*Articles*

# Light-Induced Trimer to Monomer Transition in the Main Light-Harvesting Antenna Complex of Plants: Thermo-Optic Mechanism†

Győző Garab,*,‡ Zoltán Cseh,§,|| László Kovács,‡,|| Subramanyam Rajagopal,‡,|| Zsuzsanna Várkonyi,‡,|| Mark Wentworth,||,▽ László Mustárdy,‡ András Dér,⊥ Alexander V. Ruban,▽ Elemér Papp,§ Andreas Holzenburg,∞ and Peter Horton▽

*Institutes of Plant Biology and Biophysics, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary, Robert Hill Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN, United Kingdom, Department of Biological Physics, Faculty of Science, Eötvös Loránd University, Budapest, Hungary, and Microscopy and Imaging Center, Department of Biology and Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843-2257*

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ABSTRACT: The main chlorophyll *a/b* light-harvesting complex of photosystem II, LHCIIb, has earlier been shown to be capable of undergoing light-induced reversible structural changes and chlorophyll *a* fluorescence quenching in a way resembling those observed in granal thylakoids when exposed to excess light [Barzda, V., et al. (1996) *Biochemistry 35,* 8981−8985]. The nature and mechanism of this unexpected structural flexibility has not been elucidated. In this work, by using density gradient centrifugation and nondenaturing green gel electrophoresis, as well as absorbance and circular dichroic spectroscopy, we show that light induces a significant degree of monomerization, which is in contrast with the preferentially trimeric organization of the isolated complexes in the dark. Monomerization is accompanied by a reversible release of Mg ions, most likely from the outer loop of the complexes. These data, as well as the built-in thermal and light instability of the trimeric organization, are explained in terms of a simple theoretical model of thermo-optic mechanism, effect of fast thermal transients (local *T*-jumps) due to dissipated photon energies in the vicinity of the cation binding sites, which lead to thermally assisted elementary structural transitions. Disruption of trimers to monomers by excess light is not confined to isolated trimers and lamellar aggregates of LHCII but occurs in photosystem II-enriched grana membranes, intact thylakoid membranes, and whole plants. As indicated by differences in the quenching capability of trimers and monomers, the appearance of monomers could facilitate the nonphotochemical quenching of the singlet excited state of chlorophyll *a*. The light-induced formation of monomers may also be important in regulated proteolytic degradation of the complexes. Structural changes driven by thermo-optic mechanisms may therefore provide plants with a novel mechanism for regulation of light harvesting in excess light.

The photosystem II light-harvesting system of higher plants contains the most abundant chlorophyll-binding proteins in nature. The structural model of the main complex, LHCII,[1] shows the features that allow its efficient function in light harvesting (*1*). These proteins are also the sites of major physiological regulatory mechanisms that give plants the ability to respond to changing environmental conditions (*2*−*5*). The plasticity of the function appears to depend on their inherent structural flexibility, the details of which, however, are largely unknown. LHCII is found to be a trimer both in vitro and in vivo (*6, 7*). The complexes in isolated lamellar aggregates, in lipid-reconstituted lamellae, and in the native, granal membranes are assembled in large, ordered arrays (*8*−*10*). Spectroscopic methods have revealed that this order is perturbed upon illumination (*11*−*13*). Thylakoid membranes, upon illumination with moderate and high light intensities (60−640 W $m^{-2}$, 2 min), have been shown to undergo reversible structural changes, which affected the

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* Corresponding author: e-mail Gyozo@nucleus.szbk.u-szeged.hu; phone 36-62-433131; fax 36-62-433434.

‡ Institute of Plant Biology, Hungarian Academy of Sciences.

§ Eötvös Loránd University.

|| These authors contributed equally to the work.

⊥ University of Sheffield.

▽ Institute of Biophysics, Hungarian Academy of Sciences.

∞ Texas A&M University.

[1] Abbreviations: Chl, chlorophyll; CD, circular dichroism; LHCII, light-harvesting chlorophyll *a/b* pigment−protein complex of photosystem II; NPQ, nonphotochemical quenching of chlorophyll fluorescence; PSII, photosystem II.

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long-range chiral order of the chromophores (*11*). Originally these changes were thought to arise from transmembrane and local proton and other ion gradients generated by the photosynthetic charge transport system. In other terms, these reorganizations were thought to resemble or to be directly associated with other, well-established, photochemically driven feedback mechanisms via ΔpH or through redox sensors. Such mechanisms are known to regulate the utilization and dissipation of light energy absorbed in the antenna (*2*−*5*). However, it turned out that the structural changes were largely independent of the photochemical activity of thylakoids, and the rate of the changes increased approximately linearly with the light intensity above the saturation of photosynthesis (*12*). Further, lamellar aggregates of LHCII have also been shown to be capable of undergoing very similar structural reorganizations in the long-range chiral order of the chromophores. These were also fully reversible and exhibited a linear light-intensity dependence in the whole range tested (100−500 W $m^{-2}$). It has been hypothesized that the structural reorganizations in LHCII originate from a thermo-optic effect—fast, local thermal transitions due to the dissipation of the excess excitation energy in the antenna system (*12*). Recent experimental data obtained in thylakoids on thermal and light stabilities of macroarrays of different structural complexity are fully consistent with this mechanism (*14*).

The exact nature of the structural changes and the series of molecular events underlying the reorganizations in lamellar aggregates of LHCII and thylakoid membranes have not been identified. Since it is known that the organization of LHCII in crystalline arrays of the isolated complexes (*7*) and in ordered arrays of PSII particles (*9*) depends on the trimeric state, it was possible that the structural changes could involve or even be triggered by a monomerization of LHCII trimers. We sought to test this hypothesis by measuring the content of monomers and trimers in dark-adapted and light-treated samples. We found that light induces a trimer-to-monomer transition of LHCII both in vitro and in vivo and that this can be accounted for by a thermo-optic effect in the light-harvesting antenna complexes.

