Changes in membrane lipids and carotenoids during light acclimation in a marine cyanobacterium *Synechococcus* sp.

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Time course of carotenoid and membrane lipid variation during high light (HL) acclimation (about 85 μmol m^{-2} s^{-1}), after transfer from low light (LL) (5–10 μmol m^{-2} s^{-1}), was determined in a marine *Synechococcus* strain. High-performance liquid chromatography (HPLC) coupled to diode array detector (DAD) or electrospray ionization mass spectrometry (ESI-MS) was used for compound separation and detection. Myxoxanthophyll rose within a time interval of 8 h to 24 h after the onset of exposure to HL. β-carotene content started to decrease after 4 h of the onset of exposure to HL. Zeaxanthin content rose with exposure to HL, but it was only significant after 24 h of exposure. Carotenoid changes are in agreement with a coordinated activity of the enzymes of the myxoxanthophyll biosynthetic pathway, with no rate-limiting intermediate steps. Lipid analysis showed all species with a C18:3/C16:0 composition increased their content, the changes of PG(18:3/16:0) and MGDG(18:3/16:0) being primarily significant. Major lipid changes were also found to occur within 24 h. These changes might suggest reduction and reorganization of the thylakoid membrane structure. Hypotheses are also drawn on the role played by lipid molecule shape and their possible effect in membrane fluidity and protein accommodation.

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1. Introduction

Light acclimation of cyanobacterial cells is a process that involves a reduction of chlorophyll and phycobiliprotein contents, besides a change in the relative proportion of carotenoids (Riethman *et al.* 1988; Hirschberg and Chamovitz 1994; Falkowsky and Raven 1997; McIntyre *et al.* 2002; Schagerl and Müller 2006). In *Synechocystis* strains, the increase in the glycocarotenoid Myx that is elicited by high light exposure has been explained by the need for protection against ROS that are generated in the photosynthetic light reactions (Lagarde and Vermaas 1999; Steiger *et al.* 1999; Schäfer *et al.* 2005). The rise in Myx is also observed in some *Synechococcus* strains under high light or nitrogen deficiency (Masamoto and Furukawa 1997; Graham and Bryant 2008; Montero *et al.* 2011). A biological function for myxoxanthophyll alternative to that of photoprotection has been suggested by Mohamed *et al.* (2005). Using a mutant of *Synechocystis* sp. strain PCC6803 that lacks fucose synthetase activity and, thereby, myxoxanthophyll, a functional

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Abbreviations used: Car, β-carotene; Chl, chlorophyll *a*; HL, high light; LL, low light; Lyc, lycopene; MGDG and DGDG, mono- and di-galactosyl-diacyl-glycerol; Myx, myxoxanthophyll (myxol-2′-fucoside); PG, phosphatidyl-diacyl-glycerol; PRCs, photosynthetic reaction centers; SQDG, sulfoquinovosyl-diacyl-glycerol; Z, zeaxanthin

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role for myxoxanthophyll was proposed by these authors in relation to thylakoid membrane stabilization and S-layer formation. Nonetheless, experimental evidence available till date does not clearly highlight on the actual biological function of this glycocarotenoid.

Recent research has unraveled the myxoxanthophyll biosynthetic pathway almost completely (Graham and Bryant 2009). The involvement of the same β-carotene hydroxylase (*crtR* gene) that participates in Z biosynthesis through 3-hydroxylation was early demonstrated by Lagarde and Vermaas (1999). The Myx biosynthetic pathway proposed by Graham and Bryant (2009) encompasses 1′-hydroxylation of Lyc as the first step (CruF enzyme) (figure 1); thereby, since Car is formed through two cyclization steps from Lyc, a competition for the Lyc pool is likely to arise between the two pathways. Furthermore, the Z content is also dependent on the Car pool that is available for the β-carotene hydroxylase enzyme. Hence, it is expected high light acclimation to involve a transition interval during which the cellular contents of Car and Z are reduced. In particular, since Z has been reported to increase with exposure to high light in Cyanobacteria (Masamoto and Furukawa 1997), Lyc content should also increase to satisfy the demand from the two pathways. To our knowledge, there is not report yet on the time course of Myx rise and other carotenoid variation during light acclimation *in vivo*.

Membrane lipids have long been regarded as having a structural function only, but recent research is pointing out

