Thermal stability of trimeric light-harvesting chlorophyll $a/b$ complex (LHCIIb) in liposomes of thylakoid lipids

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Abstract

The major light-harvesting chlorophyll $a/b$ complex (LHCIIb) of photosystem (PS) II functions by harvesting light energy and by limiting and balancing the energy flow directed towards the PSI and PSII reaction centers. The complex is predominantly trimeric; however, the monomeric form may play a role in one or several of the regulatory functions of LHCIIb. In this work the dissociation temperature was measured of trimeric LHCIIb isolated from Pisum thylakoids and inserted into liposomes made of various combinations of thylakoid lipids at various protein densities. Dissociation was measured by monitoring a trimer-specific circular dichroism signal in the visible range. The LHCIIb density in the membrane significantly affected the trimer dissociation temperature ranging from 70 °C at an LHCIIb concentration comparable to or higher than the one in thylakoid grana, to 65 °C at the density estimated in stromal lamellae. Omitting one thylakoid lipid from the liposomes had virtually no effect on the thermal trimer stability in most cases except when digalactosyl diacylglycerol (DGDG) was omitted which caused a drop in the apparent dissociation temperature by 2 °C. In liposomes containing only one lipid species, DGDG and, even more so, monogalactosyl diacylglycerol (MGDG) increased the thermal stability of LHCIIb trimers whereas phosphatidyl diacylglycerol (PG) significantly decreased it. The lateral pressure exerted by the non-bilayer lipid MGDG did not significantly influence LHCII trimer stability.

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Keywords: Light harvesting complexes of photosystem II (LHCIIb); Liposome; Thermal stability; Thylakoid lipids; Trimer

1. Introduction

The major light harvesting chlorophyll complex (LHCIIb) of photosystem (PS) II comprises more than half of the chlorophylls (Chl) in the chloroplast and is the most abundant membrane protein on Earth. The function of LHCIIb is to absorb solar energy, to keep the energy balance between PSI and PSII in the thylakoid membrane under different incident light conditions [1], and to maintain the grana structure [2,3]. LHCIIb is also very important in the photoprotection of PSII by dissipating excess energy under light stress conditions [4].

LHCIIb is composed of three different apoproteins (Lhcb 1–3) which are nuclearly encoded by a gene family [5] and, as cofactors, each bind 8 Chl $a$, 6 Chl $b$, 4 carotenoid molecules and 2 lipids as revealed by LHCIIb structural analysis at near-atomic resolution. [3,6]. The basic functional unit of LHCIIb is the trimer. During the greening process, LHCIIb first appears in the form of monomers which are then assembled into trimeric complexes [7]. It has been proposed that trimeric LHCIIb in mature green leaves undergoes trimer–monomer transition during regulatory processes such as state 1–state 2 transition [8] and non-photochemical quenching [4]. The dissociation of trimeric LHCIIb may also be of significance for the degradation of the complex under various physiological conditions. A protease involved in LHCIIb breakdown, FtsH6, is constitutively present in the thylakoid membrane, its activity possibly being

Abbreviations: CD, circular dichroism; Chl, chlorophyll; DGDG, digalactosyl diacylglycerol; L/P, lipid/protein ratio; LHCII, major light-harvesting chlorophyll $a/b$ complex of photosystem (PS) II; LM, n-dodecyl-$β$-D-maltoside; MGDG, monogalactosyl diacylglycerol; PG, phosphatidyl diacylglycerol; PS, photosystem; SQDG, sulphoquinovosyl diacylglycerol; TL, thylakoid lipids; Tm, apparent dissociation temperature

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regulated by variable structural properties of its substrate [9]. Since earlier work has shown that monomeric but not trimeric LHCIIb is targeted for proteolysis [10], dissociation of LHCIIb into monomeric complexes may be such a structural signal for breakdown. Therefore, identifying the physical conditions under which LHCIIb trimer dissociation occurs is an important issue.

