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Thermo-optically induced reorganizations in the main light harvesting antenna of plants. I. Non-Arrhenius type of temperature dependence and linear light-intensity dependencies

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Abstract

Thermo-optically induced structural reorganizations have earlier been identified in isolated LHCII, the main chlorophyll a/b light harvesting complexes of Photosystem II, and in granal thylakoid membranes [Cseh et al. (2000) Biochemistry 39: 15250–15257; Garab et al. (2002) Biochemistry 41: 15121–15129]. According to the thermo-optic mechanism, structural changes can be induced by fast, local thermal transients due to the dissipation of excess excitation energy. In this paper, we analyze the temperature and light-intensity dependencies of thermo-optically induced reversible and irreversible reorganizations in the chiral macrodomains of lamellar aggregates of isolated LHCII and of granal thylakoid membranes. We show that these structural changes exhibit non-Arrhenius type of temperature dependencies, which originate from the 'combination' of the ambient temperature and the local thermal transient. The experimental data can satisfactorily be simulated with the aid of a simple mathematical model based on the thermo-optic effect. The model also predicts, in good accordance with experimental data published earlier and presented in this paper, that the reorganizations depend linearly on the intensity of the excess light, a unique property that is probably important in light adaptation and photoprotection of plants.

Abbreviations: CD – circular dichroism; Chl – chlorophyll; LHC II – light-harvesting chlorophyll *a*/*b* pigment-protein complex; NPQ – non-photochemical quenching; psi – polymer- and salt-induced; PS II – Photosystem II

Introduction

The main chlorophyll *a*/*b* light harvesting complex of Photosystem II, LHCII, in addition to its primary function, absorbing light and transferring the excitation energy toward the reaction centers, is also involved in a number of short and long term regulatory mechanisms in response to changes in the environment, e.g. in the spectral composition and intensity of illumination and in the ambient

temperature (Anderson and Andersson 1988). In particular during light adaptation, plants can reversibly adjust their light harvesting capabilities. This evidently requires reorganizations in the molecular architecture of the complexes. Upon reversible phosphorylation of LHCII, e.g. due to an imbalance in the excitation of the two photosystems, a subset of LHCII is transferred from the stacked to the unstacked region of the thylakoid membranes and thus controls the relative light-harvesting capability of the two photosystems (Allen and Forsberg 2001; Aro and Ohad 2003; Lunde et al. 2000). In high light, the singlet excited state of Chl *a* can be quenched non-photochemically (NPQ), which thus down-regulates PSII. NPQ contains a short-term (1–2 min) component, q_E, which depends on the transmembrane ΔpH (Horton et al. 1996), as well as on the presence of PsbS protein (Li et al. 2000) and on the formation of zeaxanthin (Horton et al. 1999). Several observations suggest that q_I, the more sustained form of NPQ, also involves reorganization in the antenna system (Horton et al. 1996).

Although the mechanisms of these reorganizations have not been fully explored, the roles of redox regulations and the transmembrane ΔpH have been well established, and it is most widely accepted view that the structural reorganizations in the antenna system of the thylakoid membranes are regulated via feedback mechanisms through these two factors. In other terms, it is believed that the reorganizations in the antenna system are driven by 'photosynthetic functions', rather than by reactions directly in the light harvesting antenna.

There are, however, a number of observations, which show that this picture is incomplete, and the antenna complexes play more active role in adjusting their own functions, and thus in the feed-back regulation of the photosynthetic functions. It has been shown that isolated LHCII complexes exhibit light-induced reversible fluorescence quenching, which resembles to a certain extent the NPQ in chloroplasts (Jennings et al. 1991), and which is associated with structural reorganizations (Barzda et al. 1999; Grudzinski et al. 2002). It has also been shown that loosely stacked lamellar aggregates of LHCII, which retain substantial amounts of lipids, possess the ability of undergoing gross light-induced reversible structural reorganizations dramatically affecting the long-range chiral order of their chromophores (Barzda et al. 1996). These reorganizations in the chiral macrodomains have been revealed by changes in the main, psi-type circular dichroism (CD) bands; psi-type CD is given rise by the long-range chiral order of the chromophores. Similar structural changes were earlier observed in thylakoid membranes and were originally thought to be driven by transmembrane and local proton and other ion gradients (Garab et al. 1988b). It has, however, been shown that also in thylakoid membranes these reorganizations are largely independent of the operation of the photochemical apparatus of the membranes (Barzda et al. 1996; Istokovics et al. 1997), and originate from a novel, biological thermo-optic effect, structural changes provoked by fast, local thermal transients due to the dissipation of the excess excitation energy (Cseh et al. 2000). Structural reorganizations associated with the isomerization of carotenoid components have also been observed upon heat and excess light of isolated LHCII (Grudzinski et al. 2001, 2002). Consistently with the thermo-optic mechanism, light induced conformational changes affecting the proton buffering capacity of LHCII have been detected in model lipid membranes containing purified LHCII (Iwaszko et al. 2004). It has recently been shown that phosphorylation of LHCII, in addition to the well established redox control, is also regulated by light at the substrate level, both in the isolated antenna complexes and in the thylakoid membranes (Zer et al. 1999, 2003). This is due to changes in the outer loop segments of the complexes, which are provoked by the absorbed light energy and can be accounted for by a thermo-optic mechanism. The basic oligomerization state (trimers vs monomers) of LHCII has also been shown to be regulated by heat and excess light, and can be explained within the framework of a simple model of the thermo-optic effect both in the isolated complexes and in intact native systems (Cseh et al. 2000; Garab et al. 2002). The quenching, as shown on LHCII macroaggregates, depends on the size of the domains (Barzda et al. 2001). Monomerization of the complexes also affects the ability of LHCII to participate in NPQ (Wentworth et al. 2004). Monomerization is essential for proteolytic removal of LHCII (Yang et al. 2000) and thus in the acclimation of plants to high light. It has been