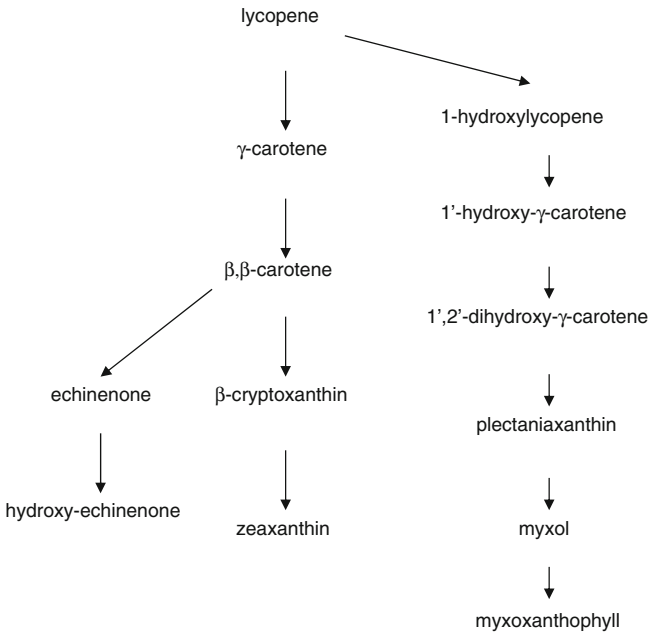

Figure 1. Biosynthetic pathways for echinenone, zeaxanthin and myxoxanthophyll from lycopene in cyanobacteria, according to Lagarde and Vermaas (1999) and Graham and Bryant (2009).

lipids are involved in different physiological processes in a specific manner, which accounts for interaction with membrane proteins and participation in different membrane functions (Lee 2004; Palsdottir and Hunte 2004; McIntosh and Simon 2006; Jones 2007; Hölzl and Dörmann 2007). Cyanobacterial membrane lipids have shown changes in their contents that relied on environmental factors like temperature, light, salt stress and drought (Riethman *et al.* 1988; Sakamoto *et al.* 1997; Hölzl and Dörmann 2007; Sato and Wada 2009). The lipid profile of cyanobacterial membranes is composed of mono- and di-galactosyl-diacyl-glycerol (MGDG and DGDG, respectively), sulfoquinovosyl-diacyl-glycerol (SQDG), and phosphatidyl-diacyl-glycerol (PG). C18 chains are preferred at the *sn-1* position of the glycerol backbone, while C16 chains are preferred at the *sn-2* position (Zepke *et al.* 1978; Hölzl and Dörmann 2007; Sato and Wada 2009). An essential role for PG has been found in photosynthesis (Sato *et al.* 2000; Domonkos *et al.* 2004; Sato 2004; Wada and Murata 2007) and regulation of carotenoid biosynthesis (Domonkos *et al.* 2009); SQDG and/or DGDG seem to substitute PG under phosphate limitation (Güler *et al.* 1996; Dörmann and Benning 2002; Sato 2004; Frentzen 2004; Awai *et al.* 2007; Kobayashi *et al.* 2009); and MGDG or the MGDG/DGDG ratio has been bound to the upkeep of the PSII complex functional configuration and efficient energy transfer between the photosystem II antenna complexes and core complex (Grzyb *et al.* 2008; Leng *et al.* 2008; Zhou *et al.* 2009).

Till date, changes in lipid composition have been primarily determined by gas chromatography coupled to electron impact mass spectrometry (GC-EI-MS), with separation of the different lipid classes by thin-layer chromatography and further fatty acid saponification with KOH in methanol (methylation). This procedure imposes some constrains to the ascertaining of glycerolipid variations at the species level, which may be avoided by analysis with mass spectrometry techniques as they are implemented at present, namely, electrospray ionization coupled to ion-trap or triple-quadrupole analysers (Welti *et al.* 2003; Yao *et al.* 2006; Okazaki *et al.* 2009). A high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) method was used in this study that enabled lipid isobaric species separation as well as ascertaining their content time course during light acclimation. Time course variation of carotenoids was simultaneously determined using HPLC-DAD measurements.

2. Material and methods

2.1 *Culture and experimental setup*

The *Synechococcus* sp. strain 01/0202 from the Marine Microalgae Culture Collection of the Institute for Marine Sciences of Andalusia (ICMAN, CSIC) was used in this

study. This strain was previously found to be closely related to the model strain *Synechococcus* sp. PCC7002 according to 16S rDNA, although lacking the carotenoid synechoxanthin (Montero *et al.* 2011). Cells were grown in 100 mL erlenmeyer flasks containing 60 mL of culture volume, and were placed in a thermostated culture chamber (Friocell-111, MMM Medcenter Einrichtungen GmbH, Gräfelging/München, Germany). Light was provided by daylight fluorescent tubes. Culture medium was natural seawater filtered through 1.0 μm filter, sterilized in an autoclave (120°C, 1 kg pressure, 60 min), and enriched with F/2 medium with double nitrate and phosphate concentrations. Culture temperature was 20°C, and irradiance was 5–10 μmol photons m^{-2} s^{-1} for LL conditions and 85–90 μmol photons m^{-2} s^{-1} for HL conditions. These irradiances were chosen because previous experiments on growth pointed out there was a significant difference between both irradiances regarding growth but without stress.

For experiments, cultures were freshly prepared by adding 5 or 10 mL of the stock culture to autoclaved seawater with F/2 medium, and kept at low irradiance for 48 h in order to get the cells acclimated again to the LL conditions. Following, the cultures were moved to high light (HL conditions, time 0), and 2 mL samples from 3 independent cultures taken at different time intervals. Cell density was determined by measuring the absorbance of 1 mL samples at 750 nm (A_{750}) (Sorokin 1973; Montero *et al.* 2002); and stock cultures used for inoculums were diluted to give an absorbance (r.u.) between 0.1 and 0.2 when appropriate. Two independent experiments were conducted with each culture in triplicate.

