Digalactosyl-diacylglycerol-deficiency lowers the thermal stability of thylakoid membranes

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Abstract We investigated the effects of digalactosyl-diacylglycerol (DGDG) on the organization and thermal stability of thylakoid membranes, using wild-type Arabidopsis thaliana and the DGDG-deficient mutant, dgd1. Circular-dichroism measurements reveal that DGDG-deficiency hampers the formation of the chirally organized macrodomains containing the main chlorophyll a/b light-harvesting complexes. The mutation also brings about changes in the overall chlorophyll fluorescence lifetimes, measured in whole leaves as well as in isolated thylakoids. As shown by time-resolved measurements, using the lipophylic fluorescence probe Merocyanine 540 (MC540), the altered lipid composition affects the packing of lipids in the thylakoid membranes but, as revealed by flash-induced electrochromic absorbance changes, the membranes retain their ability for energization. Thermal stability measurements revealed more significant differences. The disassembly of the chiral macrodomains around 55°C, the thermal destabilization of photosystem I complex at 61°C as detected by green gel electrophoresis, as well as the sharp drop in the overall chlorophyll fluorescence lifetime above 45°C (values for the wild type—WT) occur at 4–7°C lower temperatures in dgd1. Similar differences are revealed in the temperature dependence of the lipid packing and the membrane permeability: at elevated temperatures MC540 appears to be extruded from the dgd1 membrane bilayer around 35°C, whereas in WT, it remains lipid-bound up to 45°C and dgd1 and WT membranes become leaky around 35 and 45°C, respectively. It is concluded that DGDG plays important roles in the overall organization of thylakoid membranes especially at elevated temperatures.

Keywords Arabidopsis mutants · Digalactosyl-diacylglycerol · dgd1 mutant · Thermal stability · Thylakoid lipids · Thylakoid membranes

Abbreviations

\( \begin{align*}
\tau_{\text{ave}} & \quad \text{Average fluorescence lifetime} \\
\text{Chl} & \quad \text{Chlorophyll} \\
\text{CD} & \quad \text{Circular dichroism} \\
\text{DGDG} & \quad \text{Digalactosyl-diacylglycerol} \\
\Delta A515 & \quad \text{Electrochromic absorbance change} \\
\text{FLIM} & \quad \text{Fluorescence lifetime imaging microscopy} \\
\text{LHCI} & \quad \text{Light-harvesting antenna complexes of photosystem I} \\
\text{LHCCI} & \quad \text{Major light-harvesting complex of photosystem II}
\end{align*} \)
MGDG    Monogalactosyl-diacylglycerol  
MC540    Merocyanine 540    
PSI      Photosystem I    
PSII     Photosystem II    
TCSPC    Time-correlated single photon counting    
WT       Wild type

Introduction

Digalactosyl-diacylglycerol (DGDG) is one of the main bilayer-prone lipid species in thylakoid membranes of higher plants, constituting about 30% of the total lipid content (Douce and Joyard 1996). Its structural importance is well established for several (super)complexes of the photosynthetic machinery. It has been shown to be bound to photosystem II (PSII) (Loll et al. 2005, 2007), it forms photosynthetic machinery. It has been shown to be bound to photosystem II (PSII) (Loll et al. 2005, 2007), it forms hydrogen bonds with tyrosine in PSII (Gabashvili et al. 1998), and it is important for the binding of extrinsic proteins required for the stabilization of the oxygen-evolving complex (Sakurai et al. 2007). DGDG was resolved in the crystal structure of major light-harvesting complex of photosystem II (LHCII), the major light-harvesting complex of PSII. The head groups of two DGDG molecules are simultaneously hydrogen bonded to the lumenal-surface amino acids from two adjacent LHCII trimers, functioning as a bridge (Liu et al. 2004; Yan et al. 2007). DGDG appears to be required for the formation of 2D and 3D crystals of LHCII (Nuβberger et al. 1993). The functional significance of this lipid was studied employing a genetic approach—a mutant of Arabidopsis (Arabidopsis thaliana) was generated which lacks more than 90% of the DGDG content of the membranes (dgd1, Dörmann et al. 1995). This results in a change in the chloroplast ultrastructure—the thylakoid membranes are highly curved and displaced from the central stroma area toward the envelope, the length of both grana and stroma membranes and the total length of the thylakoid membrane are increased in the mutant (Dörmann et al. 1995). This is accompanied by a decrease of the total chlorophyll (Chl) content on a fresh weight basis of about 25%, in the Chl alb ratio by about 20% and a 1.7 times higher xanthophyll content (Härtel et al. 1997); however, the amount of metabolic intermediates (products of the dark reactions of photosynthesis) were found to be indistinguishable from those of the wild type (WT) (Härtel et al. 1998). Ivanov et al. (2006) have established that the DGDG deficiency has a larger effect on the structure of photosystem I (PSI) than on PSII: the relative abundance of the reaction center protein of PSII (PsbA) and the light-harvesting proteins associated with PSII (Lhcb1, Lhcb2, Lhcb3 and Lhcb5) are not changed in the mutant, whereas the reaction center proteins of PSI (PsaA and PsaB) are significantly reduced (by about 50%) and the abundance of the PsaC, PsaL, and PsaH subunits is also substantially decreased compared to the WT (Ivanov et al. 2006). Moreover, unlike the WT, in dgd1 PSI has been shown to be less stable against treatment with chaotropic salts and the light-harvesting antenna complexes of PSI (LHCI) could more easily be detached from the core complex (Guo et al. 2005).

The modified protein content in dgd1 is accompanied by differences in various functional parameters. For example, the amount of non-photochemical quenching in dgd1 is increased at the expense of PSII photochemistry (Härtel et al. 1998); the linear electron transport between the two photosystems is restricted, the plastoquinol pool is more reduced, PSI has an increased capacity for cyclic electron transfer and the capacity for state transitions is reduced by 50% as compared to WT (Ivanov et al. 2006).

The emergence of these specific but nonetheless rather diverse effects of DGDG deficiency might be correlated with the multiplicity of DGDG-binding sites. However, as shown by Hendrickson et al. (2006) cold acclimation of the dgd1 mutant, while not affecting the lipid composition, led to the recovery of PSII and PSI photochemistry as well as the CO2 uptake capacity, and even the pigment composition became equivalent to that of WT. Based on these results, it was suggested that DGDG deficiency affected the global physical properties of the membranes, which in turn exerted specific effects in a temperature-dependent fashion. As discussed by Hendrickson et al. (2006) and can be inferred from literature data (e.g., Williams 1998; Harwood 1998; Garab et al. 2000) temperature-dependent modifications in the global properties can arise from the altered ratio of the bilayer to non-bilayer lipid contents.

