Thermooptic Effect in Chloroplast Thylakoid Membranes. Thermal and Light Stability of Pigment Arrays with Different Levels of Structural Complexity[†]

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Received July 11, 2000

ABSTRACT: In chloroplast thylakoid membranes, chiral macrodomains, i.e., large arrays of pigment molecules with long-range chiral order, have earlier been shown to undergo light-induced reversible and irreversible structural changes; such reorganizations did not affect the short-range, excitonic pigment−pigment interactions. These structural changes and similar changes in lamellar aggregates of the main chlorophyll *a*/*b* light-harvesting complexes exhibited a linear dependence on the intensity of light that was not utilized in photosynthesis. It has been hypothesized that the light-induced rearrangements are driven by a thermooptic effect, i.e., thermal fluctuations due to the dissipation of excess excitation energies [Barzda, V., et al. (1996) *Biochemistry 35*, 8981−8985]. To test this hypothesis, we have utilized circular dichroism (CD) spectroscopy to investigate the structural stability of the chiral macrodomains and the constituent bulk pigment−protein complexes of granal thylakoid membranes against heat and prolonged, intense illumination. (i) In intact thylakoid membranes, the chiral macrodomains displayed high stability below 40 °C, but they were gradually disassembled between 50 and 60 °C; the thermal stability of the chiral macrodomains could be decreased substantially by suspending the membranes in reaction media that were hypotonic or had low ionic strength. (ii) The chiral macrodomains were also susceptible to high light: prolonged illumination with intense white light (25 min, 2500 μE m^{-2} s^{-1}, 25 °C) induced similar, irreversible disassembly to that observed at high temperatures; in different preparations, lower thermal stability was coupled to lower light stability. (iii) The light stability depended significantly on the temperature: between about 5 and 15 °C, the macrodomains in the intact thylakoids were virtually not susceptible to high light; in contrast, the same preillumination at 35−40 °C almost completely destroyed the chiral macrodomains. (iv) As testified by the excitonic CD bands, the molecular organization of the pigment−protein complexes in all samples exhibited very high thermal stability between about 15 and 65 °C, and virtually total immunity against intense illumination. These data are fully consistent with the hypothesis of a thermooptic effect, and are interpreted within the frame of a simple model.

A basic feature of the photosynthetic system of higher plants is its ability to be regulated by short-term variations in the external environmental conditions such as temperature and illumination. This is achieved by multilevel regulatory processes, in which structural rearrangements in the thylakoid membranes evidently play an important role (*1*). Upon redox-regulated reversible phosphorylation of membranes, a subset of LHCII[1] is transferred from the stacked to the unstacked region of the thylakoid membranes [LHCII is the main chlorophyll (Chl) *a*/*b* complex of photosystem II (PSII)]. This mechanism controls the relative light-harvesting capability of the two photosystems, which can vary due to spectral variations of the absorbed light (*2*, *3*). When the illumination experienced by a plant exceeds the level that can be used in photosynthesis, protective mechanisms are induced, which prevent this excess from causing sustained damage to the plant. In thylakoids exposed to high light, different components of nonphotochemical quenching of the singlet excited states of Chl *a* can down-regulate the photosynthetic energy conversion. The short-term (1−2 min) component of the regulated energy dissipation has been shown to depend on ΔpH and on the conformational changes affecting the aggregation state of the LHCII in vivo (*4*−*6*). Mutants lacking the PsbS protein of PSII have recently been isolated with a significantly reduced capability for nonphotochemical quenching (*7*). These mutants lack ΔA_{535}, the ΔpH- and zeaxanthin-dependent conformational changes that are detected as light-induced reversible light-scattering transients. Significant structural rearrangements involving dissociation and movement of LHCII have also been induced by heat

[†] This work was supported by grants from the Hungarian Research Foundation, OTKA (T019226 and T030324).

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

10.1021/bi001600d CCC: $19.00
Published on Web 11/10/2000

treatment of thylakoid membranes (*8*). During the long-term acclimation of plants, the LHCII content of the membranes often changes, which in turn affects the ultrastructure of the granum (*9*).

