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The phospholipid-deficient *pho1* mutant of *Arabidopsis thaliana* is affected in the organization, but not in the light acclimation, of the thylakoid membrane

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Abstract

The *pho1* mutant of *Arabidopsis* has been shown to respond to the phosphate deficiency in the leaves by decreasing the amount of phosphatidylglycerol (PG). PG is thought to be of crucial importance for the organization and function of the thylakoid membrane. This prompted us to ask what the consequences of the PG deficiency may be in the *pho1* mutant when grown under low or high light. While in the wild-type, the lipid pattern was almost insensitive to changes in the growth light, PG was reduced to 45% under low light in the mutant, and it decreased further to 35% under high light. Concomitantly, sulfoquinovosyl diacylglycerol (SQDG) and to a lesser extent digalactosyl diacylglycerol (DGDG) increased. The SQDG increase correlated with increased amounts of the SQD1 protein, an indicator for an actively mediated process. Despite of alterations in the ultrastructure, mutant thylakoids showed virtually no effects on photosynthetic electron transfer, O_2 evolution and excitation energy allocation to the reaction centers. Our results support the idea that PG deficiency can at least partially be compensated for by the anionic lipid SQDG and the not charged lipid DGDG. This seems to be an important strategy to maintain an optimal thylakoid lipid milieu for vital processes, such as photosynthesis, under a restricted phosphate availability.

Keywords: Diacylglycerol; Light acclimation; Membrane stacking; Phosphate deficiency; Thylakoid lipid; Sulfolipid

Abbreviations: DGDG, digalactosyl diacylglycerol; DPS, de-epoxidation state of xanthophyll-cycle pigments; Φ_{PSII}, actual PSII efficiency; GSA-AT, glutamate 1-semialdehyde aminotransferase; HL, high light of 450 μmol m^{-2} s^{-1}; LHCII, light-harvesting pigment–protein complex of PSII; LL, low light of 75 μmol m^{-2} s^{-1}; MGDG, monogalactosyl diacylglycerol; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PPFD, photosynthetic photon flux density; *q*E, high-energy state quenching; *q*N, non-photochemical quenching; *q*P, photochemical quenching; σ, photoreduction efficiency of the primary stable quinone electron acceptor of PSII Q_A; SQDG, sulfoquinovosyl diacylglycerol

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1. Introduction

Lipids are highly conserved components of thylakoid membranes and are considered to play an important role in the ordered assembly and structural maintenance of the photosynthetic apparatus (for reviews see [1,2]). Thylakoid membranes are unique among membranes in their lipid composition. They are composed of the not charged galactolipids monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG), which together represent 60–80% of total lipids, and of the negatively charged lipids phosphatidyl glycerol (PG) and sulfoquinovosyl diacylglycerol (SQDG). While there is an advanced understanding of the role of proteins and pigments for the assembly and function of the photosynthetic apparatus, the function of lipids is still poorly understood [3,4]. A significant body of evidence implicates specific associations between certain lipids and the heterogeneously distributed protein complexes within the thylakoid membrane (e.g. [5–9]). A highly specific interaction of PG with proteins of photosystem (PS)II and PSI is suggested by electron spin resonance studies [10]. Additionally, PG and DGDG seem to be of major importance with regard to the structural organization of the main light-harvesting pigment–protein complexes (LHCs) of PSII (LHCII) [4,11]. LHCII binds about 65% of the chlorophyll and accounts for more than one-third of the overall thylakoid proteins [11]. Crystallization studies with LHCII indicate the presence of stoichiometric amounts of PG and DGDG in trimeric LHCII [12]. Treatment of isolated LHCII with phospholipase A_2 resulted in the dissociation of trimeric LHCII into monomeric forms [12,13]. In contrast to PG, DGDG was easily removable and was not required for maintenance of trimerization. It is proposed that the positively charged residues of Arg-21 and/or Lys-23 provide specific PG-binding sites in LHCII by interacting with the negatively charged PG head group [11]. Electrophoretic separation of the inner (Lhcb3–Lhcb6) and the peripheral (Lhcb1 and Lhcb2) LHCs of PSII showed a significant enrichment in PG [8,9,14]. It is thought that the ubiquitous thylakoid fatty acid *trans*-Δ^3-hexadecenoic acid (t-16:1), an unusual fatty acid that is exclusively esterified to the *sn*-2 position of the glycerol backbone of PG, plays a special role in the assembly of LHCII. In etiolated tissue, t-16:1 containing PG is barely detectable, but accumulates during the process of light-induced chloroplast differentiation [15,16]. The presence of t-16:1-PG has been correlated with both LHCII oligomerization [5,13,17,18], the extent of grana stacking [18,19] and the efficiency of energy transfer from LHCs to the core complexes of PSII and PSI [13,20]. Moreover, the ratio of saturated to unsaturated fatty acids of PG is believed to be an important determinant for cold stress acclimation [21]. Finally, PG depletion was shown to considerably inhibit photosynthetic electron transport activities [22,23]. The latter finding may be related to results of Murata et al. [7], who showed that about 30% of the lipid associated with the reaction center/core complex of PSII in spinach is PG. Taken together, all these findings suggest a critical role of PG in maintaining proper assembly and function of the photosynthetic apparatus.

It has been shown that phosphate deficiency during growth strongly affects the lipid composition of the thylakoid membrane by reducing the relative proportions of the phospholipids PG, phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) in both photosynthetic bacteria [24,25] and in *Arabidopsis thaliana* [26]. This finding offers a new method to selectively manipulate the lipid composition of thylakoid membranes. Such experiments may be particularly useful to evaluate the structural and functional significance of PG for the photosynthetic apparatus under in-vivo conditions. In this regard, the *pho1* mutant of *A. thaliana* provides a promising novel tool. The mutant is thought to be defective in phosphate loading of the xylem, which results in a strongly restricted accumulation of inorganic and organic phosphate in the aerial parts [27]. It has been shown that in the *pho1* mutant the same changes in lipid composition occur as in the *A. thaliana* wild-type grown in medium with reduced phosphate [26]. These results further corroborated that plants are able to respond to the phosphate limitation by a selective accumulation of SQDG and to a lesser extent DGDG [26]. This prompted speculations of an important function of SQDG in substituting PG within the photosynthetic apparatus [28], and suggest a conditional role for SQDG with regard to the structural and functional integrity of the photosynthetic apparatus. The present study was conducted to

evaluate the consequences of the PG deprivation in the *pho1* mutant on the thylakoid membrane organization and function.

2. Materials and methods

2.1. Plant material

Surface sterilized seeds of *A. thaliana* ecotype Columbia (Col-2, wild-type) and the six-times-backcrossed *pho1* mutant [27] were germinated on 0.7% (w/v) agar-solified MS medium [29] supplemented with 1% (w/v) sucrose. The seedlings were kept on agar for 10 days prior to the transfer to pots containing a soil mixture as described [30], where plants were exposed to light of a photosynthetic photon flux density (PPFD, 400–700 nm) of 75 μmol m^{-2} s^{-1}, designated as low light (LL). After further 10 days on soil, one set of plants was transferred to a PPFD of 450 μmol m^{-2} s^{-1}, designated as high light (HL) under which plants were grown for additional 10 days, while the other set of plants continued to grow under LL conditions. Leaves of plants were usually harvested after 30 days. Irrespective of the PPFD that plants experienced during growth, a 14-h light/10-h dark regime was applied. The day/night temperature was controlled at 23/18°C. Light was provided by an array of halogen lamps (SON-T AGRO 400, Phillips, Eindhoven, Netherlands). PPFD incident upon the top of plants was determined using a quantum sensor (LI-189A, Li-Cor, Lincoln, NE). If not stated otherwise, for measurements leaves were harvested 5 h after initiation of light exposure.

