corning 2-64 and 4-96) against stray light from the beam of a 650 W tungsten lamp, which also passed through a heat filter of 10 cm water (Garab et al., 1988b). Before the measurements, the samples were kept in the dark for 20 min. ΔCD in different spectral regions occurred with essentially the same kinetics and were sensitive to inhibitors to the same extent (Garab et al., 1988b).

Absorbance changes of neutral red (20 μM) for determining the transmembrane ΔpH (Junge et al., 1979) were measured at 553 nm in a Shimadzu UV 3000 spectrophotometer equipped with a side-illumination attachment. Fluorescence induction kinetic measurements were performed in a Walz PAM 103 Chl-fluorometer. The Chl concentration was 5 μg/mL. Chl content was determined as described by Arnon (1949).

RESULTS AND DISCUSSION

Light-Induced Reorganizations and Energy Dissipation in Thylakoid Membranes. As shown in Figure 1A, in isotonic buffer supplemented with cations the CD spectra of pea thylakoid membranes, similarly to barley and spinach thylakoids (Garab et al., 1988a, 1991), exhibit characteristic bands with psi-type features: intense, anomalously shaped bands accompanied with long tails outside the absorbance bands (Keller & Bustamante, 1986). This type of CD signal has been shown to originate from chiral macrodomains of

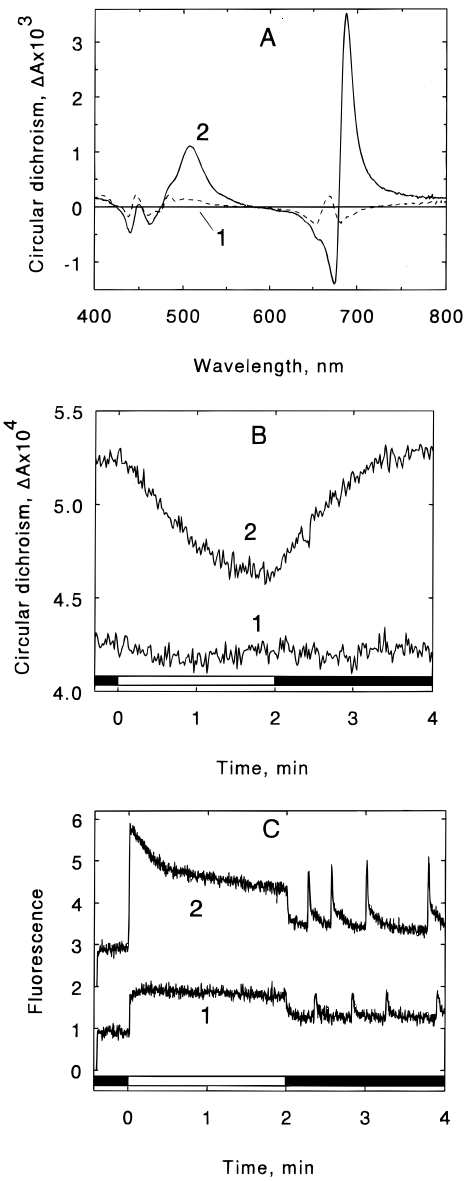

FIGURE 1: CD spectra (A), light-induced changes in the CD at 510 nm (B), and Chl fluorescence induction curves (C) in pea thylakoid membranes suspended in 30 mM tricine (pH 7.8) (curve 1) and in the same buffer supplemented with 330 mM sorbitol and 5 mM $MgCl_2$ (curve 2). The same batches of membranes were used in panels A through C. Black and white horizontal bars in panels B and C indicate dark and light (500 W/m^2) periods, respectively. In order to measure the recovery of the fluorescence yield, in panel C, the dark period was interrupted by short (1−2 s) flashes of the actinic light, but otherwise the protocol was the same as in panel B.

PSII particles (Garab et al., 1988a; Barzda et al., 1994). In contrast, in low-salt, hypotonic medium, the psi-type CD bands are absent, and the remaining bands can be attributed to excitonic interactions inside the particles (Garab et al., 1991).

