Investigations on the Reaction Pattern of Photosystem II in Leaves from *Arabidopsis thaliana* Wild Type Plants and Mutants with Genetically Modified Lipid Content†

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ABSTRACT: The role of digalactosyldiacylglycerol (DGDG) for the functional competence of photosystem II (PS II) has been analyzed in leaves of *Arabidopsis thaliana* plants where the lipid composition was selectively modified by genetic mutations. Measurements with a newly developed laser flash fluorometer and data evaluation within the framework of an extended "3-quencher" model lead to the following results: (i) the normalized fluorescence transients $F(t)/F_0$ induced by an actinic laser flash in dark adapted leaves are virtually the same in wild type (WT) and mutants with diminished (about 50%) monogalactosyldiacylglycerol (MGDG) content (*mgd1* mutant); (ii) significant changes of the $F(t)/F_0$ curves are observed in mutants with a severely reduced DGDG content; (iii) in mutants *dgd1* and *dgd1 dgd2-1* with DGDG contents of 1/15 of the control and below the detection limit, respectively, the probability of the dissipative recombination reaction between $P680^{+\bullet}$ and Q_A^- increases by factors of about two and four, respectively; (iv) the acceptor side reactions are only slightly affected; (v) excitation with actinic laser flash energies above the saturation level of photosynthesis gives rise to elevated carotenoid triplet formation in mutants *dgd1* and *dgd1 dgd2-1*; and (vi) the relationship between DGDG content and functional effect(s) on PS II is strikingly nonlinear. A small fraction of DGDG molecules of the total pool is inferred to be specifically bound to PS II as an essential constituent for its functional competence.

In all living cells, the spatial separation of metabolic processes into specialized cell compartments is a fundamental principle in free energy transduction and the regulated interplay between a high number of individual reactions. This compartmentalization is achieved by means of lipid membranes that contain integral proteins as functionally active complexes. The physical and chemical properties of these membranes are determined by their lipid and protein composition. Apart from establishing a well-defined barrier for solutes and electrochemical free energy transport, lipids can also selectively affect membrane-associated reactions through specific interaction with membrane-bound proteins. Biological membranes comprise a variety of lipid classes. Phospholipids are abundant in animal and yeast cells. In marked contrast, the lipid composition of thylakoid membranes in photosynthesizing organisms is unique and characterized by its high content of glycolipids. Major glycolipids found in these membranes are the two galactolipids monogalactosyldiacylglycerol (MGDG)[1] and digalactosyldiacylglycerol (DGDG) and the sulfolipid sulfoquinovosyldiacylglycerol (SQDG) (*1*, *2*). The amounts of phospholipids, however, are very low in thylakoid membranes. Phosphatidylglycerol (PG) is the only phospholipid found in thylakoids, whereas phosphatidylcholine (PC), an abundant lipid of all extraplastidial membranes, is virtually absent from thylakoid membranes (*3*). Glycolipids where found to exert specific interactions of functional relevance with different operational units of the photosynthetic apparatus. Illustrative examples are (i) the effect of DGDG and phosphatidylglycerol (PG) on the trimer formation of light harvesting complex II (LHC II) (*4*−*7*); (ii) the role of MGDG for the activity of violaxanthin-deepoxidase (VDE), an essential component of the adaptive machinery to light stress (*8*); (iii) the influence of SQDG and DGDG on the reaction properties of the oxygen evolving photosystem II (PS II) in cyanobacteria and plants (*9*−*17*); and (iv) the regulation of the aggregation state of photosystem I (PS I) in cyanobacteria by PG (*18*). The biosynthesis of galactolipids is catalyzed by specific synthases which are localized to chloroplast membranes. In *Arabidopsis thaliana*, functional MGDG synthases are encoded by three different genes (*MGD1*, *MGD2*, and *MGD3* (*19*)). Likewise, for DGDG two genes (*DGD1* and *DGD2*) have been identified (*20*, *21*), and a third galactolipid

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[1] Abbreviations: Chl, chlorophyll; Car, carotenoid; PS II, photosystem II.; P680, photoactive Chl of the reaction center of PS II; Q_A and Q_B, primary and secondary plastoquinones of PS II; WOC, water-oxidizing complex; Y_Z, tyrosine 161 of the PSII D1 polypeptide; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; fwhm, full width at half-maximum; EET, electronically excited energy transfer; LHC, light harvesting complex; PAR, photosynthetic active radiation.

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synthase activity has recently been reported (*22*, *23*). Different mutants deficient in one or two of these synthases have been isolated from *A. thaliana*. The *mgd1* mutant (*24*) carries a T-DNA insertion in the promoter region of the *MGD1* gene. As a consequence, *MGD1* mRNA abundance is strongly reduced, and the MGDG content is decreased to about 25% of total lipids as compared to 45% in wild type. The contents of other lipids in *mgd1* are not significantly altered. Furthermore, the 16:3 to 18:3 ratio is slightly changed in *mgd1*, but the overall degree of fatty acid unsaturation is very similar to wild type. The *mgd1* mutant is characterized by a severely reduced content of chlorophyll per seedling. However, total amounts and composition of carotenoids were very similar to wild type. The chloroplasts in *mgd1* leaves show an altered thylakoid structure, and they are smaller, show a more spherical shape, and contain fewer thylakoids. This finding emphasizes the importance of MGDG production for normal chloroplast development (*24*).