2.2. Lipid analysis

Harvested leaves were immediately frozen in liquid nitrogen, and lipids were extracted as previously described [30]. Lipid extracts were analyzed on activated ammonium sulfate impregnated silica gel TLC plates using a solvent system of acetone–toluene–water (91:30:8, v/v/v). Lipids were visualized with iodine vapor and identified by co-chromatography with lipid extracts of known composition. For quantitative analysis, individual lipids were isolated from TLC plates and used to prepare fatty acid methyl esters. The methyl esters were quantified by GLC using myristic acid as internal standard [30].

2.3. Pigment and protein assays

Leaves were rapidly frozen in liquid nitrogen for subsequent pigment and protein determination. Pigments were extracted and analyzed by high-performance liquid chromatography [31]. The de-epoxidation state of xanthophyll-cycle pigments (DPS) was calculated as DPS = (antheraxanthin+zeaxanthin)/(violaxanthin+antheraxanthin+zeaxanthin). Immunoblotting was performed as in Härtel et al. [32].

2.4. Transmission electron microscopy

Rosette leaves of 30-day-old plants were fixed in 4% (v/v) glutaraldehyde, 0.1 M sodium phosphate (pH 7.2) on ice followed by a secondary fixation in 1% (w/v) OsO_4 in the same buffer [33]. The specimens were dehydrated in a graded series of acetone and embedded in Spurr's epoxy resin. Ultrathin sections (80 nm) were stained with uranyl acetate and viewed in a Phillips 400 transmission electron microscope. Chloroplasts were counted in the spongy parenchyma. Dimensions were determined from photographs of two to three independent preparations of three plants.

2.5. Oxygen evolution and chlorophyll fluorescence measurements

In vivo O_2-evolution rates under saturating CO_2 were determined as described [32]. Low temperature (77 K) chlorophyll fluorescence emission spectra of thylakoid suspensions were recorded as previously described [32]. Thylakoids were isolated as described [32]. In vivo room temperature chlorophyll fluorescence was monitored as described [34]. The empirical fluorescence parameters for photochemical quenching (qP), non-photochemical quenching (qN), high-energy state quenching (qE), for the quantum yield of linear electron transfer via PSII (Φ_{PSII}) and the maximum photochemical efficiency of PSII, i.e. the ratio of variable to maximum fluorescence (F_v/F_m), were determined as in Härtel et al. [34]. The photoreduction efficiency of the primary stable quinone electron acceptor of PSII Q_A (σ) was derived from

light dosage response curves of the fluorescence yield increase induced by a single turn-over flash [35] as previously described [32]. For all room temperature chlorophyll fluorescence measurements plants were dark-adapted for at least 1 h prior to the measurements.

3. Results

3.1. The pho1 mutant shows a strongly altered lipid composition depending on the growth PPFD

Plants were usually grown under a moderate PPFD of 75 μmol m^{-2} s^{-1}, designated here as LL. To gather information on the role of individual lipids for structural and functional integrity of the photosynthetic apparatus, one set of plants was also grown under a six-fold higher PPFD of 450 μmol m^{-2} s^{-1}, designated as HL. The rationale behind this experiment was to force additional structural changes in the photosynthetic apparatus, because its composition is known to be highly sensitive to long-term changes in the PPFD. It is well established that growth under different PPFDs can result in dramatic changes in the chloroplast architecture [3,36–38]. Leaves of about 30-day-old plants were used for all measurements. At this time wild-type plants were at a stage just prior to the bolting of the primary inflorescence stalk. Usually, bolting in the mutant lagged behind by about 2–3 days. Leaves of the *pho1* mutant were always smaller than those of the wild-type, and more circular in shape. Almost no macroscopic differences of the phenotypes were observed in the *pho1* mutant plants grown under the two PPFDs.

Under LL, the relative amount of all phospholipids was reduced in the *pho1* mutant. PG, that is predominantly found in thylakoids, was reduced to 45% of the wild-type amount, whereas the relative amounts of SQDG and DGDG increased to 240 and 160%, respectively (Table 1). The lipid composition was indistinguishable in the wild-type grown under LL and HL conditions. However, under HL exposure in the *pho1* mutant PG decreased to 35% of the wild-type level, while the proportion of SQDG further increased to 285%. Thus, the proportion of SQDG significantly increased (Students *t*-test, $P < 0.05$) by a factor of 1.2 in HL- versus LL-grown *pho1* mutant plants. The SQDG increase was accompanied by a further increase in the proportion of DGDG to 180% of the HL-grown wild-type. Irrespective of the changes in the lipid composition, the sum of SQDG and PG remained nearly unchanged in the *pho1* mutant as compared to the wild-type with 8.0% versus 9.3% for LL-grown and 8.2% versus 9.0% for HL-grown plants, respectively, suggesting a near equimolar exchange and a correlation between the changes in these negatively charged lipid classes. A quantitative inspection of the fatty acid compositions of PG and SQDG revealed only slight differences (Table 2A,B). There seems to be an increase in the proportion of 18:1 PG and a decrease of 18:1 SQDG in *pho1* mutant plants. The fatty acid pattern of the two neutral lipids MGDG and DGDG did also not change under the different PPFDs which

Table 1
Lipid composition of mature leaves of *A. thaliana* wild-type and the *pho1* mutant grown either under low light (LL) or high light (HL) conditions

Lipid class	LL		HL	
	Wild-type	*pho1*	Wild-type	*pho1*
MGDG	50.4 ± 5.2	56.9 ± 4.0	48.4 ± 3.5	53.8 ± 5.1
PG	7.5 ± 0.4[a]	3.5 ± 0.4[a,b]	7.1 ± 0.4[a]	2.5 ± 0.3[a,b]
DGDG	14.3 ± 1.0[a]	23.3 ± 1.3[a]	15.5 ± 0.7[a]	28.1 ± 3.0[a]
SQDG	1.8 ± 0.3[a]	4.5 ± 0.2[a,c]	1.9 ± 0.4[a]	5.7 ± 0.2[a,c]
PE	8.6 ± 1.7[a]	4.1 ± 0.4[a,c]	10.0 ± 1.4[a]	3.0 ± 0.4[a,c]
PC	17.4 ± 4.2[a]	7.7 ± 0.8[a]	17.1 ± 2.3[a]	6.9 ± 1.5[a]

All values are given as mol% and represent the means (± S.E.) of three different determinations with individual plants.
[a,c]Values are significantly different based on Student's *t*-test ($P < 0.05$).
[b]Values are significantly different based on Student's *t*-test ($P < 0.1$).

