The Functional Significance of the Monomeric and Trimeric States of the Photosystem II Light Harvesting Complexes†

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Received June 6, 2003; Revised Manuscript Received November 13, 2003

ABSTRACT: The main light harvesting complex of photosystem II in plants, LHCII, exists in a trimeric state. To understand the biological significance of trimerization, a comparison has been made been LHCII trimers and LHCII monomers prepared by treatment with phospholipase. The treatment used caused no loss of chlorophyll, but there was a difference in carotenoid composition, together with the previously observed alterations in absorption spectrum. It was found that, when compared to monomers, LHCII trimers showed increased thermal stability and a reduced structural flexibility as determined by the decreased rate and amplitude of fluorescence quenching in low-detergent concentration. It is suggested that LHCII should be considered as having two interacting domains: the lutein 1 domain, the site of fluorescence quenching [Wentworth et al. (2003) *J. Biol. Chem. 278*, 21845−21850], and the lutein 2 domain. The lutein 2 domain faces the interior of the trimer, the differences in absorption spectrum and carotenoid binding in trimers compared to monomers indicating that the trimeric state modulates the conformation of this domain. It is suggested that the lutein 2 domain controls the conformation of the lutein 1 domain, thereby providing allosteric control of fluorescence quenching in LHCII. Thus, the pigment configuration and protein conformation in trimers is adapted for efficient light harvesting and enhanced protein stability. Furthermore, trimers exhibit the optimum level of control of energy dissipation by modulating the development of the quenched state of the complex.

The light harvesting antenna of photosystem II (PSII)[1] is a multiprotein complex comprised of an inner antenna of CP43/47, which forms the core complex with the D1/D2 reaction center, surrounded by a peripheral antenna, which includes the Lhcb proteins as well as a number of LHC-related proteins, including PsbS and the ELIPs (*1*, *2*). There are six Lhcb proteins (Lhcb1−6) organized into two groups. The first group, Lhcb4−6, forms the minor antenna complexes, CP29, CP26, and CP24, which are monomeric and bind ∼10% of the chlorophyll in PSII. The second group forms the major antenna complex LHCII, which binds ∼60% of the PSII chlorophyll and is a heterotrimer of Lhcb1−3.

There is currently no sufficient explanation for this level of complexity in the molecular design of the PSII antenna. For the light harvesting complexes, it is not known why trimeric complexes form the major population of complexes. Trimerization is a specific process that requires binding of phospholipid (*3*, *4*) and depends on particular protein sequences (*5*, *6*). Trimers are formed from monomers during the last stages of thylakoid development (*7*). In the thylakoid membrane, the core complex, the minor antenna complexes, and most of the LHCII trimers are organized into the PSII supercomplexes (*8*), which may form large semicrystalline arrays in the granal membranes of the chloroplast (*9*). The dimeric PSII supercomplex binds up to four trimers, and additional trimers have been located in membrane domains deficient in PSII (*9*). Oligomers of trimers have been observed by electron microscopy of solubilized membranes (*10*) and on sucrose gradient fractionations (*7*, *11*).

Spectroscopic investigation of LHCII trimers and monomers has revealed alterations in absorption and CD spectra of chlorophyll *a*, chlorophyll *b*, and lutein (*12*, *13*). However, it is not known if these alterations have any significance in terms of light harvesting. Rather, they indicate an altered structure of the monomer compared to the trimer. Trimerization may therefore be important in increasing the stability of the protein complex, or it may have a structural role in the macroorganization of the grana membrane. Alternatively, LHCII trimers may be important in the structural and functional flexibility of the system that provides physiological regulatory mechanisms (*14*, *15*). Trimeric LHCII is phosphorylated and relocated to the unappressed membranes giving rise to state transitions. Light-induced monomerization of LHCII trimers has also been observed, although the physiological role of this is uncertain (*16*). Only monomers of LHCII are degraded upon photoacclimation to high light (*17*), indicating a possible site of control at the trimer/monomer conversion.

† Supported by grants from the U.K. BBSRC (C11581 and C18674) to P.H.

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[1] Abbreviations: DM, *n*-dodecyl *β*-maltoside; Lhcb, proteins encoded by the Lhcb genes; LHCII, the major light harvesting complex of photosystem II; NPQ, nonphotochemical quenching; PSII, photosystem II; qE, nonphotochemical quenching dependent upon the ΔpH; ΔpH, transthylakoid pH gradient, PLA, phospholipase A.