## MATERIALS AND METHODS

*Preparation of Plant Materials, Sucrose Gradient Centrifugation, and Nondenaturing Green Gel Electrophoresis.* Thylakoid membranes and PSII-enriched grana membranes (BBY particles) were prepared from spinach (*15*). Type II, loosely stacked lamellar aggregates of LHCII and delipidated, microcrystalline (type IV), aggregated, disordered (type III), and solubilized trimeric LHCII preparations were prepared from pea as described earlier (*16*). Trimeric LHCIIb was prepared as previously described (*15*). LHCII monomers were prepared according to ref *17* by treatment of trimers (at 50 μg/mL Chl content) with 3 μg/mL phospholipase $A_2$ from bee venom (Sigma) or, with identical results, phospholipase A (Serva, snake venom from *Crotalus atrox*, research grade) at 3.3 μg/mL concentration for 36 h at room temperature. Oligomerization state of LHCII in thylakoid membranes, PSII membranes, and isolated LHCII preparations were analyzed by sucrose gradient centrifugation at 4 °C (*15*). Essentially the same results were obtained by using green gel electrophoresis (*18*). *Arabidopsis thaliana* plants (Col-0) were grown at 35 μmol of photons $m^{-2}$ $s^{-1}$ at 20 °C with an 8 h photoperiod; immediately after light treatment, excised leaves were homogenized in 0.45 M sucrose, 20 mM Tricine, 10 mM EDTA, 10 mM $NaHCO_3$, 0.1% bovine serum albumin, and 2 mM sodium isoascorbate, pH 8.4, and thylakoids were pelleted by centrifugation. The thylakoids were washed, solubilized in dodecyl maltoside (2.5%), and subjected to sucrose gradient centrifugation as above. Because the trimeric and monomeric LHCII were poorly separated on the gradient following solubilization from *Arabidopsis* leaves, the LHCII-containing fractions were collected and analyzed by nondenaturing green gel electrophoresis (*18*). This procedure gave clear separation of the trimeric and monomeric forms.

*Spectroscopy and Photoelectric Measurement.* Absorption spectra were recorded in a Shimadzu UV-160 and Aminco DW 2000 spectrophotometer (room temperature, 1 cm path length, approximately 10 μg/mL Chl content). Circular dichroic (CD) spectra were recorded on a Jobin-Yvon CD6 and a Jasco 720 dichrograph, which were equipped with regulated thermostated sample holders; optical path length was 1 cm.

Light-induced reversible CD changes were recorded as described before (*12*) at 495 nm at room temperature in a Jobin-Yvon CD6 dichrograph equipped with a side illumination attachment and crossing filters. For light treatments, if not stated otherwise, samples were incubated at 20 °C for between 5 and 120 min with white light at 1000−2500 μmol of photons $m^{-2}$ $s^{-1}$ as described in the figure captions. Photon flux intensity of the photosynthetically active radiation was measured with a Li-Cor LI 250 light meter. The light-intensity dependence of the reversible release of Mg ions from isolated lamellar aggregates of LHCII was measured spectrophotometrically in a Shimadzu UV-3000 dual-wavelength/double-beam spectrophotometer, equipped with the same side illumination attachment that was used for light-induced reversible CD changes (*12*). The measurements were performed at 554−592 nm in the presence of 100−150 μM Eriochrome Blue SE (Sigma). Calibration for Mg ion concentration, both in the presence and absence of LHCII, was carried out spectrophotometrically, between 400 and 700 nm. Light-induced electric signals on oriented LHCII lamellae trapped in polyacrylamide gel were measured by a method described earlier (*19*).

## RESULTS AND DISCUSSION

As reported in preliminary form (*20*), analysis of negative-staining electron microscopy on loosely stacked multilayers of lipid−LHCII lamellar aggregates, which exhibited a substantitial degree of long-range order of chromophores and large reversible changes in the chiral macroarray of chromophores (*12*, *16*), clearly showed that preillumination led to the disappearance of trimers. In these lamellar aggregates in the dark, LHCII trimers were surrounded by lipids and loosely organized in small (100−200 nm) hexagonally ordered patches. Following 1−2 min of preillumination with weak white light (500 μmol of photons $m^{-2}$ $s^{-1}$), the occurrence of such ordered structures was severely diminished, and in light-treated sample there was no indication of the presence of trimeric organization. Instead, a structural analysis suggested the presence of monomers. However, the poor quality of the quasi-crystalline lattice, which is probably