Table 2
Fatty acid composition of PG (A) and SQDG (B) from mature leaves of *A. thaliana* wild-type and the *pho1* mutant grown either under low light (LL) or high light (HL) conditions

Fatty acid	LL		HL	
	Wild-type	*pho1*	Wild-type	*pho1*
(A)				
16:0	26.7 ± 1.8	25.8 ± 1.3	24.6 ± 0.6	21.7 ± 4.9
16:1	28.4 ± 4.9	27.2 ± 6.0	25.6 ± 3.8	26.8 ± 5.0
16:2	1.9 ± 0.9	2.8 ± 1.4	n.d.[a]	2.4 ± 0.7
16:3	2.7 ± 1.8	2.5 ± 1.2	3.4 ± 1.1	3.4 ± 1.6
18:0	1.3 ± 0.3	3.3 ± 0.7	1.3 ± 0.3	3.2 ± 1.3
18:1	6.2 ± 1.6	12.0 ± 2.4	7.5 ± 1.8	12.1 ± 4.3
18:2	7.1 ± 0.6	7.4 ± 1.7	6.1 ± 2.0	6.1 ± 1.3
18:3	25.7 ± 4.0	19.0 ± 3.8	31.5 ± 1.4	24.3 ± 5.0
(B)				
16:0	54.5 ± 7.2	51.8 ± 0.6	48.8 ± 3.5	47.8 ± 2.4
16:1	6.3 ± 1.1	6.7 ± 1.4	7.8 ± 2.1	5.8 ± 2.0
16:2	3.4 ± 1.2	2.4 ± 0.9	4.1 ± 1.4	2.3 ± 0.6
16:3	n.d.	n.d.	n.d.	n.d.
18:0	3.9 ± 1.6	3.5 ± 0.3	4.9 ± 1.6	3.1 ± 0.4
18:1	8.1 ± 1.5	5.6 ± 0.6	8.3 ± 1.6	5.6 ± 1.2
18:2	4.1 ± 2.2	5.5 ± 0.9	6.1 ± 2.9	4.8 ± 0.9
18:3	19.3 ± 4.9	24.4 ± 3.0	20.1 ± 5.9	30.6 ± 6.3

All values are given as mol% and represent the means (± S.E.) of three different determinations with individual plants.
[a]Not detectable.

plants experienced during growth, neither in the wild-type nor in the mutant (data not shown). When related to a fresh weight basis virtually no differences were found in the sum of total fatty acids between wild-type and the *pho1* mutant.

3.2. The pho1 mutant shows an altered pigment pattern, but acclimates to a high PPFD in a similar manner as the wild-type

Leaves of the *pho1* mutant showed slightly higher chlorophyll contents per unit leaf area than wild-type leaves under both PPFDs (Table 3). Wild-type leaves exhibited lower chlorophyll *a/b* ratios when grown under LL than when grown under HL (3.2 versus 3.7), which is a typical difference found between LL- and HL-acclimated leaves [37–40]. Although mutant leaves were able to respond similarly to the changes in the PPFD by increasing their chlorophyll *a/b* ratio, the ratios were clearly lowered under both PPFDs in comparison to the wild-type. Since chlorophyll *b* is enriched in LHCII and only minor amounts are located in other LHCs and it is not present in the reaction center/core complexes [41], the ratio of chlorophyll *a/b* is a good measure of the proportion of LHCII to other chlorophyll-binding pigment–protein complexes. Hence, the decreased chlorophyll *a/b* ratios imply a relative increase in LHCII compared to the core antenna proteins under both PPFDs in the *pho1* mutant.

Although the content of β-carotene decreased by 25% in the *pho1* mutant on a total chlorophyll basis, no appreciable differences were found in the contents of the xanthophylls in LL-acclimated plants (Table 3). Upon HL exposure, the contents of the xanthophylls lutein and neoxanthin did not change, whereas the contents of β-carotene increased proportionally by 20% in the wild-type and the mutant. In addition, the content of xanthophyll-cycle pigments increased 1.7-fold (wild-type) and 2.2-fold (mutant). Increasing accumulation of xanthophyll-cycle pigments is a well established acclimatory response of plants to higher growth PPFDs [39,40,42]. As apparent from the DPS values, under LL conditions, a similar proportion of 60% violaxanthin was available for de-epoxidation, while in HL-exposed plants more violaxanthin was convertible into antheraxanthin and zeaxanthin in the mutant.

Table 3
Pigment composition of mature leaves of *A. thaliana* wild-type and the *pho1* mutant grown either under low light (LL) or high light (HL) conditions

Pigments	LL		HL	
	Wild-type	*pho1*	Wild-type	*pho1*
Chlorophyll *a*	19.3 ± 2.9	25.9 ± 4.1	24.4 ± 3.9	33.8 ± 2.3
Chlorophyll *b*	6.3 ± 0.9	9.4 ± 1.5	6.7 ± 1.0	10.7 ± 0.8
Chlorophyll (*a*+*b*)	25.6 ± 3.9	35.4 ± 5.5	31.1 ± 4.8	44.6 ± 3.1
Chlorophyll *a*/*b*	3.1 ± 0.10	2.8 ± 0.10	3.7 ± 0.10	3.2 ± 0.08
Lutein	116.5 ± 7.1	116.6 ± 12.9	114.4 ± 8.3	111.2 ± 8.2
Neoxanthin	41.8 ± 4.5	46.5 ± 5.1	44.1 ± 5.7	44.5 ± 1.5
β-Carotene	60.3 ± 7.5	46.0 ± 5.0	71.6 ± 6.4	54.2 ± 6.3
V+A+Z[a]	31.9 ± 3.8	29.0 ± 5.5	53.7 ± 6.2	63.8 ± 4.5
Maximum DPS[b]	0.60 ± 0.039	0.61 ± 0.062	0.70 ± 0.050	0.76 ± 0.053

All chlorophyll data are given as μmol m^{-2} leaf area. To facilitate comparison, carotenoid contents were given as mmol mol^{-1} chlorophyll. All values represent the means (± S.D.) of at least eight different determinations with individual plants.
[a]V, violaxanthin; A, antheraxanthin; Z, zeaxanthin.
[b]Obtained at photosynthesis saturating PPFD.

3.3. Enhanced PPFD-dependent accumulation of SQD1 in the pho1 mutant

To gain further information on the mechanism that controls the assembly of the photosynthetic apparatus in the *pho1* mutant, total leaf extracts of wild-type and the *pho1* mutant were examined by Western-blot analysis for the abundance of proteins that might be critical for the structural integrity of *pho1* thylakoids. Equal amounts of leaf protein were blotted with polyclonal antisera specific for glutamate 1-semialdehyde aminotransferase (GSA-AT) and SQD1. GSA-AT catalyzes the last step in 5-aminolevulinate formation, which reflects a key regulatory step in the biosynthesis of chlorophylls (see [43] and references cited therein). SQD1 is the protein product of a gene that is directly involved in SQDG synthesis [26]. An antiserum directed against a protein involved in DGDG synthesis is currently not available. Whereas SQD1 protein levels were barely detectable in the wild-type, they dramatically increased in the *pho1* mutant (Fig. 1). Moreover, there was an additional increase in the amount of SQD1 in extracts of the HL-grown *pho1* mutant as compared with those grown under LL. Differences were not observed for GSA-AT protein levels, suggesting a specific effect on lipid synthesis in the *pho1* mutant.