The physical state of the lipid membrane, can influence a number of different global parameters of the thylakoid membrane, such as the macro-organization of the complexes, the packing of lipids, energy migration and trapping, the energization and permeability of membranes—parameters which have not been studied in this mutant. In this study, we focused our attention on the role of DGDG for the overall structural organization of the thylakoid membrane and its thermal stability. Taking into account that DGDG participates in both the lipid matrix and in the protein structures, we investigate DGDG’s effects on the properties of these two environments separately. Our results reveal significant alterations in the overall organization of the thylakoid membranes in dgd1 and decreased thermal stability of the chirally organized LHCII-containing protein macroaggregates and also of the PSI supercomplexes. These changes are accompanied by changes in the fluorescence lifetimes of chlorophyll a. Furthermore, the lipid packing in the thylakoid membrane appears to be
different for the WT and dgd1, especially at elevated temperatures, where the energization of dgd1 membranes is hampered by an increased permeability.

Materials and methods

Plant material

Both the WT Arabidopsis thaliana (Arabidopsis) ecotype Columbia and the dgd1 mutant were grown under 16-h-light/8-h-dark cycle at 20/18 °C (day/night), light intensity of 200–250 W m⁻² at about 70% humidity. The plants used in the experiments were 28–35 days old.

Isolation of thylakoid membranes

Dark-adapted leaves were homogenized in a medium containing 50 mM Tricine (pH 7.5), 400 mM sorbitol, 5 mM MgCl₂ and 5 mM KCl; the suspension was filtered through four layers of cheese cloth and centrifuged for 4 min at 4,000×g. The chloroplasts were osmotically shocked in a hypotonic medium containing 50 mM Tricine (pH 7.5), 5 mM MgCl₂ and 5 mM KCl, and centrifuged for 5 min at 6,000×g. After washing in the same medium supplemented with 400 mM sorbitol, the pellet was resuspended in this isotonic medium and used for the fluorescence and circular-dichroism measurements.

Green (native) gel electrophoresis

Isolated thylakoid membranes from WT and dgd1 were loaded on a polyacrylamide gel, as described in De Bianchi et al. (2008). The samples were incubated for 10 min at defined temperatures. Densitometry analysis was performed using Gel-pro analyser 3.1 software.

Circular-dichroism measurements

Circular dichroism (CD) was measured on isolated thylakoid membranes between 400 and 800 nm using a Jasco J-715 spectropolarimeter. The Chl content of the samples was adjusted to 15 μg ml⁻¹, the optical pathlength of the cell was 1 cm. The spectra were recorded in steps of 1 nm with an integration time of 2 s, a band-pass of 2 nm, and scanning speed of 100 nm min⁻¹. The samples were sequentially thermostated for 10 min at each temperature starting from 3°C up to 80°C. Each experiment was repeated five times with freshly isolated thylakoids.

The amplitudes of the different CD bands were determined using reference wavelengths, e.g., by the subtraction of the maximum intensity of the positive signal at a specified wavelength and the corresponding minimum of the negative signal (for example the amplitude of the 448–459 nm band was obtained by subtracting the CD at 459 nm from the signal at 448 nm). For strongly overlapping CD bands, such as the CD band at 685 nm and at 650 nm, the amplitude was estimated by subtracting a reference zero-value CD signal (CD(685–730) and CD(610–650)). The transition temperature (T_m) is defined as the temperature at which the intensity of the CD band or band-pair is decreased by 50% of its value at 25°C, similar to Cseh et al. (2000).

Chl a time-resolved fluorescence measurements

Fluorescence lifetime imaging microscopy

Fluorescence lifetime imaging microscopy (FLIM) was performed in vivo on detached leaves of WT and dgd1, using the setup described previously (Borst et al. 2005). In short, two-photon excitation pulses (860 nm, 150 fs pulse duration, 76 MHz repetition rate) were focused into the sample with a 60× water immersion objective lens. Fluorescence was detected via non-descanned single photon counting detection, through two band-pass filters of 700 nm (75 nm width). Images of 64 × 64 pixels were obtained, with 1024 time channels of 12 ps. The fluorescence was collected for 30 min; low excitation power (of the order of 600 μW average power) was used to keep the reaction centers open and to minimize photodamage. The fluorescence decay were analyzed by software provided by Becker & Hickl (SPCImage). All measurements were performed at 22°C. The plants were dark-adapted at 20°C for 30 min before the measurements.

Time-correlated single photon counting

Time-correlated single photon counting (TCSPC) was used to perform time-resolved fluorescence measurements using a setup described earlier (Borst et al. 2005). For the fitting procedure, the dynamic instrumental response of the experimental setup was recorded using the fast and single-exponential fluorescence decay (6 ps) of the reference compound pinacyanol in methanol (van Oort et al. 2008). Data analysis was performed using the computer program described earlier (Digris et al. 1999; Novikov et al. 1999). The fit quality was evaluated from χ², and from the plots of
the weighted residuals and the autocorrelation thereof (Visser et al. 2008). Typical values of $\chi^2$ were 1.0–1.1.

For Chl $a$ fluorescence measurements, the samples were excited at 470 nm, and the emission was collected using an interference filter at 688 nm with a bandwidth of 10 nm. The samples were sequentially thermostatted at increasing discrete temperatures, between 7 and 70°C, for 10 min at each temperature. The decay curves were analyzed by a four-exponential model; for each decay trace, the average lifetime ($\tau_{\text{ave}}$) was calculated by the formula:

$$
\tau_{\text{ave}} = \sum_{i=1}^{n} x_i \tau_i
$$

$\tau$ being the fluorescence lifetime and $x$ the pre-exponential factor proportional to the fractional population, with $\sum_{i=1}^{n} x_i = 1$. For the calculation of $\tau_{\text{ave}}$, the minor contribution (typically about 1–2%) of a component with a lifetime above 1 ns, originating from closed reaction centers, was not taken into account. The mean value of $\tau_{\text{ave}}$ and its standard error presented in this article were determined from five different decay curves measured on different samples.