proposed that the oligomerization state of LHCII in plants plays an important regulatory role in variable light environment (Leng et al. 2003).

It is also interesting to note that this type of ability of structural reorganizations is not confined to higher plant antenna systems. We have shown that energy migration from the cyanobacterial external antenna, phycobilisome, to the membrane can also be regulated thermo-optically (Zsiros et al., unpublished). Recently, several reports have appeared on light-induced structural changes in other antenna complexes. For instance, substantial light-induced reorganizations, conformational jumps, have also been detected by means of microspectrometry in isolated bacterial antenna complexes (Pandit A et al. 2002; Rutkauskas et al. 2004). The mechanism of these changes has not been investigated. However, they can be accounted for by a thermo-optic origin (van Grondelle, personal communication). The above data strongly suggest that structural flexibility of the light-harvesting antennae and thermo-optically induced changes in antenna systems are of physiological significance.

The thermo-optic mechanism is based on the 'appearance' of a heat package, due to the dissipation of the excess excitation energy; this is followed by heat conductance, a spreading of the dissipated energy. Thermal transients of this origin can induce elementary structural transitions. This type of elementary structural changes can occur if around the site of dissipation there is an inherent thermal instability in the structure. In most structures, and in LHCII-containing systems in particular, there are well discernible structural changes that can be induced by elevating the temperature above the physiologically relevant temperatures but below the temperature of denaturation. The same, or very similar changes can also be induced by light (Cseh et al. 2000; Dobrikova et al. 2003; Garab et al. 2002). In contrast to the heat-induced structural changes, thermo-optic transitions are confined both temporally and spatially, and might thus result in more specific structural reorganizations. These light-induced reorganizations might be reversible or irreversible, depending on the experimental conditions.

Evidently, thermo-optically driven reorganizations differ significantly from other types of structural changes driven e.g. enzymatically or by the transmembrane ΔpH. Indeed, it has been demonstrated that they exhibit peculiar temperature dependencies, which can be accounted for by the 'combination' of the local heat and the ambient temperature (Cseh et al. 2000; Garab et al. 2002). Also, it has been demonstrated that thylakoids exhibit an apparently non-saturable, approximately linear light-intensity dependence above the saturation of photosynthesis (Barzda et al. 1996), a unique feature that is potentially important in the protection of plants against excess light. It is of interest to analyze in more detail the temperature and light-intensity dependencies, and to test experimentally the validity of some fundamental predictions on these dependencies based on the thermo-optic mechanism.

In the present work, we analyze the temperature and light-intensity dependencies of reversible and irreversible structural changes in thylakoid membranes and in lamellar aggregates of isolated LHCII. We show that thermo-optically induced structural transitions, in very good agreement with theoretical expectations, exhibit (i) non-Arrhenius type of temperature dependences, and (ii) an approximately linear dependence on the intensity of excess light. Our data, presented in this paper and published earlier, also show that the dependencies in thylakoid membranes and lamellar aggregates of the isolated complexes are very similar to each other. This will be further elaborated, and explained in terms of the recent model of LHCII-only macrodomains in granal thylakoids (Boekema et al. 2000; Dekker and Boekema 2005), in the consecutive paper (Holm et al. 2005).

Materials and methods

Isolation of thylakoid membranes and LHCII

Thylakoid membranes were isolated from either market spinach or 2-week-old pea leaves grown in the greenhouse. The isolation was carried out as described earlier and the membranes were suspended in a medium containing 5 mM $MgCl_2$, 5 mM KCl and 20 mM Tricine, pH 7.6 (Garab G et al. 1988a). Loosely stacked lamellar (type II) aggregates of LHCII were isolated from 2-week-old pea as described by Simidjiev et al. (1997).