Mainly through the use of circular dichroism (CD) spectroscopy, PSII and LHCII in the granum have been shown to form chirally organized macrodomains, large arrays with diameters of several hundred nanometers and long-range chiral order (cf. *10*). These chiral macrodomains have also been shown to be capable of undergoing light-induced reversible structural changes (*11*). These structural reorganizations were confined to the macroorganization of the complexes, and as shown by the invariability of the short-range, excitonic CD, did not affect the organization of the pigment molecules in the pigment−protein complexes that constituted the macrodomains. These rearrangements were originally thought to be driven by proton and other ion gradients generated by the photochemical apparatus. More recently, however, they have been proved to be largely independent of the photochemical activity of the membrane. Further, isolated lamellar aggregates of LHCII have been shown to possess the ability to undergo similar structural rearrangements in the chiral macroorganization of the pigment molecules (*12*, *13*). These findings ruled out the possibility that the structural changes are driven by a feedback mechanism from the photosynthetic electron transport. The facts that the rearrangements of the chiral macrodomains in thylakoid membranes exhibited a nearly linear light intensity dependence above the saturation of photosynthesis and that the reversible structural changes were also accompanied by marked alterations in the yield of Chl *a* fluorescence (*12*) strongly suggested that the reorganizations in the chiral macrodomains were linked to the adaptation of plants to high light and can therefore play a role in the protection of plants against excess light. Moreover, in lamellar aggregates of LHCII, both the structural changes (Vianelli, Barzda, Jennings, and Garab, unpublished) and the fluorescence quenching transient, which was also associated with structural rearrangements, depended linearly on the light intensity (*14*). These observations clearly revealed that there is a physical mechanism that can regulate the dissipation of the absorbed excitation energy in the macroarray of LHCII, above the saturation of photosynthesis without direct involvement of the photochemical apparatus.

As concerns the physical mechanism, it has been proposed that the reorganizations in the chiral macroarray of complexes are driven by a thermooptic effect (*12*). This hypothesis postulates that thermal fluctuations induced in the sample by dissipation of a proportion of the absorbed photons affect the macroorganization of the complexes. More specifically, *T*-jumps near the dissipating centers are thought to induce changes in the association of the complexes, but not in the short-range pigment−pigment interactions. In other terms, they are assumed to perturb the long-range chiral order of the chromophores, while exerting virtually no effect on the molecular organization of the individual complexes that constitute the macrodomains. In the present paper, we have inspected this hypothesis by testing experimentally some of the predictions based on the assumptions involved. To characterize the changes in the organization of the chiral macrodomains and of the constituent complexes, CD spectroscopy was used. CD is a very sensitive tool for the detection of changes at different levels of structural complexity. In particular, CD has been shown to provide unique structural information on both the molecular organization of the individual complexes and their macroassembly (*10*). Inside the complexes, split conservative CD bands originate from short-range excitonic interactions. Chiral macrodomains, i.e., densely packed, large arrays of pigment−protein complexes, can give rise to very intense, anomalously shaped, CD bands that carry information on the long-range chiral order of the chromophores.

If the hypothesis concerning the effect of thermal fluctuations on the chiral macrodomains is correct, the thermal stability of these macrodomains must be expected to be substantially weaker than that of the pigment−protein complexes. This can be tested by analyzing the CD spectra of different preparations via measurement of the irreversible structural changes that can be induced by elevating the temperature. Further, it may be expected that samples with a lower heat stability will exhibit a lower stability in light. To test this, the thermal stabilities of different samples must be compared with their light stabilities, which can be characterized most conveniently by the extent of the irreversible disassembly of the macrodomains, that is induced by prolonged illumination with high light (*15*). Taking into account the proposed role of *T*-jumps, it can also be expected that, below a certain temperature, the thermal fluctuations will not be able to induce structural changes in the sample. This can be tested by measuring the light sensitivity of the chiral macroorganization as a function of temperature. In the present paper, we report on the results of these tests and provide a simple model that accounts qualitatively for the basic experimental observations.

EXPERIMENTAL PROCEDURES

Isolation of Thylakoid Membranes. Thylakoid membranes were isolated from 2-week-old pea (*Pisum sativum*) grown in the greenhouse by a procedure described earlier (*16*). The membranes of 1−2 mg of Chl/mL were suspended in a medium containing 350 mM sorbitol, 5 mM $MgCl_2$, and 30 mM Tricine (pH 7.8), and stored on ice in the dark until use within 4−6 h after the isolation. Aliquots from this stock were suspended in the same medium (intact thylakoids) or washed once and suspended in Tricine buffer (hypotonic, low-salt) or in media which did not contain sorbitol (hypotonic) or $MgCl_2$ (low-salt).

Circular Dichroism Measurements; Temperature Dependence and Preillumination Protocols. CD was measured in a Jobin Yvon CD6 dichrograph. The Chl content of the samples was adjusted to 10 μg/mL. The optical path length of the cell in a thermostated sample holder was 1 cm, and the distance of the sample from the photomultiplier was 5 cm. The spectra were recorded in 1 nm steps with an integration time of 0.3 s and a band-pass of 2 nm.

CD was measured in absorbance units. However, for easier comparison, data are plotted in relative units: in each series, the intensity of the main band at around 690 nm at 20 °C was taken as 100; in membranes suspended in hypotonic, low-salt medium, when this band was essentially absent, the CD is plotted in relative units, compared to intact thylakoids.