Time-resolved fluorescence measurements of Merocyanine 540

For studying the lipid packing the lipophilic fluorescence probe, Merocyanine 540 (MC540, purchased from Sigma–Aldrich) was added, from a 1 mM ethanol stock solution (to a final concentration of 0.2 lM), to a suspension of thylakoid membranes (containing 20 μg Chl ml$^{-1}$) and incubated for 30 min before the experiments. During this time, the sample was gently stirred and kept on ice in the dark. Longer incubation with MC540 did not result in increased incorporation of the probe (see Krumova et al. 2008a and references therein).

For fluorescence lifetime measurements, the TCSPC setup described in the previous section was used. The excitation wavelength was set to 570 nm, and the emission was collected between 610 and 630 nm using a Schott OG 610 nm (3 nm) cut-off filter and a Balzers K60 interference filter. During the measurements the samples were sequentially thermostatted at increasing discrete temperatures, between 7 and 45°C, for 15 min at each temperature. The MC540 measurements, as in the more systematic study using the same lipid probe in isolated thylakoids (Krumova et al. 2008a), are confined to this temperature interval with no protein degradation (Dobrikova et al. 2003) but significant changes in the lipid packing (detected also by $^{31}$P-NMR, Krumova et al. 2008b); above 45°C, thylakoid lipids segregate in large quantities from the membrane and form extended non-bilayer structures (Gounaris et al. 1984).

For the analysis of the fluorescence decay, the three-exponential model introduced earlier (Krumova et al. 2008a) was used, which assumes a partition of MC540 between the aqueous phase with short (<200 ps) lifetime and the two lipid phases with $\sim$1- and $\sim$2-ns lifetimes.

Since these three types of microenvironments are the same for WT and dgd1, the MC540 fluorescence lifetimes for the five different WT or dgd1 samples were linked during the fitting procedure (resulting, at a given temperature, in equal lifetime values for both samples) whereas their relative amplitudes were left free. In this way, the changes in the distribution of MC540 over the different environments can be followed for WT and dgd1.

Electrochromic absorbance transients

Electrochromic absorbance changes ($\Delta A$15), induced by saturating single turnover flashes, were measured at 515 nm on detached leaves, in a setup described earlier (Büchel and Garab 1995). The plants used for the measurements were dark-adapted at 20°C for 30 min, and detached leaves of WT and dgd1 were infiltrated with water, incubated for 10 min at different temperatures, and then measured at 25°C; 64 kinetic traces were collected with a repetition rate of 1 s$^{-1}$ and averaged; the duration of the flashes was about 5 μs; the time constant of the measurements was adjusted to 100 μs. The measurements were repeated five times with leaves from different plants.

Results

Pigment–protein complexes: (macro-)organization, excitation energy migration, and trapping

Circular dichroism

The CD spectra of thylakoid membranes isolated from WT and dgd1 are presented in Fig. 1a. It can be seen that at 25°C, the amplitudes of the (~)650 nm band, arising from excitonic interactions of Chl $b$ in monomeric and trimeric LHCII, were approximately identical in WT and dgd1. Also, the Chl $a$ CD signals between 400 and 450 nm were not affected significantly by the deficiency of DGDG. In contrast, the intensities of the main $\Psi$-type CD bands, between 660 and 700 nm and at around 505 nm, were substantially smaller for dgd1 (Fig. 1a). (For the origin of the main CD bands in thylakoids, see, e.g., Garab and van Amerongen 2009).

The thermal destabilization of different protein complexes was monitored via the amplitudes of their corresponding CD bands. The (~)650 nm band exhibited the same temperature dependence for WT and dgd1 and
displayed essentially identical transition temperatures \( T_m \) at \( \sim 60^\circ \text{C} \) (Table 1). On the other hand, the mutation substantially affected the thermal stability of the Chl a excitonic bands at around 450 nm, determined either as CD \(_{448-438}\) (not shown) or CD \(_{448-459}\) (Fig. 1b). The \( T_m \) values were lower by \( \sim 6^\circ \text{C} \) for the mutant than for the WT (Table 1). The \( \Psi \)-type signal (CD \(_{685-730}\)) also exhibited different temperature dependencies for WT and \( \textit{dgd1} \) (Fig. 1c). The transition temperature for this band was \( 54 \pm 2^\circ \text{C} \) for the WT, whereas for \( \textit{dgd1} \) it was found at \( 48 \pm 1^\circ \text{C} \) (Table 1).

**Green (native) gel electrophoresis**

In order to discriminate between the thermal behavior of the different photosynthetic complexes, green gel electrophoresis of heat-treated thylakoid membranes from WT and \( \textit{dgd1} \) was performed (Fig. 2a) and analyzed for the contents of PSI supercomplexes (Fig. 2b) and LHCI trimers (Fig. 2c). The data show that the PSI supercomplex in \( \textit{dgd1} \) is less stable upon heat treatment than the WT—the intensity of the corresponding green gel band decreases by 50% at \( 57^\circ \text{C} \) for \( \textit{dgd1} \) and at \( 61^\circ \text{C} \) for WT, respectively (Fig. 2b). In contrast, the destabilization of LHCI trimers follows the same pattern in both the WT and \( \textit{dgd1} \) up to \( 65^\circ \text{C} \) (Fig. 2c).

**Chlorophyll a fluorescence lifetime**

The functional activity of the photosystems was studied with the aid of Chl a fluorescence lifetime measurements, using microscopic (FLIM) and macroscopic (TCSPC) measurements.

The FLIM images are plotted in Fig. 3a, b (WT) and c, d \( \textit{dgd1} \). The recorded fluorescence originates from Chls in the chloroplasts. Thus, the bright spots in the intensity images (Fig. 3a, c) originate from distinct chloroplasts. Their shape is not well defined in the FLIM images due to the fact that the brightness of the individual organelles is proportional to the intensity of the fluorescence emission.

| CD signal (nm) | Assignment | \( T_m \) °C (WT) | \( T_m \) °C (\( \textit{dgd1} \)) |
|----------------|------------|------------------|------------------|
| 685–730        | \( \Psi \)-type | 54 ± 2           | 48 ± 1           |
| 685–671        | \( \Psi \)-type | 54 ± 1           | 49 ± 1           |
| 505–550        | \( \Psi \)-type | 56 ± 1           | 51 ± 1           |
| 610–650        | Excitonic (Chl b, LHCI) | 61 ± 2 | 58 ± 2 |
| 448–459        | Excitonic (Chl a) | 59 ± 2           | 54 ± 1           |
| 448–438        | Excitonic (Chl a) | 57 ± 1           | 50 ± 1           |

The membranes were thermostated for 10 min at different temperatures in the range between 5 and 80°C before recording the CD spectra at the given temperature; the amplitudes for the individual bands were calculated from the difference in the intensity at specific wavelengths (see also the text). \( T_m \) is defined as the temperature at which the intensity of the CD band is decreased to 50% of its value at 25°C. The values for \( T_m \) and their standard errors are determined from five independent experiments.
Therefore, the chloroplasts being located in the focal plane are observed as bright objects, whereas the lower intensity pixels probably represent somewhat out-of-focus chloroplasts. The fluorescence decay traces recorded for each pixel were analyzed by a three-exponential model from which an average lifetime per pixel was calculated. These average lifetimes are plotted in Fig. 3b and d for the WT and dgd1, respectively. The sum of the decay curves recorded for all the pixels in the image of WT and dgd1 leaves is presented in (b) and (c), respectively. The samples were treated for 10 min at the specified temperatures before loading on the gel.