The *dgd1* mutant of *Arabidopsis* was isolated using a biochemical screening approach (*25*). This plant is strongly decreased in the amount of DGDG (1% compared to 15% of total lipid in wild type) with the contents of the other membrane lipids remaining very similar to wild type. The content of 16:3 fatty acid of MDGD in *dgd1* is reduced, with a concomitant increase in 18:3. Isolation of the *DGD1* gene revealed that it carries a point mutation in *dgd1* introducing a premature stop codon (*20*). For this reason, *dgd1* represents a null mutation. The mutant is affected in growth and photosynthetic efficiency. Slightly fewer chloroplasts were observed in *dgd1* mesophyll cells, and the thylakoids were curved and displaced from the central stroma area toward the chloroplast envelopes (*25*). Photosynthesis and thylakoid structure of this mutant were subjected to detailed analysis (*13*, *25*−*27*). The photosynthetic efficiency as deduced from measurements with a PAM fluorometer and the total chlorophyll content are reduced in *dgd1*. Furthermore, the chlorophyll a-to-b ratio in *dgd1* is reduced, indicating an increase of LHCII antenna size relative to PSII.

The *dgd2-1* mutant carrying a T-DNA insertion in the DGDG synthase gene *DGD2* was isolated via reverse genetics. The insertion is localized in the third exon of the *DGD2* gene and thus also represents a null allele. Interestingly, the *dgd2-1* mutation does not result in an alteration of lipid composition or reduced growth (*22*). In the double mutant *dgd1 dgd2-1*, the amount of DGDG is even further reduced as compared to *dgd1*. Therefore, *dgd1 dgd2-1* represents the first higher plant that is virtually devoid of this important galactolipid class. Growth and photosynthetic quantum yield of *dgd1 dgd2-1* were more severely impaired than in *dgd1*, indicating that the residual amount of DGDG in *dgd1* is functionally essential (*22*).

The *A. thaliana* galactolipid mutants provide a most appropriate material to study the role of membrane lipids in the PS II reaction pattern. Likewise, self-assembled laser equipment permits the "in-planta" monitoring of single turnover laser flash induced changes of normalized fluorescence quantum yield with high time resolution in whole leaves, and simultaneously allows covering a wide time range of 7 orders of magnitude. In this study, we report investigations on the influence of lipid deficiency on the electron transport reactions of photosystem II by analyzing laser flash induced fluorescence yield changes (*28*) in whole leaves of *A. thaliana* wild type plants and mutants with modified galactolipid content. Complementary investigations were performed using the saturation pulse (multiple turnover) method. The results obtained in this study provide clear evidence that (i) DGDG plays a crucial role in maintaining normal PS II activity and (ii) further reduction of the DGDG content in *dgd1 dgd2-1* below the detection limit has a drastic influence on the reaction pattern of PS II.

MATERIALS AND METHODS

Plant Material and Growth Conditions. A. thaliana wild type plants (ecotype Columbia-2, Col-2, and ecotype Wassilewskija, Ws) and galactolipid mutants were germinated in Petri dishes containing solidified medium (MS salts, 1% [w/v] sucrose, 1% [w/v] agarose) for 2 weeks before transfer to soil (*29*). The light regime for all growth conditions was 16 h of light/8 h of dark at a light intensity of 150 μmol/(m^2 s). Plants were dark adapted before each measurement for 1 h.

The *mgd1* (ecotype Col) plant is a T-DNA insertion mutant identified in a screen for plants with deficiency in Chl content (*24*). *dgd1* is an ethylmethane sulfonate mutant (ecotype Col) isolated during a screen for lipid mutants as described in ref *25*. *dgd2-1* (ecotype Wassilewskija, Ws) was identified in a T-DNA-tagged population as described in ref *22*. The homozygous double mutant *dgd1 dgd2-1* (*22*) results from a cross between *dgd1* and *dgd2-1* and is constantly selected from the segregating progeny of a homozygous *dgd2-1*, but heterozygous *dgd1* mutant.

Photosynthetic Pigment Analysis. Pigments were isolated from frozen leaves with 80% acetone and analyzed by reverse phase HPLC according to Thayer and Björkman (*30*). Total chlorophyll was extracted from leaves with 80% acetone and was measured photometrically (*31*).

Measurements of Transient Fluorescence Yield Changes. Laser flash induced changes of the chlorophyll fluorescence yield have been measured as described in detail in ref *28*. All measurements were performed at room temperature on whole leaves.

Saturation Pulse Measurements. Saturation pulse measurements were performed using a PAM 101/102/103 chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany) connected to a digital storage oscilloscope (Delta 9500A, Gould Nicolet). Saturation pulses of white light (16900 μmol/(m^2 s) PAR, 800 ms) were provided by means of a Schott KL-1500 lamp. Fluorescence induction was induced by means of PAM 102L light emitting diode (81 μmol/(m^2 s) PAR). The recorded traces were transferred to a computer by home built acquisition software and stored for further data processing.

RESULTS AND DISCUSSION

The different *A. thaliana* plants analyzed in this study and their respective galactolipid composition are compiled in Table 1. Two wild type (WT) plants of ecotypes Columbia-2 (WT Col-2) and Wassilewskija (WT Ws) have been used as reference systems.

Since only the content of the galactolipids MGDG and DGDG is significantly altered in the mutants, only they will be of relevance in the following analyses. In this respect it is important to note that, under normal growth conditions, MGDG, DGDG, and SQDG are exclusively found in

Table 1: *A. thaliana* Plants Analyzed in This Study and Their Respective Total Leaf Lipid Content[a]

	WT (Col-2 and Ws)	*mgd1*	*dgd1*	*dgd2-1*	*dgd1 dgd2-1*
MGDG	49	25	46	44	38
DGDG	15	23	1	16	*b*
PG	9	15	10	10	12
SQDG	2	5	2	2	4
PE	7	13	13	9	15
PC	19	20	27	18	29

[a] Data are in mol % and were taken from ref *22* for WT, *dgd1*, *dgd2-1*, and *dgd1 dgd2-1*, and from ref *24* for *mgd1*. [b] Not detectable.

chloroplast membranes, whereas PG and PC are constituents of extraplastidial membranes. In addition to the latter membranes, PG is also present in thylakoids and envelopes of chloroplasts, PC in envelopes but not in thylakoids. PE is absent from chloroplast membranes (*3*, *32*). Furthermore, in leaves of higher plants a large fraction (about 90%) of lipids is localized in the thylakoids of chloroplasts as the largest membrane system in the cell.