2.2 *Sample preparation*

After a 2 mL aliquot of culture was taken, 100 μL of 4% paraformaldehide was added to each culture sample, and the samples were placed in ice. Cells were pelleted by centrifugation at 3500 rpm (2818*g*) for 10 min at 4°C, and then the supernatant was discarded and 1 mL of cold 0.9% ammonium formate was added to the pellet for salt removal, and the suspension centrifuged again. Then 1 mL of methanol was added to the pellet after the new supernatant was discarded while the sample container was kept in ice. Following, the methanol suspension was sonicated for 1 min while being kept in ice. The methanolic extract was collected after a new centrifugation, and kept at −20°C under nitrogen atmosphere until analysis.

2.3 *Pigment chromatography*

HPLC was used for pigment determination (Montero *et al.* 2005). Briefly, gradient elution was based on two solvent mixtures that were: (A) methanol : water (8:2 v/v) and (B) methanol : acetone (1:1); and the elution sequence was time 0 min 75%A+25%B, time 4.5 min 25%A+75%B, time 7.0 min 25%A+75%B, time 12.0 min 10%A+90%B, time 15 min 100%B, time 17 min 100%B, time 22.0 min 75%A+25%B, and then kept in these conditions for another 5 min to let column re-equilibration. Flow rate was 1 mL min^{-1}, with UV detection at 441 nm. A Nucleosil 120 C18 4.0×150 mm, 5 μm particle size, column with a guard column, ODS 4.6×10 mm (Teknokroma, Barcelona, Spain), was used for reversed phase separation. The chromatographic equipment was a LaChrom Elite® HPLC system from VWR-Hitachi, which was constituted by an L-2130 binary pump equipped with a low pressure gradient accessory and in-line degasser, an L-2420 UV-Vis detector and an L-2200 autosampler. Pigments were quantified as in Montero *et al.* (2005).

2.4 *Liquid chromatography coupled to mass spectrometry*

The same LaChrom Elite® HPLC system from VWR-Hitachi and column configurations as above were used for HPLC separation. The gradient elution used for MS analysis was as follows: (1) initial 80% A+20% B, (2) 5 min linear gradient to 15% A+85% B, (3) 8 min isocratic, (4) 10 min linear gradient to 0% A+100% B, (5) 16 min isocratic, (6) 20 min linear gradient to 80% A+20% B, (7) 30 min linear gradient to 2% A+98%C, (8) 50 min isocratic, (9) 60 min linear gradient to 80% A and 20% B, and (10) 75 min isocratic; where solvent A is methanol/acenotrile/5 mM aqueous ammonium acetate (75:15:10), solvent B is acetonitrile/5 mM aqueous ammonium acetate (90:10), and solvent C is 100% methanol. The flow rate was 0.3 mL min^{-1}, and 50 μL were injected (Montero 2011).

Column effluent was connected online to the UV detector (L-2420 from Hitachi-VWR), with detection set at 450 nm, and then an ion-trap mass spectrometer (IT-MS) Esquire® 6000 from Bruker Daltonics S.A. (Bruker Daltonik GmbH, Bremen, Germany), which was equipped with an electrospray ionization source (ESI). Compounds were detected in the negative (ESI−) and positive (ESI+) ion mode in full scan. Mass spectrometer parameters for the analysis were nebulizer 45 psi, dry gas (N_2) 9.0 Lmin^{-1}, dry temperature 320°C, capillary current 3500 V, scan 50–1200 *m/z*, and collision gas was helium. Data were afterwards analysed by using the Data Analysis® software from Bruker Daltonik GmbH (Montero 2011).

2.5 *Statistical analysis*

Data were analysed statistically by using the sofware StatGraphics 5.0. Paired or unpaired tests were conducted according to similarity of variances of each variable. For

multiple comparison, one-way ANOVA was used. Statistical differences were accepted for $P<0.05$.

3. Results

3.1 *HPLC-UV analysis of pigments*

As in previous studies, major pigments found in this *Synechococcus* sp. strain were Chl, Z and Car. Myx exhibited a very low content under LL conditions, but it substantially rose after 48 h of exposure to HL conditions (see below). The time course of variation in the different pigment content during HL acclimation is shown in figure 2 at different time intervals. Chl content was substantially reduced by about 40% in the HL-exposed cultures (figure 2A), whereas it rose in cultures kept under LL conditions after 24 h and 48 h of the experiment onset. Statistical analysis showed significant ($P<0.05$) differences between the Chl contents under HL at every sampling period except for 0 and 2 h, and 4 and 8 h. The Chl contents under LL were also found to be significantly different to those under HL at equivalent sampling periods (24 and 48 h).