Figure 1B shows that thylakoid membranes containing the chiral macrodomains exhibit ΔCD (curve 2) similar to those reported earlier for spinach thylakoids (Garab et al., 1988b). In contrast, the membranes suspended in hypotonic, low-salt medium display no ΔCD (curve 1).

Fluorescence measurements also revealed significant differences between the two samples (Figure 1C). With macrodomains present, ΔCD was accompanied with significant changes in the fluorescence yield, which were gradually

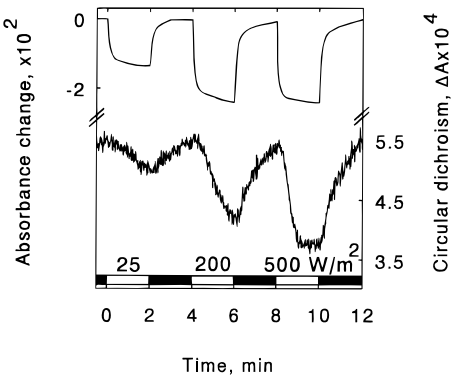

FIGURE 2: Light-induced absorbance changes of neutral red at 553 nm (upper curve) and light-induced changes in the CD at 510 nm (lower curve) in thylakoid membranes at three different intensities of the exciting light. The light and dark cycles are indicated by white and black bars, respectively. (The samples were changed after each illumination period, and the curves were "glued" together in the computer.)

albeit not fully restored during the dark interval. These data are consistent with a gradual generation of quenching centers by excess radiation [see, e.g., Horton et al. (1994)]. In contrast, in samples which displayed neither psi-type CD bands nor ΔCD, the reversible fluorescence transients were absent (curves 1). This cannot be explained by differences in the photochemical activity, which was essentially identical in the two samples (data not shown). Thus, it appears that both the light-induced reversible structural changes and the associated alterations in the photophysical pathways require the presence of chiral macrodomains.

Earlier we have shown that ΔCD originates from an overall structural rearrangement rather than from changes affecting a specific set of transition dipoles (Garab et al., 1988b). The observation that no ΔCD occurs in the absence of psi-type bands lends support to this conclusion. Thus, it appears that short-range, excitonic interactions inside PSII particles cannot be held responsible for the structural rearrangements in the macroaggregates.

According to the theory of psi-type aggregates, CD carries information on the handedness, size, and macrohelical parameters of the macroaggregates (Keller & Bustamante, 1986; Kim et al., 1986). The overall decrease in the CD amplitudes [Figure 1B; see also Garab et al. (1988b)] would be consistent with a disintegration of the macrodomains into smaller units, or with a diminishment of the long-range chiral order in the macroaggregate (Kim et al., 1986; Barzda et al., 1994). However, it is equally possible that light induces changes in the macrohelical parameters. Such changes can be envisioned, e.g., via proximity changes between adjacent lamellae. Previous thin section electron microscopic data showed shrinking and swelling of granum membranes in the light and dark, respectively (Murakami & Packer, 1970). The preliminary observation that divalent cations play an important role in ΔCD supports the hypothesis that ΔCD reflects changes in the proximity of the stacked membranes (Istokovics and Garab, in preparation). The observed slow relaxation of ΔCD is in reasonable accordance with such a mechanism.

Figure 2 shows that ΔpH builds up and decays much faster than ΔCD. Thus, we confirm our earlier conclusion (Garab et al., 1988b) that ΔCD, although sensitive to nigericin, is not directly correlated with ΔpH. A similar lag phase of

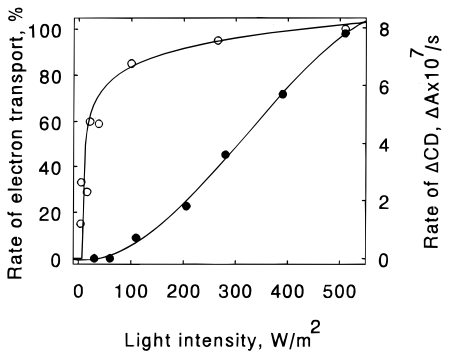

FIGURE 3: Dependence of the rate of linear electron transport (H_2O→ferricyanide) (open circles) and the initial rate of the light induced changes of CD at 510 nm (full circles) on the intensity of the exciting light in thylakoid membranes. (In this experiment, ΔCD was measured in the presence of 1 mM ferricyanide.)