As a consequence of this specific distribution of the lipids and the abundance of thylakoid membranes, their galactolipid composition is directly reflected by the corresponding contents of MGDG and DGDG in whole leaves.

A set of transient changes of the normalized flash induced fluorescence yield was monitored under conditions of excitation with actinic flashes of four different energies (the highest energy of 28 mJ/(cm^2 flash) corresponds to 7.5×10^{16} photons/(cm^2 flash)). The traces obtained for the six different plants are summarized in Figure 1.

Three parameters normalized to the fluorescence level F_0 before the actinic flash can be defined that phenomenologically characterize the observed fluorescence yield changes: the normalized maximum fluorescence yield, F_M/F_0, and the normalized fluorescence yields at 100 ns and 2 μs after the actinic flash $F_{100\,ns}/F_0$ and $F_{2\,\mu s}/F_0$, respectively.

Under native conditions the normalized maximum fluorescence yield F_M/F_0 is expected to increase with increasing flash energy until a saturation level is reached at high laser flash energies due to photosynthetic charge separation in all PS II complexes. Under the conditions used for our experiments the normalized maximum fluorescence yield is usually reached at about 50 μs after the actinic flash. Thereafter the normalized fluorescence yield decays predominantly in the time domain of hundreds of microseconds owing to Q_A^- reoxidation in PS II complexes with a Q_B site occupied by PQ (see ref *33* and references therein).

A different dependency on the energy of the actinic laser flash is expected for $F_{100\,ns}/F_0$ and $F_{2\,\mu s}/F_0$. The values of $F_{100\,ns}/F_0$ should decrease with increasing flash energy due to fluorescence quenching by ^{3}Car that superimposes the increase originating from the disappearance of the quencher $P680^{+\bullet}$ via reduction by Y_Z in the nanosecond time domain (see also ref *28*). Accordingly, above the saturation level of PS II charge separation $F_{100\,ns}/F_0$ reaches values below 1.0 at sufficiently high energies of the actinic flash. The dependency on flash energy is different for $F_{2\,\mu s}/F_0$. In this case a significant fraction of ^{3}Car formed by the actinic flash already decayed into the nonquenching ground state and the extent of $P680^{+\bullet}$ reduction by Y_Z is larger than at 100 ns. Therefore, $F_{2\,\mu s}/F_0$ should increase to a maximum level at moderate oversaturation followed by a decrease at higher energies of the actinic flash. These features are discernible in the traces of Figure 1. For a better illustration, Figure 2 shows the levels of $F_{100\,ns}/F_0$, $F_{2\,\mu s}/F_0$, and F_M/F_0 as a function of the photon density of the actinic flash.

The expected dependencies of the normalized fluorescence parameters can readily be observed for the wild type plants. A further inspection of the data reveals that the mutants *mgd1* and *dgd2-1* exhibit similar features as the WT plants. For these plant systems F_M/F_0 varies between values of 1.9 and 2.2 that are reached at the two highest photon densities of the actinic laser flashes used in this study. The results also indicate that the electron transport reactions in PS II are marginally affected in the mutant *mgd1* with a MGDG content that is reduced by about 50%, a decreased Chl content, and an altered thylakoid structure (see introductory text).

In marked contrast, clearly different features are observed for the mutants *dgd1* and *dgd1 dgd2-1* compared to WT Col-2, WT Ws, and the mutant *mgd1* and *dgd2-1* (in the latter mutant the amounts of MDGD and DGDG are very similar to those of WT, see Table 1). The maximum level of the normalized fluorescence yield in those mutants is significantly diminished with $F_M/F_0 = 1.6$ and is already reached at laser flash photon densities of 3.0×10^{15} photons/(cm^2 flash) and 6.2×10^{15} photons/(cm^2 flash). In addition, the value F_M/F_0 does not remain constant above a photon density of 6.2×10^{15} photons/(cm^2 flash) that saturates photosynthesis, but starts to decline at actinic flashes of 7.5×10^{16} photons/(cm^2 flash). The most severe effect is observed for the mutant *dgd1 dgd2-1* where F_M/F_0 is only about 1.2 at the highest photon density. This feature is accompanied by a prominent shift of the position of F_M/F_0 in the time domain to about 90 μs for this mutant.

As outlined in more detail in ref *28* the numerical data evaluation of the transient changes of the normalized fluorescence yield induced by the actinic flash can be described within the framework of the extended "3-quencher" model which accounts explicitly for the back reaction between $P680^{+\bullet}$ and Q_A^-. With respect to fluorescence emission from excited singlet chlorophylls not coupled to PS II via EET, it was outlined in ref *28* that the fluorescence emission of PS I at room temperature can be neglected. Furthermore, fluorescence lifetime measurements revealed that the fraction of LHC complexes disconnected from excitation energy transfer to either PS II or PS I is rather small in WT and all mutants analyzed (Huyer et al., unpublished results). Therefore, as a reliable approximation, u_0 in eqs 2 and 3 of ref *28* is assumed to be negligibly small. The time-dependent fluorescence yield is then given by the following equation:

$$\Phi(t) = \frac{k_f}{k_f + k_\Sigma + k_{Car}[^3Car(t)] + k_{PC}[Q_A(t)] + k_{P680}[P680^{+\bullet}(t)]} \quad (1)$$

where k_f is the emissive rate constant of chlorophyll, k_Σ is the sum of all radiationless deactivation processes, k_{Car}, k_{PC}, and k_{P680} are the rate constants of fluorescence quenching, and $[^3Car(t)]$, $[Q_A^-(t)]$, and $[P680^{+\bullet}(t)]$ are the time-dependent concentrations of the corresponding fluorescence