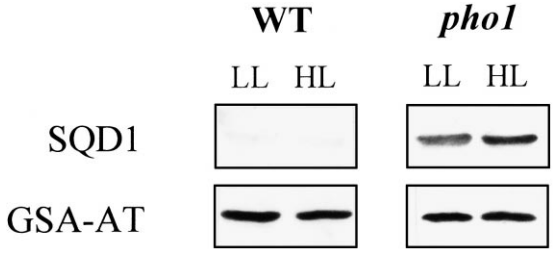

Fig. 1. Quantification of the protein contents for SQD1 and GSA-AT in total leaf extracts of wild-type and *pho1* mutant plants that were grown either under low (LL) or high light (HL). Proteins were separated by SDS-PAGE and blotted onto nitrocellulose filters. The filter-bound proteins were identified using polyclonal antibodies. Each lane was loaded with 16 μg total protein.

3.4. Thylakoid membrane ultrastructure is altered in the pho1 mutant

To further evaluate the consequences of the changed lipid composition, we analyzed the thylakoid ultrastructure of *pho1* mutant and wild-type chloroplasts by electron microscopy of thin sections of whole leaves. As evident from the micrographs in Fig. 2, the supramolecular architecture is distinctly different in chloroplasts from wild-type and mutant plants grown either under LL (Fig. 2A,B) or HL conditions (Fig. 2C,D). A morphometric analysis reveals that, irrespective of the growth PPFD, the number of plastids per cell cross-section was invariant between wild-type and the *pho1* mutant within the statistical limitations (Table 4). However, the

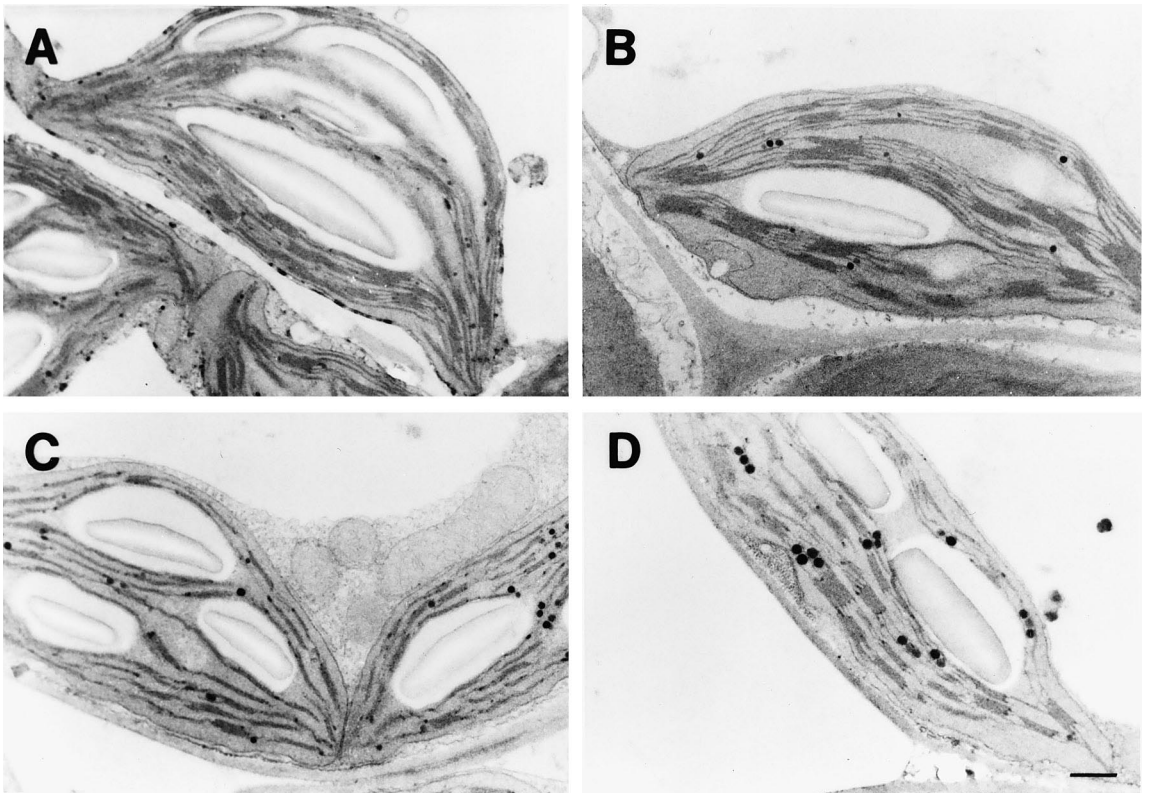

Fig. 2. Chloroplast ultrastructure of the *A. thaliana* wild-type (A,C) and the *pho1* mutant (B,D) grown under low light (A,B) or high light (C,D). Representative sections from wild-type or mutant chloroplasts are shown. Scale bar in D: 500 nm.

amount of total thylakoid membranes per plastid increased from 145.7 μm in the wild-type to 233.0 μm in the mutant grown under LL conditions. The differences become even more pronounced under HL. The enhanced amount of thylakoid membranes in the *pho1* mutant seems to be correlated with the increased chlorophyll contents per unit leaf area (Table 3). While in the wild-type the proportion of appressed membranes makes up 58% (LL) and 42% (HL), in the *pho1* mutant this proportion is 70% (LL) and 64% (HL) of the total thylakoid membranes, revealing that both lines respond similarly to the HL conditions by decreasing their amount of appressed membranes relative to LL conditions. Independent of the line and the PPFD exposed, the total amount of non-appressed membranes per plastid did not appreciably vary. Hence, the ratio of appressed to non-appressed membranes, which is an index of the degree of grana stacking, increased in the *pho1* mutant by a factor of 1.7 under LL and 2.5 under HL conditions as compared with the wild-type.

3.5. Excitation energy transfer to the reaction centers is not compromised under PG deficiency

To investigate further consequences of the structural arrangement of the pigment–protein complexes and the efficiency of excitation energy transfer to the reaction centers, low temperature (77 K) fluorescence emission spectra were recorded for thylakoid preparations of *A. thaliana* wild-type and the *pho1* mutant. Excitation was at 472 nm, the excitation maximum of chlorophyll *b* (Fig. 3). Three distinct emission maxima at 685, 693 and 730 nm can be separated, which are ascribed to emanate primarily from the PSII core antenna complexes CP43, CP47 and from PSI with its antenna, respectively [44]. By comparison of the spectra, three major features emerge: (1) there is no shift in the fluorescence emission maxima positions indicative for complete assembly of PSII and PSI complexes in both wild-type and mutant; (2) the magnitude of the 693-nm fluorescence slightly increased relative to the 685-nm fluorescence emission in the *pho1* mutant; and (3) there is a similar

Table 4
Ultrastructural analysis of chloroplasts of *A. thaliana* wild-type and *pho1* mutant leaves grown under low light (LL) or high light (HL) conditions