In the protocol for the measurements of thermal stability, the samples were preincubated in a water bath for 5 min in

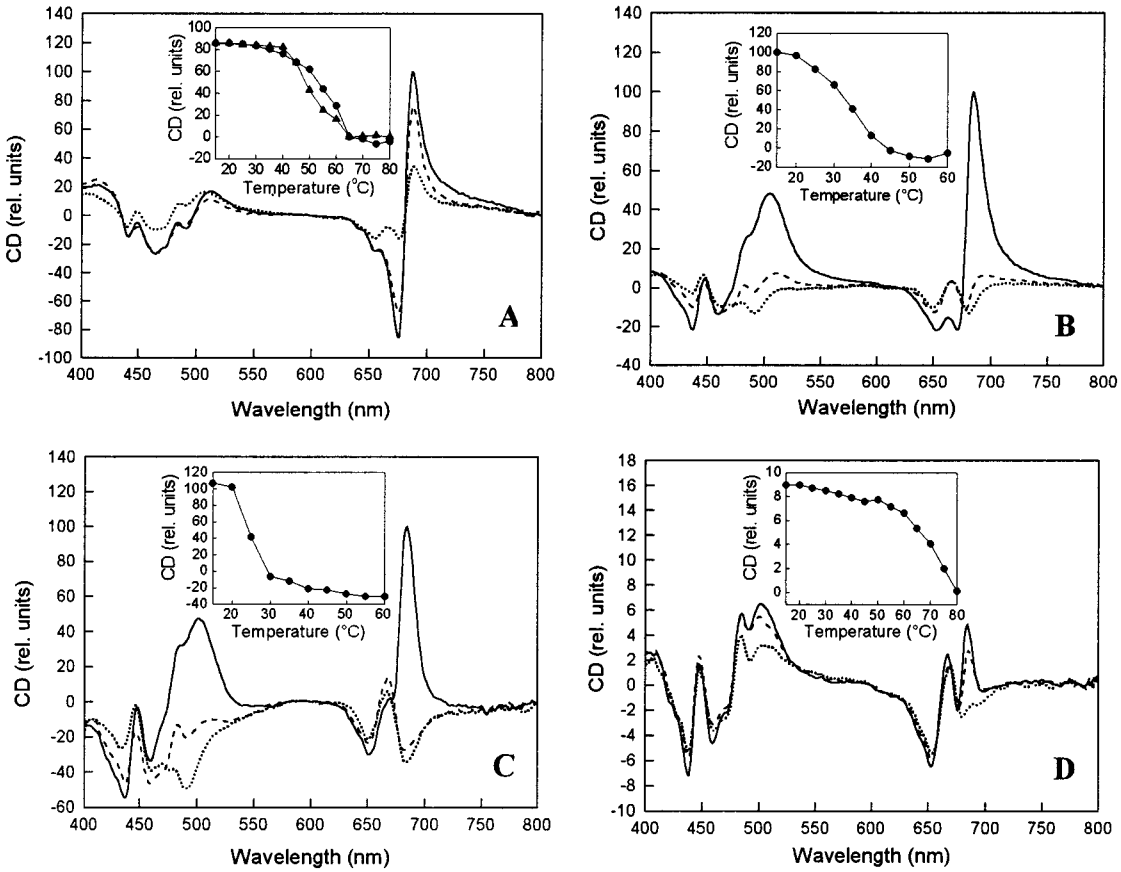

FIGURE 1: CD spectra of pea thylakoid membranes at different temperatures, and the temperature dependence of characteristic CD amplitudes (insets). (A) Intact thylakoids (in 30 mM Tricine, pH 7.8, 350 mM sorbitol, and 5 mM $MgCl_2$). (B and C) Thylakoid membranes suspended in a hypotonic and a low-salt medium, in the absence of sorbitol and $MgCl_2$, respectively, and (D) in 30 mM Tricine (pH 7.5). Continuous, dashed, and dotted lines, spectra recorded at 20, 45, and 60 °C, respectively. Insets: (A) $CD_{689-750}$ (●) and $CD_{600-676}$ (▲); (B and C) $CD_{684-750}$; (D) $CD_{667-653}$.

the dark at each temperature between 15 and 80 °C, and measured at the same temperature. Since the gross changes in the CD amplitudes were irreversible, especially at higher temperatures, essentially the same results were obtained if the measurements were performed at room temperature.

Each experiment was repeated on at least three independent batches, with identical tendencies and similar results. However, the thermostabilities of preparations from different batches of thylakoids differed somewhat from each other, as indicated, for example, by the variations in the transition temperature in a relatively broad (5−10 °C) interval. (The transition temperature for a given CD band is defined as the temperature where the amplitude decreases to 50% of its value at 20 °C.)