Myx content started to rise at about 8 h of exposure to HL conditions (figure 2B), but significant differences ($P<0.05$) with regard to the initial content were only shown for its contents at 24 and 48 h, which rose to about fourfold the initial. Under LL conditions, Myx also exhibited an increase in its content with time in regard to the initial content, but it was lower than under HL conditions, and only after 48 h its content was significantly ($P<0.05$) different to the initial one (about 2.8-fold the initial one). The contents of two minor carotenoids that elute a few minutes later than Z are shown in figure 2B as well. One of these carotenoids ($\lambda = 460$ nm) has been shown to be hydroxy-echinenone (Graham and Bryant 2009; Montero *et al.* 2011). Absorption spectrum of the other carotenoid (unkn1) has absorption maxima at 448/475 and a spectrum peak III/II ratio of about 40%, which might be indicative of this carotenoid being an intermediate in the Z or Myx biosynthesis pathway. The content of hydroxy-echinenone dropped after 4 h of HL exposure to about 60% of the initial one. An increase in the content of the second minor carotenoid (unkn1) in relation to the initial one could be only observed after 24 h of exposure to HL. Appreciable accumulation of any other intermediates of the Myx biosynthesis pathway of those shown in figure 1 could not be detected.

The time course of Car and Z content evolution is depicted in figure 2C. Initially, the Car content was higher than the Z content, but this situation reversed just after 8 h of exposure to HL conditions, which is coincident with the time interval to which the Myx content apparently started to increase. After 24 h and 48 h of HL exposure, the Z content clearly surpassed the Car content, whereas after 24 h under LL conditions Car content still remains above the Z content. Nonetheless, statistical analysis showed that Z content was significantly different between the LL and the HL treatment at 48 h sampling period. Within the HL treatment, Z content increased significantly after 24 h, from 7.58 (μmol/mL)/A_{750} to 10.60 (μmol/mL)/A_{750}. The Car content showed a significant depletion after 4 h of exposure to HL, with no significant variation afterwards; thus after 48 h of exposure to HL, the Car content had decreased by about 3.88 (μmol/mL)/A_{750}. In contrast, the Car content under LL conditions showed a significant rise after 24 h with regard to the initial content, and it was also significantly different with regard to the Car content under HL conditions. The depletion of Car content after 48 h under HL, which is 3.88 (μmol/mL)/A_{750}, was closely equivalent (within error) to the sum of Z and Myx content increase, which was 3.68 (μmol/mL)/A_{750}.

3.2 *HPLC-MS analysis of lipids*

The different glycerolipid species found in *Synechococcus* sp. Syn02 were identified by MS/MS data and published literature (Sakamoto *et al.* 1997; Kim *et al.* 1999; Montero 2011). Because analysis conditions were the same in both HL and LL, and cell density was the only parameter that changed from HL to LL, for comparative purposes quantification can be approached within each lipid class by using the peak area normalized to the absorbance measured at 750 nm (A_{750}), this latter being considered as a relative measurement of the cell density for cyanobacterial cells (Sorokin 1973; Montero *et al.* 2002). The SQDG and MGDG species content dropped in HL-exposed cells as compared to LL-exposed cells (table 1). As a general feature, the content of species containing a C18:3 fatty acyl residue rose or remained at the same level. Remarkably, MGDG(18:2/16:1), with *m/z* 770 in positive mode, was almost completely depleted while its isobaric species MGDG(18:3/16:0) rose to approximately the same level as MGDG(18:2/16:1) had under LL conditions (figure 3).

Taking these changes into consideration, it was found of interest to determine the time course of the variation observed in those species that experienced an apparently relevant change, which included MGDG(18:2/16:1), MGDG (18:3/16:0), PG(18:3/16:0), PG(18:2/16:0), MGDG(18:3/16:1) and DGDG(18:3/16:1). The results of this experiment are illustrated in figure 4b. Depletion of MGDG(18:2/16:1) with concurrent increase in MGDG(18:3/16:0) seems to have occurred after about 8 h of exposure to HL (figure 4b), when significant differences were statistically shown; however, the sum of these two species content after 48 h of HL exposure (c.a. 3.91×10^9) was lower than the sum of their initial content (c.a. 4.80×10^9). No significant differences were found in MGDG(18:3/16:1) content over the 48 h period, apart from the value at 24 h. PG(18:3/16:0)