The FLIM setup used can only be applied for measurements at 22°C. In order to check the temperature dependence of the average Chl a fluorescence lifetime (τave), it was determined for isolated intact thylakoid membranes using the TCSPC technique. The fluorescence decay curves for WT and dgd1 are shown in Fig. 4a and the parameters obtained from the fit are plotted as a table in the figure. At 25°C, the fitting analysis results in longer fluorescence lifetimes for dgd1 than for WT. Interpretation of these results is beyond the scope of this study. The only aspect of the temperature dependence that we want to point out is the strong decrease of the average lifetime above 50°C (reaching 83 ps at 65°C). For dgd1 the same sharp drop in τave occurs at lower temperatures and begins at around 45°C (Fig. 4b).

Lipid matrix: lipid packing and membrane permeability

In order to study the global physical properties of the lipid matrix of thylakoids, two methods were applied: (i) time-resolved fluorescence of MC540 in thylakoid membranes, which reports on the packing of the lipid molecules; and (ii) electrochromic absorbance transients on whole leaves, which probe the energization and the permeability of thylakoid membranes.

**Partition of MC540 in thylakoid membranes**

Using the three-exponential model for the analysis of the fluorescence decay of MC540 (see also “Materials and methods”), lifetimes of 0.19–0.23 ns (Fig. 5a), 0.66–1.08 ns (Fig. 5b), and 1.71–2.15 ns (Fig. 5c) were obtained; the lifetimes shorten with the increase of...
temperature. In this article, they are referred to as 200-ps, 1-ns, and 2-ns components, respectively.

As shown in Fig. 5a–c, the relative amplitudes of the different lifetime components of MC540 differ for WT and dgd1. The relative amplitude of the 200 ps component remains constant up to 45°C for WT, whereas for dgd1 it increases with the increase of temperature, evidently at the expense of the 1-ns component. This appears to occur especially above 25–30°C (Fig. 5a). A comparison of the relative amplitudes of the 1- and 2-ns components in dgd1 and WT) reveals that for WT the relative amplitude of the 2-ns component is slightly larger than that of the 1-ns component, indicating that the amounts of MC540 incorporated into the bilayer and located on the surface are almost equal (Fig. 5b, c). In contrast, for dgd1, the relative amplitude of the 1-ns component is significantly larger than that of the 2-ns component (Fig. 5b, c).

If the two slow components originate from a broad distribution of lifetimes (cf. Krumova et al. 2008a), then their weighted average lifetime is a more appropriate parameter to consider. As can be seen in Fig. 5d, at 7°C this average lifetime is shorter for dgd1 (1.35 ± 0.1 ns) than for WT (1.52 ± 0.01 ns). The average lifetime for both WT and dgd1 is decreasing with the increase of temperature, but the average lifetime of dgd1 remains shorter at all temperatures between 7 and 35°C; at 45°C the two lifetimes become almost identical, about 1.1 ns.

**Electrochromic absorbance changes (ΔA515) in WT and dgd1**

In order to test the membrane permeability, electrochromic absorbance change (ΔA515) measurements were performed.

On the time scale of the experiment, the rise of ΔA515, due to primary charge separations, is instantaneous. The initial amplitude of ΔA515 (for samples with identical Chl concentration) differs for WT and dgd1, as can be seen in Fig. 6a and b. At 25°C, the decay time of ΔA515 for the mutant (t1/2 = 226 ± 15 ms) is essentially the same as for the WT (t1/2 = 227 ± 19 ms). For the 35°C-treated sample, the decay of ΔA515 is significantly faster for the dgd1 mutant (Fig. 6b); the corresponding halftimes are 237 ± 16 ms for WT and 154 ± 19 ms for dgd1. No change in the decay rate was observed for the WT leaves exposed to the same temperature; only at 40°C, the decay becomes faster (t1/2 = 36 ± 12 ms) for WT; at this latter temperature no ΔA515 signal can be discerned for dgd1.

**Discussion**

In this article, we investigated the role of one of the major thylakoid lipids, DGDG on the global organization and thermal stability of the membranes. To this end, we used the Arabidopsis lipid mutant dgd1, with substantially decreased DGDG content (Dörmann et al. 1995), and measured...
different physical parameters, such as CD of the thylakoid membranes Chl a fluorescence lifetimes and the fluorescence lifetime distribution of the lipophylic dye MC540 as well as the electrochromic absorbance changes along with their temperature-dependencies in WT and dgd1 samples.

Effects of DGDG on the global organization of thylakoid membranes

Dörmann et al. (1995) have revealed major ultrastructural differences in the organization of the thylakoid membranes between the dgd1 and the WT such as increased number of thylakoids per granum and longer granal and stromal thylakoids. It is well known that the stacking of thylakoids and the lateral macro-organization of the pigment–protein complexes in the membrane are interrelated (reviewed by Mustárdy and Garab 2003; Dekker and Boekema 2005) but dgd1 is poorly characterized in this respect.

In order to obtain information on the global organization of pigment–protein complexes in dgd1 thylakoid membranes, we performed CD spectroscopic measurements. We also performed Chl fluorescence lifetime measurements to provide an insight into the energy migration and trapping capabilities of the membranes in relation to the altered composition of the membranes and the macro-organization of the complexes. The effect of DGDG deficiency on the packing of lipids and the energization of membranes were tested with the aid of MC540 fluorescence lifetime measurements and by measuring electrochromic absorbance transients.