Parameters	LL		HL	
	Wild-type	*pho1*	Wild-type	*pho1*
Plastids/cell cross-section (n=25)	11.1 ± 0.4	11.2 ± 0.6	12.7 ± 0.5	12.8 ± 0.6
Grana/plastid (n=25)	42.8 ± 1.6	50.9 ± 1.9	52.3 ± 2.0	53.1 ± 2.7
Thylakoids/granum (n=100)	5.3 ± 2.2	8.0 ± 3.5	3.1 ± 0.9	6.3 ± 2.8
Grana thylakoid length (n=100; μm)	0.46 ± 0.01	0.46 ± 0.01	0.41 ± 0.01	0.42 ± 0.01
Stroma thylakoids/plastid (n=15)	102.1 ± 6.3	119.5 ± 8.5	93.4 ± 8.4	108.3 ± 5.3
Stroma thylakoid length (n=100; μm)	0.22 ± 0.01	0.19 ± 0.01	0.21 ± 0.02	0.19 ± 0.02
Appressed membranes (μm/plastid)	84.1[a]	163.6[a]	44.8[a]	118.0[a]
Non-appressed membranes (μm/plastid)	61.6[a]	69.4[a]	62.2[a]	65.3[a]
Total thylakoid membranes (μm/plastid)	145.7[a]	233.0[a]	107.0[a]	183.3[a]
Appressed/non-appressed membranes	1.4[a]	2.4[a]	0.7[a]	1.8[a]

Values represent the means (± S.E.) of at least six different determinations with individual plants.
[a]These values were calculated from measured parameters in the table.

relative increase in PSII- to PSI-associated fluorescence emission in HL-grown wild-type and mutant plants. The latter finding may be due to the fact that plants usually adapt to higher growth PPFDs by increasing their number of PSII units, while the number of PSI units remains almost the same [3,37,38]. Together, these results are taken as evidence that the molecular environment of the PSII core complexes and the excitation energy transfer from LHCII to the reaction centers of PSII and PSI is not or only marginally affected in the mutant.

3.6. PG-deficient mutant plants are photochemically highly competent under low and high PPFDs

The light–response curves of O_2 evolution are comparable between wild-type and mutant leaves (Fig. 4). However, HL-acclimated plants showed about 2-fold higher maximum photosynthetic O_2-evolution rates than LL plants, implying that both wild-type and the mutant adapt to the higher growth PPFD by increasing the light utilization efficiency to a similar extent.

In order to further analyze possible effects on the functional integrity of thylakoid membranes, the ef-

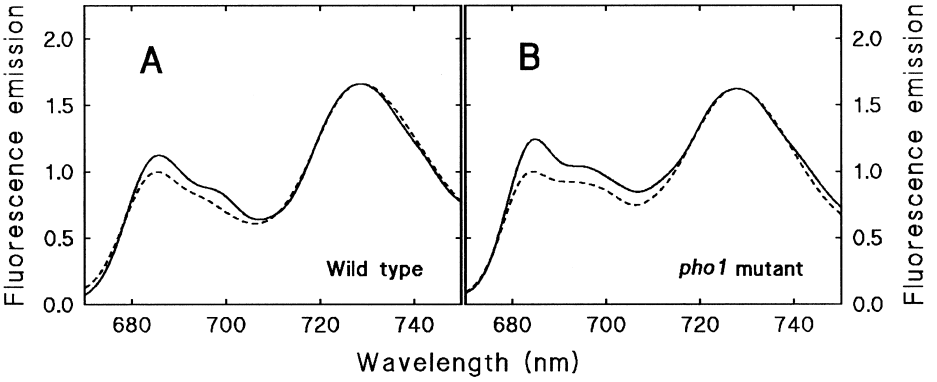

Fig. 3. Low temperature (77 K) chlorophyll fluorescence emission spectra of thylakoid suspensions of *A. thaliana* wild-type (A) and the *pho1* mutant (B) grown under low light (dashed line) or high light (continuous line). The chlorophyll concentration was 2.5 μg ml^{-1}. To facilitate comparison, the spectra were normalized to the red-most PSI emission maximum. Excitation was at 472 nm (chlorophyll *b* excitation). The spectra were corrected for the sensitivity of the measuring system. The experiments were repeated three times with thylakoid extracts isolated from leaves of different plants with the same outcome.

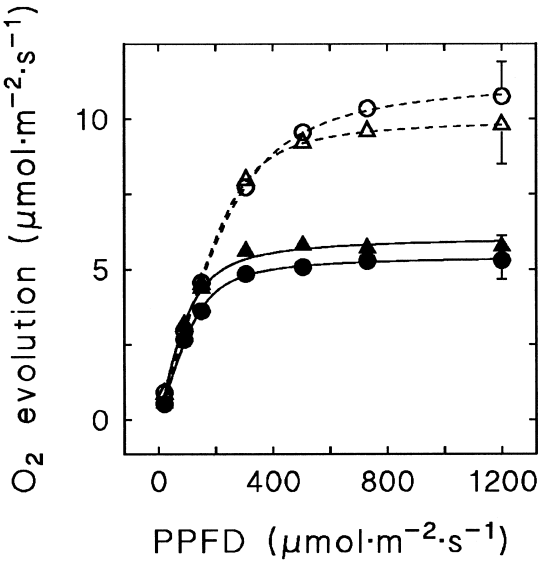

Fig. 4. Light–response curves of CO_2-saturated O_2 evolution in leaves of *A. thaliana* wild-type (△) and the *pho1* mutant (○) grown either under low light (closed symbols) or high light (open symbols) on a leaf area basis. Values represent the means of at least eight measurements. S.D. are as indicated.

ficiency of flash-induced photoreduction of the primary stable quinone electron acceptor of PSII Q_A, σ, was determined (Fig. 5). For dark-adapted leaves, σ is considered to reflect the maximum effective absorption cross section for PSII photochemistry [35]. The fluorescence yield data per single-turnover flash as a function of flash intensity could be fitted to a cumulative single-hit Poisson distribution function (Fig. 5A,B). In the wild-type, σ decreased from 9.3 in LL-grown plants to 6.3 in HL-grown plants, suggesting a 48% reduction in the relative size of the PSII antenna in HL-acclimated plants (Fig. 5A). Similarly, the *pho1* mutant showed a 32% reduction in the PSII antenna size upon HL-acclimation. However, σ was increased by 17 and 30% in LL- and HL-grown *pho1* mutant plants, respectively, as compared to the wild-type (Fig. 5B). Thus, regardless of the growth PPFD, the relative PSII antenna size is clearly increased in the *pho1* mutant.

A comparison of the values gathered from steady-state chlorophyll fluorescence measurements reveals that *pho1* mutant leaves exhibit under both PPFDs slightly lower values of intrinsic PSII efficiency, measured as the ratio of F_v/F_m, in comparison to the wild-type (Table 5). To estimate the electron flux via PSII, fluorescence quenching analyses were carried out after a 15-min exposure of leaves to 1200 μmol m^{-2} s^{-1}, a PPFD largely saturating for photosynthesis (see Fig. 4). When comparing wild-type and the *pho1* mutant that were grown under the same PPFD, only subtle differences were found in the empirical fluorescence parameters Φ_{PSII} and *q*P, which are indicative for steady-state linear electron flux through PSII [45]. Thus, the higher values in HL-