Circular dichroism spectroscopy

CD spectra were recorded in a Jobin-Yvon CD6 dichrograph, as described earlier (Barzda et al. 1994). The chlorophyll content of the samples was adjusted to 20 μg ml^{-1}, and the optical path length was 1 cm. CD was measured in absorbance units. However, for easier comparison, data are plotted in relative units: in each series, the intensity of the strongest band (and in temperature dependence measurements at 20 °C) was taken as 100.

Light-induced reversible changes (ΔCD) were measured as described earlier in the same dichrograph equipped with a side illumination attachment (Barzda et al. 1996). CD was continuously monitored at 510 and 495 nm for thylakoid membranes and LHCII aggregates, respectively.

Results and discussion

In the subsequent paragraphs we shall analyze the mechanism of thermo-optically induced reorganizations with regard to their expected temperature and light-intensity dependencies. The analysis will be based on previously published semi-quantitative model calculations (Cseh et al. 2000; Garab et al. 2002), also summed up in the Appendix. The predictions derived from the model will be compared with experimental data published earlier on lamellar aggregates of LHCII and granal thylakoid membranes, and will be complemented with new data on the same systems.

Temperature dependencies

According to the thermo-optic mechanism, fast local thermal transients (heat jumps), which appear due to the dissipation of the excess excitation energy, can induce structural changes in the close vicinity of the site of dissipation (Cseh et al. 2000; Garab et al. 2002). Evidently, the excitation which is in excess, i.e. not utilized in photosynthesis, and is not resulting in light emission, will be dissipated. This dissipation leads to the appearance of a fast jump in the local temperature which, as pointed out earlier, can be of substantial magnitude. The lowest estimate for a red photon in a 1 nm^3 volume is nearly 70 °C (Cseh et al. 2000). The excess heat is rapidly spread in the environment, i.e. transferred to the medium of an essentially infinite heat capacity. Hence, it must be stressed, dissipation of the excess excitation energy, with the exception of extreme high intensity excitations, does not lead to an overall increase in the temperature of the sample. Nonetheless, as simple heat-conductance calculations (Cseh et al. 2000) and ultrafast absorbance transient measurements (Gulbinas, Varkonyi, Garab and Valkunas, unpublished) show, in the close vicinity of the dissipation and for a short time interval thermal transients of significant magnitude occur. Simple calculations show that in a 1–2 nm radius a thermal transient of 10–20 ps length and a magnitude of about 10 °C can occur (Cseh et al. 2000). If in this radius there is a heat-sensitive structural element elementary structural changes might occur. In the simplest case, one can assume that thermally induced overall changes in the sample (in the dark) are composed of this kind of elementary structural changes, or at least contain these structural changes. Vice versa, accumulation of thermo-optically induced structural changes during the illumination of the sample can eventually lead to overall reorganizations, which might thus resemble to a considerable extent the heat-induced transitions.

Earlier we have shown that samples of isolated LHCII and granal thylakoid membranes contain well defined heat inducible structural changes between about 45 and 65 °C, with three well discernible structural changes (Dobrikova et al. 2003). This means that these samples contain transition temperatures (T_t) that fall in the range above the physiologically relevant temperatures and below the temperature of denaturation. Heat treatments of barley leaves above 45 °C induce irreversible changes (Tóth et al. 2005), and denaturation usually occurs at around and above 70 °C (cf. e.g. Dobrikova et al. 2003). We have also shown earlier that essentially the same transitions that are inducible by elevating the temperature could also be induced by light under thermostated conditions at temperatures well below 40 °C (Cseh et al. 2000; Garab et al. 2002). Hence, local heat due to dissipation can indeed result in specific, well discernible structural changes, and they can be accumulated during the illumination of the sample.

As it also follows from the above arguments and from model calculations (Cseh et al. 2000, see also Appendix), the temperature dependency of

the thermo-optically induced structural transitions is determined by two main factors: (i) the temperature dependence associated with the transition between the initial and final states, and (ii) the magnitude of the local thermal transient at the site of the reaction (ΔT). The former is defined by the free energy gap (ΔG) between the two states, the transition temperature (T_t) and its width (T_w) (Cseh et al. 2000), while ΔT is governed by various factors, such as the exact site of the dissipation and the mechanism and time course of heat conductance on the microscopic scale. T_t and T_w, associated with the temperature dependency of the structural transition in the dark, can readily be determined experimentally, and ΔG can also be obtained on a relative scale from the fitting of the 'dark' curve. These parameters and ΔT, as a variable parameter, can be used for the fitting of the temperature dependence curve in the light (Garab et al. 2002).