Circular-dichroism (CD) spectroscopy in the visible range is a valuable tool for probing the molecular architecture of the complexes and supercomplexes and their macro-organization in the membrane system (Garab and van Amerongen 2009). Two types of CD bands are relevant for the study of thylakoid membranes described a follows:

(i) Excitonic bands which originate from short-range (nanometer scale) excitonic interactions between pigments within a pigment–protein complex or on adjacent complexes (Tinoco 1962; De Voe 1965; Somsen et al. 1996; Garab and van Amerongen 2009), and can be used for testing the intactness of individual complexes or supercomplexes. Such interactions give rise to conservative band structures—i.e., the positive and negative bands of the split spectrum have equal areas. In a system as complex as the thylakoid membrane, a variety of excitonic bands is superimposed on top of each other. These are difficult to discriminate, and here, we shall use only two characteristic bands, at around 650 and 440 nm. It has been established that the (−)650 nm band originates from Chl b and is regarded as a fingerprint of the LHCII complexes (van Metter 1977; Georgakopoulou et al. 2007), while the CD bands that appear between 400 and 450 nm mainly originate from Chl a (Garab et al. 1991). The intensity of the (−)650 nm CD band remains unchanged in dgd1, which demonstrates that the molecular architecture of LHCII is not significantly affected by the mutation.

**Fig. 4** a Chlorophyll a fluorescence decay traces for isolated thylakoid membranes from WT (thick line) and dgd1 (dashed line), recorded by TCSPC. The presented curves are the sums of five independent measurements on different preparations. The excitation wavelength is 430 nm, and the emission is recorded at 688 nm at 25°C. The corresponding fits (fluorescence lifetimes (τ) and relative amplitudes, given in brackets) are also presented. b Temperature dependence of the average fluorescence lifetime for the WT (filled square) and dgd1 (open circle). Details about the fitting procedure are described in “Materials and methods”. The lines (solid for WT and dashed for dgd1) serve as a guide to the eye. The average lifetime values and their standard errors are determined from five independent experiments.

**Fig. 5** Temperature dependencies of the parameters, obtained after the analysis of the fluorescence decays recorded for MC540 in WT and dgd1 thylakoid membranes. a–c Lifetime components (blue symbols) and their respective amplitudes in WT (full black symbols) and dgd1 (open black symbols). d Weighted average lifetimes of the two long-lived components for WT (filled circle) and dgd1 (open circle). The samples were thermostated for 10 min at each temperature before starting the measurements. For further details for the fitting model see also “Materials and methods” and text.
(ii) $\Psi$-type CD bands—high-intensity bands, originating from long-range order (hundreds of nanometers) of the chromophores in chirally-organized macroarrays. They are very intense and “anomalously shaped” (Garab 1996); in thylakoids and isolated LHCII-aggregates they appear at around (+)685, (−)671 nm and (+)505 nm (Barzda et al. 1994; Dobrikova et al. 2003); these bands are also associated with long tails outside the principal absorbance bands, which originate from differential scattering of the left and right circularly polarized light (Garab 1996). $\Psi$-type bands correlate with the macro-organization of the main Chl a/b light harvesting complexes, e.g., in LHCII-only domains, as indicated by correlations between the intensity of these bands and the LHCII-content of the sample (e.g., Garab et al. 1991; Garab and Mustárdy 1999). The arrays of PSII-supercomplexes might also contribute to the $\Psi$-type CD signal. For example, in a mutant lacking one of the minor light-harvesting complexes, namely, CP24, the macro-organization of the PSII-supercomplexes is modified as compared to WT. This results in the loss of the main $\Psi$-type band in the red at around (+)690 nm (Kovács et al. 2006).
The intensities of the Ψ-type CD bands between 660 and 700 (Fig. 1a) differ for WT and dgd1 thylakoids. These CD signals are shown to be determined by the long-range organization of the pigment–protein complexes, in particular LHCII (e.g., Garab et al. 1991; Garab and Mustárdy 1999) and PSII-supercomplexes (Kovács et al. 2006). Thus, the reduced intensity of the main Ψ-type CD bands (CD_{685-703} and CD_{685-671}) in the mutant (Fig. 1a) might either be due to a smaller size of the chiral macrodomains or to a different organization of the complexes affecting the pigment–pigment interactions. It should be noted that DGDG has been found to be required for the formation of ordered 3D crystals of LHCII (Nußberger et al. 1993). Hence, our CD data strongly suggest that also in vivo in the thylakoid membranes DGDG modulates the macroorganization of the main light-harvesting complexes of PSII.

As shown by Chl fluorescence lifetime measurements, alterations in the macroorganization in dgd1 affected only marginally the energy migration and trapping (Figs. 3, 4). The mutant exhibited a somewhat longer average Chl a fluorescence lifetime (Figs. 3f, 4).

The assignment of the fluorescence lifetimes to particular protein complexes or macr assemblies is a rather complicated task for intact chloroplasts and isolated thylakoids, where a large variety of complexes and supercomplexes co-exist. For example, most studies on whole chloroplasts and intact thylakoid membranes suggested average values for the trapping time in PSII between ~300 and ~500 ps (e.g., Roelofs et al. 1992; Gilmore et al. 1996; Vasile’v et al. 1998). A very detailed study of the fluorescence kinetics of thylakoid membranes with varying composition was recently performed, using different combinations of excitation and detection wavelengths to assign the various lifetimes to PSI and PSII but this is not a trivial task (van Oort et al. 2010). For isolated BBY particles (containing only the granal thylakoid membranes) two major lifetimes of about 74–80 and 175–212 ps were observed (Broess et al. 2006, 2008), whereas three major decay times are found for PSI: 5–20, 60–130 ps (Turconi et al. 1994; Croce et al. 2000; Ihalainen et al. 2002, 2005; van Oort et al. 2008; Slavov et al. 2008). Because of the various complications, only the average lifetimes (τ_{ave}) measured for WT and dgd1 thylakoid membranes and intact leaves are compared in this article.

The longer lifetime in dgd1 can most easily be explained by taking into account the lower PSI content of the membranes (Ivanov et al. 2006)—this photosystem exhibits short lifetimes (e.g., van Oort et al. 2010). Furthermore, excess amounts of LHCl might also contribute to the longer lifetimes—according to Ivanov et al. (2006) the amount of LHCl was unchanged; hence, a fraction of these antenna complexes might not be connected to the reaction center.

As reported by the lipophilic fluorescence probe MC540, alterations in the lipid composition in dgd1 bring about changes in the lipid packing. The spectroscopic properties of MC540 are determined by the dielectric constant of its local environment (Lelkes and Miller 1980). Thus, it exhibits different fluorescent lifetimes when present in different environments (interacting with lipids or solubilized in the aqueous phase). Earlier it has been shown that the shortest lifetime (200 ps) component originates from dyes in aqueous environment and the 1- and 2-ns components from MC540 in hydrophobic environments, i.e., in the lipid phase (Krumova et al. 2008a). These lifetimes might be assigned either to two discrete populations of the molecules, reflecting two different microenvironments or to a broad distribution of lifetimes due to incorporation of MC540 in a variety of environments with small differences in their physical properties.