Differential scanning calorimetry of isolated thylakoid membranes [11] suggested dissociation of trimeric LHCIIb at 60 °C. This temperature probably is near the upper limit of leaf temperatures that are transiently reached at high ambient temperatures and intense illumination without irreversibly damaging the tissue. A thermo-optical mechanism has been proposed for light-induced trimer-to-monomer transitions of LHCIIb, due to local thermal transients arising from the dissipation of excess excitation energy. These transitions were observed both in isolated complexes and in thylakoid membranes or whole plants and regulated by the amount of excess excitation [12].

The temperature at which trimeric LHCIIb dissociates is likely to depend on the molecular environment of the complex. If trimer dissociation is a reversible process, then high concentrations of LHCIIb in the membrane would be expected to raise the dissociation temperature since they stabilize the trimeric complex by facilitating re-association of monomers into trimers. Also the lipid composition of the surrounding membrane may exert an influence. The lipid composition of the thylakoid membrane drastically changes under various stress conditions such as heat, water, or salt stress; the changed properties include the ratio of the major lipids, monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) [13], the chain length and the number of double bonds in fatty acids [14,15]. MGDG is a non bilayer-forming lipid since it is conically shaped and exerts a lateral pressure within the hydrophobic space of the membrane; this lateral pressure has been proposed to influence the stability of membrane-inserted proteins [16]. This lipid usually makes up about half of the lipid content of the thylakoid; however, lower MGDG contents are observed under various stress conditions [14]; thus, the MGDG: DGDG ratio has been found to decrease from 3.4 to 1.4 in a frost-hardy variety of wheat [14] and from 2.2 to 1.1 in Arabidopsis thaliana under drought stress [15]. Moreover, it has been proposed that MGDG molecules that cannot be accommodated in lamellar lipid structures, for instance at high lipid protein ratios, are sequestered into inverted-hexagonal lipid phases [17,18] thus lowering the MGDG partition in the remaining lamellar membranes. In order to investigate the influence of membrane properties on the thermal stability of LHCIIb trimers, we have inserted LHCIIb into artificial liposomes of thylakoid lipids and in thylakoid membranes or whole plants and regulated by the amount of excess excitation [12].

2.2. Preparation of preformed liposomes

2.2.1. Preparation of preformed liposomes

Thylakoid lipids phosphatidylglycerol (PG), DGDG, MGDG, sulfoquinovosyl diacylglycerol (SQDG), isolated from spinach, were purchased from Lipid Products Company (Redhill, UK). Lipid aliquots were dissolved and mixed in chloroform. Total thylakoid lipids contained (all w/v) 50.0% MGDG, 31.0% DGDG, 10.7% PG, 8.3% SO4. If a single lipid was omitted then the lipid mixture contained 50.0% MGDG, 10.0% SQDG, 40.0% DGDG; and those lacking MGDG contained 50.0% MGDG, 21.9% SQDG, 28.1% PG; those lacking PG contained 50.0% MGDG, 10.0% SQDG, 40.0% DGDG; and those lacking SQDG contained 50.0% MGDG, 12.8% PG and 37.2% SQDG. Uni-lamellar liposomes were made according to [20]. The chloroform solution of lipids was dried by using a rotary evaporator (Heidolph, Kelheim, Germany) at 40 °C, 200 mbar for 15 min and then 10 mbar for 30 min, so that a thin lipid layer was formed on the wall of the flask. The lipids were suspended in reconstitution buffer (10 mM NaCl, 10 mM Tris/NaOH (pH 7.8)) at 5 mg ml−1 and vortexed vigorously until a homogeneous suspension was formed. The lipid suspension was extruded (LiposoFast, Avanti) through a polycarbonate membrane (pore diameter 100 nm).