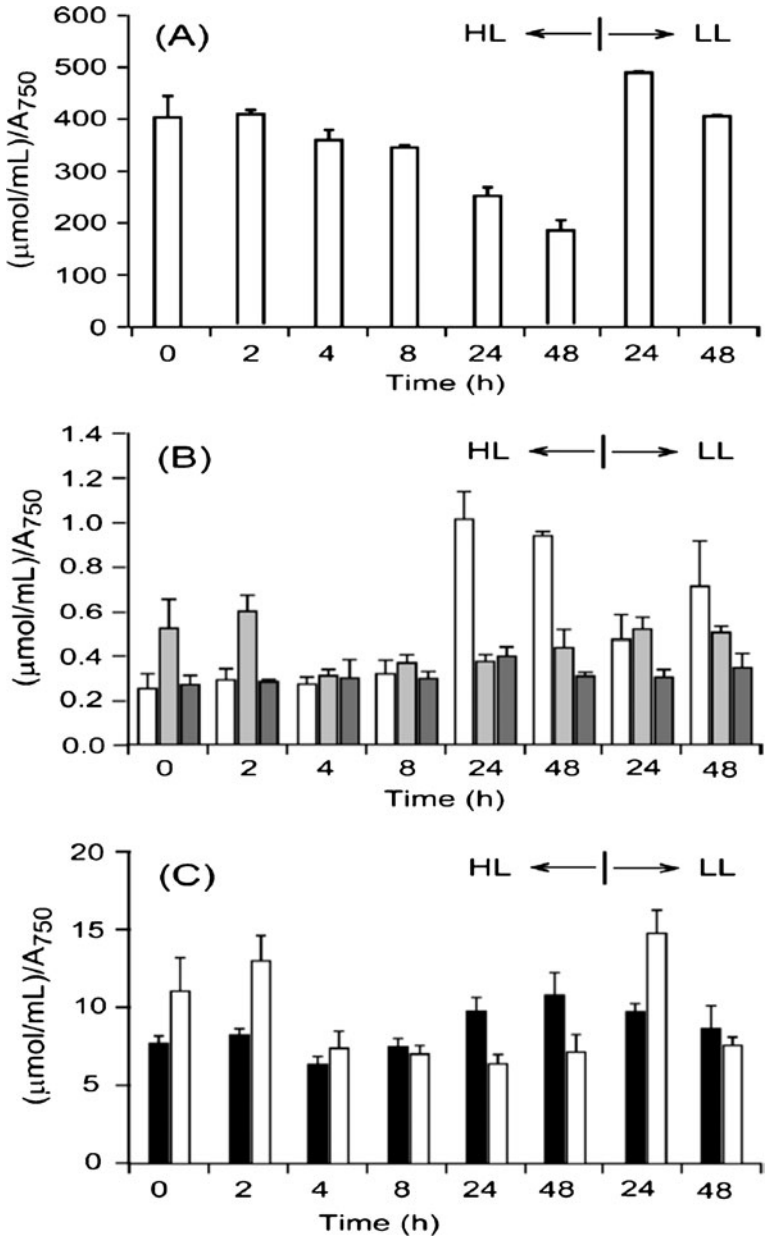

Figure 2. Time course evolution of different pigment content in cultures exposed to high light (HL) and cultures kept in low light (LL) as controls. The content is expressed as ((μmol of compound)/(mL of culture))/A_{750}, where A_{750} is the *in vivo* absorbance at 750 nm. Panels: (**A**) chlorophyll *a*; (**B**) myxoxanthophyll (open bars), hydroxy-echinenone (clear grey bars), and unknown carotenoid (dark grey bars); and (**C**) zeaxanthin (dark bars), and β-carotene (open bars).

content rose to about 3.83-fold the initial content only after 48 h of HL exposure, while PG(18:2/16:0) exhibited a significant content depletion after 24 h of HL exposure. DGDG (18:3/16:1) content increased to 1.76-fold the initial content after 48 h of HL exposure. Reversal of the MGDG(18:2/16:1)/MGDG(18:3/16:0) ratio to that observed under LL conditions was slow and took more than 72 h after the cultures were transferred back to LL conditions (data not shown).

4. Discussion

4.1 *Pigments*

Even though an apparent rise in Myx under HL conditions seems to have occurred after 8 h, statistically significant differences were only shown for the values at 24 h and 48 h, while the Car content started to decrease after 4 h; this

Table 1. Comparative content (mean ± standard error, n=3) of each lipid class under high light (HL) and low light (LL), expressed as the sum of the chromatographic peak area (r.u.) of all species and normalized to the *in vivo* absorbance at 750 nm (A_{750}), from positive (ESI+) and negative (ESI-) electrospray ionization mass spectrometry

Light	Ionization	SQDG	PG	MGDG	DGDG
LL	ESI+	9.67±0.38 10^9*	2.09±0.70 10^9	15.00±0.76 10^9*	3.76±0.90 10^9
	ESI−	9.54±3.96 10^9	2.45±0.87 10^9	3.82±0.67 10^9**	0.12±0.07 10^9
HL	ESI+	5.1±0.45 10^9*	2.01±0.11 10^9	8.37±0.61 10^9*	2.62±0.03 10^9
	ESI−	4.73±1.22 10^9	3.10±0.48 10^9	1.54±0.36 10^9**	0.08±<0.01 10^9

All other conditions were the same. SQDG: sulfoquinovosyl-diacyl-glycerol, PG: phosphatidyl-diacyl-glycerol, MGDG and DGDG: mono- and di-galactosyl-diacyl-glycerol, respectively. The symbols * and ** denote which values calculated from positive or negative ionization are significantly different ($P<0.005$) within each lipid class.