Our data reveal significant differences in the lipid packing between dgd1 and WT membranes. Most prominently, the increased amplitude of the 200 ps component suggest that in dgd1 the MC540 molecules are more exposed to the aqueous phase than in WT (Fig. 5). The lower extent of incorporation of MC540 in the thylakoid membranes isolated from dgd1 in comparison with WT membranes might be due to two factors: (i) tighter lipid packing in dgd1, which could be the consequence of modified lipid–protein interactions and changes in the macroorganization, and/or (ii) modified surface charge of the membrane, i.e., due to conformational changes in the protein complexes or to differences in lipid–protein interactions.

Despite the altered lipid composition (increased nonbilayer:bilayer lipid ratio) and alterations in the lipid packing, the ΔA515 measurements indicate that the dgd1 thylakoid membranes are perfectly adjusted to generate and maintain the transmembrane electrochemical potential difference at 25°C (Fig. 6a). ΔA515 is a voltmeter of thylakoid membranes. Light-induced primary charge separation in the reaction centers, followed by vectorial transport of charges, generate a transmembrane electrochemical potential difference, which consists of a pH gradient and an electric potential difference. By a reverse flow of protons, the electrochemically stored energy is used for ATP synthesis (Mitchell 1966). The potential gradient can also be dissipated by the basal ion efflux, which depends on the electrical permeability of the membranes. The rise and decay of the transmembrane electrical difference can be followed by the electrochromic absorbance changes (ΔA515) of the pigments embedded in the membrane, which correlates with the transmembrane electric field (Junge 1977; Witt 1979).

We have obtained ΔA515 decay times comparable with those observed for barely under similar conditions (Garab
et al. 1983). The initial amplitude of ΔA515 is lower for 
dgd1 than for WT, but this can be attributed to the
decreased content of PSI reaction centers in the mutant
(Ivanov et al. 2006). These data are also in line with the
data of Härter et al. (1997) showing that dgd1 is capable of
maintaining a low luminal pH, needed for the xanthophyll
cycle operation.

Effects of DGDG on the thermal stability of thylakoid
membranes

The temperature dependencies of the various CD bands
reveal that whereas LHCII (characterized by (−)650 nm
Chl b excitonic band) preserved its stability, the Ψ-type
(CD 685–730) and CD 685–671) and the excitonic Chl a CD
bands (CD 448–459 and CD 448–438) are significantly less
stable in the mutant (Fig. 1; Table 1). The latter two Chl
bands most probably originate from the core com-
plexes of PSII and/or PSI which bind only Chl
a absorption bands (CD (448–459) and CD (448–438)) are significantly less
stable in the mutant (Fig. 1; Table 1). The latter two Chl
a CD signals most probably originate from the core com-
xplexes of PSII and/or PSI which bind only Chl
b. Changes in the thermal stability of pigment–protein complexes, including the LHCII, exhibit
a close correlation between the ability of plants to acquire
thermotolerance and the increase in the DGDG level and
in the DGDG:MGDG ratio, while no correlation was found
with the accumulation of heat-shock proteins.

The differences in the temperature dependencies of the
lipid packing in WT and dgd1 might (at least in part) be
due to the increased non-bilayer propensity of the bulk
lipids in comparison to the WT. Previously, it has been
shown, by means of 31P-NMR, that non-bilayer lipid
structures are present in spinach thylakoid membranes
(Krumova et al. 2008b). Analogous 31P-NMR studies
would provide valuable information for the phase proper-
ties of WT and mutant thylakoid membranes. However,
given the fact that 31P-NMR measurements require isolated
thylakoid membranes of 50–100 mg Chl content, it is not
feasible with Arabidopsis.

While at 25°C, the kinetic patterns of the electrochromic
absorbance transients in dgd1 and WT leaves do not differ
from each other, in the mutant, the membranes become
permeable to ions even at 35°C (Fig. 6b), in contrast to
WT, which becomes leaky only above 40°C. Dependence
of the membrane permeability on the lipid content of
thylakoids was also demonstrated for a mutant of Arabid-
opsis (mgd1-1, Jarvis et al. 2000) with decreased amount of
MGDG—the thylakoid membranes of mgd1-1 were shown
to exhibit increased conductivity at high light intensities,
which resulted in inefficient operation of the xanthophyll
cycle (Aronsson et al. 2008) and which further demon-
strates the importance of the lipid phase behavior for the
electric properties of the membrane.

Conclusion

It has become clear in this study that the DGDG deficiency
substantially influences both the overall organization and
functioning of the thylakoid membrane and its thermal sta-
ility. At room temperature (25°C) the arrangement of the
pigment–protein complexes in dgd1 differs from that in WT:
the Ψ-type CD bands, originating from large macrodomains
of pigment–protein complexes, including the LHCII, exhibit
significantly lower amplitudes for dgd1. Experiments using
the fluorescent lipid probe MC540 reveal differences in the
packing of the lipid molecules, indicating a tighter packing
or a modified surface charge density in the mutant thylakoid
membranes. These alterations, together with a reduced PSI
content of the membranes, albeit that they exert some well
discernible effects on the energy migration and trapping
properties as well as on the energization of the membranes,
do not disturb dramatically the functions, at least as long as
the plants are kept in a temperature range not higher than
25°C. At moderately elevated temperatures, however,
dramatic differences emerge, which are manifested in increased thermal susceptibilities in \textit{dgd1} compared to WT: the LHCII–PSII containing macromodules disassemble, PSI complexes degrade, the excitation energy is quenched, large amounts of lipids are protruded from the membranes, and the thylakoids become leaky for ions—in all these cases, the changes occur 5–7°C lower in \textit{dgd1} than in WT. Hence, these data strongly suggest that the lipid matrix of \textit{dgd1} is not able to maintain the functional state of the protein molecules at moderately elevated temperatures.