10.1021/bi034975i CCC: $27.50
Published on Web 12/17/2003

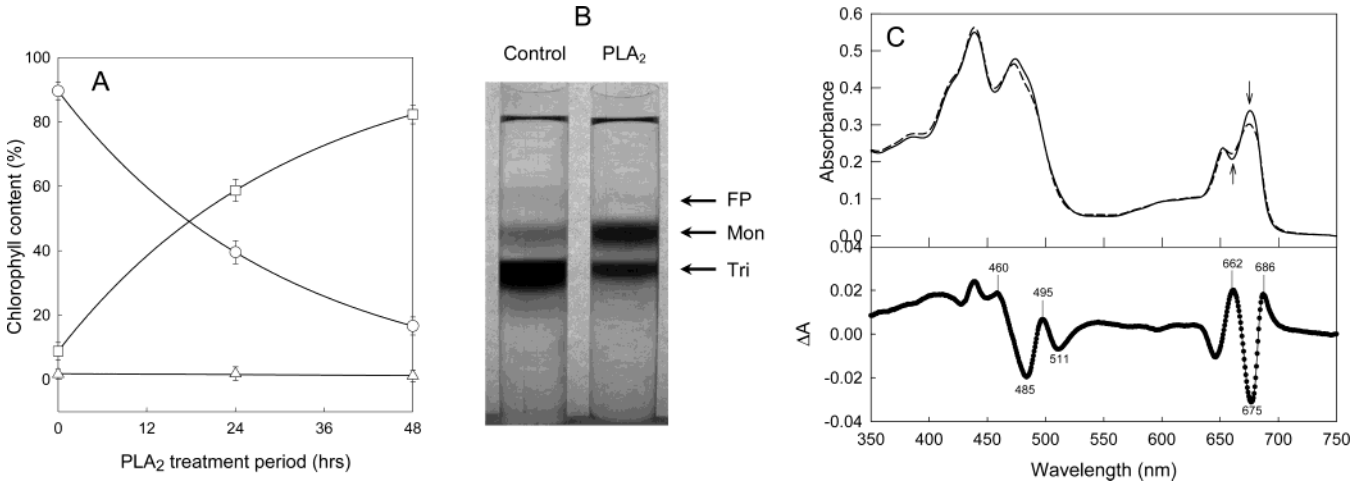

FIGURE 1: Preparation and isolation of monomeric LHCII following PLA treatment. (A) Chlorophyll distribution on a sucrose gradient following different periods of PLA treatment: (○) LHCII trimers, (□) LHCII monomers, and (△) free pigment. (B) Typical sucrose gradient of LHCII following 24 h of PLA treatment. All gradients were loaded with 1 mg of total Chl. Abbreviations: FP, free pigment, Mon, LHCII monomers; Tri, LHCII trimers. (C, top) Comparison of absorption profiles from sucrose gradient prepared trimeric (whole line) and monomeric (dashed line) LHCII. Arrows illustrate the major changes in absorption following monomerization. (C, bottom) Absorption difference spectra following monomerization of LHCII trimers. The difference spectrum was calculated from unnormalized spectra as monomeric LHCII − trimeric LHCII.

Table 1: Pigment Composition of LHCII Fractions Following 24 h PLA Treatment[a]

		pigment composition						
		Lut	Neo	XC	Car/Chl	Chl *a*	Chl *b*	Chl *a/b*
control (trimer)	free pigment (%)	58.5 ± 0.2	7.7 ± 0.6	33.8 ± 0.6		ND	ND	
	LHCII (%)	58.3 ± 1.7	31.8 ± 1.5	9.9 ± 0.3	0.25 ± 0.01	54.9 ± 0.4	45.0 ± 0.4	1.22 ± 0.1
	MR	1.89	1.03	0.32		7.14	5.86	
PLA treated (monomer)	free pigment (%)	58.1 ± 0.3	20.7 ± 1.0	21.2 ± 0.7		ND	ND	
	LHCII (%)	59.3 ± 0.6	21.7 ± 1.3	18.9 ± 0.7	0.27 ± 0.02	55.4 ± 0.5	44.6 ± 0.5	1.24 ± 0.2
	MR	2.05	0.75	0.66		7.20	5.80	

[a] Abbreviations: Lut, lutein; Neo, neoxanthin; XC, total xanthophyll cycle pigments (violaxanthin + antheraxanthin); %, pigment content expressed as a percentage of either total carotenoid (for Lut, Neo, and XC) or total chlorophyll (for Chl *a* and Chl *b*); Chl *a*/*b*, chlorophyll *a*/*b* ratio; MR, estimated molar ratio, normalized on 13 chlorophylls per monomer; ND, nondetectable. Values represent the mean ± SE ($n = 6$).

LHCII trimers have also been suggested to be involved in the inducible increase in the level of energy dissipation that gives rise to the nonphotochemical quenching of chlorophyll fluorescence (NPQ) and which provides protection against photodamage (*18*, *19*). The major proportion of NPQ, called qE, has been shown to be obligatorily dependent on the transthylakoid ΔpH and controlled by the deepoxidation of the carotenoid violaxanthin into zeaxanthin through the action of the xanthophyll cycle (*20*, *21*). The exact site of energy dissipation within the antenna remains uncertain. Using an antisense approach to remove either CP26 or CP29 from the PSII antenna, it was shown that their absence led to only small reductions in the level of qE (*22*). In *Chlamydomonas*, elimination of one of the major LHCII-type complexes had a significant effect on qE, although in plants the lack of Lhcb1 and Lhcb2 only reduced qE capacity by about 20% (*23*). Mutants lacking the Lhc-related protein PsbS almost completely lack qE (*24*), although the exact role of PsbS is unclear, partially due to uncertainties of its pigment binding capabilities (*25*−*30*). It has been suggested that PsbS may be a regulatory subunit in qE rather than being the site of quenching per se (*31*), providing binding sites for protons (*32*) and zeaxanthin (*33*).

Investigation of the quenching of fluorescence in isolated antenna complexes has given many insights into the mechanism of qE, particularly concerning the possible mode of action of the xanthophyll cycle. Recently, an investigation of the temperature dependency of quenching has led to specific proposals for how a conformational change in a light harvesting complex can give rise to quenching (*34*). It was found that the trimeric LHCII quenched more slowly and to a lesser extent than either of the monomeric minor antenna complexes CP29 and CP26 (*35*, *36*, *37*). Initial observations made on LHCII monomers suggested that this difference arose directly from trimerization, rather than from differences in protein sequence or pigment binding (*34*, *37*). This apparent functional difference between trimeric and monomeric complexes may provide an understanding of the biological significance of trimerization. In this paper, we report a detailed comparison between carefully prepared intact monomeric LHCII and LHCII trimers and confirm the differences in fluorescence quenching. On the basis of the analysis of the changes in pigment composition and absorption spectra, we propose a model in which energy dissipation in LHCII is regulated by interdomain communication within the monomeric LHCII subunit, a process that is modulated by trimerization.