finding could be indicative of the fact that demand of Lyc (and/or γ-carotene) for Myx biosynthesis is initiated without an apparent increase in Lyc synthesis occurs. This hypothesis is also underpinned by the delay in Z content rise, which did not take place until 24 h of HL exposure as well. Because substantial accumulation of any Myx biosynthetic pathway intermediates could not be detected, apart from that shown for the second minor carotenoid (unkn1), even at very short times after HL exposure (data not shown), together with the long delay required for Myx to show an appreciable increase, it can be suggested that, at least *in vivo*, the enzymes of the Myx biosynthetic pathway act sequentially, with no limiting steps in any of the intermediates and tightly coupled reactions (Graham and Bryant 2009). Indeed, the intermediates 1-hydroxylycopene, 3-deoxy-myxol (plectaniaxanthin) and myxol have been solely detected in previous studies using mutant strains with any enzyme silenced (Maresca *et al.* 2007; Graham and Bryant 2009). It may be also considered that demand for Car, which has been shown to be the only carotenoid in PRCs (Loll *et al.* 2005), is likely reduced because of high light acclimation involves a decrease in PSI units (Murakami and Fujita 1991; Fuhrmann *et al.* 2009), and hence, a fraction of the Lyc pool would be deviated towards Myx biosynthesis without the need for it being increased. Accordingly, the following event sequence is suggested to occur: (i) within a time interval of 2–4 h the synthesis of Car is reduced, whose content is likely reduced because of the synthesis of Z, with Lyc being primarily directed towards the Myx biosynthesis pathway; meanwhile, Chl is degraded and its biosynthesis substantially reduced; (ii) after about 8 h of HL exposure the activity of the β-carotene hydroxylase for Z synthesis is moderately enhanced again, which leads to the rise in Z content to start, whereas Car synthesis remains in a steady state; concurrently, the activity of the enzymes of the Myx pathway is also enhanced; and (iii) after 24 h of HL exposure a steady state activity of enzymes involved in Car, Z and Myx biosynthesis is attained, and the relative proportion of these carotenoids is maintained.

4.2 *Membrane lipids*

The lipid configuration in which C18:3 and C16:0 fatty acyls esterify the glycerol backbone seems to be the predominant one under HL conditions in this *Synechococcus* strain. Indeed, the SQDG, PG and MGDG species with the above configuration exhibited a substantial increase in their contents, whereas other species were almost depleted. Only MGDG(18:3:/16:1) and DGDG(18:3/16:1) contents did not show a significant modification during light acclimation. Therefore, it may be suggested that C18:3 acyl chains bound to the *sn-1* position of the glycerol backbone could have significance in regard to glycerolipid functionality. In a similar sense, the physiological relevance of C16 acyl chains at the *sn-2* position of the glycerol backbone was shown by Okazaki *et al.* (2006). According to the present knowledge, this change in fatty acyl composition of the glycerolipid species towards species with more unsaturated acyl chains is likely to have been motivated by the need of higher membrane fluidity, which may be triggered by the HL-induced enhancement of photosynthetic activity, i.e. O_2 release, plastoquinone diffusion and PSII repair cycle.

Functional roles of lipids in photosynthetic membranes, in addition to structural roles, are now being outlined by recent experimentation, and in particular for PG (Frentzen 2004; Lee 2004; Jones 2007; Hölzl and Dörmann 2007; Wada and Murata 2007; Sato and Wada 2009). The results obtained in the present study suggest that within PG species, only PG (18:3/16:0) seems to be the most relevant species in membrane configuration for an efficient photosynthesis performance under HL conditions. These changes in PG species could be also related to the concomitant increase in myxoxanthophyll that was observed, as pointed out by Domonkos *et al.* (2009).

MGDG is the most abundant lipid class in cyanobacterial membranes (Hölzl and Dörmann 2007; Sato and Wada 2009). In this study, MGDG species were shown to undergo a significant reduction in their total content under HL conditions in relation to LL conditions (table 1). Since MGDG