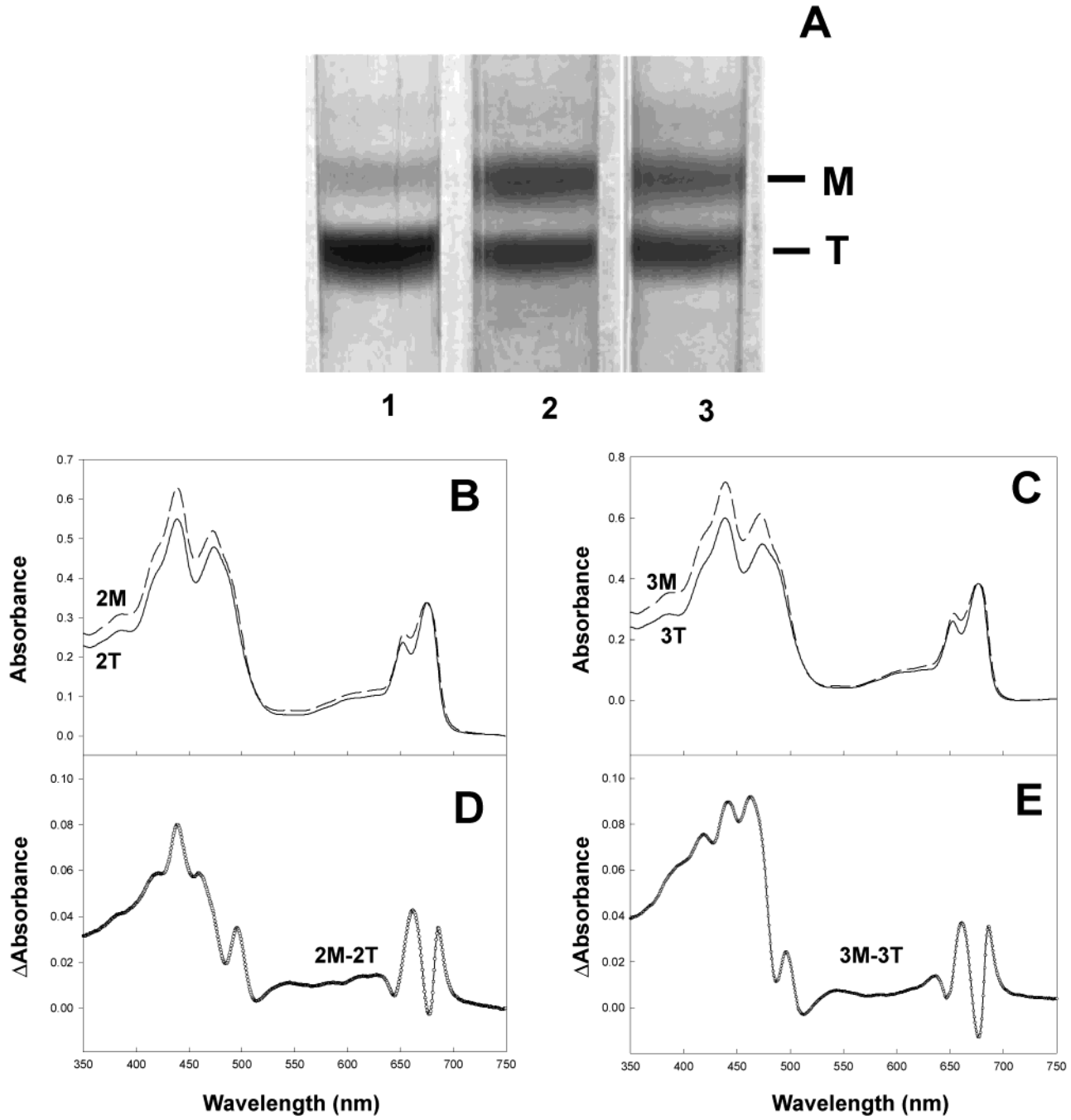


FIGURE 1: Analysis of the oligomerization state of LHCIIb by sucrose gradient centrifugation (A) and comparison of the absorption spectrum of LHCIIb trimers and monomers (B, C). (A) (1) Dark-adapted spinach LHCIIb solubilized in 200 $\mu$M dodecyl maltoside; (2, 3) monomers obtained from phospholipase $A_2$ treatment (36 h, room temperature) and preillumination (60 min, white light, 2500 $\mu$mol of photons $m^{-2}$ $s^{-1}$), respectively. (B, C) Absorbance spectra of monomers produced by phospholipase $A_2$ and light treatments, respectively, compared to the untreated trimers; (D, E) Corresponding monomer minus trimer difference spectra. (The samples were eluted from the sucrose gradient bands and normalized to the redmost absorbance peak.)

correlated with its profound structural flexibility, does not permit firm conclusion concerning the reversible disassembly of trimers in the light. While these investigations and the improvement of the quality of the negative-staining electron-microscopic images of lipid−LHCII multilayered lamellae are in progress, substantiation of the results by independent techniques has been achieved, which are to be presented in the following sections. The main advantage of these analyses, sucrose gradient centrifugation and green gel electrophoresis, complemented with absorbance and CD spectroscopy, is that the oligomerization state of LHCII can be analyzed not only in well-ordered 2D arrays of the complexes but also in detergent-solubilized complexes, PS II membranes, intact thylakoids, and whole plants that are less readily amenable for structural analyses.

As expected, in the dark, detergent-solubilized sample, over 80% of the Chl was in the heavier, trimeric band of the sucrose gradient (Figure 1A, tube 1). Tube 2 shows, for comparison, the conversion of trimers into monomers as a result of treatment in the dark with phospholipase. Upon preillumination, a significant portion of the solubilized trimers were transformed into monomers (tube 3). This shows that indeed the trimeric organization can be disrupted by light, and this occurs not only in lamellar aggregates but also

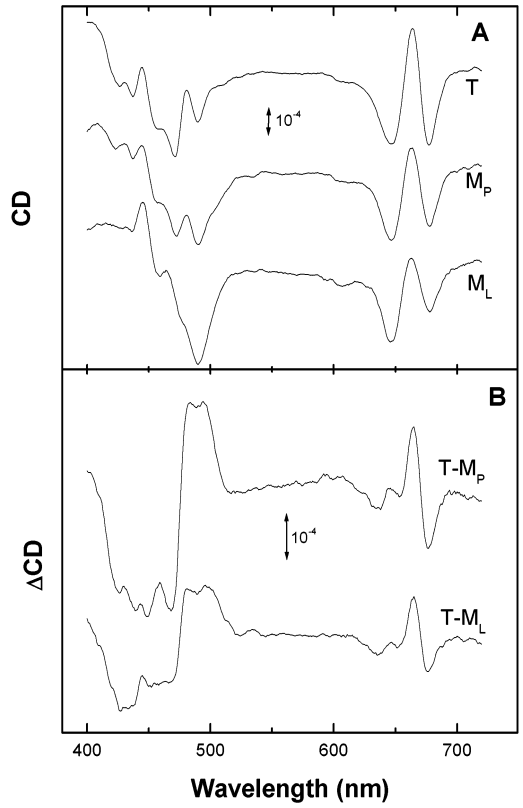


FIGURE 2: Circular dichroic spectra (CD, panel A) and difference spectra (ΔCD, panel B) of detergent-solubilized trimers of pea LHCII (T) obtained from isolated lamellar aggregates and of monomers, obtained after phospholipase A treatment (36 h, room temperature) ($M_P$) and preillumination (15 min, 1000 $\mu$mol of photons $m^{-2}$ $s^{-1}$) ($M_L$) followed by gradient centrifugation. The spectra were normalized at 648 nm.