MATERIALS AND METHODS

Light harvesting complexes were purified from spinach leaves as described previously (*38*). LHCII monomers were prepared by PLA treatment of IEF-isolated trimers as

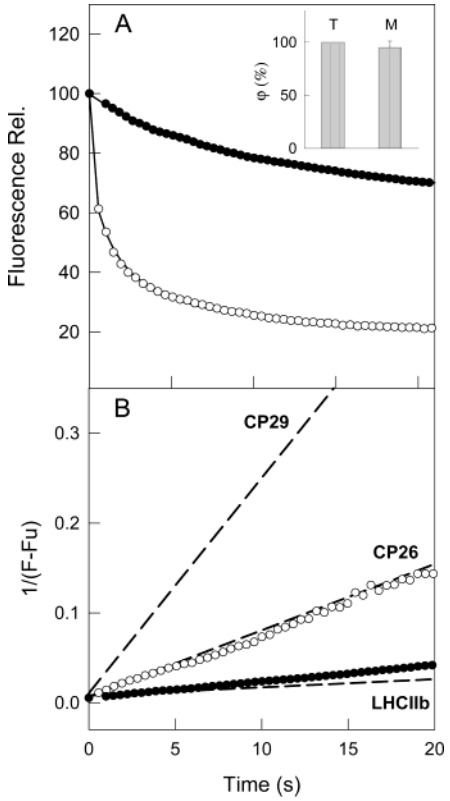

FIGURE 2: Kinetics of chlorophyll fluorescence quenching in isolated LHCII trimers and monomers: (●) LHCII trimers; (○) LHCII monomers. (A) Decrease in fluorescence. Insert: Maximum fluorescence yield (φ) of LHCII trimers and monomers measured in 200 μM DM. Isolated antenna complexes were used at a final concentration of 3 μM chlorophyll, and values were normalized to 100% against trimeric LHCII. (B) Second-order reciprocal plot of the data ($r^2 = 0.99$ in both cases) compared to that of isolated CP29, CP26, and LHCII (dashed lines; taken from ref *36*).

previously described (*39*), modified as in ref *40*. The pigment composition of isolated samples was determined by reverse-phase HPLC according to the method of Faber et al. (*41*). Standard absorption spectra were measured using an SLM DW2000 spectrophotometer as described previously (*35*). Samples were suspended in 25 mM HEPES, 10 mM MES, and 200 μM DM, pH 8.0.

Investigation of the quenching of chlorophyll fluorescence followed two well-documented protocols. In the first, the samples were dissolved in 200 μM DM and diluted into a detergent-free medium at a specified pH and temperature to give a final DM concentration of approximately 6 μM (*35*, *42*). Where modulators were used, they were added at a specific concentration (indicated in the figure) to the detergent-free medium prior to the addition of the antenna complexes. In the second protocol, isolated samples were incubated for 2 min in buffer containing 200 μM DM, and quenching was induced by the addition of the modulator (*37*). To investigate the effect of temperature, the detergent-free medium was incubated at the desired temperature before sample addition, and the temperature was monitored throughout each experimental run using an integrated thermocouple. Chlorophyll fluorescence yield was measured using a PAM 101 fluorometer (Waltz, Germany) as described previously (*42*) and displayed on a chart recorder. Data points were obtained by digitization of chart recorder traces using Ungraph (Cambridge Soft). Kinetic analysis was carried out as described previously (*36*), and the data were fitted to a simple hyperbolic decay, $F = 1/(kt + 1/F_q) + F_u$, where k is the second-order rate constant, F_q is the amplitude of quenchable fluorescence, and F_u is the amplitude of unquenchable fluorescence. Unless stated otherwise, isolated antenna complexes were used at a final chlorophyll concentration of 2 μM.

Measurements of the absorption change occurring during the quenching reaction at different temperatures were carried out using a Cary 500 UV−visible spectrophotometer (Varian) at a spectral resolution of 1 nm as in Wentworth et al. (*34*). Temperature was controlled using an attached Peltier system. The sample buffer was equilibrated at the desired temperature before the addition of sample and induction of quenching. Measurements of the changes in both the chlorophyll and protein CD that occurred during quenching at different temperatures were carried out using a J810 (Jasco) spectropolarimeter (*34*). Temperature was maintained by an attached PFD425S Peltier system, and spectra were recorded at a resolution of 2 nm. After sample addition quenching was allowed to proceed until a steady-state fluorescence yield was achieved (~30−60 s after induction depending on the complex) before absorption and CD spectra were measured. Activation energies for the quenching reaction were calculated by fitting the rate data to the Arrhenius equation, $k = \ln[A - (E_a/RT)]$, where k is the rate constant for the quenching reaction, A is the preexponential factor, R is the gas constant, T is the absolute temperature, and E_a is the activation energy.

RESULTS

Characterization of an Intact Monomeric LHCII Preparation Produced by PLA Treatment. It has been well documented that digestion of the phospholipid bound by trimeric LHCII with PLA leads to disassociation of the complex, releasing the individual LHCII subunits (*12*, *13*, *16*, *39*, *40*). Figure 1 demonstrates the effectiveness of the procedure used here. As found previously with this method, monomerization results in no detectable release of chlorophyll (*16*). In fact, the level of free pigment in the PLA-digested sample was much less than in the undigested control, as seen in a sucrose gradient profile (Figure 1B), and this is entirely due to differences in carotenoid content. Table 1 shows the pigment composition of the free pigment band from untreated and PLA-treated LHCII. Upon monomerization, there was little change in the lutein content of the free pigment zone, but the level of neoxanthin increased approximately 2.5-fold and the (Vio + Anth) content decreased 33%.