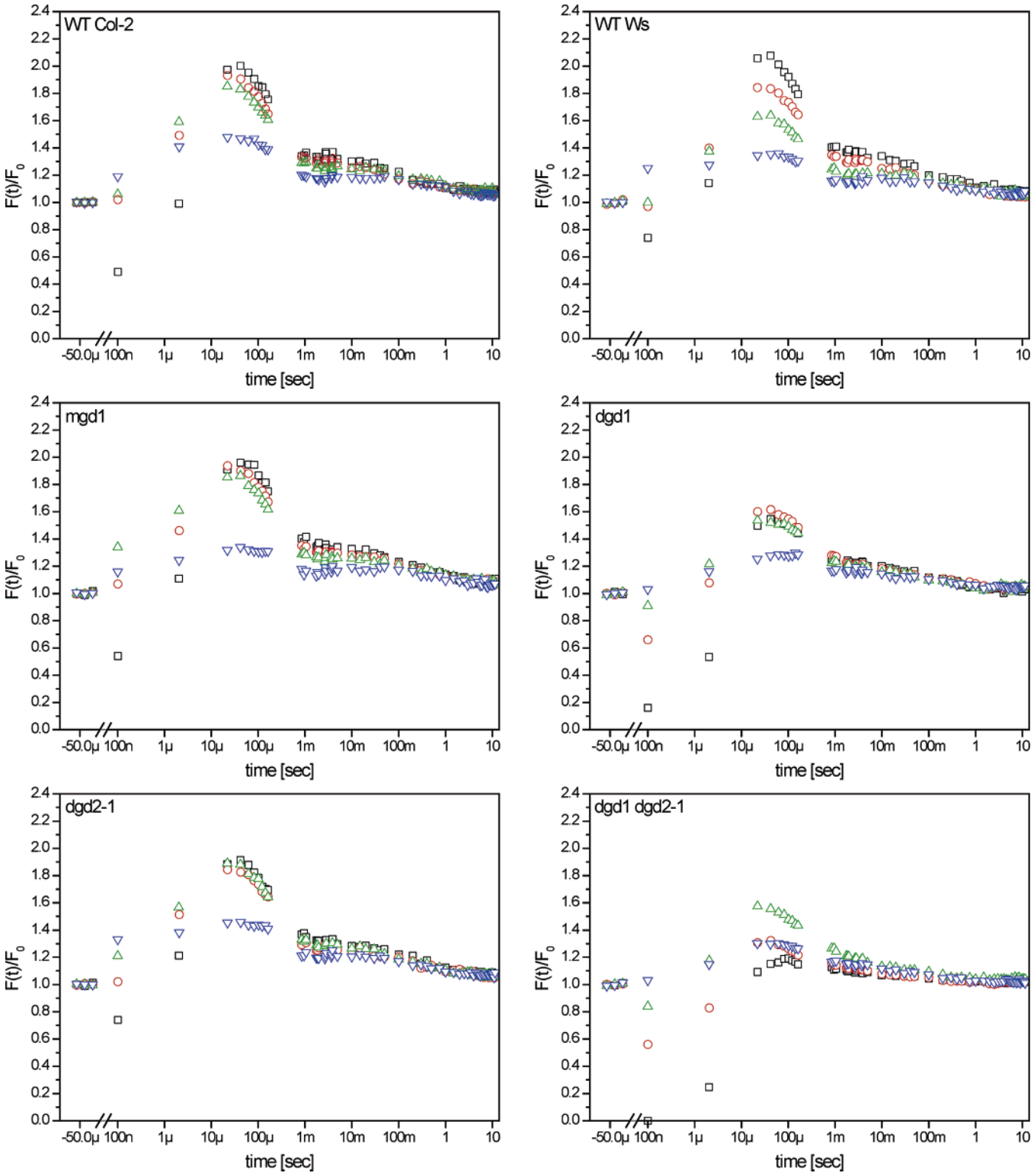

FIGURE 1: Laser flash induced transient changes of the normalized fluorescence yield at different actinic laser flash energies in each of the *A. thaliana* plants listed in Table 1 (black squares 7.5×10^{16} photons/(cm^2 flash), red circles 6.2×10^{15} photons/(cm^2 flash), green triangles up 3.0×10^{15} photons/(cm^2 flash), blue triangles down 5.4×10^{14} photons/(cm^2 flash)).

quenchers. The analytical expressions used for the time-dependent concentrations of quenchers $P680^{+\bullet}$, Q_A^-, and ^3Car have been derived in ref *28* with a lifetime of $\tau_{recomb} = 160$ μs for the recombination reaction between $P680^{+\bullet}$ and Q_A^-. For the rate constants the following values are used: $k_f = (15\ ns)^{-1}$ (*34*), $k_\Sigma = (6\ ns)^{-1}$, $k_{P680} = k_{Car} = 7\ (4.3\ ns)^{-1}$ and $k_{PC} = 7/2\ (4.3\ ns)^{-1}$, as described in ref *28*. To describe the kinetics of $[P680^{+\bullet}(t)]$ and $[Q_A^-(t)]$ three-exponential kinetics are used, as outlined and rationalized in the accompanying report (*28*). The former study also revealed that the whole system is overparametrized as is reflected by a correlation between the parameters $a_{P680,f}$ and p_{recomb}. This correlation especially complicates the evaluation of the mutant plant measurements. In order to cope with this problem the parameter $a_{P680,f}$ was kept constant at a value of 0.8 (deduced from measurements with WT) and the