In the protocol for light stability, we compared the irreversible effects induced in the sample by intense white light (25 min, 2500 μE m^{-2} s^{-1}). In this series, the samples were thermostated at 25 °C. After the illumination period, the samples were incubated for 5 min in the dark at the same temperature, and then measured in the dichrograph at room temperature.

In the measurements relating to the temperature dependence of the light stability, the illumination (25 min, 2500 μE m^{-2} s^{-1}) was performed in a water bath thermostated between 5 and 75 °C, and the CD was then measured after a 5 min dark-adaptation at room temperature. In the dark-adapted control, the variations in the CD amplitudes between 5 and 40 °C did not exceed 10%; these were neglected when the magnitudes of the light-induced irreversible CD changes were determined at different temperatures.

RESULTS AND DISCUSSION

Thermal Stability of the Macrodomains and the Constituent Complexes. As may be seen in Figure 1A, the CD spectrum of intact granal thylakoids contains intense psi-type bands at around (+) 689 and (−) 676 nm, and long tails due to differential scattering outside the principal absorbance bands (psi, polymer, or salt-induced). This type of CD has been shown to originate from LHCII-containing chiral macrodomains, macroarrays of PSII, and LHCII ($d \sim 200-400$ nm) (*10*, *17*, *18*). Below 40 °C, the amplitude of the psi-type bands exhibited very little sensitivity on the elevation of the temperature. Above this temperature, however, the psi-type bands rapidly diminished. At 60 °C, although some psi-type features were retained, the CD was predominated by the excitonic bands, which can be seen, for instance, at around (+) 450 and (−) 650 nm. The excitonic bands disappeared only at around 65−70 °C; as a result, a weak, broad (−) 675 nm band appeared, which evidently originated from the intrinsic chirality of the Chl molecules (data not shown). These data indicate that, in accordance with our expectations, the sensitivities of the psi-type and excitonic bands, i.e., the heat stabilities of the chiral macrodomains and the constituent complexes, differ significantly from each other.

As concerns the psi-type bands, the amplitude of the (−) 676 nm band, measured as $CD_{676-600}$, decreased more sharply than that of the (+) 689 nm ($CD_{689-750}$) band. The transition temperatures, CD at half-maximum value, were found at around 50 and 55 °C, respectively. Since the former band has been preferentially associated with stacking (*11*), this may indicate that the disassembly of the macrodomains is initiated by a destacking of the membranes. This sounds plausible because, without destacking, the lateral movement of the complexes and/or particles would be largely restricted.

We have earlier shown that two external factors influence the long-range chiral order of the chromophores in granal thylakoid membranes: (i) electrostatic screening by divalent cations, which facilitates the stacking of membranes, and (ii) the osmotic pressure of the medium, which influences mainly the lateral packing density of the complexes (*16*). It is reasonable to assume that these factors also influence the heat stability of the chiral macrodomains. Indeed, following a hypotonic treatment of the membranes, i.e., washing and suspending intact thylakoids in the absence of sorbitol, but in the presence of 5 mM $MgCl_2$, the transition temperature was shifted to about 35 °C, and the long-range chiral order of the chromophores was already lost at around 45−50 °C (Figure 1B). An even larger heat sensitivity was seen in membranes that were suspended in an isotonic medium (350 mM sorbitol) but at low ionic strength. In this sample, the transition temperature was found at around 25 °C, and the chiral macrodomains were already disassembled at around 30−35 °C (Figure 1C).

For all samples, the excitonic CD bands appeared essentially invariable to the variations in temperature in the range between 15 and 60 °C. This was confirmed by measuring the thermal sensitivity of CD in thylakoid membranes suspended in hypotonic, low-salt medium (Figure 1D). Under these conditions, discounting some small contributions from psi-type bands at around (+) 689 and (+) 510 nm, the CD can be attributed to the weighted sum of the spectra due to the individual complexes, in which short-range pigment−pigment interactions give rise to excitonic bands (*10*). In these samples, most bands indeed exhibited only very small variations with the temperature (Figure 1D, inset).

These data reveal that the thermal stability of the chiral macrodomains is considerably lower than that of the pigment−protein complexes, which is consistent with our hypothesis. Further, they demonstrate that the susceptibility of the macrodomains to elevated temperatures cannot be directly correlated with any sizable instability in the constituent complexes. It must be stressed, however, that alterations may (and are likely to) occur at the level of the constituent particles, but these clearly do not noticeably affect the pigment−pigment interactions.

Light Stabilities of Samples with Different Thermal Stabilities. To find a more direct correlation between the light-induced structural changes and the sensitivity of the molecular organization of the pigment system, we measured the light stabilities of different preparations. The hypothesis of the thermooptic effect in thylakoids forecasts that samples with lower heat stabilities will exhibit lower stabilities when exposed to intense illumination.