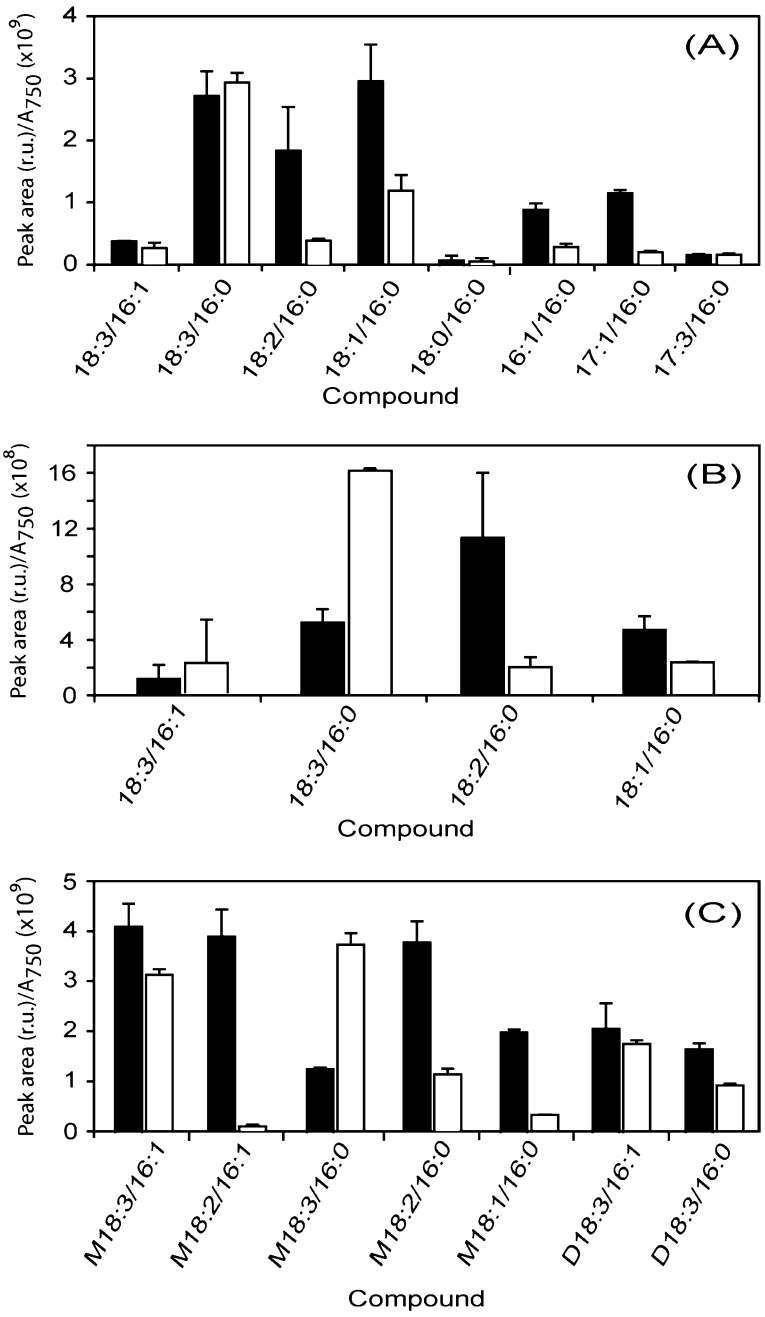

Figure 3. Comparative content from positive ionization of the different membrane lipid species, expressed as the chromatographic peak area and normalized to the *in vivo* absorbance at 750 nm (A_{750}). High light: \open bars, and low light: dark bars. Panels: (**A**) SQDG, (**B**) PG, and (**C**) MGDG and DGDG (M and D in X-axes mean MGDG and DGDG, respectively).

species are known to be located on both leaflets of the thylakoid membrane (Guskov *et al.* 2009; Kern *et al.* 2009), whose lipids account for up to 90% of the cyanobacterial membrane lipids (Murata *et al.* 1981), the reduction in the total MGDG content might suggest a decrease in the membrane area, which could in turn be related to the reduction in PSI that has been documented to take place under high light in cyanobacteria (Murakami and Fujita 1991; Fuhrmann *et al.* 2009). This suggestion would be in accordance with the reduction in β-carotene and Chl*a* contents indicated above. Furthermore, considering that PG and SQDG species are known to be located on the stromal side of the thylakoid membrane (Fuhrmann *et al.* 2009; Guskov *et al.* 2009), where MGDG species are also present, it may