2.2.2. Reconstitution of LHCIIb into preformed liposomes

Preformed liposomes (2.5 mg ml−1) were mixed with Triton X-100 to a final detergent concentration of 0.05% (w/v). Purified LHCIIb in 0.05% Triton was added dropwise with sonication in a bath sonicator to the required lipid/protein ratio (L/P). The Triton X-100 was brought to a final concentration of 0.7% (w/v). The mixture was incubated at room temperature, and finally the mixture was incubated under constant rotating overnight at 4 °C to remove Triton X-100. The mixture was centrifuged briefly, the supernatant removed to a new tube, fresh Biobeads were added to the supernatant, and the mixture was incubated at 4 °C for 1 h. This procedure was repeated except the 1-h incubation was at room temperature, and finally the supernatant was collected.

2.3. CD measurements and temperature treatment:

CD spectra were recorded on a J-810 spectropolarimeter (Jasco, Gross-Umstadt, Germany) equipped with a temperature control set. The spectral range was from 400 to 750 nm, data pitch 1 nm, response time 2 s, band width 2 nm and scanning speed 500 nm/min. Temperature scans were from 20 °C to 80 °C at through three layers of cheesecloth, the suspension was centrifuged at 7000×g for 10 min at 4 °C. The pellet was resuspended in cold buffer containing 50 mM sorbitol, 5 mM EDTA (pH 7.8) and centrifuged at 10,000×g for 10 min at 4 °C. The pellet was resuspended in cold water and the density of the suspension adjusted to 0.8 mg Chl/ml. Upon dissolving the thylakoid membrane by adding 5% (w/v) Triton X-100 stock solution to a final detergent concentration of 0.7% (w/v), the LHCIIb was precipitated by adding 20 mM MgCl2 and 100 mM KCl. The pellet was suspended in water again and the process was repeated until the Chl/ab ratio of the LHCIIb extract was about 1.4.

The isolated native LHCIIb was further purified by sucrose density gradient ultracentrifugation. Sucrose density gradients were generated by freezing (−20 °C) and thawing (4 °C) a 0.4 M sucrose solution containing 0.1% (w/v) n-dodecyl-β-D-maltoside (LM), 5 mM Tris/HCl (pH 7.8). For preparing LHCIIb for proteoliposome reconstitution, 0.05% (w/v) Triton was used instead of 0.1% LM. After a 17-h ultracentrifugation at 170,000×g, 4 °C, the trimer-containing band was collected. For circular dichroism (CD) measurements, the concentration of the sample was adjusted to about 200 μg Chl /ml. For preparing proteoliposomes, LHCIIb in 0.05% Triton was concentrated about 20 fold to ca 1.5 mg Chl /ml by Centricon centrifugal filter units (30 kDa, Millipore, Billerica, Mass., USA). The concentrated LHCIIb was diluted about 20 fold with reconstitution buffer (10 mM NaCl, 10 mM Tris/NaOH (pH 7.8)), and concentrated again; this process was repeated twice so that the succrose was almost completely removed. For the last step, a 100 kDa Centricon centrifugal filter unit was used to reduce the Triton X-100 concentration.

2.4. Isolation and characterization of LHCIIb

LHCIIb was isolated from 2-week-old pea seedlings according to [19] with the following modifications. Leaves were homogenized in 400 mM sorbitol, 5 mM EDTA and 50 mM tricine/NaOH (pH 7.8). After filtering the homogenate.
a rate of 1 °C min⁻¹. The scans were stopped at every 5 °C for 0.7 min, in order to record CD spectra. The CD signal at 473 nm was recorded continuously during a temperature scan at time intervals of 0.2 s. The data were fitted to a sigmoid function with the software Table Curve 2D 4.0 (SPSS Inc, Chicago, USA) based on a Marquardt algorithm procedure. The quality of the fits was assessed using residual R² criteria and plots of residuals. The inflection points of the sigmoid functions were taken as apparent dissociation temperatures of the pigment–protein complexes. All data were averaged from 3 to 7 independent measurements.