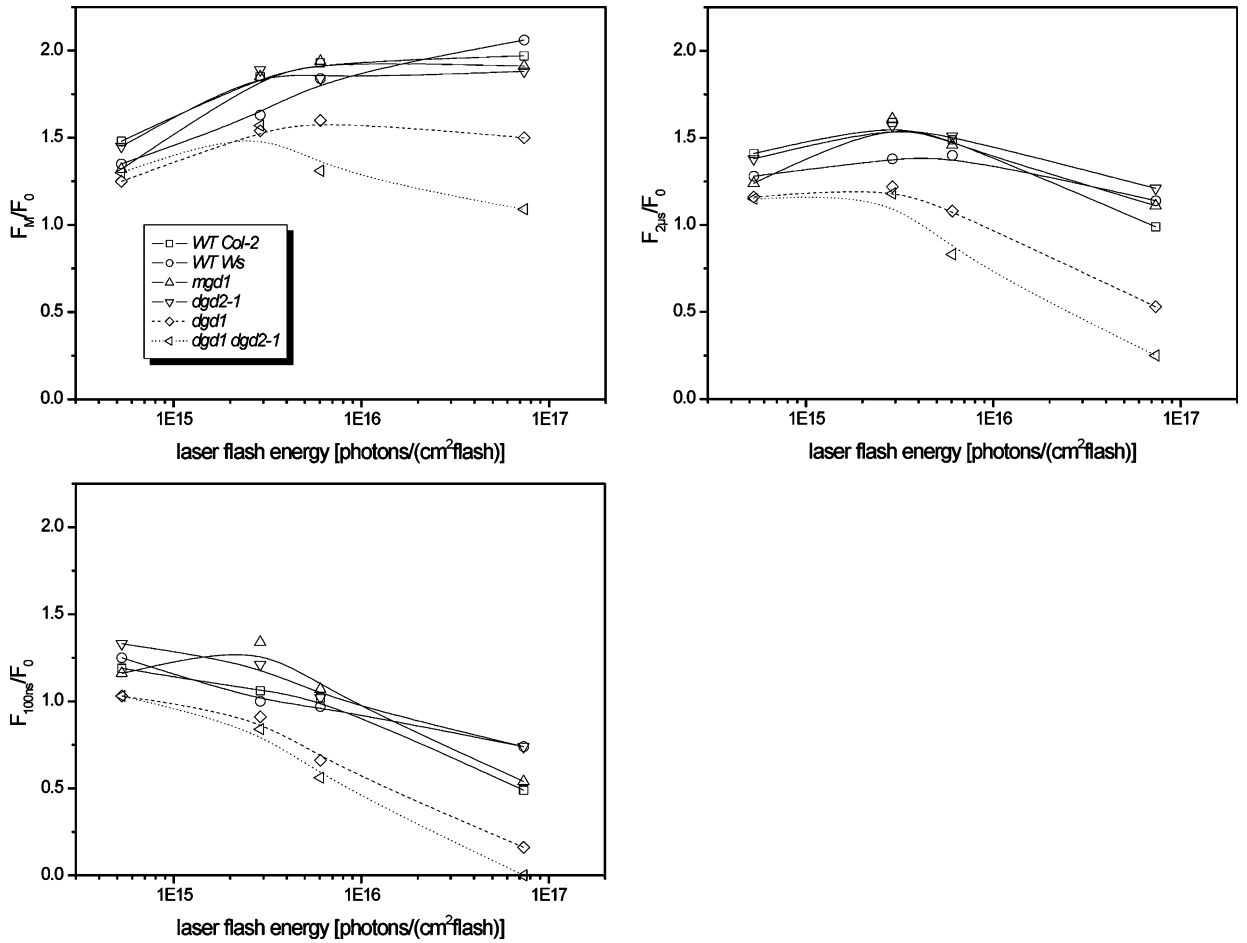

FIGURE 2: Normalized fluorescence parameters F_M/F_0, $F_{2\ \mu s}/F_0$, and $F_{100\ ns}/F_0$ as a function of the photon density of the actinic flash.