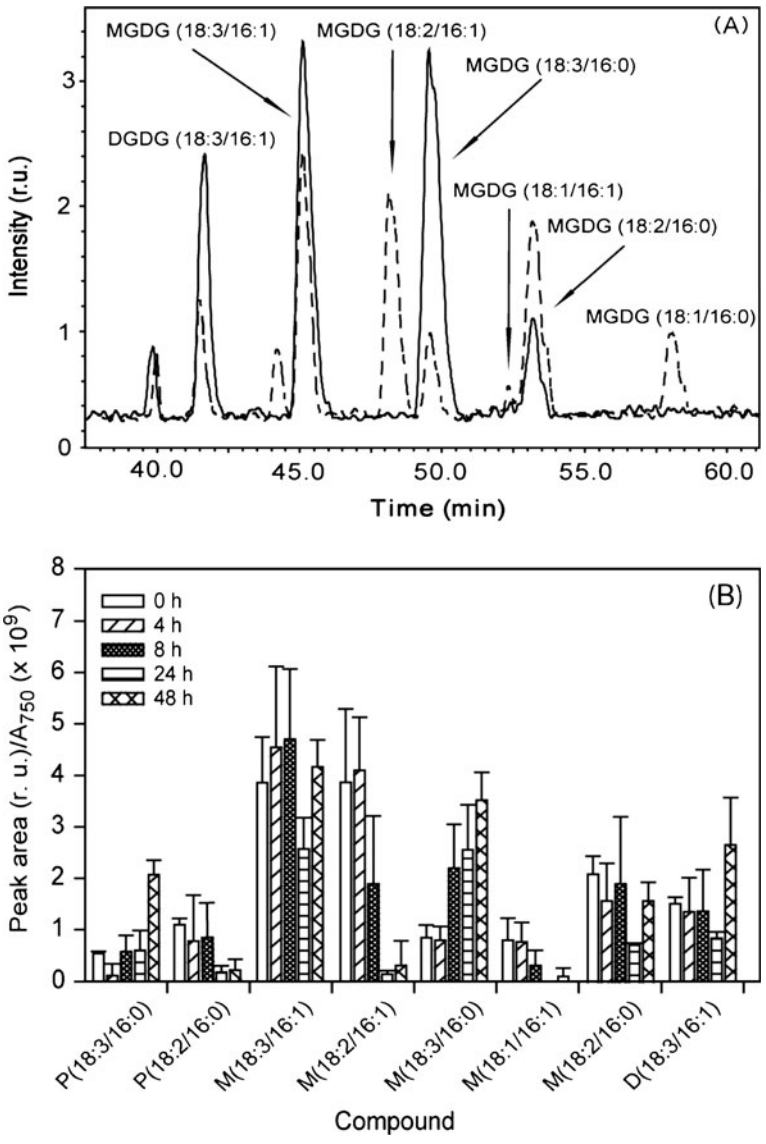

Figure 4. (**A**) Chromatograms showing the different relative proportion of MGDG(18:3/16:0) and MGDG(18:2/16:1) contents in cells exposed to high light (continuous line) in relation to cells exposed to low light (dashed line). (**B**) Time course evolution of different lipid species content, expressed as the chromatographic peak area determined from analysis with positive ionization and normalized to the *in vivo* absorbance at 750 nm (A_{750}). X-axes: P means PG, and M and D mean MGDG and DGDG, respectively.

be suggested that primary changes in membrane conformation and size elicited by HL conditions are to take place in the membrane leaflet facing its stromal side, with the anionic PG augmenting its content significantly. This feature might contribute to facilitate proton translocation from the lumen to the stroma.

Of particular concern is the depletion of MGDG(18:2/16:1) as concomitant rise in MGDG(18:3/16:0) occurred after cells were exposed to HL conditions, although this change in MGDG species may also be envisaged within the general changes discussed above. Two factors are currently described to affect membrane fluidity, which are the geometry of the glycerolipid and the number of double bonds. The content change in these two MGDG species encompasses neither variation in glycerolipid-associated geometry nor double bond number modification, apart from the fact that the three double bonds are located within the same acyl chain in MGDG(18:3/16:0). Therefore, irrespective of these two factors, we find that the only feature that could be relevant in the MGDG configuration change from C18:2/C16:1 to C18:3/C16:0 in regard to membrane properties is that it implies a more linear and hydrophobic residue at the *sn*-2 position and a more bended residue at the *sn*-1 position of the glycerol backbone, with the three double bonds

supporting an electron-rich nodule. Therefore, we are prone to hypothesize on the fact that this MGDG composition change is to be likely related to an acyl chain-induced effect on the glycerolipid shape, this hypothesis being similar to what Barkan *et al.* (2006) explained as an unexpected chilling-resistance phenotype after the replacement of C16:3 in MGDG by the saturated acyl group C16:0 in the *fad5-2* mutant of *Arabidopsis* plants. Hence, it is likely that glycerolipid functionality regarding membrane fluidity and photosynthetic component diffusion is dependent not only on the head group but also on the particular acyl chains (Mullineaux 2008). Indeed, changes in the shape of lipid molecules have been reported to be of importance for different membrane functions (Gounaris and Barber 1983; Simidjiev *et al.* 2000). Contrary to other membrane lipid classes, MGDG is a non-bilayer forming glycerolipid because of its 'cone-like' geometry (Gounaris and Barber 1983). Diverse authors have shown the importance of non-bilayer lipids for full protein functionality (Simidjiev et al. 2000; de Kruijff 1997; Garab *et al.* 2000). This feature has been attributed to the high lateral pressure exerted by the acyl groups of the non-bilayer lipids on the proteins embedded in the membrane, which could contribute to keep the protein subunits in the proper arrangement. It could then be hypothesized on the highly bended C18:3 acyl chain being the responsible for such lateral pressure because of the alternating three double bonds acting like a 'stress tensor', while the linear C16:0 acyl chain acts like a 'nail' and functions in keeping the lipid embedded within the bilayer. Nonetheless, hypotheses drawn above are devoted of experimental testing.

Regarding the time course of variation in both pigment and membrane lipid contents, it is noteworthy that major changes seem to have been accomplished in a time interval of about 24 h, which has also been reported to be the time necessary for phycobilisome degradation (DeRuyter and Fromme 2008). Hence, it may be suggested that the reorganization of the photosynthetic apparatus is a coordinated process, which simultaneously encompasses both protein embedding into the membrane and lipid species modification, with specific lipid species contributing to the targeting signal for protein sorting after translocation (Spence *et al.* 2003).

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