3. Results

3.1. Temperature-dependent changes of LHCIIb CD spectra

Circular dichroism (CD) in the visible spectral region is used to monitor the organizational state of LHCIIb at different temperatures. The CD spectrum of trimeric LHCIIb in detergent solution (Fig. 1A, bottom spectrum) exhibits negative signals at 493, 650, and 680 nm, that are all seen in monomeric LHCIIb (empty arrows), and an additional negative band at 473 nm (and, sometimes less clearly seen, a shoulder near 648 nm) that only appear in trimeric LHCIIb (black arrows) and, thus, are indicative of this organizational state [21]. When the temperature is raised gradually between 20 and 75 °C, the trimer-specific CD signal at 473 nm is the first one to vanish at 40–60 °C. The correlation between the signal change at 473 nm and LHCIIb trimer dissociation into monomers has been verified by analysing the complexes on a partially denaturing “green” gel (not shown). Between 60 and 70 °C all the other signals flatten out, indicating disintegration of LHCIIb monomers. The CD spectrum of LHCIIb in liposomes at room temperature (Fig. 1B, bottom spectrum) is similar to that of detergent-dissolved LHCIIb except that it contains an additional prominent negative signal at around 438 nm. Such a signal has been observed with aggregated LHCIIb produced from the solubilized complex by lowering the detergent concentration [22] and therefore most likely reflects the formation of some aggregated state of LHCIIb in liposomes. Both near this peak and near the trimer-specific negative CD signal at 473 nm there are additional negative peaks at 426 and 457 nm, respectively, which are not present in detergent-dissolved LHCIIb solution but have consistently been observed in liposome-inserted LHCIIb, independently of lipid composition or L/P and, therefore, suggest slight differences in the arrangement of LHCIIb-bound pigments. Upon raising the temperature, the aggregate peak at 438 nm disappears between 40 and 50 °C, before the trimer-specific CD signal at 473 nm vanishes between 60 and 70 °C. Comparison of Fig. 1A and 1B shows that trimeric LHCIIb in liposomes withstands higher temperatures than in detergent solution where the trimer-specific CD signal disappears between 40 and 60 °C. Monomeric LHCIIb, too, is more stable in liposomes since the monomer-specific CD signals disappear between 70 and 80 °C compared to 60–70 °C in detergent solution. Upon raising the temperature to 55 °C and then lowering it back to room temperature, the signal change at 473 nm is largely reversed (not shown) indicating a temperature-dependent trimer–monomer equilibrium in lipid-bound LHCIIb. It should be noted, however, that no true equilibrium was reached at any given point when LHCIIb was subjected to a continuous temperature gradient; therefore, the temperatures at which the trimer signal in the CD spectrum disappeared should be regarded as apparent dissociation temperatures. The spectral changes observed at higher temperatures are irreversible as has been described by Wentworth et al. [23]. The temperature-dependent CD signal changes stayed the same upon dilution of the liposome suspension up to 4 fold.

In order to assess the thermal stability of trimeric LHCIIb more precisely, we continuously monitored the CD signal at 473 nm during the temperature gradient. This is shown for a liposome-inserted LHCIIb in Fig. 2. The trace was then fitted with a sigmoidal function (Y=a/(1+exp(-(X-b)/c))), and the inflection point (b) was taken as the apparent dissociation temperature (Tm) for LHCIIb trimers. The residuals (Fig. 2B) indicate that the sigmoidal function fits the data reasonably well. The apparent Tm taken from Fig. 2 is 66.9 °C which is consistent with the dissociation temperature estimated from Fig. 1B.

3.2. Lipid/protein ratios

LHCIIb was inserted into preformed liposomes made of thylakoid lipids at different L/P. At L/P=85 and below the protein formed 2D-crystalline arrangements similar to the ones described by Kühlbrandt and Thaler [24] as seen by electron microscopy (data not shown). In order to assess the influence of the protein density in proteoliposomes on the thermal stability of trimeric LHCIIb, the temperature-dependent decline of the trimer-specific CD signal was measured at varying L/P (Table 1). Table 1 confirms that trimers are more stable towards thermal dissociation in a lipid membrane environment than in detergent solution, since the Tm values of all the liposome-inserted LHCIIb trimers are significantly higher than those of the detergent-dissolved LHCIIb. The L/P of the proteoliposome influenced the thermal stability of LHCIIb trimers, at least at
higher protein densities. The Tm value of about 65 °C at the highest L/P does not significantly change going to L/P=340 but then significantly rises to almost 70 °C when going to lower L/P.