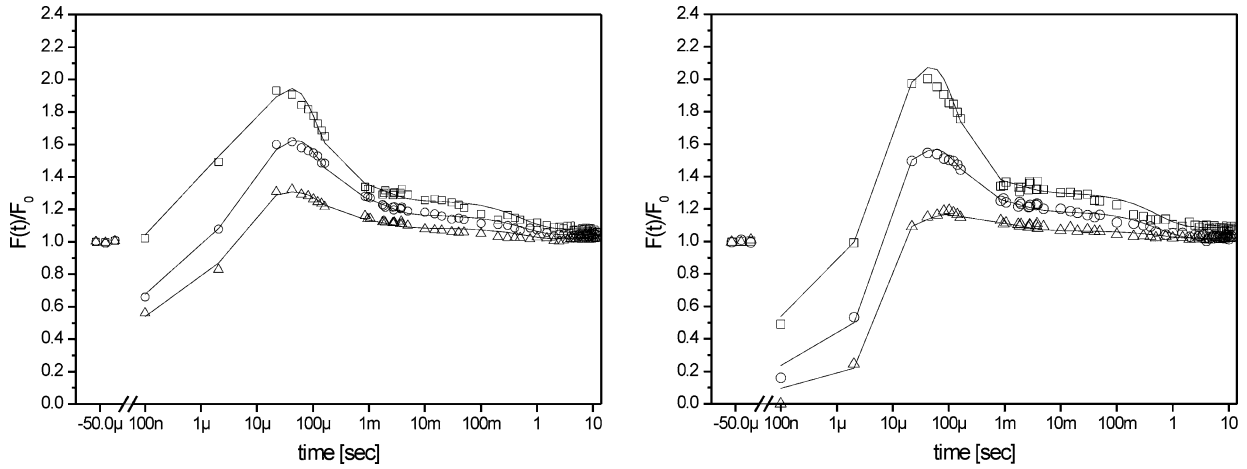

FIGURE 3: Numerical fits for WT Col-2 (squares) and the mutants *dgd1* (circles) and *dgd1 dgd2-1* (triangles up) at saturating actinic flash energies of 6.2×10^{15} photons/(cm^2 flash) (left panel) and 7.5×10^{16} photons/(cm^2 flash) (right panel).

probability of the back reaction p_{recomb} taken as variable. An equivalent description leading to the same overall conclusion could be achieved when p_{recomb} is set to be constant and $a_{P680,f}$ is variable. For a better illustration of the effect(s) the former alternative was preferred.

The numerical fits of the experimental data for WT Col-2, *dgd1*, and *dgd1 dgd2-1* are shown in Figure 3 for saturating laser flash energies. These results show that all transients of the flash induced fluorescence yield changes could consistently be described by the extended "3-quencher" model.

The model parameters gathered from the numerical fit procedure are compiled in Figures 4 and 5. Two main features emerge from an inspection of this data: (i) the probability of the recombination between $P680^{+\bullet}$ and Q_A^- (p_{recomb}, see Figure 4, panel A) and the relative extent of ^{3}Car quenching (see Figure 5) exhibit much higher values in *dgd1* and especially in *dgd1 dgd2-1* as compared to WT Col-2; and (ii) the parameters are almost independent of the actinic flash energy except of ^{3}Car that is characterized by a significant dependence on this energy. The most interesting conclusion can be drawn when the change of the parameter

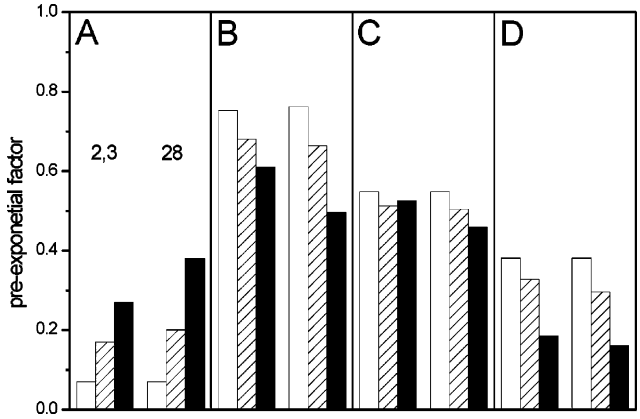

FIGURE 4: Relative extent of parameters p_{recomb} (panel A), $A_{P680,f} + A_{P680,m}$ (panel B), $A_{Q,f}$ (panel C), $A_{Q,m} + A_{Q,s} + A_{Q,offset}$ (panel D) of *A. thaliana* leaves excited with actinic flashes of 6.2×10^{15} photons/(cm^2 flash) (left set of columns of each panel) and 7.5×10^{16} photons/(cm^2 flash) (right set of columns of each panel). The parameters $A_{K,I}$ are the corresponding preexponential factors $A_{K,I} = a_{K,I}(1 - p_{recomb})$ with (K = P680 or Q and I = f, m, s, or offset) of the fits as described in ref *28*. The differently marked columns represent WT Col-2 (open), *dgd1* (hatched) and *dgd1 dgd2-1* (solid).

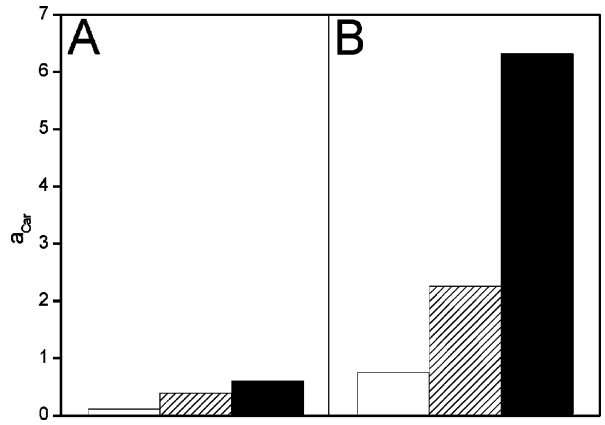

FIGURE 5: Relative extent of fluorescence quenching by ^{3}Car in *A. thaliana* leaves excited with actinic flashes of 6.2×10^{15} photons/(cm^2 flash) (panel A) and 7.5×10^{16} photons/(cm^2 flash) (panel B). The differently marked columns represent WT Col-2 (open), *dgd1* (hatched), and *dgd1 dgd2-1* (solid).

p_{recomb} of different plant species is compared with their lipid content. In the mutant *dgd1*, where the content of MGDG remains virtually unaffected while that of DGDG is diminished by a factor of 15 compared to the WT control (see Table 1), the value of p_{recomb} increases by a factor of about 2 whereas $a_{Q,f}$ is nearly constant. These results indicate that the bulk of DGDG molecules is nearly without effect on the PS II acceptor side reaction pattern but that DGDG depletion below the level of about 10% of the control causes marked effects on the PS II donor side. The present findings nicely confirm our former conclusions on the modulation of the PS II reaction pattern near the water-oxidizing complex (WOC) by DGDG reduction in the *dgd1* mutant (*13*).