In simple cases, ΔG of a given heat-inducible transient is largely independent of the temperature below and above T_t, and has a sharp decrease (small T_w) around this particular temperature. This means that the considered structure possesses two stable conformations, one below and one above T_t. We have earlier presented examples on such temperature dependencies. Chiral macrodomains are quite stable up to about 40 °C and disassemble into smaller aggregates with a T_t of about 45 °C (Cseh et al. 2000). Isolated trimers are stable below about 50 °C and exhibit a sharp transition around 55 °C; above this transition temperature, up to 65 °C, only monomers are found (Garab et al. 2002). Accordingly, in the dark near T_t, the reaction rates of the structural changes are determined by the temperature dependency of ΔG, and only outside this region they are governed by the 'usual' Arrhenius-type of $\exp(-1/kT)$ dependence.

For the calculation of the rate constants in the light, evidently, the knowledge of the ambient (basal) temperature and the temperature dependence of ΔG is not sufficient. This is because of the presence of fast local thermal transients due to energy dissipation; hence ΔT must be added to the basal temperature. Evidently, this will further complicate the temperature dependency of the reaction kinetics. Hence, it will not follow the patterns for Arrhenius-type of reactions.

Earlier, we have analyzed the temperature dependency of the heat-induced irreversible structural changes of the chiral macrodomains in isolated, type II lamellar aggregates of LHCII. We have shown that based on the thermal behavior of the chiral macrodomains their light-induced disassembly can be understood within the framework of a thermo-optic model (Garab et al. 2002). We have also shown that chiral macrodomains of granal thylakoid membranes exhibit very similar temperature dependences both in the dark and in the light (Cseh et al. 2000). Thus, we can conclude that, as concerns the irreversible changes, the basic processes and parameters in the two systems are essentially identical.

For LHCII, it has been shown that T_t, T_w and ΔG can be obtained from a fit of the temperature dependence of the irreversible disassembly of the macrodomains in the dark. Hence, we have been able to describe the reaction kinetics, i.e. the k_{ij} and k_{ji} rates ($i = 1,2$ and $j = i+1$) in the dark (Garab et al. 2002). For the light-induced irreversible disassembly of the macrodomains, a ΔT of 15 °C was introduced as a fitting parameter of the temperature dependency. In the following section, based on these results, we extend the application of the thermo-optic model for reversible changes affecting the long-range chiral order of the chromophores in lamellar aggregates of LHCII and in granal thylakoid membranes. This can be done because in the model we assumed that the ΔCD was associated mainly with the reversible state. (In the model we assume that the $A_2 \rightarrow A_3$ transition does not affect the CD amplitude. According to the model, the irreversible state, A_3, is reached via the reversible state A_2 during prolonged illumination with intense light. For shorter illumination periods and with moderate light intensities, A_3 cannot accumulate and thus the reversibility is retained.

As shown in Figure 1a, the light induced reversible changes in the psi-type circular dichroism signals (ΔCD) of LHCII exhibit a strong temperature dependency. Below about 10 °C, the changes are small and slow. In this range, quite often, it is difficult to induce any sizeable ΔCD. There is a range, usually between 15 and 30 °C, where the changes can be provoked relatively easily and the rates increase rapidly with the increase of the temperature. Above 35 °C ΔCD abruptly disappears. These data obtained on lamellar aggregates of isolated LHCII are very similar to those in granal thylakoids (Figure 1 in

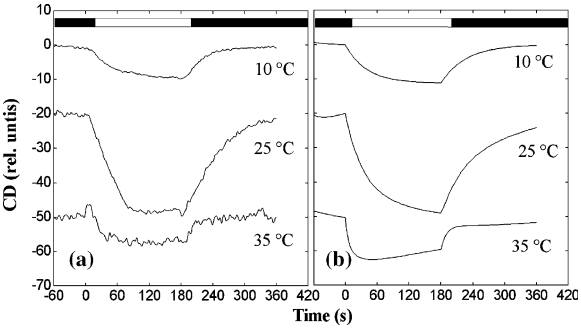

Figure 1. Light-induced reversible changes in the psi-type CD band at 495 nm of type II, lamellar aggregates of isolated LHCII at different temperatures. Experimental data obtained upon an excitation with red light of 1200 μmol of photons m^{-2} s^{-1} (a), and simulated kinetic traces using a thermo-optic model (b). Dark and light periods are indicated by filled and open bars, respectively. In the simulation, we used the set of parameters derived earlier from the temperature dependence of the main, psi-type CD signals of type II, lamellar aggregates of isolated LHCII (Figure 6 in Garab et al. 2002). Main parameters: T_t = 37 °C; ΔT = 15 °C, and the reaction rates between the two states, A_1 and A_2, k_{12} = 3, k_{21} = 980. The same reaction rates at 65 °C are 520 and 75, respectively. We also use the simple assumption that the amplitude of the psi-type CD is 100 for state A_1 and it vanishes for A_2. (The reaction rates k_{23} and k_{32} at 0 °C are 0.000196 and 0.001, and at 65 °C, 316 and 0.0153, respectively. These rate constants do not influence the reversible changes).