fluorescence quenching compared to ΔpH was observed for qE [see, e.g., Johnson et al. (1994)]. In unreported experiments, we observed that, although nigericin inhibited ΔCD for the first few excitations (Garab et al., 1988b), upon continued light/dark cycles the membranes regained their ability to undergo structural changes even in the presence of 5 μM nigericin, albeit with diminished amplitude. Thus, ΔpH cannot be held directly responsible for the structural changes. In line with this conclusion, we show that the amplitude and the initial rate of ΔCD increase with the increase of the light intensity also in the range when ΔpH is already saturated (Figure 2).

Figure 3 shows the initial rate of the linear electron transport and that of ΔCD as a function of the exciting light intensity. The measurements were performed in the presence of ferricyanide, on the same thylakoid membranes, under the same experimental conditions. (The use of the same preparation is important as both the rate and the amplitude of ΔCD vary broadly from batch to batch.) It can be seen that at intensities where the electron transport is nearly saturated, around 100 W/m^2, ΔCD can be hardly detected. On the other hand, at higher light intensities, the initial rate of ΔCD rose approximately linearly with the increase of the intensity of the exciting light. ΔCD could not be saturated in the range studied.

It is to be noted that the operation of qE is also observed at high light intensities, and in fact this protective mechanism is thought to be activated in excess light [cf. Walters and Horton (1991)], i.e., when the absorbed energy can no more be utilized for photochemistry. Investigations are underway to clarify the exact correlation between ΔCD and the mechanisms of fluorescence quenching.

Light-Induced Reversible Changes in LHCII Macroaggregates. Figure 4A shows that ΔCD occurs not only in thylakoids but surprisingly also in macroaggregates of purified LHCII. Ultrastructural investigations revealed that in all cases when ΔCD was observed, the complexes were found to form stacked lamellae [data not shown; see McDonnel and Staehelin (1980); Kühlbrandt (1994)]. Conversely, ΔCD could easily be eliminated by disintegrating the macroaggregates into smaller particles of 100−200 nm in diameter. [The state of aggregation of the sample was tested by negative staining electron microscopy and fluorescence spectroscopy (Barzda et al., 1994).] The fact that in small aggregates light induces no ΔCD suggests that the changes in the chiral macroorganization in the large ag-

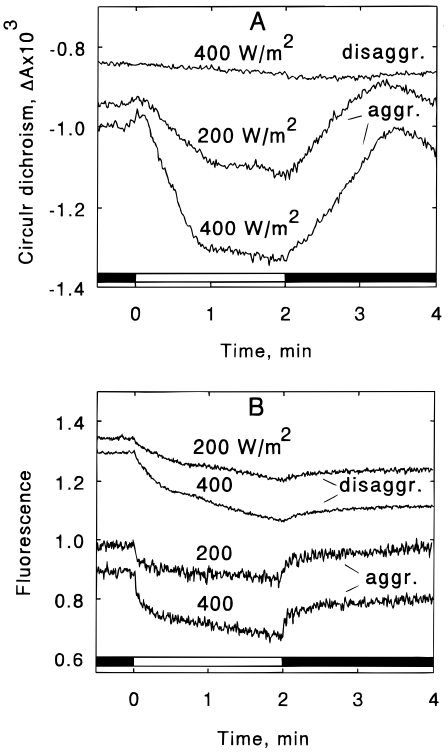

FIGURE 4: Light-induced changes in the CD at 495 nm (A) and in the fluorescence yield (B) of purified LHCII preparations in macroaggregated and small aggregate forms at two different light intensities. The light and dark cycles are indicated as white and black bars, respectively.

gregates do not stem from structural rearrangements in the trimers. These observations resemble those in thylakoid membranes (see above).

In analogy with thylakoid membranes, we put forward the hypothesis that ΔCD originates from changes in the proximity between the stacked lamellae of LHCII. This hypothesis can be substantiated or falsified by X-ray and/or neutron scattering experiments, which are in preparation.