3.3. Lipid composition of liposomes

To assess the effect of the non-bilayer lipid MGDG on the thermal stability of LHCIIb, Tm values were measured of LHCIIb inserted into preformed liposomes made of mixed thylakoid lipids at different L/P either containing or lacking MGDG. Fig. 3 shows that the omission of MGDG has a slight effect on Tm only at the highest protein density measured (L/P=34) where the trimers are somewhat more stable in the absence of MGDG. At lower protein densities, there are no significant differences between liposomes with or without MGDG.

We extended the analysis of lipid-dependent thermal stabilities of trimeric LHCIIb to other thylakoid lipids by measuring the apparent dissociation temperature of LHCIIb trimers inserted into liposomes either lacking one of the thylakoid lipids or containing only one lipid species (Fig. 4). Tm values of LHCIIb trimers in single-lipid liposomes vary substantially depending on the lipid species. DGDG and, even more so, MGDG make the complexes more stable towards thermal dissociation than the native lipid mixture (Tm=(72.9±0.5) °C and (74.7±0.8) °C vs. (67.5±0.8) °C, respectively) whereas PG clearly destabilizes the trimers (Tm=(60.4±1.5) °C vs. (67.5±0.8) °C). SQDG liposomes behave similarly to those with all native lipids (Tm=(67.5±0.7) °C vs. (67.5±0.8) °C). On the other hand, the omission of only one of the lipid species of the mixture caused little or no effect. Only the lack of DGDG caused a drop in Tm by about 2 °C. All the other lipid mixtures led to dissociation temperatures not significantly different from the one in the control liposomes. It should be noted that in all these experiments only the lipid composition was varied of the preformed liposomes that the LHCIIb trimers were inserted to. No effort was made to remove the tightly bound lipids from the complexes so that the intrinsic PG and DGDG molecules seen in the crystal structure [3,6] presumably are in place.

Table 1

<table>
<thead>
<tr>
<th>L/P</th>
<th>Tm (°C)±SD</th>
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<tbody>
<tr>
<td>34</td>
<td>69.4±0.4</td>
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<tr>
<td>85</td>
<td>67.5±0.8</td>
</tr>
<tr>
<td>340</td>
<td>65.6±0.5</td>
</tr>
<tr>
<td>850</td>
<td>64.8±1.0</td>
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<tr>
<td>LHCIIb in 0.1% LM</td>
<td>59.4±0.3</td>
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*L/P indicates the molar ratio of lipid to protein in the proteoliposomes. Tm values are given as the average of 3–7 individual measurements±standard deviation.*
4. Discussion

The dependence of the LHCIib oligomerizational state on the lipid environment of the complex is difficult to measure in vivo or in isolated thylakoid membranes since the lipid composition of these membranes cannot easily be varied and the impact of the lipid environment would be hard to distinguish from the impact of other thylakoid proteins present at high densities. On the other hand, studying LHCIib trimer stability in mixed detergent-lipid micelles has the disadvantage that the micellar environment is physically quite different from a lamellar membrane environment. Therefore, we chose to insert LHCIib into artificial liposomes made of thylakoid lipids. LHCIib-containing liposomes have been prepared by Moya et al. [25] for time-resolved fluorescence analysis of the complexes. Chlorophyll fluorescence lifetimes in liposome-bound LHCIib were shorter than those in detergent solubilized complexes and dependent on the protein density in the liposomes, indicating, in accordance with data in the present paper, that physical properties of LHCIib change upon going from detergent micelles to the membrane bilayer.