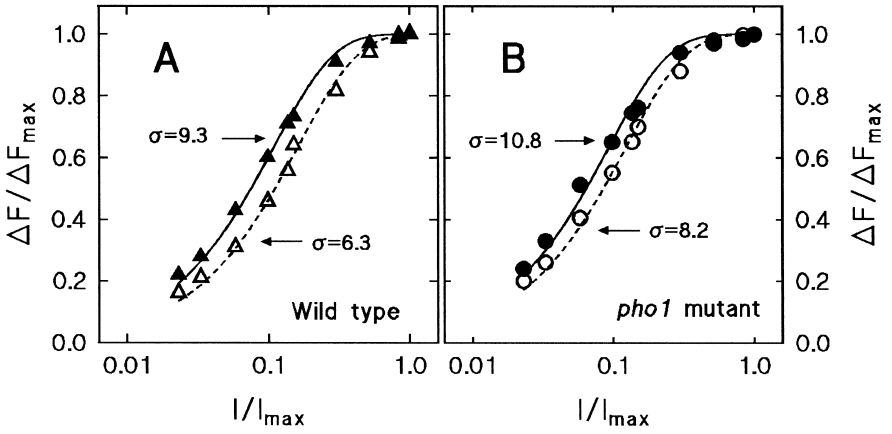

Fig. 5. Flash energy–response curves of the fluorescence yield increase in leaves of *A. thaliana* wild-type (△) and the *pho1* mutant (○) grown either under low light (closed symbols) or high light (open symbols). Changes in fluorescence yield are expressed as $\Delta F/\Delta F_{max}$, where ΔF is the fluorescence yield induced with a single-turnover flash of an intensity I, and ΔF_{max} is the maximum fluorescence yield produced by the non-attenuated flash of I_{max}. The plot of $\Delta F/\Delta F_{max}$ versus I/I_{max} can be described by an exponential saturation function $\Delta F/\Delta F_{max} = 1-e^{\sigma I/I_{max}}$, which can be fitted to the data with r^2 values of ≥ 0.98. Values represent the means of at least three measurements.

Table 5
Chlorophyll fluorescence parameters obtained with mature leaves of *A. thaliana* wild-type and the *pho1* mutant grown under low light (LL) or high light (HL) conditions after 1 h darkening (F_v/F_m) or after a 15-min exposure to light of a PPFD of 1200 μmol m^{-2} s^{-1}

Parameters	LL		HL	
	Wild-type	*pho1*	Wild-type	*pho1*
F_v/F_m	0.817 ± 0.008	0.785 ± 0.020	0.826 ± 0.009	0.795 ± 0.008
Φ_{PSII}	0.203 ± 0.028	0.195 ± 0.010	0.338 ± 0.012	0.370 ± 0.033
*q*P	0.363 ± 0.042	0.405 ± 0.012	0.615 ± 0.019	0.671 ± 0.031
*q*N	0.718 ± 0.025	0.737 ± 0.011	0.772 ± 0.022	0.739 ± 0.013
*q*E	0.543 ± 0.008	0.560 ± 0.014	0.664 ± 0.019	0.629 ± 0.035

Values represent the means (± S.D.) of at least four different determinations with individual plants.

as compared to LL-acclimated plants indicate a higher capacity for PSII-mediated electron transfer in both lines consistent with the enhanced O_2-evolution rates. These results suggest that the slightly diminished F_v/F_m ratios in the *pho1* mutant are not due to an altered PSII efficiency, but most probably due to the relative increased PSII antenna size, which gives rise to an increased F_o level and thereby decreases F_v and hence F_v/F_m. Finally, there are rather marginal differences in the capacity of leaves for non-radiative energy dissipation as indicated by *q*N, and its high-energy-dependent component *q*E under the same growth PPFD.

4. Discussion

4.1. PG deficiency is compensated for by increased accumulation of SQDG and DGDG

In the present study, we used the *pho1* mutant of *A. thaliana* as a tool to gain more insight into the role of specific lipids for the structural and functional integrity of the photosynthetic machinery. The mutant has a reduced ability to accumulate phosphate in aerial parts [27], which was shown to decrease the phospholipid PG [26]. Of the total leaf PG, about 85% is located within chloroplasts [46]. PG is thought to be of crucial importance for assembly and function of the thylakoid membrane [2,4]. Hence, the question arose what the consequences of PG deficiency may be for thylakoid membrane organization and function in the *pho1* mutant.

In the *pho1* mutant, a relative reduction in PG content to 45 and 35% of the wild-type content under LL and HL conditions, respectively, was associated with simultaneously increased proportions of SQDG and, to a smaller extent, of DGDG (Table 1), reflecting a shift towards the non-phosphorous lipids SQDG and DGDG at the expense of the amount of phospholipids. The increase in SQDG and DGDG are not simply brought about by a concentration effect due to reduced phospholipids, because MGDG did not change significantly. Moreover, the commensurate accumulation of SQDG with SQD1 protein levels in the *pho1* mutant under both LL and HL conditions (Fig. 1) implies that the SQDG increase is due to an active regulation of the expression of a gene involved in SQDG synthesis as it has been recently suggested [26]. It remains to be shown whether the DGDG increase is also due to such a regulatory process. Independent of the PPFD that plants experienced during growth, PG reduction in the *pho1* mutant was apparently compensated for by increased amounts of SQDG and DGDG. This confirms and extends previous data obtained with *A. thaliana* wild-type plants, where a correlation between PG abundance and the concentration of exogenous phosphate was shown [26]. The dramatic shift in whole lipid classes was with almost no influence on the proportion of fatty acids within the individual lipid classes (Table 2A,B), suggesting that for PG compensation, apparently lipid head groups are the determining factor and not, as frequently found with other lipid mutants, the degree of fatty acid saturation.

4.2. Lipid changes affect the organization, but not light acclimation, of the thylakoid membrane

Irrespective of the strongly decreased PG contents in the *pho1* mutant, the amount of chlorophyll (*a*+*b*)

per unit leaf area was increased accompanied by diminished chlorophyll *a*/*b* ratios under both PPFDs applied. This indicates that the total amount of chlorophyll *b*-binding LHCs was increased in the mutant both relative to the chlorophyll *a*-binding reaction center/core complexes and to the leaf area. Of the carotenoids, the content of β-carotene that is enriched in the reaction center/core complexes was decreased in the mutant, while xanthophylls that are mainly bound to LHCs [41], were present in stoichiometric amounts relative to chlorophylls under LL conditions (Table 3). The increase in β-carotene and the pool size of the xanthophyll-cycle carotenoids upon HL exposure shows that the mutant did not lose its competence for light acclimation by changing its pigment composition. Owing to its proportional reduction in comparison with the wild-type under both PPFDs, the altered β-carotene content might be linked to the altered lipid composition. Moreover, the lower chlorophyll *a*/*b* ratios associated with an increase in the effective absorption cross section of PSII (Fig. 5) are clearly indicative for a larger PSII antenna size in the *pho1* mutant than in the wild-type. Regardless of the differences in the PSII antenna size, the photochemical efficiency of PSII did not appreciable vary (Table 5), indicating that the photochemical competence of PSII reaction centers is not compromised in the *pho1* mutant.