Light-induced reversible changes in the fluorescence yield of LHCII were described by Jennings et al. (1991), who suggested that such changes may be involved in the regulation of the dissipation of excess excitation energy in thylakoid membranes. In agreement with these authors, we found that the extent of fluorescence quenching depended on the intensity of the actinic light. We found, however, that whereas in small aggregates the changes in the fluorescence yield are large and essentially irreversible, large aggregates exhibit smaller but almost fully reversible changes (Figure 4B). Although the correlation between the fluorescence changes and ΔCD is likely to be complex and the data require further analysis, it can be concluded that the light-induced reversible changes in the chiral macroorganization are associated with characteristic reversible changes in the photophysical pathways.

The physical mechanism of the light-induced changes in the chiral macroorganization of the lamellar LHCII(-containing) aggregates is not known, and it is open for different considerations. It is also not clear whether or not similar changes occur in other biological systems, e.g., in psi-type aggregates or in other stacked membrane systems.

According to the theory of psi-type aggregates, in densely packed macroaggregates with long-range chiral order the excitation energy can delocalize over large domains (Keller & Bustamante, 1986). Comparative studies with nonlinear spectroscopic methods revealed significant differences in the energy migration patterns between small and large aggregates of LHCII (Lokstein et al., 1995; Barzda et al., 1996). However, these differences in the excitation energy of migration are highly unlikely to be responsible for the light-induced reversible changes in the macrostructure of LHCII macroaggregates and granal thylakoid membranes. The lifetime of the excited state is too short, the intensity of the actinic light is too weak, and the decay of changes is far too slow to explain the observed changes in terms of the electronic excited state of the molecules. Thus it seems more plausible that the ΔCD stems from thermally-induced structural changes.

As CD is sensitive to the orientation of the particles (Charney, 1979; Garab et al., 1988a), ΔCD could arise from a reorientation of the lamellae, e.g., via convection currents in the cell. This mechanism, however, can be ruled out. Thylakoid membranes show strong ΔCD both in the absence and in the presence of external magnetic field of 1 T, which is sufficient to "firmly" align the membranes (Garab et al., 1988b). Furthermore, ΔCD can be inhibited by various agents, such as ionophores (Garab et al., 1988b) and quinone antagonists (Istokovics et al., 1992). (These latter agents, as will be shown elsewhere, also inhibit ΔCD of LHCII.)

Excess light may induce changes in the vicinity of the excited molecule, e.g., via local heat produced via internal conversion. This can change the mobility of charged groups, which in turn perturbs the ion distributions around the particles. In lamellar macroaggregates, it is reasonable to assume that long-range order brings about an efficient heat-conductance inside the lamellae. Heat-conductance is expected to "smear" the heat over large domains, which may bring about changes in the electrostatic stacking of the lamellae. This proposed mechanism is reminiscent to the thermo-optic effects in certain liquid crystals in which texture changes can be induced at relatively low light intensities (Janossy, 1991). However, elucidation of the physical mechanism(s) underlying light-induced structural changes in lamellar aggregates of LHCII and granal thylakoid membranes requires further studies.

Based on the present data, we conclude that LHCII-containing lamellar aggregates possess the ability to undergo light-induced reversible structural rearrangements which influence the photophysical pathways in the antenna system.

ACKNOWLEDGMENT

We are indebted to Profs. P. Horton and R. Jennings for valuable comments and suggestions during the preparation of the manuscript, and to Profs. A. Scherz and L. Bata and Drs. A. Vianelli and L. Zimányi for stimulating discussions. We thank Dr. S. Demeter for the use of the PAM fluorometer. We are grateful to Dr. L. Mustárdy for the electron microscopic investigations.

REFERENCES

Arnon, D. I. (1949) *Plant Physiol. 24*, 1−15.
Barzda, V., Mustárdy, L., & Garab, G. (1994) *Biochemistry 33*, 10837−10841.
Barzda, V., Garab, G., Gulbinas, V., & Valkunas, L. (1996) *Biochim. Biophys. Acta 1273*, 231−236.
Breton, J., & Vermeglio, A. (1982) in *Photosynthesis* (Govindjee, Ed.) pp 153−193, Academic Press, New York.