The carotenoid composition of LHCII trimers and monomers was different, as found before (*11*). There was an increase in carotenoid content accounting for the difference in the free pigment band, but most striking was the change in carotenoid composition. There was an increase of almost 2-fold in the xanthophyll cycle content in the monomers, accompanied by a clear reduction in the content of neoxanthin. There was no change in the chlorophyll *a* to chlorophyll *b* ratio.

As found previously (*12*, *13*, *16*, *43*), monomerization led to characteristic changes in the absorption spectrum. Figure 1C shows a comparison of the unnormalized absorption spectra of LHCII trimers and monomers. There was a striking

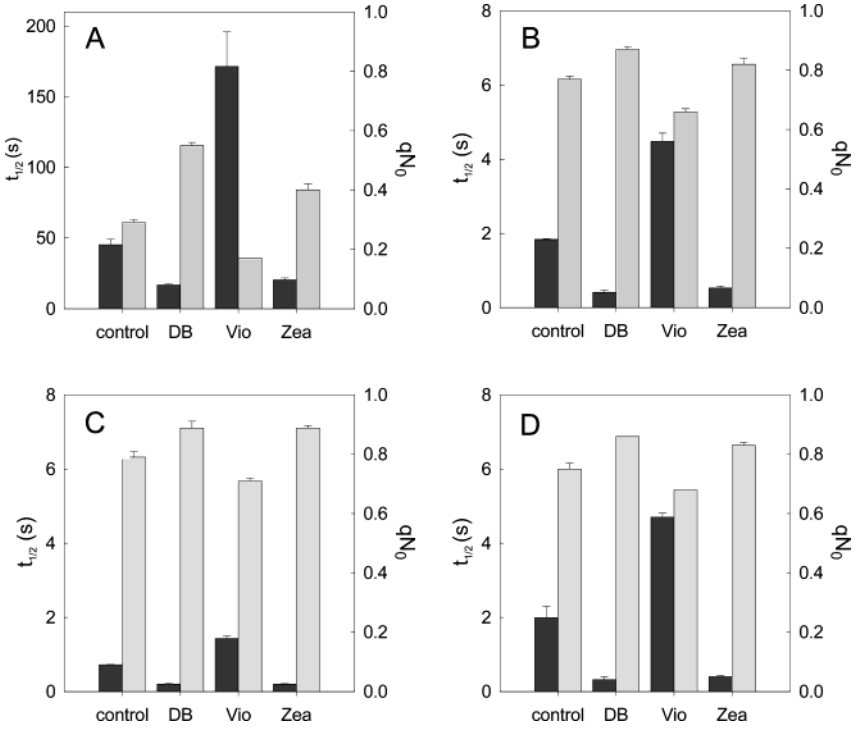

FIGURE 3: The half-time and maximum potential quenching achieved by (A) trimeric LHCII, (B) monomeric LHCII, (C) CP29, and (D) CP26. Data for trimeric LHCII, CP26, and CP29 represent control data collected as described previously (*36*). $\tau_{1/2}$, quenching half-time (dark gray bars) calculated as $1/(F_q k)$; qN_0, maximum potential quenching (light gray bars) calculated as $F_q/(F_q + F_u)$; DB, 100 μM dibucaine; Vio, 20 μM violaxanthin; Zea, 20 μM zeaxanthin. Errors represent the standard error from the mean and were calculated from at least six independent quenching curves.

decrease in the chlorophyll *a* absorption around 675 nm following monomerization and an increased absorption around 660 nm. As illustrated by the difference spectra (lower panel) in the Soret region, there were changes in the absorption of the carotenoids, with a loss of absorption centered around 511 nm and a clear increase at 495 nm. In the red region of the spectrum three new bands appeared in the difference spectrum following monomerization. A negative band was clearly apparent centered around 675 nm, flanked by two positive bands at 662 and 686 nm.

In Vitro Chlorophyll Fluorescence Quenching Characteristics of LHCII Monomers. Figure 2A shows a typical chlorophyll fluorescence trace for trimeric and monomeric LHCII following dilution into detergent-free buffer. For trimeric LHCII dilution into detergent-free medium initiated quenching and led to a 20% decrease in the fluorescence yield after 20 s. However, as found previously (*16*) under exactly the same conditions, quenching in the monomer led to an 80% decrease in its fluorescence yield. As can be seen in the inset figure, there was no significant change in the maximum fluorescence yield of LHCII following monomerization.

It has been shown that the kinetics of quenching can be fitted to a second-order reaction (*36*, *44*, *45*). It is evident from the linear reciprocal plots presented in Figure 3B that monomerization of LHCII does not affect the second-order nature of the reaction. Additionally, since the gradient of the reciprocal plot represents the rate of reaction, it was possible to compare the rates of quenching of trimeric and monomeric LHCII with those of other antenna complexes. The dashed lines in Figure 2B represent the linear regressions of second-order plots and therefore the rates of reaction for untreated LHCII, CP26, and CP29. As expected, the rate of quenching for trimeric LHCII is almost identical to that shown for the untreated LHCII. However, while the quenching reaction remains second order following monomerization, the rate and depth of quenching increased dramatically, becoming almost identical to that of the minor antenna complex CP26 but much slower than that observed for CP29.