In intact thylakoids, as shown in Figure 2A, prolonged illumination with intense light (25 min, 2500 μE m^{-2} s^{-1})

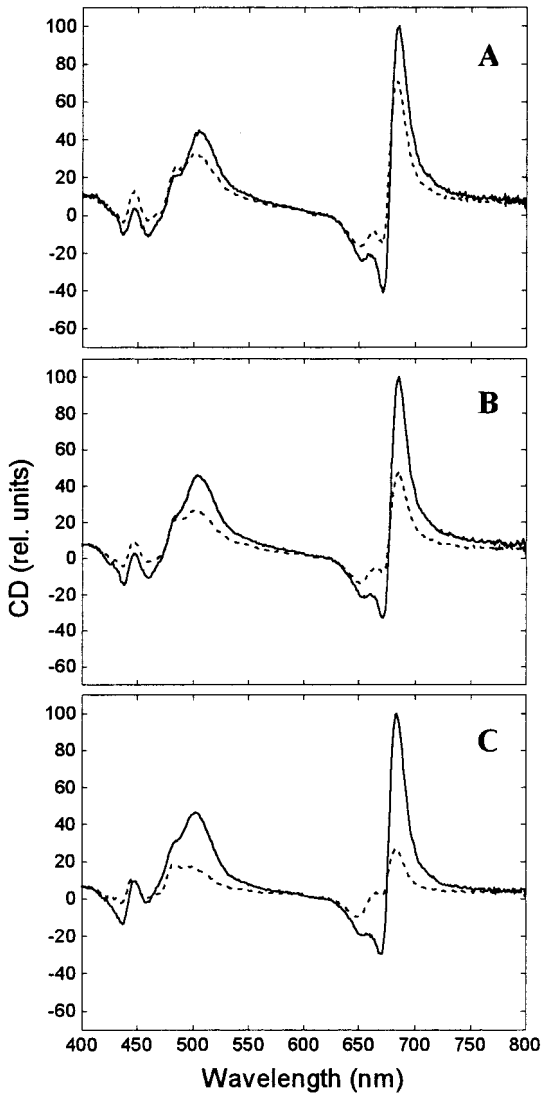

FIGURE 2: Effect of prolonged, intense preillumination (25 min, white light of 2500 μE m^{-2} s^{-1}, 25 °C) on the CD spectra of different thylakoid preparations. (A) Intact thylakoids; (B and C) thylakoid membranes suspended in a hypotonic and a low-salt medium, respectively (cf. Figure 1). Continuous and dashed lines, spectra of dark-adapted and preilluminated samples, respectively.

induced a decrease in the psi-type bands: about one-third of the amplitude of the (+) 689 nm band was lost; again, a somewhat more pronounced decrease was observed at (−) 676 nm. These data are in perfect agreement with the data published earlier, which revealed that photoinhibitory illumination led to an irreversible disassembly of the chirally organized macrodomains (*15*). As indicated by these experiments, following the photoinhibitory preillumination the domain size decreased below the threshold level of detectability, about 100 nm (cf. *10*). A recent analysis of electron micrographs of negatively stained grana has shown indeed a considerable fragmentation of the large ($d \sim 300-400$ nm) domains after photoinhibitory treatment of the membranes (*19*). The same analysis revealed some alterations in the structure of PSII particles. These latter modifications were not resolved in our measurements [data not shown, see (*15*)]: membranes suspended in a hypotonic, low-salt medium suffered almost no changes in their CD spectrum, which was dominated by the excitonic bands of LHCII (*10*).

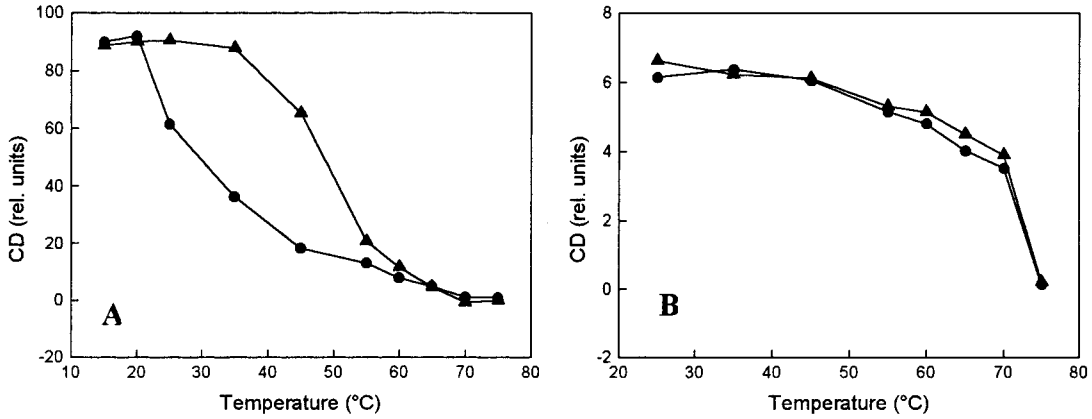

FIGURE 3: Effect of prolonged, intense preillumination (25 min, white light of 2500 μE m^{-2} s^{-1}, 25 °C) on characteristic CD amplitudes of thylakoid membranes at different temperatures. (A) $CD_{690-670}$ in intact thylakoids; (B) $CD_{446-460}$ in thylakoid membranes suspended in 30 mM Tricine (pH 7.5); (●)dark; (▲), preilluminated.