4.1. Protein density

The apparent transition temperature for the dissociation of trimeric LHCIib into the monomeric complexes clearly is dependent on the density of the complexes in the lipid bilayer. This indicates an equilibrium between the trimeric and monomeric form which, according to the law of mass action, is shifted towards the oligomeric form at higher LHCIib concentrations. In detergent solution, too, the dissociation of LHCIib trimers into monomers has been shown to be reversible: When the detergent concentration is reduced by dialysis, the trimers re-associate [26]. The lower dissociation temperature of trimeric LHCIib in detergent solution than in liposomes presumably is due to the larger “dilution” of the complexes in the hydrophobic phase of the detergent micelles, i.e. a lower probability of monomers getting close enough to each other to re-form trimeric complexes.

At the highest protein density studied here, at an L/P of 34, the LHCIib trimers form extended 2D crystals in the lipid membrane. It should be noted that these crystalline arrays are different from LHCIib arrangements formed in the thylakoid since the unit cell of planar 2D LHCIib crystals is made up of proteins of opposite orientation in the membrane [3] whereas in the thylakoid all LHCIib proteins are uniformly oriented in the membrane. However, the orientation of LHCIib in the membrane is not likely to have a strong impact on its dissociation equilibrium and, therefore, we regard liposomes made of thylakoid lipids as a useful model system for studying the oligomerization behavior of LHCIib. Another difference between thylakoids and LHCIib liposomes is that thylakoids but not liposomes are crowded with other proteins. Whilst these other proteins do not directly affect the LHCIib trimer–monomer equilibrium, they may have an indirect influence by limiting lateral diffusion in the membrane.

A ratio between lipids and monomeric LHCIib of 58 has been measured in thylakoid membranes from spinach [27]. Since LHCIib makes up only about one third of total thylakoid protein [28], the overall L/P is about 20. As these figures are averaged over the entire thylakoid, it needs to be taken into account that most of LHCIib resides in the grana fraction, next to the PSIα particles, and only part of the mobile fraction of LHCIib, amounting to a maximum of 15–20% of total LHCIib [29], can migrate to the non-appressed membranes in the grana margins and the stromal lamellae. The grana comprises 80% of the thylakoid, and 40% of the grana is made up by the margin [30]. Consequently, at least 80% of the LHCIib is concentrated in the grana to a lipid/LHCIib ratio of 35, whereas in the other thylakoid domains, this ratio amounts to 150 at maximum state transition and to much higher values otherwise. Therefore, our data indicate that the lower LHCIib density in non-appressed thylakoid regions causes a drop in the dissociation temperature of the trimeric complex by about 5 °C in comparison to stacked grana thylakoids. This does not take into account the possible effect of N-proximal phosphorylation of the mobile LHCIib fraction during state transition [31]; whether phosphorylation has an effect on LHCIib trimer stability still needs to be established. Phosphorylation-induced trimer dissociation has been proposed as a key step during state transition [8]. On the other hand, a PSI–LHCl–LHCIib supercomplex containing one LHCIib trimer has been detected in Arabidopsis thylakoids isolated under state-2 conditions [32].

4.2. Lipid composition

The lipid composition of LHCIib-containing liposomes has only a limited effect on trimer stability. Only when the liposomes are made of single lipid species does the trimer–monomer transition temperature change substantially compared to the one seen in the native thylakoid lipid mixture. The strongly reduced thermal stability in PG (60 °C vs. 68 °C in mixed thylakoid lipids) is not easily explained. PG contains a different fatty acid mixture (predominantly C16) than the galactosyl lipids (mostly C18) [33,34]. The shorter fatty-acid chain length of PG results in a reduced thickness of the membrane layer which may not be sufficient to accommodate LHCIib, resulting in a hydrophobic mismatch [35,36] that would destabilize the protein complex. However, SQDG has a fatty acid composition similar to that of PG and, even so, does not exhibit a destabilizing effect on LHCIib trimers. The major difference between these two lipid classes is the unique C16:1 (Δ3-trans) fatty acid found only in PG and exclusively in the thylakoid [37]. This lipid has been suggested to bind to LHCIib specifically and to stabilize the trimeric complex [38]. The tightly bound PG molecules presumably stay bound to LHCIib throughout the isolation procedure used in this work, and it is not known whether C16:1(Δ3-trans) PG in the PG-only liposomes has a stabilizing or de-stabilizing effect on trimeric LHCIib. Another possibility would be that the negative charge of the lipid molecule that distinguishes PG from the galactosyl lipids exerts a de-stabilizing effect on LHCIib trimers; however,
again SQDG like PG carries a negative charge but does not lower the dissociation temperature of LHCIIb trimers.