A 70–80% depletion in the thylakoid PG content after phospholipase A_2 digestion inhibited both PSII and whole-chain electron transport activity by more than 50% in pea [22] and spinach [23]. This has lead to the conclusion that PG is important for maintaining the structural and functional integrity of the components involved in photosynthetic electron transfer. Upon HL acclimation the amount of PG was similarly reduced by 65% in the *pho1* mutant, with no appreciable effect on overall PSII electron transfer, as judged from the similar Φ_{PSII} values and light–response curves of photosynthetic O_2 evolution (Table 5 and Fig. 4). This shows that the thylakoid membrane possess the inherent potential to compensate for the loss in PG throughout long-term acclimation. Furthermore, it is thought that the protonation of carbonyl resides at the luminal surface of the LHCs of PSII and violaxanthin de-epoxidation together provide the basis for *q*N, and in particular *q*E formation [47]. The fact that *q*E was apparently insensitive to the changes in lipid composition (Table 5) suggests that the molecular mechanism giving rise to *q*E and, hence, the molecular structure of the LHCs that mediates *q*E build-up, was not affected. The finding that *q*E was similarly enhanced in HL plants further indicates that the changes in the organization of LHCs, that were brought about by the higher growth PPFD, were of similar nature in both lines. The changes in the lipid pattern in *pho1* mutant plants were accompanied by only subtle changes in the 77 K fluorescence characteristics, indicating that the excitation energy distribution to the reaction centers of the two photosystems was not compromised. Taken together, the changes in the wild-type and the *pho1* mutant point to a similar adaptive response in thylakoid organization during LL/HL acclimation under PG deficiency. This implies that at least a large fraction of PG is not absolutely required for light acclimation of the photosynthetic apparatus. We conclude that adjusting a certain glycerolipid composition allows to maintain the function of essential processes that are restricted to the thylakoid membrane, such as photosynthesis, even under severe PG deficiency.

4.3. *Grana stacking is increased in PG-deficient mutant thylakoids*

It is still a matter of debate whether or not PG may have a role (directly or indirectly) in thylakoid membrane stacking. In particular, t-16:1 containing PG has been frequently correlated with thylakoid membrane stacking [18,19,48]. On the other hand, the *fadA* (*fad4*) mutant of *A. thaliana*, which compensated for the complete lack of t-16:1-PG by increased amounts of 16:0-PG showed almost no changes in thylakoid membrane stacking [49,50], questioning at least an obligatory role of t-16:1 PG. In the *pho1* mutant, the extent of grana stacking and the amount of appressed thylakoid membranes were markedly increased relative to the wild-type control under both PPFDs (Fig. 2 and Table 4). Because t-16:1-PG decreased proportionally with the overall PG content in the mutant, these results are not consistent with a tight coupling between t-16:1-PG and thylakoid membrane stacking and suggest that it may be only of conditional significance. However, if one accepts that PG is, irrespective of

the presence of t-16:1 or 16:0, one decisive determinant in mediating grana stacking, either directly or via its role in LHC stabilization [5,8,9,13,48], the increased grana stacking in the *pho1* mutant (Fig. 2 and Table 4) is best explained by the idea that SQDG and/or DGDG can functionally substitute PG.

Grana formation has been shown to be related to the presence of PSII because the stacking is likely mediated by the LHCs of PSII [51]. This is not in contradiction with the *pho1* mutant data, where increased grana formation was correlated with reduced chlorophyll *a*/*b* ratios, indicative for an increase in LHCs of PSII relative to the core complexes. The relative amounts of the anionic lipids PG and SQDG have been frequently found to be enriched in the LHCs of PSII [8,9,13,48], and a high ratio of MGDG to DGDG has been correlated with enhanced grana formation [16,30,52,53], suggesting that DGDG is less relevant in this process. Hence, the more likely candidate for substituting PG is SQDG. In fact, the nearly inverse relationship between the relative amounts of PG and SQDG in the *pho1* mutant, that were found irrespective of the stronger PG reduction under HL exposure (Table 1), suggests that the anionic lipid SQDG is able to replace the anionic lipid PG in the thylakoid membrane of *A. thaliana*. This would imply that specific sites exist within the thylakoid membrane that can be occupied by either PG or SQDG. At present, this idea remains speculative because the interpretation of our data is complicated by the fact that concomitant with SQDG also DGDG increased, though less pronounced. However, a strong argument in favor of a more specific role for SQDG is provided by a recently isolated cyanobacterial mutant which mainly compensated for the complete lack in SQDG with increased amounts of PG, with only subtle changes in DGDG [25]. On the other hand, the *dgd1* mutant of *A. thaliana* responded to the 90% decrease in DGDG without any selective increase in PG or SQDG [30]. Together, these findings are consistent with the idea that indeed SQDG (rather than DGDG) may substitute for PG in the thylakoid membrane. This does not exclude the possibility that DGDG may have another function in compensating for PG deprivation within the photosynthetic apparatus.

The marked changes in the thylakoid ultrastructure together with the fact that growth was delayed and the appearance of the *pho1* mutant was altered (this study, [27]) indicates that neither SQDG nor DGDG are able to complement PG exactly and that other factors have to be considered, too. It is clear that the limited phosphate availability has more far-reaching consequences for cell metabolism as it is also indicated by the reduced proportions of the extra-plastidic phospholipids PC and PE (Table 1). In particular, the well-established effect on carbon metabolism (e.g. [54–56]) may be critical for both total lipid and membrane synthesis under phosphate limiting conditions. Regarding the structural changes in the *pho1* mutant, it should be noted that the negatively charged PG and SQDG have a distinct fatty acid composition, differing particularly in the relative amounts of the different fatty acids 16:1 and 16:0. About 27% of the fatty acids of PG is present as 16:1, and 25% as 16:0 (Table 2A). In the case of SQDG, about 50% were found as 16:0, whereas *cis*-16:1 makes up only 6% (Table 2B). In this context, it is worth mentioning that DGDG, which contains a particular high proportion of 16:0-DGDG, was successfully applied in reconstitution experiments with PG-depleted LHCII, whereas MGDG with very low proportions of 16:0 (and 16:1) was not [5]. Although, not specifically tested, SQDG, with its similarly high proportion of 16:0, seems likely to exert a similar effect as DGDG did. In view of its different biophysical properties, it seems reasonable to assume that the changes in the thylakoid ultrastructure in the *pho1* mutant are a direct consequence of the altered fatty acid composition towards 16:0 fatty acids at the expense of the proportion of 16:1.

In summary, the loss of 65% of all the PG in the thylakoid membrane of the *pho1* mutant neither compromised photosynthetic electron transfer, O_2 evolution, or excitation energy allocation to the reaction centers of the two photosystems, suggesting that the phosphate-mediated changes in the thylakoid structure of the *pho1* mutant did not compromise the thylakoid function. Moreover, despite of considerable structural changes, the thylakoid membrane did not lose its competence for light acclimation. Thus, the increase in the amounts of SQDG and DGDG with decreasing PG point to a special

significance of these lipids in the adaptation process of the thylakoid membrane to phosphate deprivation. In future experiments on *A. thaliana*, including plants with *SQD1* and *DGD1* cDNA antisense RNA expression and plants that overexpress the *SQD1* cDNA as they become available, attempts will be made to further unravel the specific roles of SQDG and DGDG in maintenance of the structural and functional integrity of the thylakoid membrane under phosphate-limiting conditions.