As pointed out above, within the frame of the thermal fluctuations induced by dissipation of the excess excitation energy, it can be expected that, if the heat stability of a sample is lower, it will be easier to induce structural changes by light. Indeed, removal of the sorbitol from the suspending medium, which resulted in a substantial (about 20 °C) shift in the transition temperature (cf. Figure 2B), was accompanied by a substantial increase in the extent of the light-induced irreversible changes. The loss of the main CD band was more than 50%, as compared with about 30% in the control (Figure 2B). The removal of Mg ions, which shifted the transition temperature even more, resulted in an even more dramatic increase in the sensitivity of the chiral macroorganization of the complexes, which was manifested as an almost complete loss of the psi-type bands in high light (Figure 2C). Unreported experiments have disclosed (Barzda and Garab, unpublished) that the extent of the irreversible changes gradually increases in response to a gradual decrease of the concentration of $MgCl_2$ in the suspension medium. It has also been shown that the changes develop gradually during the illumination period.

It must be stressed that in these experiments the temperature of the sample was kept constant. Furthermore, even without thermostating of the cell, the temperature of the sample rose only very moderately. Thus, a direct heat effect of the light could be ruled out.

Temperature Dependence of the Light-Induced Irreversible Changes. The hypothesis of thermal fluctuation allows the prediction that the light sensitivity of the macrodomains must be temperature-dependent. In simple terms, this is expected because toward lower temperatures, the significance of thermal fluctuations becomes negligible relative to the transient temperature. Thus, it is expected that, below a certain temperature, intense preillumination will not be able to raise the local temperature sufficiently. Vice versa, at higher temperatures, but still in the range of high thermal stability, the effect is expected to increase.

Indeed, as shown in Figure 3A, the magnitude of the irreversible light-induced changes (25 min, 2500 μE m^{-2} s^{-1}) depends strongly on the temperature. Below 15−20 °C, no or only minor irreversible changes could be induced in intact thylakoids. In contrast, at higher temperatures, despite the considerable thermal stability of the sample in the dark, the amplitude of the main CD bands could easily be decreased. While the psi-type bands were stable up to 40 °C in the dark, in the light the transition temperature, as shown by $CD_{690-670}$, was reached at around 30 °C. [In this representation, i.e., by plotting the sum of the two main psi-type bands, the difference in behavior between the two bands is not resolved. Again, the (−) 670 nm band was more sensitive than the (+) 690 nm band, as indicated by the transition temperatures at around 25−30 °C and somewhat below 35 °C, respectively (data not shown).] In broad terms, the substantial, 15−20 °C shift in the transition temperature can be instructive as concerns the magnitude of the thermal fluctuation in the membranes. Although the *T*-jump cannot be calculated from this shift, it may be concluded that the effect of heat on the macroorganization can be 'replaced' by intense, prolonged illumination of the sample. Since it may be assumed that the magnitude of the *T*-jumps depends only on the absorbance, which can be considered constant, the temperature dependence in the light can be understood in terms of an additional effect of the ambient temperature and the (effective) *T*-jump.

In this context, it is interesting to note, and is fully in line with the hypothesis, that the rate of the reversible structural changes in thylakoids (*12*), and in isolated LHCII (Vianelli, Barzda, Rajagopal, and Garab, unpublished), increased substantially with the temperature, and the changes were essentially arrested below 10 °C.

It could not be ruled out a priori that a 'combined' effect of high temperature and intense light can lead to an enhanced destabilization of the pigment−protein complexes. In fact, it has been suggested that the bleaching of bacteriorhodopsin in high light and at elevated temperature can be ascribed to a thermal dissociation of the chromophore from the protein (*20*). The reaction center complex of PSII is also known to undergo specific structural changes upon prolonged illumination with high light (*21*). However, as shown by the intensity of the main excitonic bands, the majority of the pigment−protein complexes suffered no detectable structural change (Figure 3B). Hence, albeit other effects, such as photooxidative damage of some components and cleaving by proteases, cannot be ruled out, these data show that the architecture of the bulk complexes permits a harmless energy dissipation inside the complexes, even at high temperature.