in isolated, solubilized trimers. [However, in contrast to 2D lamellae, in dilute suspension no spontaneous reassembly of trimers from monomers can occur. The ability of the light-induced momoners to reassemble into trimers has not been tested; this could be done by lowering the detergent concentration and/or reaggregation by salts or reconstitution in lipid vesicles (*21*).] The monomers formed by illumination appeared to be very similar to those prepared by phospholipase treatment: their absorption spectra, with the exception of the 420−460 nm interval, were almost identical (Figure 1B−E). The difference spectrum for monomerization in both cases showed bands around 510 nm due to changes in lutein and around 660 and 680 nm from Chl *b* and *a*, respectively. Such changes have been documented previously (*22*). CD spectroscopy on the monomer and trimer bands also demonstrated the presence of characteristic excitonic band structures, revealing the previously documented alterations in the pigment system found in monomers compared to trimers (Figure 2). In the red region, decrease of the long-wavelength CD amplitudes, and alterations in the band structure between 460 and 500 nm, were very similar to those reported earlier (*21*). Loss of the fine structure of Chl *b* bands around 650 nm, detected at low temperature (*17*), was not resolved but could be revealed in the difference spectra (Figure 2B). These latter spectra also show that, apart from minor differences between 430 and 460 nm, the two types of monomers, produced by phospholipase treatment and preillumination, are virtually indistinguishable. Very similar differences were seen before the separation of the bands,

Table 1: Chlorophyll Fluorescence Quenching in Trimers and Monomers of LHCIIb[a]

| sample | half-time (s) | total quenching |
|---|---|---|
| LHCII trimer | 13.2 ± 2.2 | 0.60 ± 0.04 |
| LHCII monomer (PL) | 1.3 ± 0.1 | 0.77 ± 0.01 |
| LHCII monomer (30 min L) | 1.8 ± 0.1 | 0.79 ± 0.01 |
| LHCII monomer (120 min L) | 1.8 ± 0.1 | 0.78 ± 0.02 |

[a] PL, prepared by phospholipase $A_2$ treatment, and L, prepared by light treatment, as in Figure 1A. Quenching was initiated by dilution into low-detergent buffer as described before (*38*). Total quenching is expressed as the maximum extent of quenching as a proportion of the fluorescence maximum, and quenching half-time was calculated following the fitting of the quenching data to a second-order kinetic plot, exactly as described previously (*38*).

which shows that sucrose gradient centrifugation introduced no significant artifact.

As shown in Table 1, the ability of monomers to quench the singlet excited state of Chl *a* (*23*) was also essentially the same in the two types of LHCII monomers. Hence, it can be concluded that there is no significant structural or functional difference between the monomers formed upon phospholipase and light treatments.

The extent of monomerization depended on the length (Figure 3A) and intensity (not shown) of illumination as well as on the preparation (see below). Although preillumination induced some disruption of the pigment system and photobleaching of chlorophylls, the magnitude of the destruction of complexes, below and around 25 °C, remained low even after prolonged illumination.

Earlier it has been shown that in thylakoid membranes the light-induced reorganizations detected by CD could be accounted for by a thermo-optic effect: fast, local thermal transients due to the dissipated excitation energy. Thermal transients (local *T*-jumps) can readily induce structural changes because of the built-in thermal instability of the macroarrays in intact thylakoids between 45 and 55 °C (*14*). It is possible that the light-induced trimer-to-monomer transition can be similarly explained and that LHCII itself may have a thermally inducible trimer-to-monomer transition, and a threshold temperature above which only monomers are stable. Indeed, Figure 3B shows that conversion of trimers into monomers could also be induced by elevating the temperature. This transition can be characterized by a relatively sharp decline of the trimeric state at 45 °C and the accumulation of monomers between 50 and 60 °C. It is important to note that heat-induced monomerization of LHCII, similarly to the effect of light, does not cause a substantial accumulation of free pigments and is therefore not likely to be accompanied by protein thermal denaturation (Figure 3B).

As pointed out above, in dilute, detergent-solubilized samples reassembly of monomers to trimers is severely limited, and thus, no reversibility can be expected. On the other hand, reversibility of the structural changes, under essentially the same conditions of illumination can be demonstrated in loosely stacked lamellar aggregates. As shown in Figure 4, the light-induced changes in these lamellae can remain fully reversible even after relatively long exposures to intense light (>20 min, 2500 $\mu$mol of red photons $m^{-2}$ $s^{-1}$). In these samples, the CD spectrum (Figure 4A) is dominated by the so-called $\psi$-type bands that arise

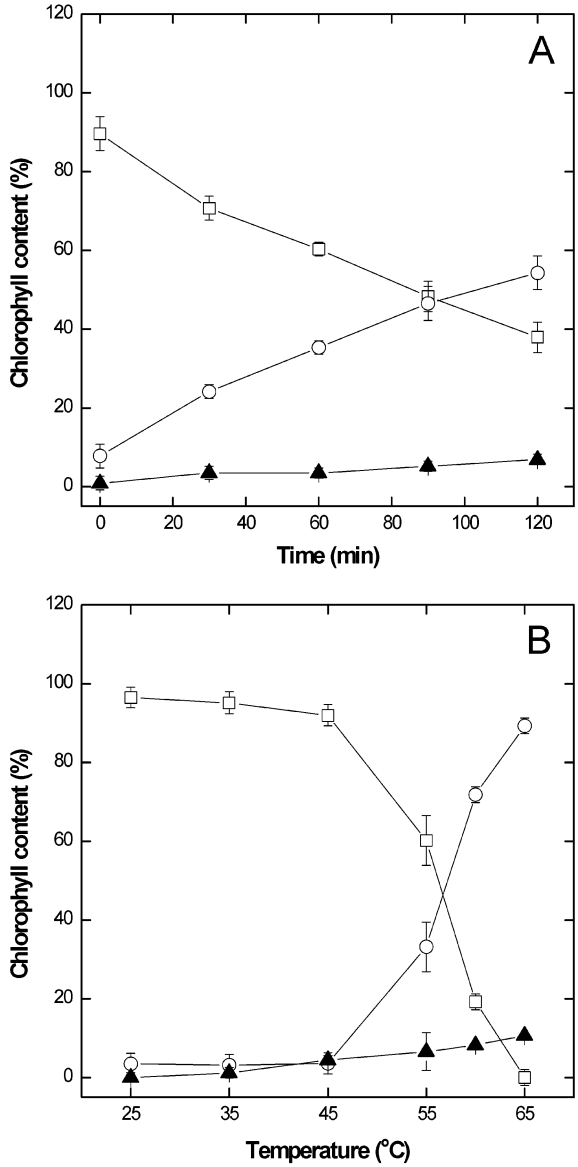


FIGURE 3: Chlorophyll distribution on sucrose gradient following light (A) and heat (B) treatment of LHCIIb trimers isolated from spinach thylakoid membranes. (□) Trimers, (○) monomers, and (▲) free pigments isolated on the sucrose gradient are shown. Chlorophyll distribution was determined by determination of the Chl concentration and volume of the eluted bands. Data are the means of triplicate tubes ± SEM, normalized at 648 nm. Light treatment (A) (2500 $\mu$mol of photons $m^{-2}$ $s^{-1}$) was performed at 20 °C; incubation time for heat treatment (B) was 30 min.