A variety of agents strongly affect the kinetics of the quenching reaction of antenna complexes in vitro when added to the reaction mix (*36*, *37*, *40*, *46*, *47*). Figure 3 shows the calculated half-time and maximum level of quenching for LHCII trimers (A), LHCII monomers (B), CP29 (C), and CP26 (D) in the presence of the quenching modulators dibucaine, violaxanthin, and zeaxanthin. It is clear that the quenching modulators have the same effect on monomeric LHCII (Figure 3B) as they do on the other antenna complexes (Figure 3A,C,D); i.e., the quenching activators dibucaine and zeaxanthin decreased the half-time and increased the total level of quenching, while the inhibitor violaxanthin had the opposite effect. It was also apparent that the effect of the modulators on monomeric LHCII was almost identical to that shown by CP26 when data were analyzed by the *T*-test (data not shown). This was most noticeable when the half-times of the quenching reaction were compared. In the presence of violaxanthin, the half-times were found to be 78, 4.4, 1.4, and 4.7 s for LHCII trimers, LHCII monomers, CP29, and CP26, respectively, whereas the activator zeaxanthin caused a decrease in the half-times to 20.3, 0.53, 0.2, and 0.4 s.

We have shown previously that fluorescence quenching in isolated antenna complexes is pH dependent (*11*, *36*). The extent of quenching recorded after dilution of the antenna complex into detergent-free medium can be titrated against pH (Figure 5). The titration curves for LHCII monomers and

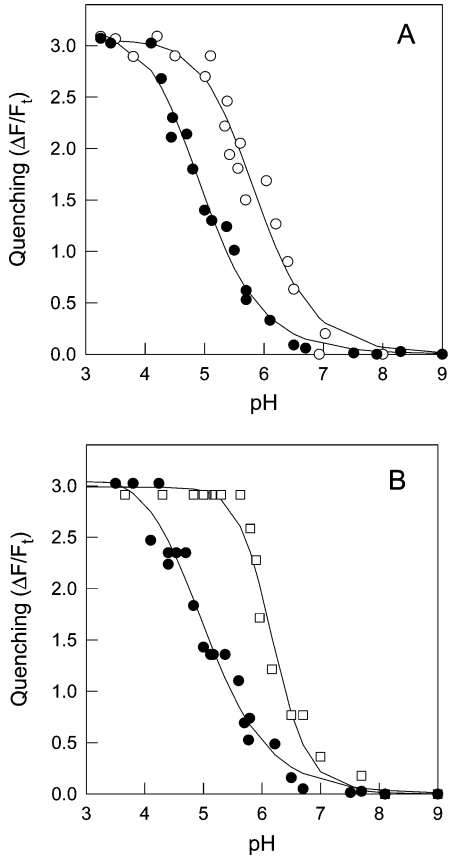

FIGURE 4: Quenching of chlorophyll fluorescence in isolated LHCII as a function of medium pH. (A) Comparison of IEF-isolated LHCII trimers (●) and CP26 (○). (B) Comparison of sucrose gradient isolated LHCII trimers (●) and LHCII monomers (□). Quenching was calculated as $(F_{max} - F_t)/F_t$, where F_{max} is the maximum unquenched fluorescent yield of the sample and F_t is the steady-state fluorescence yield measured 30 s after quenching induction.

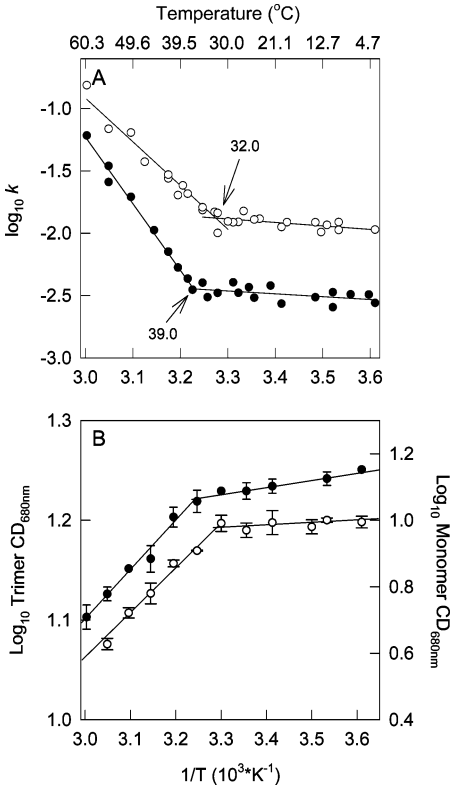

FIGURE 5: Arrhenius plots of quenching of chlorophyll fluorescence in isolated LHCII. (A) Rate of chlorophyll fluorescence quenching. Quenching was induced by dilution at each particular temperature, and the rate of quenching (k) was calculated as described in Materials and Methods for LHCII trimers (●) and LHCII monomers (○). (B) Effect of temperature on the amplitude of the CD signal at 680 nm during chlorophyll fluorescence quenching in LHCII trimers (●) and monomers (○). Data represent the mean ± SE ($n \geq 3$).

trimers are different (Figure 4A), the pK_a of the quenching reaction shifting from pH ~5.0 to pH 6.0 following monomerization. The apparent pK_a of CP26 was 6.2, and that for untreated LHCII was 5.1 (Figure 4B).