Interpretation. Model Calculations. A qualitative interpretation of our data can be presented in terms of a simple

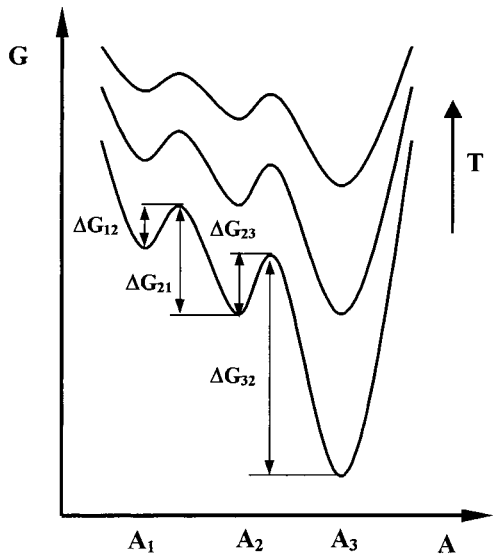

FIGURE 4: Schematic representation of the potential profile of the proposed three-state model, and its dependence on the temperature. (For further explanations, see text.)

model. The first assumption in this model is that the structural changes in the chiral macroorganization of the chromophores originate from alterations in the outer loop of the proteins. There are several examples demonstrating that changes in the outer loop of the complexes can play an important role in thylakoids and isolated LHCII. The flat surface of LHCII appears to be important in the stacking of membranes (*22*). Phosphorylation of the thylakoid membranes has been proposed to induce conformational changes in the outer loop of LHCII, which in turn leads to major structural rearrangements (*23*). In isolated LHCII, the outer loop of the polypeptide has been shown to be involved in the regulation of phosphorylation by light at the substrate level (*24*).

The model also has to satisfy the condition that the organization of the pigment molecules 'inside' the complexes is not affected. This assumption is dictated by the observations of the very high stability of the CD bands that originate from short-range, excitonic interactions (cf. also *11, 16*).

To account for the occurrence of irreversible structural changes provoked by elevated temperatures and intense prolonged illumination, let us consider a simple, three-state model:

$$A_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} A_2 \xrightarrow{k_{23}} A_3$$

A_1 is the initial state, A_3 is the irreversible state, and A_2 is the intermediate state of the macroarray; the corresponding light-induced transition rates and their dark recovery rates are indicated by k_{12}, k_{23}, and k_{21}, respectively. (Because of the irreversibility, k_{32} is not indicated.) It is assumed that the three-state model of the macroarray in thylakoids can be represented by the potential curve depicted in Figure 4. As indicated in the figure, the potential profile depends on the temperature. This can be justified by considering the gross changes in the psi-type CD as a function of temperature. It is also assumed that the potential profile depends on the ionic strength of the medium and on the osmotic pressure. Since the chiral macroorganization of the chromophores is known to be determined by these factors (*16*), this latter assumption seems plausible.

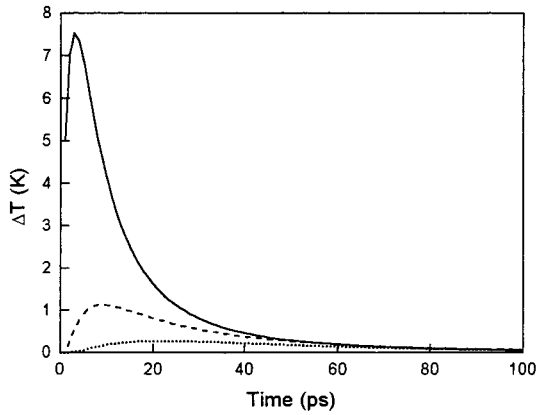

FIGURE 5: Time courses of the calculated thermal transients in a 1 nm^3 volume located at different distances from the 1 nm^3 'dissipating cell'. Center-to-center distances: 1 nm (—), 2 nm (- - -) and 3 nm (···).

The reaction rates are exponentially proportional to the corresponding energy gap: $k_{ij} = \alpha_i \exp[-\Delta G_i(T)/kT]$, where i represents the index of the different transitions, $j = i + 1$, and the α_i terms are constants. According to our model, the high stability of the macrodomains at lower temperatures (i.e., below 20 °C) and the irreversible changes at elevated temperatures can be explained by the variations in the potential profile, rather than by the temperature dependence introduced by the denominator in the exponent. Since the transition occurs in a narrow range, this assumption also seems very plausible.

For a consideration of the light-induced effects, we shall first show that, albeit for short intervals, *T*-jumps can occur in the outer loops of proteins. After giving estimates of the lengths and magnitudes of such *T*-jumps, we offer an explanation that shares common ground with the thermally induced transitions.