Istokovics et al. 1997). In fact, the data in LHCII are almost identical with those found in thylakoids. Hence, similarly to the irreversible disassembly of the chiral macrodomains in thylakoids and lamellar aggregates of LHCII, the two LHCII-containing systems bear close similarity also with regard to their reversible reorganizations, and thus can be described with the same model.

We must also stress that this ability of isolated LHCII to undergo reversible light-induced reorganization in the long-range chiral order of the chromophores is lost upon delipidation of the sample. Type IV, tightly stacked, microcrystalline aggregates of LHCII, which also possess long-range chiral order, are not capable of undergoing this type of reorganization. These samples, as tested with FTIR (Taneva et al. 1998) exhibit a high thermal stability, which explains their inability to undergo light-induced reversible structural changes (cf. also Simidjiev et al. 2004). LHCII preparations which do not contain sufficient amounts of lipids might exhibit light-induced chlorophyll degradation, as proposed by FTIR measurements (Rogl et al. 2003). However, degradation evidently cannot account for fully reversible structural changes and their strong temperature dependences in isolated LHCII and thylakoid membranes. It is also to be noted that the light-induced reversible CD changes in these samples are insensitive to oxygen (Busheva et al., unpublished). Further, it must also be pointed out that, albeit the role of lipids in these processes should not be overlooked (Simidjiev et al. 1998), such a strong and peculiar temperature dependence cannot be expected from changes in the phase behavior of lipids (Williams 1998).

The peculiar temperature dependencies in LHCII and thylakoid membranes can be explained in simple terms taking into account the thermo-optic mechanism. In the low temperature range, below a certain threshold, the transition temperature is very difficult to reach with the 'combination' of the ambient and local temperatures. Hence the reactions are very slow. Above this threshold, the probability of the transition increases rapidly with the increase of the temperature. At higher temperatures, the probability of spontaneous transitions becomes high and thus the light-induced component decreases.

As shown in Figure 1b, the kinetic traces can readily be simulated using the thermo-optic model and the parameters derived from the thermal instability and light-induced irreversible disassembly of the chiral macrodomains (Garab et al. 2002; see legend). The exact nature of the structural changes, in particular the identity of the elementary structural transitions, the site(s) of dissipation, the quantum yield etc. have not been determined. Without these parameters, any further refinement of the model seems very difficult. Nonetheless, this simulation yields a reasonably good approximation for the experimental data.

The theoretical model also gives a good fit to the observed temperature dependence of the initial rates of ΔCD (Figure 2). This dependence clearly deviates from simple Arrhenius-type of temperature dependencies, but is fully consistent with the thermo-optic mechanism of the structural changes associated with the observed ΔCD.

Light-intensity dependencies

It has been shown earlier that thylakoid membranes possess the ability of undergoing light-induced reversible structural changes affecting the long-range chiral order of the chromophores

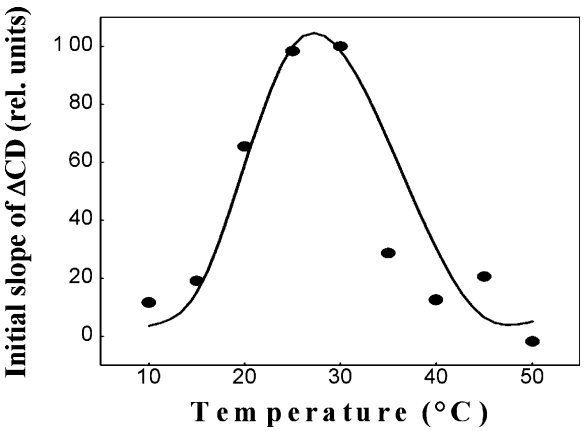

Figure 2. Initial slope of light-induced reversible changes in LHCII as a function of the ambient temperature. Data points (filled circles) represent mean values from 3 repetitions on the same batch; different batches exhibited slightly different temperature dependencies with identical trends. The curve was obtained from the thermo-optic model with the same parameters as in Figure 1.