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References

Aronsson H, Schottler MA, Kelly AA, Sundqvist C, Dörmann P, Karim S, Jarvis P (2008) Monogalactosyldiacylglycerol deficiency in Arabidopsis affects pigment composition in the prolamellar body and impairs thylakoid membrane energization and photoprotection in leaves. Plant Physiol 148:580–592. doi: 10.1104/pp.108.123372

Barzda V, Mustárdy LA, Garab G (1994) Size dependency of circular dichroism in macroaggregates of photosynthetic pigment–protein complexes. Biochemistry 33:10837–10841. doi: 10.1021/bi00020a034

Ben-Shemi A, Frolow F, Nelson N (2003) Crystal structure of plant photosystem I. Nature 426:630–635. doi: 10.1038/nature02200

Borst JW, Hink MA, van Hoek A, Visser AJWG (2005) Effects of refractive index and viscosity on fluorescence and anisotropy decays of enhanced cyan and yellow fluorescent proteins. J Fluoresc 15:153–160. doi: 10.1007/s10895-005-2523-5

Broess K, Trinkunas G, van der Weij-de Wit CD, Dekker JP, van Hoek A, van Amerongen H (2006) Excitation energy transfer and charge separation in photosystem II membranes revisited. Biophys J 91:3776–3786. doi: 10.1529/biophysj.106.085068

Broess K, Trinkunas G, van Hoek A, Croce R, van Amerongen H (2008) Determination of the excitation migration time in photosystem II. Consequences for the membrane organization and charge separation parameters. Biochim Biophys Acta 1777:404–409. doi: 10.1016/j.bbapap.2008.02.003

Broess K, Borst JW, van Amerongen H (2009) Applying two-photon excitation fluorescence lifetime imaging microscopy to study photosynthesis in plant leaves. Photosynth Res 100:89–96. doi: 10.1007/s11120-009-9431-5

Büchel C, Garab G (1995) Electromechanical absorbance changes in the chlorophyll-c-containing alga \textit{Pleuracorhizus miragensis} (Xanthophyceae). Photosynth Res 43:49–56. doi: 10.1007/BF00029462

Chen J, Burke J, Xin Z, Xu C, Velten J (2006) Characterization of the \textit{Arabidopsis} thermosensitive mutant 177 ato2 reveals an important role for galactolipids in thermostolerance. Plant Cell Environ 29:1437–1448. doi: 10.1111/j.1365-3040.2006.01527.x

Chitnis PR (2001) Photosystem I: function and physiology. Annu Rev Plant Physiol Plant Mol Biol 52:593–626. doi: 10.1146/annurev.plant.52.1.593

Croce R, Dorra D, Holzwarth AR, Jennings RC (2000) Fluorescence decay and spectral evolution in intact photosystem I of higher plants. Biochemistry 39:6341–6348. doi: 10.1021/bi992659r

Cseh Z, Rajagopal S, Tsonev T, Busheva M, Papp E, Garab G (2000) Thermooptic effect in chloroplast thylakoid membranes. Thermal and light stability of pigment arrays with different levels of structural complexity. Biochemistry 39:15250–15257. doi: 10.1021/bi001600d

De Bianchi S, Dall’Osto L, Tognon G, Morosinotto T, Bassi R (2008) Minor antenna proteins CP24 and CP26 affect the interactions between photosystem II subunits and the electron transport rate in grana membranes of Arabidopsis. Plant Cell 20:1012–1028. doi: 10.1105/tpc.107.055749

De Voe H (1965) Optical properties of molecular aggregates. II. Classical theory of the refraction, absorption, and optical activity of solutions and crystals. J Chem Phys 43:3199–3208. doi: 10.1063/1.1697294

Dekker JP, Boekema EJ (2005) Supramolecular organization of thylakoid membranes in green plants. Biochim Biophys Acta 1706:12–39. doi: 10.1016/j.bbabio.2004.09.009

Digris AV, Skakun VV, Novikov EG, van Hoek A, Claiborne A, Visser AJWG (1999) Thermal stability of a flavoprotein assessed from associative analysis of polarized time-resolved fluorescence spectroscopy. Eur Biophys J 28:526–531. doi: 10.1007/s002490050235

Dobrikova AG, Várkonyi Z, Krumova SB, Kovács L, Kostov GK, Todinova SJ, Busheva MC, Taneva SG, Garab G (2003) Structural rearrangements in chloroplast thylakoid membranes revealed by differential scanning calorimetry and circular dichroism spectroscopy. Thermo-optic effect. Biochemistry 42:11272–11280. doi: 10.1021/bi034899g

Dörmann P, Hoffmann-Benning S, Balbo I, Benning C (1995) Isolation and characterization of an Arabidopsis mutant deficient in the thylakoid lipid digalactosyldiacylglycerol. Plant Cell 7:1801–1810

Douce R, Joyard J (1996) Biosynthesis of thylakoid membrane lipids. In: Ort DR, Yacum CF (eds) Advances in photosynthesis/oxygenic photosynthesis: the light reactions. Kluwer, Dordrecht, pp 69–101. doi: 10.1007/978-94-017-1974-5

Gabashvili IS, Menik J, Segui J, Fragata M (1998) Protein structure of photosystem II studied by FT-IR spectroscopy. Effect of digalactosyldiacylglycerol on the tyrosine side chain residues. J Mol Struct 444:123–133. doi: 10.1016/S0022-1694(97)00367-0

Garab G (1996) Linear and circular dichroism. In: Amesz J, Hoff AJ (eds) Biophysical techniques in photosynthesis. Kluwer, Dordrecht, pp 11–40

Garab G, Mustárdy L (1999) Role of LHCII-containing macromodules in the structure, function and dynamics of grana. Aust J Plant Physiol 26:649–658

Garab G, van Amerongen H (2009) Linear dichroism and circular dichroism in photosynthesis research. Photosynth Res 101:135–146. doi: 10.1007/s11120-009-9424-4

Garab G, Sanchez Bargos AA, Zimányi L, Faludi-Dániel A, Gácsi K, Chmito PR (2001) Photosystem I: function and physiology. Annu Rev Plant Physiol Plant Mol Biol 52:593–626. doi: 10.1146/annurev.plant.52.1.593

Garab G, Kieczczawa J, Sutherland JC, Bustamante C, Hind G (1991) Organization of pigment–protein complexes into macromodules in the thylakoid membranes of wild type and chlorophyll b-less mutant of barley as revealed by circular dichroism.
Jarvis P, Dörmann P, Peto CA, Lutes J, Benning C, Chory J (2000) Involvement of chloroplast lipids in the reaction of photosystem II chlorophyll a fluorescence lifetimes and intensity are independent of the antenna size differences between barley wild-type and chloroplast mutants: photochemical quenching and xanthophyll cycle dependent non-photochemical quenching of fluorescence. Photosynth Res 48:171–187.