Briantais, J., Vernotte, C., Picaud, M., & Krause, G. H. (1979) *Biochim. Biophys. Acta 548*, 128−138.
Busheva, M., Garab, G., Liker, E., Tóth, Z., Széll, M., & Nagy, F. (1991) *Plant Physiol. 95*, 997−1003.
Charney, E. (1979) in *The Molecular Basis of Optical Activity*, John Wiley & Sons, New York.
Finzi, L., Bustamante, C., Garab, G., & Juang, C.-B. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8748−8752.
Garab, G., Faludi-Dániel, A., Sutherland, J. C., & Hind, G. (1988a) *Biochemistry 27*, 2425−2430.
Garab, G., Leegood, R. C., Walker, D. A., Sutherland, J. C., & Hind, G. (1988b) *Biochemistry 27*, 2430−2434.
Garab, G., Wells, K. S., Finzi, L., & Bustamante, C. (1988c) *Biochemistry 27*, 5839−5843.
Garab, G., Kieleczawa, J., Sutherland, J. C., Bustamante, C., & Hind, G. (1991) *Photochem. Photobiol. 54*, 273−281.
Gilmore, A. M., & Yamamoto, H. Y. (1991) *Plant Physiol. 96*, 635−643.
Horton, P., Ruban, A. V., Rees, D., Pascal, A. A., Noctor, G., & Young, A. J. (1991) *FEBS Lett. 292 (1,2)*, 1−4.
Horton, P., Ruban, A. V., & Walters, R. G. (1994) *Plant Physiol. 106*, 415−420.
Istokovics, A., Lajkó, F., Liker, E., Barzda, V., Simidjiev, I., & Garab, G. (1992) in *Research in Photosynthesis II* (Murata, N., Ed.) pp 631−634, Kluwer Academic Publishers, Dordrecht, Boston, and London.
Janossy, I. (1991) *Optical Effects in Liquid Crystals*, Kluwer Academic Publishers, Dordrecht.
Jennings, R. C., Garlaschi, F. M., & Zucchelli, G. (1991) *Photosynth. Res. 27*, 57−64.
Johnson, G. N., Young, A. J., & Horton, P. (1994) *Planta 194*, 550−556.
Joliot, P., Bennoun, P., & Joliot, A. (1973) *Biochim. Biophys. Acta 305*, 317−328.
Junge, W., Auslander, W., McGeer, A. J., & Runge, T. (1979) *Biochim. Biophys. Acta 546*, 121−141.
Keller, D., & Bustamante, C. (1986) *J. Chem. Phys. 84*, 2972−2979.
Kim, M., Ulibarri, L., Keller, D., Maestre, M. F., & Bustamante, C. (1986) *J. Chem. Phys. 84*, 2981−2989.
Krupa, Z., Huner, N. P. A., Williams, J. P., Maissan, E., & James, D. R. (1987) *Plant Physiol. 84*, 19−24.
Kühlbrandt, W. (1994) *Curr. Opin. Struct. Biol. 4*, 519−528.
Lavergne, J., & Trissl, H.-W. (1995) *Biophys. J. 68*, 2474−2492.
Liker, E., & Garab, G. (1995) *Physiol. Plant. 93*, 187−190.
Lokstein, H., Leupold, D., Voigt, B., Nowak, F., Ehlert, J., Hoffmann, P., & Garab, G. (1995) *Biophys. J. 69*, 1536−1543.
McDonnel, A., & Staehelin, L. A. (1980) *J. Cell Biol. 84*, 40−56.
Murakami, S., & Packer, L. (1970) *J. Cell Biol. 47*, 332−351.
Staehelin, L. A. (1976) *J. Cell Biol. 71*, 136−158.
Walters, R. G., & Horton, P. (1991) *Photosynth. Res. 27*, 121−133.
Weis, E., & Berry, J. A. (1987) *Biochim. Biophys. Acta 894*, 198−208.

BI960114G