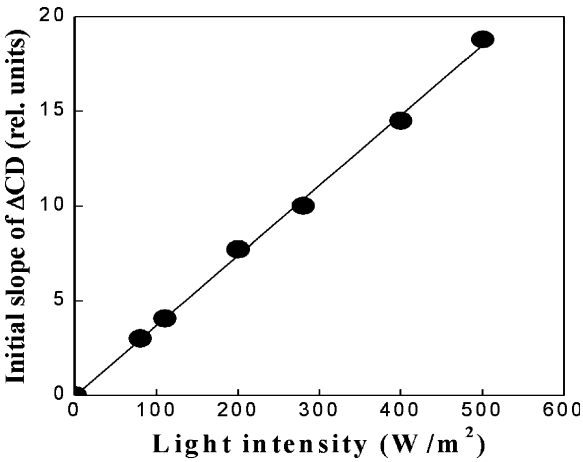

Figure 3. Light-intensity dependence of the initial rate of the light-induced reversible reorganizations (ΔCD at 495 nm induced by red light) in type II, lamellar aggregates of isolated LHCII. Experimental data (full circles) were determined from short (10–30 s) kinetic traces recorded at room temperature, with 30–60 s dark intervals between the light periods. The fitted curve (line) was obtained from the thermo-optic model presented above, which yielded a linear dependence; its slope was adjusted to match the data points. The slope varied from batch to batch but the (approximately) linear relationship was retained in all experiments. For the same sample, the variation of the slope was smaller than 10%.

(Garab et al. 1988a). It has also been revealed that the rates of these reorganizations, measured as the initial rate of ΔCD, exhibit an approximately linear light-intensity dependence above the saturation of photosynthesis (Barzda et al. 1996). This unexpected structural flexibility of the thylakoid membranes has been shown to be 'borrowed' from LHCII (Barzda et al. 1996). No light-intensity dependence has, however, been presented for LHCII, and in general no attempt has been made to interpret the light-intensity dependencies of the reversible and irreversible reorganizations in LHCII and thylakoid membranes within the framework of the thermo-optic model.

Figure 3 shows that the initial rate of ΔCD in LHCII exhibits a linear dependency on the light intensity. This, and the similar behavior of thylakoid membranes can readily be understood based on the thermo-optic effect of the excess excitation. (For thylakoids, the approximately linear relationship departs above the saturation of the photochemical reactions (Barzda et al. 1996). Hence, the reaction rates appear to be proportional with the intensity of the excess light, which is not used for photochemical energy conversion, both in thylakoids and LHCII). For explaining these linear light-intensity dependencies, we assume that the excess light elevates (locally and temporarily) the temperature inside a certain number of units; this number is proportional to the light intensity. Hence, in these units the transitions occur with rates valid for the elevated local temperature. For instance, the k_{12}, k_{21}, k_{23} and k_{32} rates at 15 °C and 35 °C change from 5 to 44, from 970 to 750, from 0.001 to 0.95 and from 0.002 to 0.005, respectively. Since the changes in the CD amplitudes are assumed to be proportional to the concentration of the initial, dark state, A_1, and it is assumed to follow the simple relation of $(dA_1/dt) = -k_{12}A_1 + k_{21}A_2$; for small changes and $A_2 \ll A_1$, this yields a constant rate, defining the initial rate of ΔCD.

The light-intensity dependencies for the irreversible changes become somewhat more complex. With prolonged illumination periods and intense light, all reaction constants must be taken into account (e.g. the magnitude and variation of A_2 during the illumination period cannot be neglected; the amount of A_2 also depends on the reaction rates between A_2 and A_3; see Appendix). Under these conditions, A_3 state is expected to gradually accumulate. The irreversible loss in the CD amplitude can be considered to be proportional to the concentration of A_3. (The spectra are measured in the dark, following the preillumination of the sample, and would allow an equilibration (relaxation) between A_2 and A_1, hence the

concentration of A_2 is nearly zero.) This loss in the psi-type CD intensity is proportional to the dimensions of the macrodomains, i.e. the changes indicate a disassembly of the chiral macrodomains (Barzda et al. 1994; Gussakovsky et al. 1997). Figure 4 demonstrates that, as expected, the accumulation of A_3 progresses gradually with the length of the illumination period.

This accumulation of the A_3 state also depends on the light intensity and the temperature, as seen in Figure 5. Evidently, at lower temperatures the changes are considerably smaller than at temperatures closer to T_t. A comparison of Figures 5 a and b shows that the preillumination of the membranes at 15 °C with 1160 W m^{-2} white light induces smaller changes than that at 35 °C with 660 W m^{-2}. These data are also fully consistent with the thermo-optic origin of these structural changes, and, as shown in Figure 6, can also be fitted with the same set of parameters as in the above examples.