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References

[1] R. Douce, J. Joyard, Annu. Rev. Cell Biol. 6 (1990) 173–216.
[2] M.S. Webb, B.R. Green, Biochim. Biophys. Acta 1060 (1991) 133–158.
[3] J.M. Anderson, C.B. Osmond, in: D.J. Kyle, C.B. Osmond, C.J. Arntzen (Eds.), Photoinhibition, Elsevier, Amsterdam, 1987, pp. 1–38.
[4] H. Paulsen, Photochem. Photobiol. 62 (1995) 367–382.
[5] R. Rémy, A. Trémolierès, J.C. Duval, F. Ambard-Bretteville, J.P. Dubacq, FEBS Lett. 137 (1982) 271–275.
[6] U. Pick, K. Gounaris, M. Weiss, J. Barber, Biochim. Biophys. Acta 808 (1985) 415–420.
[7] N. Murata, Y. Fujimura, S. Higashi, Biochim. Biophys. Acta 1019 (1990) 261–268.
[8] A. Trémolierès, P. Dainese, R. Bassi, Eur. J. Biochem. 221 (1994) 721–730.
[9] N. Sato, K. Sonoike, M. Tsuzuki, A. Kawaguchi, Eur. J. Biochem. 234 (1995) 16–23.
[10] G. Li, P.F. Knowles, D.J. Murphy, I. Nishida, D. Marsh, Biochemistry 28 (1989) 7446–7452.
[11] W. Kühlbrandt, Curr. Opin. Struct. Biol. 4 (1994) 519–528.
[12] S. Nußberger, K. Dörr, D.N. Wang, W. Kühlbrandt, J. Mol. Biol. 234 (1993) 347–356.
[13] Z. Krupa, J.P. Williams, M.U. Khan, N.P.A. Huner, Plant Physiol. 100 (1992) 931–938.
[14] A. Trémolierès, J.P. Dubacq, F. Ambard-Bretteville, R. Rémy, FEBS Lett. 130 (1981) 27–31.
[15] P.G. Roughan, N.K. Boardman, Plant Physiol. 50 (1972) 31–34.
[16] J. Bahl, B. Francke, R. Moneger, Planta 129 (1976) 193–201.
[17] N.P.A. Huner, M. Krol, J.P. Williams, E. Maissan, P.S. Low, D. Roberts, J.E. Thompson, Plant Physiol. 84 (1987) 12–18.
[18] A. Trémolierès, O. Roche, G. Dubertret, D. Guyon, J. Garnier, Biochim. Biophys. Acta 1059 (1991) 286–292.
[19] C. Tuquet, T. Guillot-Salomon, M. De Lubac, M. Signol, Plant Sci. Lett. 8 (1977) 59–64.
[20] J. Maroc, A. Trémolierès, J. Garnier, D. Guyon, Biochim. Biophys. Acta 1020 (1987) 153–162.
[21] I. Nishida, N. Murata, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 541–568.
[22] B.R. Jordan, W.-S. Chow, A.J. Baker, Biochim. Biophys. Acta 725 (1983) 77–86.
[23] P.-A. Siegenthaler, J. Smutny, A. Rawyler, Biochim. Biophys. Acta 891 (1987) 85–93.
[24] C. Benning, J.T. Beatty, R.C. Prince, C.R. Somerville, Proc. Natl. Acad. Sci. USA 90 (1993) 1561–1565.
[25] S. Güler, A. Seeliger, H. Härtel, G. Renger, C. Benning, J. Biol. Chem. 271 (1996) 7501–7507.
[26] B. Essigmann, S. Güler, R.A. Narang, D. Linke, C. Benning, Proc. Natl. Acad. Sci. USA 95 (1998) 1950–1955.
[27] Y. Poirier, S. Thoma, C. Somerville, J. Schiefelbein, Plant Physiol. 97 (1991) 1087–1093.
[28] C. Benning, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 53–75.
[29] T. Murashige, F. Skoog, Physiol. Plant 15 (1962) 473–497.
[30] P. Dörmann, S. Hoffmann-Benning, I. Balbo, C. Benning, Plant Cell 7 (1995) 1801–1810.
[31] H. Härtel, H. Lokstein, B. Grimm, B. Rank, Plant Physiol. 110 (1996) 471–482.
[32] H. Härtel, H. Lokstein, P. Dörmann, B. Grimm, C. Benning, Plant Physiol. 115 (1997) 1175–1184.
[33] S. Hoffmann-Benning, K.L. Klomparens, H. Kende, Ann. Botany 74 (1994) 563–572.
[34] H. Härtel, H. Lokstein, P. Dörmann, R.N. Trethewey, C. Benning, Plant Physiol. Biochem. 36 (1998) 407–417.
[35] P.G. Falkowski, K. Wyman, A.C. Ley, D.C. Mauzerall, Biochim. Biophys. Acta 849 (1986) 183–192.
[36] O. Björkman, in: O.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler (Eds.), Encyclopedia of Plant Physiology, NS, Vol 12A: Physiological Plant Ecology I, Springer-Verlag, Berlin, 1981, pp. 57–107.
[37] J.M. Anderson, W.S. Chow, D.J. Goodchild, Aust. J. Plant Physiol. 15 (1988) 11–26.
[38] W.R. De la Torre, K.O. Burkey, Photosynth. Res. 24 (1990) 127–136.
[39] S.S. Thayer, O. Björkman, Photosynth. Res. 23 (1990) 331–343.
[40] H. Härtel, I. Reinhardt, B. Grimm, J. Photochem. Photobiol., B. Biol. 43 (1998) 136–145.
[41] H.Y. Yamamoto, R. Bassi, in: D.R. Ort, C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, Kluwer Academic Publishers, Dordrecht, 1996, pp. 539–563.
[42] B. Demmig-Adams, W.W. Adams III, Annu. Rev. Plant Phys. Plant Mol. Biol. 43 (1992) 599–626.

[43] H. Härtel, E. Kruse, B. Grimm, Plant Physiol. 113 (1997) 1113–1124.
[44] G.H. Krause, E. Weis, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 313–349.
[45] U. Schreiber, W. Bilger, C. Neubauer, in: E.-D. Schulze, M. Caldwell (Eds.), Ecophysiology of Photosynthesis, Springer-Verlag, Berlin, 1994, pp. 49–70.
[46] J. Browse, N. Warwick, C.R. Somerville, C.R. Slack, Biochem. J. 235 (1986) 25–31.
[47] P. Horton, A.V. Ruban, R.G. Walters, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 655–684.
[48] G. Dubertret, A. Mirshahi, M. Mirshahi, C. Gerard-Hirne, A. Trémolierès, Eur. J. Biochem. 226 (1994) 473–482.
[49] J. Browse, P. McCourt, C.R. Somerville, Science 227 (1985) 763–765.
[50] P. McCourt, J. Browse, J. Watson, C.J. Arntzen, C.R. Somerville, Plant Physiol. 78 (1985) 853–858.
[51] L.A. Staehelin, G.W.M. Van der Staay, in: D.R. Ort, C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, Kluwer Academic Publishers, Dordrecht, 1996, pp. 11–30.
[52] K. Gounaris, C. Sundby, B. Andersson, J. Barber, FEBS Lett. 156 (1983) 170–173.
[53] R. Douce, R.B. Holtz, A.A. Benson, J. Biol. Chem. 248 (1973) 7215–7222.
[54] D.A. Walker, M.N. Sivak, Trends Biochem. Sci. 11 (1986) 176–179.
[55] K.-J. Dietz, L. Heilos, Plant Physiol. 93 (1990) 1219–1225.
[56] I.M. Rao, A.L. Fredeen, N. Terry, Plant Physiol. Biochem. 31 (1993) 223–231.