We have previously shown that fluorescence quenching can be initiated in isolated antenna complexes even at high DM concentration by the addition of the activators zeaxanthin and auroxanthin (*37*) or by lowering the pH of the medium (*36*). Under these conditions, protein aggregation is prevented and quenching is associated only with events occurring within individual complexes. Table 2 shows the level of Chl fluorescence quenching expressed as a percentage of the maximum yield 2 min after the addition of either zeaxanthin or auroxanthin. Upon addition of zeaxanthin to the complexes there was an immediate quenching of the chlorophyll fluorescence yield. In trimeric LHCII this led to a decrease of only 15−20%, CP26 and LHCII monomers showed quenching of around 40%, while quenching in CP29 was almost 50% of F_{max}. Similar results were observed upon the addition of auroxanthin with the fluorescence yield falling approximately 35% for LHCII trimers, 61% for LHCII monomers and CP26, and over 70% for CP29.

Thermodynamic Analysis of Quenching in Trimers and Monomers. Recent analysis of the temperature dependency of the quenching rate constant showed the presence of two distinct phases in an Arrhenius plot (*34*). These have activation energies around 7 and 80 kJ mol^{-1}, with a transition temperature around 35 °C. Figure 5A shows data obtained for LHCII trimers and monomers. The transition temperature was found to be higher in trimers, at 38.5 °C compared to 32 °C for monomers. The CD change associated with quenching (*34*) also revealed the same difference in transition temperature (Figure 5B).

Table 2: Effect of Zeaxanthin and Auroxanthin on Chl Fluorescence Quenching in Isolated PSII Antenna Complexes[a]

	quenching (% of total yield)	
complex	zeaxanthin	auroxanthin
LHCII trimer	19.0 ± 2.0	36.2 ± 3.0
LHCII monomer	40.5 ± 1.0	61.0 ± 2.0
CP26	41.4 ± 3.0	62.5 ± 3.0
CP29	49.2 ± 3.9	69.4 ± 1.6

[a] Quenching (%) calculated as $(F_t/F_{max}) \times 100$, where F_{max} is the initial steady-state amplitude of fluorescence and F_t is the amplitude of fluorescence recorded 2 min after addition of the carotenoid. Isolated antenna complexes were used at a final chlorophyll concentration of 2 μM; the modulators zeaxanthin and auroxanthin were used at a concentration of 20 and 10 μM, respectively. Data represent the mean ± SE ($n > 3$).

The extent of reversibility of fluorescence quenching is an indicator of the thermal stability of the complex. For both trimers and monomers, near 100% reversibility was obtained until around 50 °C (Figure 6A). Beyond this temperature, there was a sharp decline in reversibility. For trimers the temperature at which this instability arose was approximately 57 °C and for monomers approximately 47 °C. Reversibility

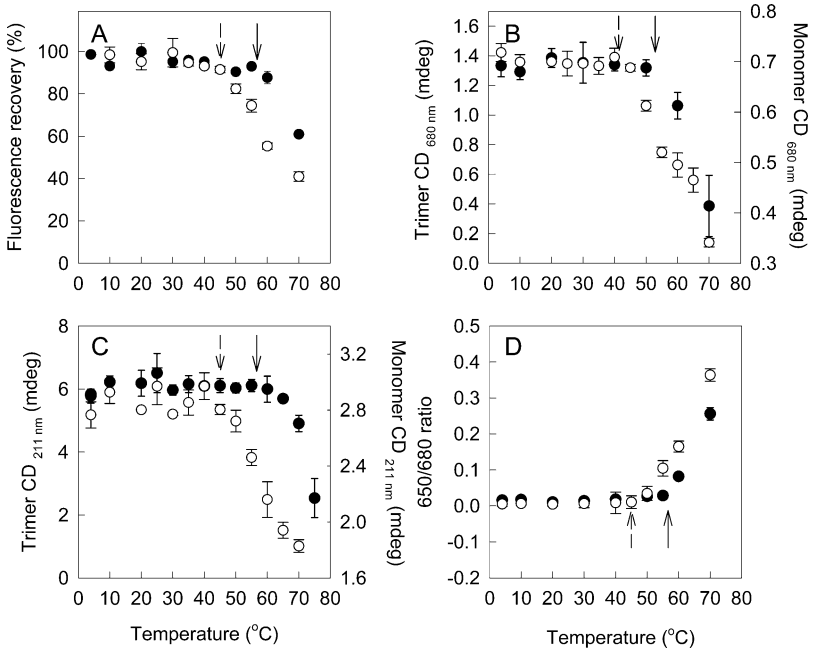

FIGURE 6: Reversibility of the spectroscopic changes in LHCII monomers (○) and trimers (●) after induction of quenching at different temperatures. LHCII was diluted into detergent-free buffer, and after 60 s the DM concentration was increased to 200 μM to test for reversal. Data represent the mean ± SE ($n \geq 3$). Fluorescence recovery (A) was measured after 2 min and percentage recovery (R) calculated as $R = 100(F_t/F_{max})$, where F_{max} is the maximum fluorescence yield of the sample in 200 μM DM and F_t is the fluorescence yield 2 min following the addition of DM. The CD changes at 680 nm (B) and 211 nm (C) and the ratio of 650/680 nm fluorescence emission measured at 77 K measured after recovery (D) were recorded. Arrows illustrate the temperature at which spectroscopic changes occur in trimers (solid arrow) and monomers (dashed arrow), respectively.

of the CD change at 680 nm showed the same temperature dependency (Figure 6B). The decrease in reversibility was associated with a loss of the CD signal at 211 nm, indicating a loss of helical structure (Figure 6C), and with in increase in fluorescence at 650 nm, from chlorophyll *b* (Figure 6D). The latter is indicative of a disruption of energy transfer from chlorophyll *b* to chlorophyll *a*, arising from disturbance of pigment binding to the denaturing protein.