Upon dissipation of a photon energy in the antenna, the temperature at and near the site of dissipation transiently increases. The magnitude and time course of the *T*-jump depend mainly on the distance from the site of dissipation. The temperature transient in each point is governed by the thermodiffusion equation:

$$\frac{\partial^2 T}{\partial \mathbf{r}^2} - \frac{1}{\kappa}\frac{\partial T}{\partial t} = 0 \qquad (1)$$

In this equation, T is the temperature, $\mathbf{r}$ is the position vector, k is the thermodiffusion constant, and t is the time. The thermal equilibrium in the sample, and the infinite heat capacitance of the system, can be taken into account by setting the boundary condition to $T(\mathbf{r}, 0) = 0$ (i.e., the initial temperature of each cell is zero), and by considering the temperature of the sample zero throughout the whole process.

It can be calculated that dissipation of the energy of a 1.8 eV red photon in a volume of 1 nm^3 results in a *T*-jump of about 65 °C. (The heat capacitance of this dissipating cell is assumed to be equal with that of water.) Because of the heat conductance, this is a transient on a picosecond time scale (cf. *25*). It can also be calculated that in a 1 nm^3 volume that is adjacent to the dissipating cell the *T*-jump amounts to about 5−10 °C and lasts for about 10 ps (Figure 5).

In the simplest case in the model, we assume that the structural rearrangement is initiated by a very fast step, faster

than the *T*-jump. With this assumption, we can treat the effect of light by considering local temperature changes. If this simplifying assumption is not applicable, the effect of transient local temperature jumps must be considered in a more complex model.

Although we are not able at present to offer experimental evidence for the involvement of such an ultrafast step, some support can be given for this assumption. For example, such fast transients can involve the small displacement of cations attached to specific cation-binding sites on the outer loop of the polypeptide. It has been shown that cation movements are involved in the light-induced reversible changes both in LHCII and in thylakoid membranes (*26*). Ultimately, this type of release of cations can be linked to *T*-jumps via effects on the conformation of proteins near the cation-binding site (*25*). An analysis of the light-induced reversible fluorescence quenching in LHCII has revealed that the generation of the quenchers is a very fast process, which can compete with other de-excitation pathways (*14*). Fast quenching transients can indeed be identified in LHCII (*27*). Further possibilities for the involvement of fast steps include thermally excited transitions in the lipidic environment (*28*). Lipids have been shown to play a significant role in the light-induced reversible structural rearrangements in LHCII (*29*). There are several examples showing that protein dynamics, and random thermal fluctuations in particular, play a key role in determining the observed low rates of processes that consist of ultrafast steps; in these types of systems, the rates are ultimately determined by the conformational transitions of the proteins that contain the active groups (*30, 31*).

Within the framework of the model (Figure 4), in the dark and at around room temperature, when ΔG_{12} is much higher than *kT*, the macrodomains remain in the A_1 state. At higher temperatures, this can be shifted toward A_3, a transition that also depends on the composition of the medium (Figure 1). Upon illumination of the sample with intense light, *T*-jumps will lead to the appearance of the A_2 and A_3 states. From A_2, the system can undergo reversal to A_1 with a probability $\alpha_{21} \exp(-\Delta G_{21}/kT)$. However, consecutive photon dissipation can lead to an $A_2 \rightarrow A_3$ transition. Because of the irreversibility of this latter state, this will lead to a gradual accumulation of the A_3 state. The time scale of this process strongly depends on the frequency of *T*-jumps, i.e., ultimately on the light intensity, and also depends on the transition temperature, i.e., the heat stability of the macroassembly (Figure 2). In high light and at elevated temperature, near the dissipating sites the local temperature transiently increases. There will then be a higher probability of the structural transitions despite the heat stability of the macrodomains in the dark. Vice versa, it can be expected that below a certain temperature, the probabilities of the transitions will be very low (Figure 3). Hence, the model appears to offer a reasonable explanation for the light-induced irreversible changes and their temperature dependences. The same explanation appears to be applicable for the characteristic temperature dependence of the reversible light-induced structural changes in thylakoids (*12*) and LHCII (Vianelli, Barzda, Rajagopal, Jennings, and Garab, unpublished). In these, 'freezing' of the light-induced reversible changes has been observed at around 5−10 °C; between about 20 and 35 °C, the rates increased sharply with the increase of temperature. As will be shown elsewhere (Cseh, Papp, and Garab, in preparation), the model also offers explanation for the nearly linear light intensity dependence of these structural changes. It can thus be concluded that the basic features of the light-induced structural rearrangements in thylakoid membranes and LHCII can be explained in terms of a thermooptic effect, i.e., of thermal fluctuations due to the dissipation of excess excitation energies.

ACKNOWLEDGMENT

We are indebted to Profs. A. Scherz and L. Zimányi for helpful discussons.

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BI001600D