Conclusions

Thermo-optically induced reversible and irreversible changes, reorganizations affecting the long-range chiral order in lamellar aggregates of isolated LHCII and thylakoid membranes, as shown earlier and in this paper, exhibit peculiar, non-Arrhenius type of temperature dependencies. This behavior is the direct consequence of the fact that the structural changes originate from a thermo-optic effect: the excess excitation energy, upon internal conversion results in ultrafast local thermal transients, which in turn induce elementary structural changes. This mechanism is based (i) on the thermal instability of the structural element involved in the reaction, and (ii) a heat dissipation in the close vicinity of the thermally instable structural element. In this particular case, the reactions are based on the thermal instability of the chiral macrodomains and the dissipation of the excess excitation in LHCII.

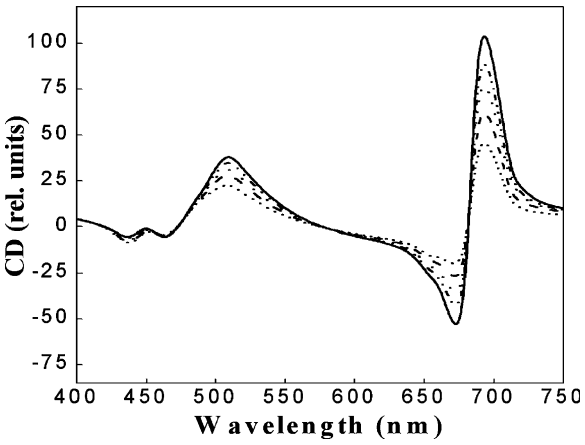

Figure 4. CD spectra of isolated spinach thylakoid membranes recorded after different periods of preillumination with intense (500 W m^{-2}, i.e. approximately 2500 μmol of photons m^{-2} s^{-1}) white light. Curves from top to bottom at 693 nm: 0 min, solid line; 15 min, dash dot dot line; 30 min, dotted line; 45 min, dashed line and 60 min, short dash dot line. The preillumination was provided in a thermostated sample holder at 25 °C, the measurements were performed at room temperature after a 5 min dark incubation.

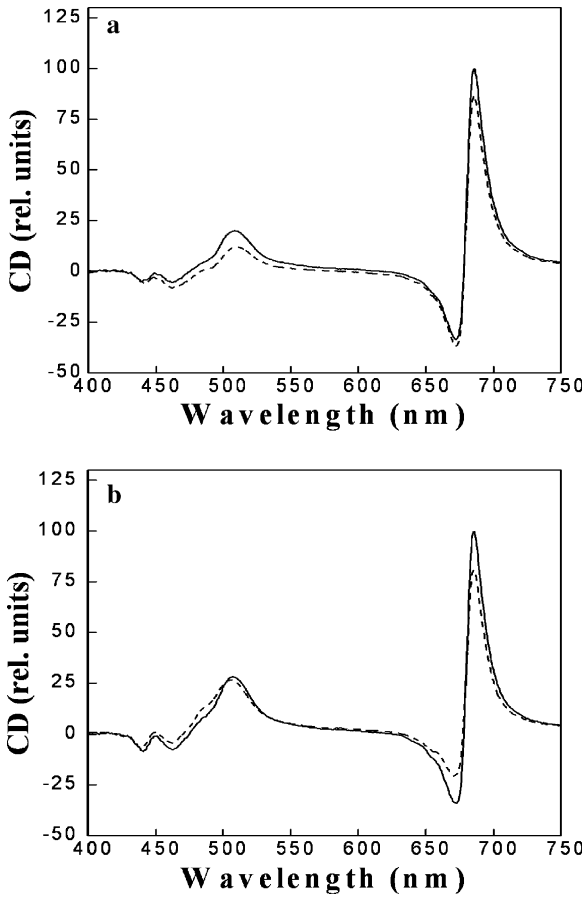

Figure 5. Typical CD spectra of dark adapted (continuous lines) and preilluminated (dashed lines) thylakoid membranes isolated from spinach leaves. The preillumination was performed in a thermostated sample holder at 15 °C (a) and 35 °C (b) with white light of 1160 W m^{-2} (a) and 660 W m^{-2} (b); the measurements were carried out at room temperature after a 5 min incubation in the dark.

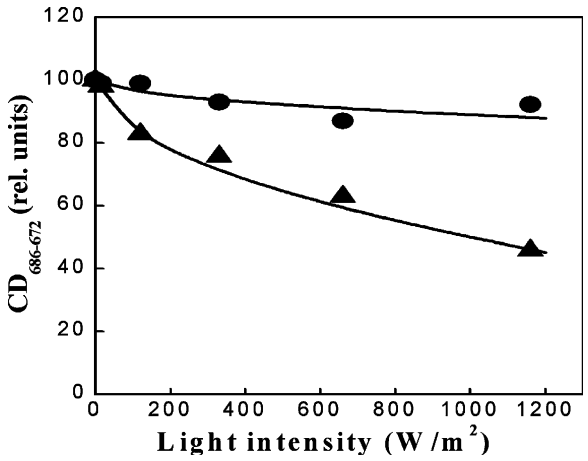

Figure 6. Light-intensity dependence of the amplitude-loss of the psi-type CD signal ($CD_{686–672}$) characteristic of the dimension of the chiral macrodomains in isolated spinach thylakoid membranes at 15 °C (circles) and at 35 °C (triangles). The data are taken from a series of measurements at different light intensities and temperatures, as in Figure 5. The magnitude of the changes depended on the plant material but the trends were very similar in all thylakoid preparations. The fitted curves were obtained using the thermo-optic model with the same parameters as above (see legend to Figure 1).