DISCUSSION

Pigment Composition of Monomeric LHCII. This paper describes the production of monomeric and trimeric LHCII that have identical content of chlorophyll. As described previously (*11*, *48*) monomerization was accompanied by a loss of neoxanthin, suggesting a decrease in the strength of the neoxanthin binding site. Accompanying the loss of neoxanthin was an increase in the affinity of the complex for the xanthophyll cycle carotenoids, antheraxanthin and violaxanthin. This increase in xanthophyll binding by the monomeric LHCII is very similar to that reported for the minor antenna complexes CP26 and CP29 (*11*, *49*). One explanation for the increased xanthophyll binding is that it replaces the lost neoxanthin because of an alteration in that site. However, the total amount of carotenoid bound to the monomer also increases, indicating the increased availability or affinity of other binding sites. A peripheral binding site for xanthophyll cycle carotenoids has been identified on LHCII trimers (*11*, *49*). This site appears to be only partially occupied, the occupancy increasing under stress conditions when the xanthophyll cycle pool is enlarged (*50*). Genetic manipulation to increase the pool size similarly leads to increased occupancy of this site (*51*). The affinity of this site for xanthophyll is relatively weak, and bound pigment can be released by washing with detergent, explaining the enrichment of xanthophyll cycle carotenoids in the free pigment band obtained after sucrose gradient centrifugation (see Figure 1B). It is suggested that the affinity of this site is increased upon monomerization.

Pigment Configuration in LHCII Monomers. Distinctive differences were found between the absorption spectra of LHCII monomers and trimers, as previously described (*12*, *13*, *43*). In the Soret region a new positive band appeared at 495 nm, and two negative bands were visible at 485 and 511 nm. In vivo neoxanthin absorbs around 485 nm (*43*), and the decrease in this band is almost certainly caused by the loss of neoxanthin. It is known that the pair of luteins (Lut1 and Lut2) present in LHCII have different absorption spectra. Lut1 and Lut2 have absorption maxima at 495 and 511 nm, respectively (*43*), the latter associated with a twisted configuration identified by resonance Raman spectroscopy. Since there was no difference in the amount of lutein bound to the complex before and after monomerization (Table 1), the loss of absorption at 511 nm and increase at 495 nm in the monomers appear to arise from a change in configuration of Lut2, making it more similar to Lut1.

In the red region of the spectrum, there was a decrease in the chlorophyll absorption around 675 nm (Figure 2B). This change had been attributed to loss of chlorophyll *a* during PLA treatment (*39*), but this did not occur in our experiments (Table 1). Thus, monomerization must result in a change in the environment of certain chlorophyll molecules, leading to an alteration of their absorption characteristics. Examination of the absorption spectrum provides further support for this idea. Two positive bands at 662 and 686 nm accompany the negative band at 675 nm, and the asymmetry of the positive band at 686 nm suggests that the bands at 662 and

686 nm arise from a blue shift and a broadening of the 675 nm band. The 675 nm absorption in the trimeric LHCII is most likely from either Chl a_4 or Chl a_5 (*52*), which appear to be in close proximity to Lut2 (*53*). Thus, these differences in absorption spectrum between LHCII trimers and monomers can tentatively be attributed to a change in the Lut2/Chl a_4/Chl a_5 domain occurring upon monomerization.

Structural Basis for Altered Pigment Binding and Configuration in Trimers and Monomers. Examination of the structural model of LHCII trimers can explain the observed differences in pigment binding and configuration between trimers and monomers (Figure 7). The monomeric unit of LHCII consists of three parts, which may be designated the lutein 2, lutein 1, and neoxanthin domains. The lutein 2 domain faces the interior of the trimer, whereas lutein 1 faces the exterior. This asymmetry imposed by trimerization is absent in monomers, and this then explains why the distinctive spectral property of Lut2 is lost, and Lut2 and Lut1 become indistinguishable. The change in configuration of Lut2 may then explain the alterations in the Chl a_4 and a_5 in the lutein 2 domain.

The binding site for neoxanthin is thought to be near the C-helix (*54*, *55*). This site occurs at the interface between adjacent monomers in the trimer (indicated by an arrow in Figure 7), and the strength of binding may arise from features established from two subunits. Hence, breaking the trimer would be predicted to weaken the neoxanthin binding, as observed. The location of the violaxanthin binding site is unknown, except that it is normally regarded as "peripheral". However, this definition is based only on the ease with which endogenous violaxanthin can be removed from LHCII trimers during their isolation and the readiness with which exogenous violaxanthin interacts with the complexes. The increased strength of violaxanthin binding to the monomer may therefore originate from any of the minor changes in conformation that occur upon monomerization.

Chlorophyll Fluorescence Quenching. Previous work has shown that trimeric LHCII always quenched more slowly than the monomeric minor antenna complexes, and the final level of quenching was less. These kinetic differences could arise from the differences in amino acid sequence and the amount of bound chlorophyll. However, here we have shown that the differences in quenching can be fully explained by the effect of trimerization, since following monomerization LHCII monomers quenched more rapidly and to a greater extent than trimers. The quenching kinetics of LHCII monomers were almost identical to those observed for the minor complexes, particularly to CP26 (*34*). Despite kinetic differences, the mechanism of quenching appears to be the same in the trimeric and monomeric LHCII. The kinetics of the quenching reaction could be fitted to a second-order kinetic model in both cases, and the kinetics were affected by modulators of quenching in the same way. The temperature dependency of quenching followed the same pattern: a phase with low activation energy until around 40 °C, above which a phase with higher activation energy was observed. The only difference was that the transition temperature was higher for trimers. These two phases have been suggested to arise respectively from minor changes in pigment configuration within a monomeric complex and from major reorganization of the conformation of the complex such as oligomerization (*34*).