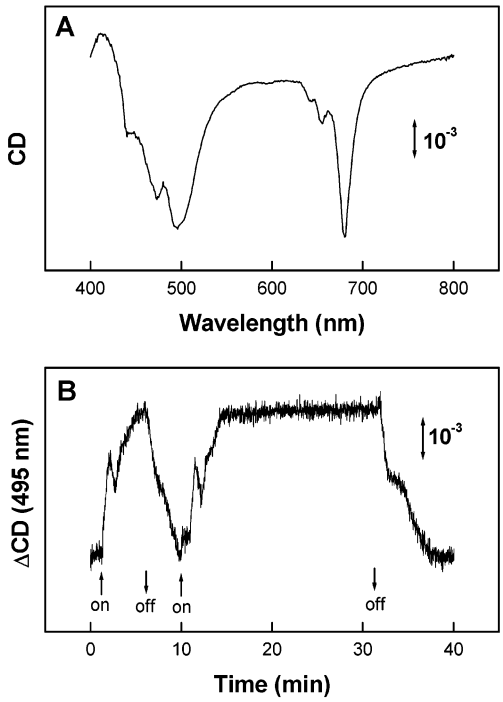


FIGURE 4: Circular dichroic spectrum (A) and light-induced CD changes at 495 nm (B) in lamellar aggregates of isolated pea LHCII. Optical path length, 1 cm; Chl content, 35 $\mu$g/mL; intensity of the actinic light, obtained from a tungsten−halogen lamp and red cutoff (>650 nm) filter, 2500 $\mu$mol of photons $m^{-2}$ $s^{-1}$; on and off, respectively, indicate the beginning and end of illumination period.

from a long-range chiral order of the chromophores ($\psi$, polymer or salt-induced) (*24*, *25*). However, variations in the intensity of these bands, which dominate the light-induced CD changes, do not carry information on the short-range excitonic interactions of pigment molecules in trimers and monomers. Thus, and because of instabilities in both the dark and light periods, the light-minus-dark difference spectra could not be used for CD fingerprinting of the trimer-to-monomer transition. (The instabilities were commensurate with the magnitude of the excitonic bands.) It is interesting to note that the light-induced kinetic traces (Figure 4B), under these conditions, indicate that there is a bistability in the system. This is in line with earlier observations (Barzda and Garab, unpublished). In addition, usually both stable conformations exhibit $\psi$-type features, i.e., a long-range chiral order of the chromophores. Although the presently available data do not allow a more precise interpretation, it is plausible to assume, but remains to be shown (e.g., by trapping the two stable conformations at low temperature and analyzing the excitonic band structure of CD), that these two states of LHCII in the lamellae are preferentially associated with trimeric and monomeric states in the dark and light, respectively. This tentative assignment is fully consistent with the conclusion from the preliminary analysis of negatively stained electron microscopic images. (*20*). The light-induced increase in the surface area occupied by the complexes in monolayers (*26*) may also be accounted for by dissociation of trimers to monomers.

As shown in Figure 5A (columns 1 and 2), lamellar aggregates of LHCII enriched in lipids exhibited a virtually full conversion of trimers to monomers within 10−15 min at moderate light intensity. (In these samples, in the dark, the complexes were often found in a diffuse, rather than a sharp band, which suggests the presence of mixed oligomers rather than pure trimers. This is most likely a consequence of the profound structural flexibility of the lamellar aggregates.) The enhanced ability of lipid-enriched lamellae for monomerization compared to solubilized LHCII, i.e., their considerably lower light intensity threshold, is consistent with the suggested key role of lipids in the structural flexibility of LHCII (*16*).

It has been noted that the light-induced CD changes in lamellar aggregates of LHCII closely resemble those observed in granal thylakoids illuminated with excess light (*12*). We therefore determined the effect of illumination of PSII-enriched grana membranes (containing trimeric LHCII) and intact thylakoids on the distribution of chlorophyll between

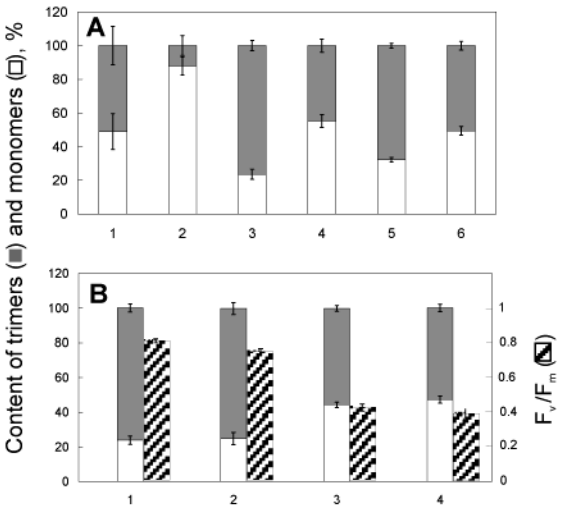


FIGURE 5: Analysis of the chlorophyll distribution between trimers and monomers in isolated, LHCII-containing artificial and native membranes (A) and in whole plants (B). (A) Lamellar aggregates of isolated pea LHCII (1, 2), PSII-enriched spinach grana membranes (3, 4), and intact thylakoid membranes (5, 6) in the dark (1, 3, 5) and after light treatments [(2) 1000 $\mu$mol of photons $m^{-2}$ $s^{-1}$, 15 min; (4, 6) 2500 $\mu$mol of photons $m^{-2}$ $s^{-1}$, 60 min]. Chlorophyll distribution was determined by measuring the chlorophyll concentration and volume of the eluted trimeric and monomeric fractions and normalizing to 100%. Data are the means of triplicate tubes ± SEM. (B) Whole *Arabidopsis* plants dark-adapted (1) and illuminated with 1000 $\mu$mol $m^{-2}$ $s^{-1}$ for 5 min (2), 1 h (3), and 2 h (4). Chlorophyll distribution was determined by densitometric analysis of the LHCII trimer and monomer bands on a green gel. $F_v/F_m$ values were determined with a Walz PAM 2000 chlorophyll fluorometer on 10-min dark-adapted leaves.