The presently available data suggest that the 'built-in' thermal instabilities are of physiological significance. It is interesting to note that they are found considerably above the physiologically relevant temperature range (see Dobrikova et al. 2003 and references therein). This means, that without the assistance of the light energy, more precisely of the local heat-jumps originating from the dissipation of excess light, the structure remains stable. Thus, these structural instabilities appear to play role mainly in the light, and via thermo-optic effect.

It is also important to stress, as it has already been pointed out in our earlier publications, that by this means there is no need to introduce an overall, uniform structural change in the system. Instead, with the thermo-optic mechanism, the structural changes which could in principle also be induced by elevating the temperature, occur locally, i.e. at the site of dissipation of the excess excitation. This might be an important factor in retaining the structural stability while allowing substantial local reorganizations. Also, reorganizations of this type might favor the reversibility of the reactions; this latter might be facilitated e.g. by cooperative interactions.

Clearly, as a consequence of the thermo-optic effect, the reaction rates are significantly accelerated with the increase of the temperature. This might be important with regard to the mechanism of light adaptation at low and high temperatures and to combinations of light and heat stresses. These questions warrant further systematic investigations, which would clarify the physiological significance of thermo-optically induced changes, in addition to those mentioned in the Introduction.

Thermo-optic mechanism also brings about a light-intensity dependency of the reaction rates that is approximately linearly proportional with the magnitude of the excess excitation. This, as shown here and in an earlier publication, is valid both for the lamellar aggregates of LHCII and, above the saturation of the photosynthetic electron and proton transport, for thylakoid membranes. This, to our knowledge, is a unique feature, which potentially plays an important role in the light adaptation and photoprotection of plants.

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Appendix

Models for thermo-optically induced structural changes have been presented earlier. For convenience, here we give a short summary; for details the reader is referred to our earlier publications (Cseh et al. 2000; Garab et al. 2002).

According to the model, at the site of the dissipation of the excess excitation a substantial jump in the temperature is given rise, which is then conducted away and rapidly transferred to the medium. Nonetheless, in the close vicinity of these sites the heat transients might induce elementary (local) structural changes which on the timescale of the thermal transients do not relax. We assume that these elementary structural changes can be described by a transition between two states, A_1 and A_2. We allow reversibility from A_2 to A_1. We also assume, however, that upon further excitation (and dissipation) during the lifetime of A_2, an irreversible state, A_3 is reached. The potential energy profiles associated with these states are chosen in a way that the probability of the transitions remain very low in the

'normal' temperature range, i.e. below 40–45 °C. Above this range, however, we assume that there is a substantial decrease of the free energy gaps (ΔGs) between the states in a narrow temperature range (this corresponds to phase transition between two states). This means that ΔGs exhibit significant and sharp temperature dependences near T_t, the transition temperature. (The transition is also characterized by its width, T_w.) Spontaneous thermal transitions occur at temperatures near T_t. (Reversibility in these, heat-induced transitions are usually not retained because of the possible involvement of other, non-specific heat-induced structural alterations; these factors are not included in the model.)

Upon dissipation, the local temperature raises to or near the temperature of elementary structural transition, T_t, and thus the reactions occur in the light despite the relatively low ambient (basal) temperature. A key factor in the model: the reaction rates, because of the temperature dependence of the free energy gaps, depend in dual way on the T temperature: $k_{ij} = \alpha_i \exp(-\Delta G_i(T)/kT$, where $i = 1, 2$ represents the index of the different transitions, $j = i + 1$, i.e. not only through kT but also via $\Delta G(T)$.

The kinetic model, as outlined above, operates with three states:

$$A_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} A_2 \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} A_3$$

with the following set of differential equations:

$$\frac{dA_1}{dt} = -k_{12}A_1 + k_{21}A_2,$$

$$\frac{dA_2}{dt} = k_{12}A_1 - (k_{21} + k_{23})A_2 + k_{32}A_3$$

and

$$\frac{dA_3}{dt} = k_{23}A_2 - k_{32}A_3$$

for $t = 0$, $A_1 = A_0 = 1$, $A_2 = A_3 = 0$

and for $t > 0$

$$A_1 + A_2 + A_3 = A_0 = 1$$

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