Dörmann P, Haertel H, Lokstein H, Grimm B, Benning C (1998) Temperature dependence of the lipid packing in thylakoid membranes studied by time- and spectrally resolved fluorescence of Merocyanine 540. Biochim Biophys Acta 1778:2823–2833. doi:10.1016/S0167-4889(08)70192-9

Kovács L, Damkjar J, Kereczie S, Illiosa C, Ruhan AV, Boekema EJ, Jansson S, Horton P (2006) Lack of the light-harvesting complex CP24 affects the structure and function of the grana membranes of higher plant chloroplasts. Plant Cell 18:3106–3120. doi:10.1105/tpc.106.045641

Krumova SB, Koehorst RMB, Bota A, Päti T, van Hoek A, Garag, van Amerongen H (2008a) Phase behavior of phosphatidylglycerol in spinach thylakoid membranes as revealed by 13C-NMR. Biochim Biophys Acta 1778:997–1003. doi:10.1016/j.bbamem.2008.01.004

Lehky PL, Miller IR (1980) Perturbations of membrane structure by optical probes: I. Location and structural sensitivity of Merocyanine 540 bound to phospholipid membranes. J Membr Biol 52:1–15. doi:10.1007/BF01869001

Liu ZF, Yang HC, Wang KB, Kuang TY, Zhang JP, Gui LL, An XM, Chang WR (2004) Crystal structure of spinach major light-harvesting complex at 2.7 Å angstrom resolution. Nature 428:287–292. doi:10.1038/nature02373

Lolli B, Kern J, Sänger W, Zouni A, Biesiada J (2005) Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. Nature 438:1040–1044. doi:10.1038/nature04224

Lolli B, Kern J, Sänger W, Zouni A, Biesiada J (2007) Lipids in photosystem II: interactions with proteins and cofactors. Biochim Biophys Acta 1767:509–519. doi:10.1016/j.bibba.2006.12.009

Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol Rev Camb Philos Soc 41:445–502.

Mustárdy L, Garab G (2003) Granum revisited. A three-dimensional model—where things fall into place. Trends Plant Sci 8:117–122. doi:10.1016/S1360-1385(03)00015-3

Novikov EG, van Hoek A, Visser AJW, Hofstraat JW (1999) Linear algorithms for stretched exponential decay analysis. Opt Commun 166:189–198. doi:10.1016/S0030-4018(99)00262-X

Nußberger S, Dörr K, Wang DN, Kühlbrandt W (1993) Lipid–protein interactions in crystals of plant light-harvesting complex. J Mol Biol 234:347–356. doi:10.1006/jmbi.1993.1591

Roofofs TA, Lee CI, Holzwarth AR (1992) Global target analysis of picosecond chlorophyll fluorescence kinetics from pea chloroplasts: a new approach to the characterization of the primary processes in photosystem II z-units and b-units. Biophys J 61:1147–1163. doi:10.1016/S0006-3495(92)81924-0

Sakurai I, Mizusawa N, Wada H, Sato N (2007) Digalactosyldiacylglycerol is required for stabilization of the oxygen-evolving complex in photosystem II. Plant Physiol 145:1361–1370. doi:10.1104/pp.107.106781

Slavov C, Ballottari M, Morosinotto T, Bassi R, Holzwarth AR (2008) Trap-limited charge separation kinetics of photosystem I complexes from higher plant. Biophys J 94:3601–3612. doi:10.1529/biophysj.107.117101

Smith PJ, Peterson S, Masters VM, Wydzynski T, Stirling S, Krausz E, Pace RJ (2002) Magneto-optical measurements of the pigments in fully active photosystem II core complexes from plants. Biochemistry 41:1981–1989. doi:10.1021/bi0111202

Somerson OJG, van Grondelle R, van Amerongen H (1996) Spectral broadening of interacting pigments: polarized absorption by photosynthetic proteins. Biochim Biophys Acta 1778:997–1003. doi:10.1016/j.bbapap.2008.09.007

Turconi S, Weber N, Schweitzer G, Strötman H, Holzwarth AR (1994) Energy transfer and charge separation kinetics in
photosystem I. 2. Picosecond fluorescence study of various PS I particles and light-harvesting complex isolated from higher plants. Biochim Biophys Acta 1187:324–334. doi:10.1016/S0006-3495(93)81552-2
van Metter RL (1977) Excitation energy transfer in the light-harvesting chlorophyll a/b protein. Biochim Biophys Acta 462:642–658. doi:10.1016/0006-3495(77)90107-4
van Oort B, Amunts A, Borst JW, van Hoek A, Nelson N, van Amerongen H, Croce R (2008) Picosecond fluorescence of intact and dissolved PSI-LHCI crystals. Biophys J 95:5851–5861. doi:10.1529/biophysj.108.140467
van Oort B, Alberts M, de Bianchi S, Dall’Osto L, Bassi R, Trinkunas G, Croce R, van Amerongen H (2010) Effect of antenna-depletion in photosystem II on excitation energy transfer in Arabidopsis thaliana. Biophys J 98:922–931. doi:10.1529/biophysj.109.110112
Vasile’v S, Wiebe S, Bruce D (1998) Non-photochemical quenching of chlorophyll fluorescence in photosynthesis. 5-Hydroxy-1,4-naphthoquinone in spinach thylakoids as a model for antenna based quenching mechanisms. Biochim Biophys Acta 1363:147–156. doi:10.1016/S0006-3495(97)00096-0
Visser NV, Westphal AH, van Hoek A, van Mierlo CPM, Visser AJWG, van Amerongen H (2008) Tryptophan-tryptophan energy migration as a tool to follow apoflavodoxin folding. Biophys J 95:2462–2469. doi:10.1529/biophysj.108.132001
Williams WP (1998) The physical properties of thylakoid membrane lipids and their relation to photosynthesis. In: Siegenthaler PA, Murata N (eds) Advances in photosynthesis. Kluwer, Dordrecht, pp 103–118
Witt HT (1979) Energy conversion in the functional membrane of photosynthesis. Analysis by light pulse and electric pulse methods. The central role of the electric field. Biochim Biophys Acta 505:355–427. doi:10.1016/0304-4173(79)90089-9
Yan H, Zhang P, Wang C, Liu ZH, Chang W (2007) Two lutein molecules in LHCII have different conformations and functions: insights into the molecular mechanism of thermal dissipation in plants. Biochem Biophys Res Commun 355:457–463. doi:10.1016/j.bbrc.2007.01.172