A more pronounced change, however, emerges in the *dgd1 dgd2-1* mutant where the remaining DGDG content falls below the detection limit. In this case the probability of dissipative recombination between $P680^{+\bullet}$ and Q_A^- exhibits a pronounced increase and reaches values of about 30% (compared to 7% in the WT) concomitant with a decrease of the total extent of $P680^{+\bullet}$ reduction by Y_Z via nanosecond kinetics. The striking nonlinear relationship between DGDG content and the effect(s) on the PS II reaction pattern (as reflected by the value of p_{recomb}) indicates that a small number of DGDG molecules are essential for a functionally fully competent PS II complex. On the basis of a comparison of both mutants (*dgd1* versus *dgd1 dgd2-1*) it can be concluded that the DGDG content exhibits a remarkable heterogeneity: a small fraction is closely associated with PS II and of functional relevance for its full activity whereas the bulk of DGDG plays no particular role for PS II.

The idea of rather specific effect(s) of a few DGDG molecules is highly supported by the finding that isolated PS II core complexes from higher plants (*Spinacea oleracrea*) deprived of most of the pigment protein complexes of the proximal and peripheral antenna and surrounded by a belt of detergent molecules still contain a limited number of about 10 natural lipid molecules per PS II monomer. It is very interesting to note that among this limited lipid content DGDG molecules are highly enriched (about 2/3 of the total number, see ref *35*) compared with the average lipid composition of the thylakoid membrane where the percentage of DGDG is only about 25% (*3*).

The consequence of a drastically increased p_{recomb} is a corresponding decrease of the efficiency of oxygen evolution (see ref *36* and references therein). This effect, however, has only a minor influence on the oxygen evolution rate under saturating CW illumination because a nonlinear relationship exists between this activity and the number of PS II complexes with a fully competent WOC (*37*).

An inspection of Figure 5 also reveals that in both mutants the quenching by ^{3}Car is significantly larger than in the WT control and that this effect is especially pronounced at actinic flash photon densities of 7.5×10^{16} photons/(cm^2 flash) with the most prominent increase in the double mutant *dgd1 dgd2-1*. The increase of a_{Car} could originate from a higher population or a higher quenching efficiency of ^{3}Car in the mutants or a combination of both effects. To address this point the pigment composition of leaves from wild type and mutant plants has been analyzed. The results obtained and compiled in Table 2 reveal that the amount of neoxanthin and the xanthophyll cycle pigments is increased in *dgd1*, and even further in *dgd1 dgd2-1* compared with the WT. The findings on the *dgd1* mutant are in correspondence with those of an earlier report (*26*). A closer inspection of the numbers in Table 2 indicates that the ratio of the xanthophyll cycle carotenoids (X) plus lutein (L) and the total Chl content (Chl_{total}), $(X + L)/Chl_{total}$, increases from WT to the *dgd1 dgd2-1* double mutant.

A comparison of the ratio $(X + L)/Chl_{total}$ with the amplitudes a_{Car} from the fluorescence measurements shows that for both laser energies a linear correlation exists between these parameters as illustrated in Figure 6. The increase in a_{Car} can therefore be assigned to the increase of carotenoid content.

The results presented so far are interpreted by a specific role of DGDG depletion on the reaction properties of the PS II complex itself and the flash induced formation of ^{3}Car. Alternatively, they could also originate from indirect effects owing to changes of the thylakoid membrane organization. The latter possibility, however, is highly unlikely as illustrated by an inspection of the traces in Figure 1. The laser flash induced transients of the normalized fluorescence yield, $F(t)/F_0$, are virtually the same for WT

Table 2: Pigment Composition of *Arabidopsis* Plants[a]

	WT				
	ol-2	Ws	*dgd1*	*dgd2-1*	*dgd1 dgd2-1*
Chl a	1007 ± 19	1068 ± 17	726 ± 11	940 ± 40	630 ± 15
Chl b	333 ± 4	358 ± 6	291 ± 7	316 ± 12	288 ± 13
Chl a/Chl b	3.0	3.0	2.5	3.0	2.2
violaxanthin	32 ± 3	33 ±1	31 ± 2	35 ± 2	41 ± 5
antheraxanthin			10 ± 1		21 ± 4
zeaxanthin	5.1 ± 0.5	4.8 ± 0.1	8.8 ± 0.5	3.5 ± 0.7	15 ± 3
neoxanthin	31 ± 2	32 ± 1	44 ± 6	35 ± 3	50 ± 8
lutein	86 ± 4	89 ± 2	85 ± 7	88 ± 4	100 ± 17
β-carotene	46 ± 1	56 ± 3	37 ± 3	44 ± 5	38 ± 3

[a] Values are given in [μg/g fresh weight] ± SD.

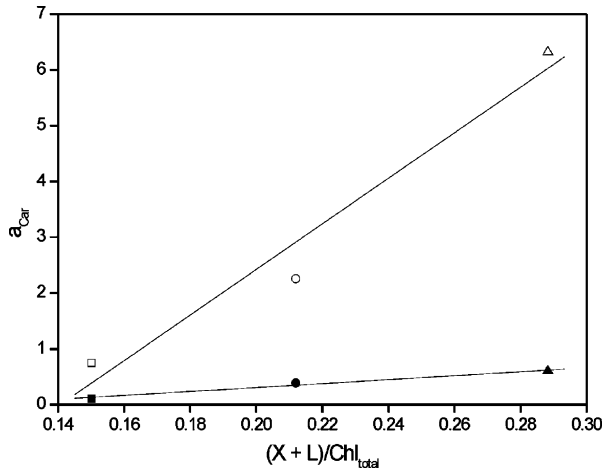

FIGURE 6: Correlation between a_{Car} and the ratio (X + L)/Chl$_{total}$ for laser flash photon densities of 6.2×10^{15} photons/(cm^2 flash) (closed symbols) and 7.5×10^{16} photons/(cm^2 flash) (open symbols): WT Col-2 (squares), *dgd1* (circles), and *dgd1 dgd2-1* (triangles).