trimeric and monomeric complexes. For PSII membranes, light caused a strong reduction in the trimeric LHCII band and a corresponding increase in the monomeric fraction (Figure 5A, columns 3 and 4). Illumination of thylakoids (Figure 5A, columns 5 and 6) caused a similar change: the intensity of the monomeric fraction increased to a statistically significant extent at the expense of the trimers. However, the extent of these changes was smaller than in isolated LHCII, possibly because of a partial re-formation of trimers in the dark following the preillumination or during the solubilization of membranes. In this context, we note that light-induced reorganizations in thylakoid membranes remain fully reversible in strong light (for >30 min, with 2 min light/2 min dark intervals, at 550 W $m^{-2}$, corresponding to about 3000 $\mu$mol of photons $m^{-2}$ $s^{-1}$) (*11*). The changes become irreversible only upon prolonged, continuous illumination with intense light (15 min of white light, 2000 $\mu$mol of photons $m^{-2}$ $s^{-1}$), but even under these conditions some reversibility was retained (*27*). It must also be noted that the main CD features of the reversible and irreversible changes were very similar to each other; they were dominated by the $\psi$-type bands, while variations in some minor excitonic bands could be suspected but were not resolved. The light treatment of thylakoids also resulted in a 30−50% nonphotochemical quenching (NPQ) of the Chl *a* fluorescence (data not shown).

Light-induced monomerization of LHCII was also found in whole plants (Figure 5B). LHCII from dark-adapted plants consisted of approximately 75% in the trimeric form. A brief period of illumination caused no detectable change in the content of monomers and induced no or little, slowly reversible nonphotochemical quenching. In contrast, 1−2 h of illumination with excess light (1000 $\mu$mol of photons $m^{-2}$ $s^{-1}$) produced a progressive decrease in the proportion of trimers, so that after 2 h the ratio of trimers:monomers was almost 1:1. During this treatment there was a significant development of the sustained qI form of NPQ, with a decline in the dark-adapted value of $F_v/F_m$ from 0.8 in the control to 0.4 for the light-treated leaves. ($F_v$ and $F_m$ denote the variable and the maximum Chl *a* fluorescence.) Although the kinetics of the reversibility of the trimer-to-monomer transition and fluorescence quenching were not studied, it is evident that, in this physiological range, the antenna system of plants readily relaxes in a few hours dark period (cf. *2*).

Examination of the thermal stability of different oligomerization states as well as the temperature dependency of the changes in CD which accompany the structural rearrangements suggests that, similar to thylakoid membranes (*14*) and by analogy with a phenomenon known for liquid crystals (*28*), the monomerization of LHCII may be caused by a thermo-optic effect. In the following section the thermo-optic model will be applied for lamellar aggregates of LHCII, mainly because they closely resemble the thylakoid membranes. Lamellar aggregates of LHCII and intact thylakoid membranes contain large ordered domains that give rise to CD bands due to long-range order of the chromophores (*9*, *10*; see also *29*). Further, both thylakoid membranes and LHCII lamellae possess the ability of undergoing light-induced reversible (and similar irreversible) rearrangements, as well as light-induced fluorescence quenching (*12*). It must be stressed that the model can also be adapted to solubilized complexes. For instance, it is easy to show that the model is fully consistent with the irreversible light- and heat-induced monomerization of isolated trimers (see above, Figure 3). We also emphasize, however, that the main aim of this model is to aid conceptualizing the physical mechanism rather than to attempt an exact description of the observations.

As shown in Figure 6A, isolated lamellar aggregates of LHCII, similar to intact thylakoid membranes (*14*), exhibit a relatively low thermal stability in the dark. This is characterized by a transition of the macroaggregation-associated CD signal in a narrow temperature interval. This means that certain LHCII conformations or assemblies exhibit well-defined heat instability. In the model, this can be accounted for by temperature dependence of the depths of potential wells belonging to different conformational states (cf. *14*). It must be stressed that these transitions, although mostly irreversible, do not lead to a destruction of complexes, which occurs only at higher (denaturing) temperatures. Dissipation of the photochemically not utilized excitation in the pigment bed transiently increases the temperature in the close vicinity of the dissipating centers. The occurrence of large, fast thermal transients, local *T*-jumps in light-harvesting antennae, has been reported earlier (*30*). Upon raising the local temperature toward the transition temperature, elementary structural transitions can be induced. These elementary structural changes are evidently of the same nature as those induced in the bulk by elevating the ambient temperature. However, thermo-optical transitions can evidently occur well below the transient temperature while the probability of similar transition in the dark is low. This results in a shift of the thermal stability toward lower temperatures