DGDG is found in contact with LHCIIb in various crystal forms of the protein [3,6]; moreover, DGDG is essential for the formation of 2D crystals of LHCIIb [26]. This makes it likely that DGDG is a preferred boundary lipid of the protein which would explain why LHCIIb trimers are more stable when they are inserted in DGDG liposomes rather than liposomes of the thylakoid lipid mixture, and less stable when DGDG is omitted from this mixture. The omission of MGDG from the liposome lipids does not change the apparent dissociation temperature of LHCIIb trimers at an L/P of 85 (or higher, Fig. 3)) and only slightly stabilizes the complexes at an L/P of 34 (Fig. 3). The complete omission of MGDG from the membrane is expected to significantly lower the lateral pressure in the membrane. The lateral pressure exerted on the hydrophobic membrane space by non-bilayer lipids such as MGDG, due to their conical shape with a small head group and a more space-demanding hydrophobic group, has been proposed to stabilize membrane proteins [16,36]. We conclude that, by contrast, the lateral pressure does not significantly influence LHCIIb trimer stability. Possibly the higher lateral pressure in purely-MGDG liposomes is responsible for the increased thermal stability of the complexes observed here.

The extreme variation of the lipid composition in liposomes used in this study, including liposomes made of only a single lipid species, certainly do not per se reflect physiologically significant situations. However, since our model system, as opposed to native thylakoid membranes, allows such extensive variations, we are able to systematically assess the influence of lipids present in the membrane and of the lateral pressure exerted by non-bilayer lipids, on the thermal stability of LHCIIb trimers. On the basis of the small changes observed in LHCIIb trimer stability upon drastic changes of the lateral pressure in the membrane, we can safely assume that the presumably smaller changes of lateral pressure encountered in vivo are highly unlikely to affect the thermal dissociation of trimeric LHCIIb.

4.3. Biological impact of LHCIIb trimer dissociation

The monomer–trimer transition temperatures measured by CD during temperature gradients must be regarded as apparent dissociation temperatures since no attempt was made to reach equilibrium at individual temperatures. Differential scanning calorimetry of isolated Pismum or barley thylakoids indicated a dissociation temperature of trimeric LHCIIb at about 60 °C [11] which suggests that the apparent dissociation temperature reported here, between 65 °C and 70 °C depending on the protein density, may in fact be somewhat over-estimated. These temperatures do not seem to be commonly reached in leaves although the temperature of a sunlit leaf on a calm day may be well over 10 °C above ambient temperature [39]. A thermooptic effect has been suggested to generate even higher local temperature changes due to the dissipation of excitation energy in photosynthetic pigments [40,41]. In fact, light-induced dissociation of LHCIIb trimers has been measured upon prolonged illumination with strong light of isolated LHCIIb, thylakoid membranes and whole plants [12].

Even at temperatures below Tm where only a fraction of LHCIIb is expected to be in its monomeric state at any given time point, this dissociation may be of importance if the monomeric state is trapped by some other process that is fast enough to compete with re-association of monomers to trimers. One such process has been suggested to be the generation of an energy-dissipating form of LHCIIb, presumably by some conformational change in the protein that can take place in the monomeric but not the trimeric form [42]. Another possible fate of monomeric LHCIIb is degradation. The monomer but not the trimeric form of LHCIIb is prone to degradation by a thylakoid-associated protease [10]. If in fact the dissipation of excess excitation triggers the dissociation of LHCIIb trimers [12] and targets the monomers for degradation, this would provide a regulatory mechanism for the reduction of the photosynthetic antenna size observed under excess light conditions [43].

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