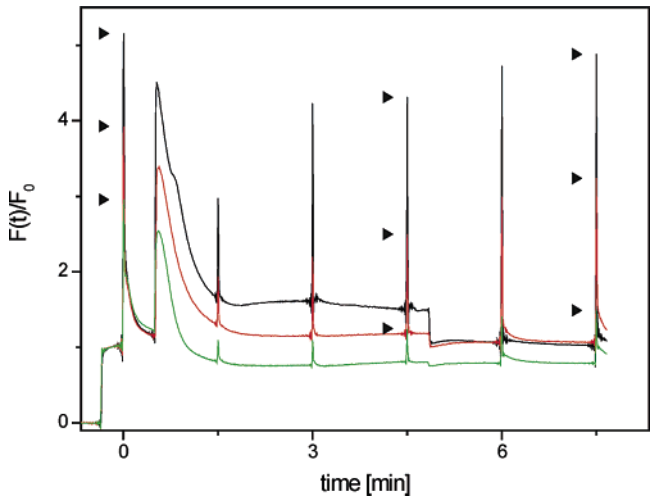

FIGURE 7: Saturation pulse measurements of WT Col-2 (black), *dgd1* (red), and *dgd1 dgd2-1* (green). Black arrows indicate the maximum fluorescence yield during individual saturation pulses (from top to bottom: WT Col-2, *dgd1*, and *dgd1 dgd2-1*).

and mutant *mdg1* whereas the pigment content and thylakoid structure of both species exhibit remarkable differences (see introductory text and ref *24*). It is therefore reasonable to extrapolate the phenomenon of a PS II that is rather independent of the overall membrane organization also to the mutants with strongly diminished DGDG content. This idea is strongly supported by an independent line of evidence. It was shown that the sensitive reactions of $P680^{+\bullet}$ reduction by Y_Z and the redox transitions in the water-oxidizing complex are very similar in PS II complexes embedded either in thylakoids or in PS II membrane fragments, or isolated as PS II cores surrounded by a surrogate detergent belt environment (*38*−*40*).

For a further characterization of the mutants, a complementary fluorometric method was applied, i.e., the saturation pulse method. It has to be emphasized that in this case the leaves were excited with multiple turnover light pulses of saturating energy and a duration of 800 ms. In order to avoid misleading conclusions it has to be stressed that the value of F_M/F_0 induced by a short laser flash, as used in the experiments described before, depends on the kinetics and the population of the different quenchers $P680^{+\bullet}$, Q_A, and ^{3}Car as shown by eq 1, but is virtually independent of other types of nonphotochemical quenching (NPQ), e.g., membrane energetization or state transition. As a consequence, in this case F_M/F_0 markedly differs from the corresponding parameter monitored under excitation with long (800 ms) saturating multiple turnover flashes, where effects due to $P680^{+\bullet}$ and ^{3}Car are negligibly small but other quenching mechanisms (NPQ) contribute substantially. Accordingly, compared to the former single turnover approach, different information is obtained. Figure 7 shows typical traces of transient fluorescence yield changes obtained with the saturation pulse approach used for measurements of WT Col-2 and both DGDG mutants.

This data reveals that the normalized maximum fluorescence yields in the dark and light adapted state, F_M/F_0 and F'_M/F'_0, respectively, are significantly reduced in the mutants compared to the WT. The feature obtained for the *dgd1* mutant corresponds with the results reported in a former study (*27*). A much more pronounced effect, however, is observed for the *dgd1 dgd2-1* mutant. The drastically diminished extent of the maximum of the normalized fluorescence yield induced by a long saturating pulse in dark adapted leaves is qualitatively in perfect agreement with the results obtained by using the single turnover flash method (vide supra). In the mutant *dgd1 dgd2-1* the fluorescence yield decreases below the level of F_0 during the induction phase, and the newly reached light adapted state has a lower fluorescence yield than the dark adapted state (i.e., $F(t > 2$ min$)/F_0 \leq 1$, see Figure 7). Furthermore, the extent of nonphotochemical quenching is increased in the mutants and its relaxation is severely retarded, as can be seen from the slower dark recovery kinetics of F'_M/F'_0 (slower recovery of qE and qT quenching (see ref *41*)) observed in the mutants. The level of nonphotochemical quenching observed at about

7.5 min (Figure 7) could still be observed after about 30 min of dark recovery (data not shown), but a full recovery was reached after 1 h of dark adaptation. Theoretically also $P680^{+\bullet}$ and ^{3}Car can give rise to fluorescence levels below F_0. However, as the decrease below F_0 in the *dgd1 dgd2-1* mutant disappears rather slowly in the dark, the latter possibility can be ruled out for kinetic reasons. Further experiments are required to clarify the origin of this particular effect of $F(t)/F_0 \leq 1$ in the *dgd1 dgd2-1* mutant.

CONCLUSIONS

On the basis of analyses of laser flash induced fluorescence transients the present study reveals that the PS II reaction pattern is significantly modified in mutants of *A. thaliana* plants which are severely deprived of the lipid DGDG. The results indicate that the total DGDG pool is highly heterogeneous in its functional relevance for PS II: only a small percentage of DGDG is essential for a fully competent PS II complex. This fraction of DGDG molecules is most likely specifically bound and affects predominantly the reaction properties of the PS II donor side that catalyzes the oxidative water cleavage into molecular oxygen and protons.

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