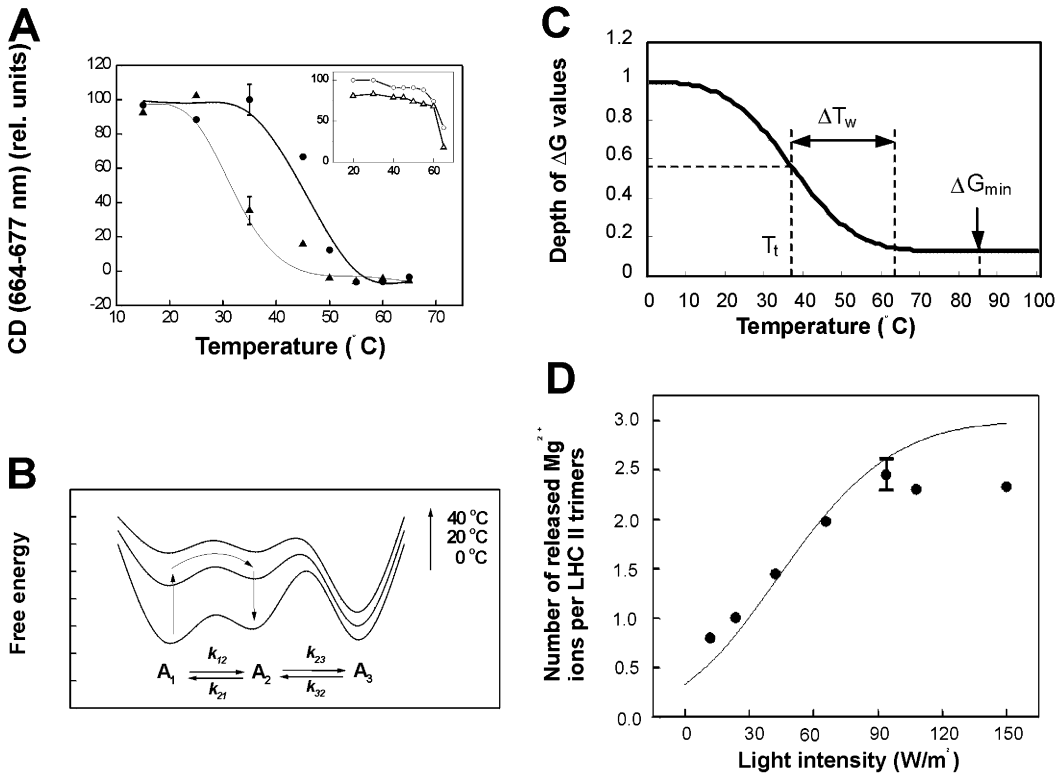


FIGURE 6: Thermo-optic model of the structural transitions of LHCII. (A) Thermal stability of lamellar aggregates of pea LHCII, measured with the aid of a characteristic CD band in the dark (●) and following a preillumination (▲) at different temperatures for 20 min with white light of approximately 1000 $\mu$mol of photons $m^{-2}$ $s^{-1}$. Error bars at 35 °C are from four independent experiments. Solid lines show theoretical curves from a model calculation based on structural transitions of thermo-optic origin. Inset, thermal stability of isolated microcrystalline LHCII in the dark (○) and light (2500 $\mu$mol of photons $m^{-2}$ $s^{-1}$, 15 min) (△), respectively; other conditions were as for lamellar aggregates. (B) Potential profile representation of the simplified, three-state model of LHCII at different temperatures. The proposed mechanism of thermo-optic transitions is illustrated by arrows: upon internal conversion of an excited-state molecule, the local temperature jumps up for a very short time interval; this step can facilitate an elementary structural transition, which at the ambient temperature will be only slowly reversible from $A_2$ or remain irreversibly in $A_3$, and thus $A_2$ and $A_3$ states can be accumulated. (C) Dependence of the depth of potential energy wells on the temperature, defined by the parameters, $\Delta G_{min}$, $\Delta T_w$, and $T_t$. (D) Light-intensity dependence of the amount of reversible release of Mg ions from isolated lamellar aggregates of pea LHCII (data points and SEM at the saturation light intensity, obtained from five independent experiments) and theoretical curve based on the thermo-optic model.

in the light compared to that in the dark (Figure 6A; cf. also *14*). For the changes are confined to small volumes (sizable *T*-jumps occur in volumes smaller than a few cubic nanometers) and very short time intervals (10−20 ps) (*14*), their reversibility can be retained. It is a plausible assumption that the changes become virtually irreversible only upon a repeated excitation (*T*-jump) before relaxation.

It is important to note that the thermo-optic mechanism of a structural change heavily relies on the presence of a well-defined heat-inducible structural transition and cannot occur in the absence of a built-in thermal instability of the structure. As shown in the inset to Figure 6A, the complexes in microcrystalline (type IV) LHCII do not possess a sizable transition below 60 °C, somewhat surprisingly, not even in strong light. These preparations are also not capable of light-induced reversible CD changes (*16*) and are entirely inactive with regard to reversible light-induced release of Mg ions (for lamellar aggregates, see below). Further, compared to other preparations, they exhibited the weakest reversible fluorescence quenching (*31*). Other, somewhat less delipidated (type III) preparations of LHCII (cf. *16*) exhibited thermal and light instabilities between the two extreme cases (lamellar aggregates and microcrystals). This qualitatively explains the differences between the susceptibility of different LHCII preparations toward heat and light treatments.

Hence, in the thermo-optic model, we assume that the array of complexes possesses a built-in thermal instability and that there are local thermal transients due to photon energy dissipation (internal conversion of excited molecules). These are assumed to induce $\Delta T$ effective *T*-jumps at the site of elementary structural changes, i.e., in the vicinity of the dissipating centers, where the respected elementary structural transition occurs. Because of the built-in thermal instability, a $\Delta T$ *T*-jump at this site can lead to a reversible elementary structural transition ($A_1-A_2$) or a series of reversible transitions ($A_1-A_4$) (for further explanation of the four states, see below). We also assume that upon prolonged illumination or at high ambient temperatures a consecutive state with a deep potential well can be reached (irreversible state). Hence, this model contains five states: $A_1$, $A_2-A_4$, and $A_5$, or initial, intermediary, and final states, respectively. For a simplified case, as above, by use of effective rate constants ($k_{12}$ and $k_{21}$), the intermediary states can be combined in one, reversible state ($A_2$) (Figure 6B). To explain the thermal instability of the system, i.e., the sharp structural transition in a narrow temperature interval, we assume a simple temperature dependence of the potential energy profiles (Figure 6C), with $\Delta G$ being temperature-independent in the ranges considerably below and above the thermal transitions and with a sharp decline around $T_t$, the transition temperature.