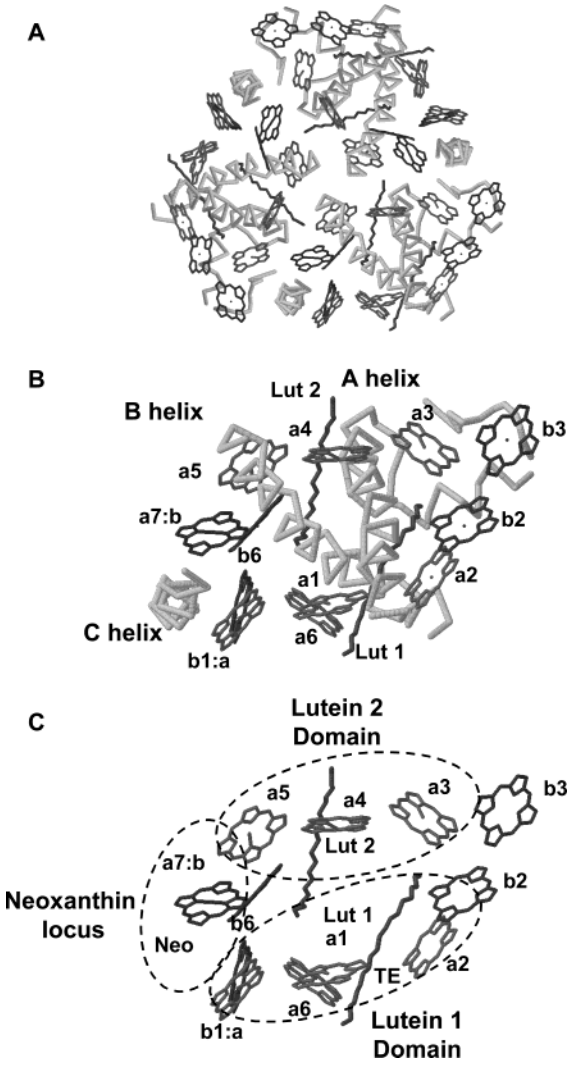

FIGURE 7: Molecular model of the LHCII trimer based on the 4 Å crystal structure (*53*) showing the location of the lutein 1, lutein 2, and neoxanthin domains. (A) Complete LHCII trimer. (B) Single LHCII monomer showing the positions of the chlorophylls, carotenoids, and α-helices. Chlorophylls are labeled on the basis of their designation in Kühlbrandt et al. (*53*). Abbreviations: Lut1 and Lut2, the positions of lutein 1 and lutein 2; Neo, the proposed position of the neoxanthin binding site as inferred from spectroscopic measurements (*54*). (C) Proposed domain structure of LHCII. For clarity the helices have been removed, and only the pigments are shown. LHCII is divided into three domains, designated lutein 1, lutein 2, and neoxanthin, each of which contains a different carotenoid. TE = the terminal emitter chlorophylls a_1 and a_2.

The differences between the quenching kinetics and temperature dependency of trimers and monomers of LHCII are best described by the stabilization of the unquenched state of the complex by trimerization. Examination of the pH dependency of the quenching reaction is also consistent with this notion. In both CP26 and LHCII monomers, the pK_a of the quenching reaction showed an alkaline shift of approximately 1.0 pH unit compared to that of the trimer. Since protonation is the driving force for the conformational change responsible for quenching, it appears that monomerization of LHCII reduces this requirement. A lower pH is required to cause the conformational change when the complexes are in the trimeric state.

What might be the structural basis for the reduction in quenching potential of the trimeric state? We suggest that

the configuration of the lutein 2 domain, which is clearly different in monomers and trimers, may provide the explanation. Recently, we have provided evidence that Chl a_1 and Chl a_2 in the lutein 1 domain form the site of quenching within LHCII (*34*). We now extend this description of the structure/function of LHCII by suggesting that the lutein 2 domain interacts with the lutein 1 domain. In the trimer, the conformation of the lutein 2 domain is different than in monomers, and we suggest that this trimeric conformation restricts the conformational flexibility of lutein 1 domain, stabilizing the unquenched pigment configuration. The lutein 2 domain therefore provides a site of allosteric control of quenching in LHCII.

Physiological Significance of Trimers. The data obtained here point to two different explanations of the role of LHCII trimerization. First, it was shown that trimers are more resistant to heat-induced denaturation than monomers, there being a 10 °C difference in the temperature at which denaturation is started. This increased stability may be important in making the photosynthetic membrane more stable at high physiological temperatures. Second, and probably more important, the data suggest that trimerization is necessary to optimize light harvesting. Stabilization of the unquenched state by trimerization is clearly an adaptation to increased efficiency of light harvesting. Thus, the molecular architecture of the PSII antenna, in which LHCII trimers are precisely assembled into supercomplexes, which then form the structural unit of the grana membranes, is the optimum structure, with the appropriate level of structural flexibility for function in low and high light (*14*). The importance of the trimeric state is best illustrated by the fact that when LHCII trimers are removed by genetic manipulation, they are replaced by another complex, which itself is assembled into trimers (*56*). The fact that this complex is CP26 is of course consistent with the strong sequence similarities between Lhcb1/2 and Lhcb5 (*2*). Moreover, this similarity explains the almost identical behavior of LHCII monomers and CP26. In evolutionary terms, it is likely that LHCII trimers evolved from a CP26-like monomer as an adaptation to provide more efficient light harvesting, the monomers having their origins in a family of stress-related proteins such as PsbS, ELIPS, and HLIPS.

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BI034975I