[The half-width of this temperature range is $\Delta T_w = T_w - T_t$; for $T_w > T_t$, $T_w$ is defined as the temperature where $\Delta G(T_w) - \Delta G_{min} = [\Delta G(T_t) - \Delta G_{min}]/e^2$; where e is the base for natural logarithm. $\Delta G_{min}$, in relative units, is the minimum depth of the given potential gap.] These parameters, $T_t$, $\Delta T_w$, and $\Delta G_{min}$, can be obtained from a fit of the temperature dependence of the characteristic CD bands in the dark; for lamellar aggregates, the thermal instability in the array of trimers can be followed by $CD_{664-672}$ that is characteristic of the long-range chiral order of the chromophores (*12*, *16*). (Gradual disappearance of this band at higher temperatures originates from a gradual destruction of the complexes.)

In the numerical calculations, as in ref *14*, we started with the determination of the parameters for the thermal instability of the complexes in the dark. From a fit we obtained $T_t = 37$ °C, $\Delta T_w = 26$ °C, and $\Delta G_{min} = 0.12$. Then the thermal stability in the light was fitted by selecting the value of the effective *T*-jump, which yielded $\Delta T = 15$ °C (Figure 6A). (Further details of the model, and its application to the light-induced reversible CD changes will be published elsewhere; Cseh, Papp, and Garab, manuscript in preparation.)

One of the basic assumptions of the proposed thermo-optic mechanism (*14*) is that the thermal transient provokes a fast event that is slowly reversible (hence, the *T*-jump per se would not explain the structural changes). It is estimated that thermal transients of about 10 °C in magnitude can last for 10−20 ps at a distance of about 1 nm from the dissipating center (*14*). Taking into account the structure of LHCII, particularly its flat outer loop segment (*7*) and the short distances of some pigment molecules from the aqueous phase, one can predict the susceptibility of cation binding sites. An increased vibrational motion of the protein due to heat jumps in the vicinity of these sites could result in release of cations, which may explain the occurrence of a fast (<200 ns, instrument-limited) electric signal (not shown), most likely from charge displacement, upon an excitation of oriented lamellae with 20 ns laser pulse. Indeed, we have found that illumination of LHCII results in the liberation of Mg ions (Figure 6D): the extent of this release is nearly linearly proportional to the light intensity between about 10 and 80 W $m^{-2}$, and upon saturation, around 100 W $m^{-2}$ (>650 nm, approximately 600 $\mu$mol of photons $m^{-2}$ $s^{-1}$), reaches a value of 2.5 Mg ions/trimer. In the model, we assume a gradual release of three Mg ions, in consecutive steps from $A_1$ to $A_2-A_4$; this stoichiometric release of Mg ions from trimers is likely to be responsible for the monomerization of the complexes. (Regarding CD, as indicated above, $A_2-A_4$ states can be combined into a single state, $A_2$.)

The involvement of the stromal surface of LHCII is consistent with the light-dependent exposure of the phosphorylation site (*32*), which is located on a threonine residue close to the N-terminus. Light-induced release of Mg ions has been documented in thylakoid membranes (*33*). It is well-known that LHCII and Mg ions play a major role in establishing the three-dimensional organization of the thylakoid grana (*34*) and that the granal structure appears necessary for the regulatory functions of the light-harvesting system in plants (*35*).

The fact that monomerization of LHCII can be caused also by phospholipase clearly indicates that there may be a role of lipids in this process. Lipids have been shown to play a key role in the structural flexibility of LHCII (*36*). In terms of the thermo-optic effect, it can be envisioned that thermal transients could induce changes in specific lipid−protein interactions, such as between acyl chain groups and apolar residues in the grooves of membrane proteins, near the aqueous surface, or via lubrication of protein−protein contacts (*37*). Then the release of Mg ions can be considered either a concerted event or the consequence of the altered protein conformation due to primary effects in the lipophilic phase.

What might be the physiological significance of the light-dependent monomerization of LHCII? It is now established that the PSII light-harvesting system can exist in two different states in vivo: an unquenched state that is functional in light-harvesting and a quenched state, induced under excess light conditions, that dissipates the excess absorbed excitation energy (*2*). This regulatory process, feedback dissipation, is observed as nonphotochemical quenching (NPQ). By use of an in vitro system of purified LHCII components, a model for the in vivo NPQ, it was found that trimeric LHCII did not quench to the same extent or as rapidly as either its monomeric form or the minor, monomeric complexes (CP29 and CP26) (*38*, *23*). Monomers prepared by the light treatment also possessed the ability of rapid and large quenching (Table 1). Hence, light-induced monomerization converts LHCII into a form that, similarly to other monomeric complexes (*38*, *23*), has the capability to be readily be quenched, and therefore, we infer, it might play a significant role in NPQ. To avoid misunderstanding, we must however point out that monomers cannot be equated with the quenchers (*23*). These data merely show that their dynamic properties (most probably for steric reasons) permit the formation of quencher more readily than in the (presumably more compactly organized) trimers. Further, photogeneration of quenchers is a fast reaction that competes with other excitation−relaxation pathways (*31*).

NPQ consists mainly of two types of quenching: a rapidly relaxing qE form dependent upon the $\Delta$pH and the xanthophyll cycle (*2*) and involving the action of the PsbS protein (*4*); and a more sustained form (qI) occurring at higher irradiance about which very little is known. The features of the light-dependent monomerization suggest that it plays a role in this latter process. Indeed, during the light treatment of thylakoids and leaves that induce monomerization of LHCII, there was significant induction of qI. Previously, high light treatment of rice leaves has been reported to increase the relative amount of monomeric LHCII (*39*). It can also be speculated that uncoupled chlorophylls that have been detected in photoinhibited thylakoids originate from monomerized LHCII (*40*). In this context, it is important to note that heat-induced alterations in the oligomerization state of PSII and LHCII have earlier been reported (*41*). High light treatment also results in the regulated proteolytic degradation of LHCII, and it is perhaps significant that only LHCII monomers are targeted (*42*). Thus light-induced monomerization could represent an important aspect of the response to high light involvement in the dissociation of the PSII macroorganization, inducing a further protective quenched state of the antenna and yielding excess LHCII for proteolytic degradation.

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