Mechanism of downregulation of photosystem I content under high-light conditions in the cyanobacterium Synechocystis sp. PCC 6803

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Downregulation of photosystem I (PSI) content is an essential process for cyanobacteria to grow under high-light (HL) conditions. In a pmgA (sll1968) mutant of Synechocystis sp. PCC 6803, the levels of PSI content, chlorophyll and transcripts of the psaAB genes encoding reaction-centre subunits of PSI could not be maintained low during HL incubation, although the causal relationship among these phenotypes remains unknown. In this study, we modulated the activity of psaAB transcription or that of chlorophyll synthesis to estimate their contribution to the regulation of PSI content under HL conditions. Analysis of the psaAB-OX strain, in which the psaAB genes were overexpressed under HL conditions, revealed that the amount of psaAB transcript could not affect PSI content by itself. Suppression of chlorophyll synthesis by an inhibitor, laevulinic acid, in the pmgA mutant revealed that chlorophyll availability could be a determinant of PSI content under HL. It was also suggested that chlorophyll content under HL conditions is mainly regulated at the level of 5-aminolaevulinic acid synthesis. We conclude that, upon the shift to HL conditions, activities of psaAB transcription and of 5-aminolaevulinic acid synthesis are strictly downregulated by regulatory mechanism(s) independent of PmgA during the first 6 h, and then a PmgA-mediated regulatory mechanism becomes active after 6 h onward of HL incubation to maintain these activities at a low level.

INTRODUCTION

While light is essential for growth of photosynthetic organisms, excess light energy leads to the production of reactive oxygen species and to eventual inactivation of photosynthesis. To avoid such damage, photosynthetic organisms must acclimatize to high-light (HL) conditions by altering their photosynthetic apparatus. For example, they decrease the amount of antenna pigments (Anderson, 1986; Melis, 1991; Anderson et al., 1995; Walters, 2005), carry out state transition (Fujimori et al., 2005a), increase the capacity of CO2 fixation (Bjorkman, 1981; Anderson, 1986) and activate the scavenging system for reactive oxygen species (Grace & Logan, 1996; Niyogi, 1999). Regulation of the amount of photosystems is another way to acclimate to HL (Anderson, 1986; Neale & Melis, 1986; Murakami & Fujita, 1991; Hihara & Sonoike, 2001). In cyanobacteria, the decrease of photosystem I (PSI) content is more prominent than that of photosystem II (PSII), leading to the decrease of photosystem stoichiometry (PSI/PSII ratio) under HL conditions (Murakami & Fujita, 1991; Hihara & Sonoike, 2001). Several components involved in the regulation of photosystem stoichiometry have been reported (Hihara et al., 1998; Fujimori et al., 2005b; Ozaki et al., 2007). The physiological significance of this regulation has been shown by the characterization of a pmgA (sll1968) mutant of the cyanobacterium Synechocystis sp. PCC 6803. This mutant has a defect in keeping PSI content at a low level under HL conditions, while its PSII content is regulated normally as in wild-type (WT) cells (Hihara et al., 1998). The large amount of PSI complex in the pmgA mutant causes higher electron-transport activity, leading to an enhanced rate of photosynthesis. Although a higher rate of photosynthesis in the pmgA mutant contributes to a growth advantage over the WT during a short-term exposure (~24 h) to HL, a disadvantage appears under prolonged HL conditions. The growth of the pmgA mutant is severely inhibited after 48 h of HL exposure, probably because of the generation of reactive oxygen species at the reducing side of PSI (Sonoike et al., 2001). Apparently, a decrease in the PSI content should be indispensable for growth under HL conditions. However, the mechanism by which the amount of PSI complex is modulated under HL has remained unknown.

Abbreviations: ALA, 5-aminolaevulinic acid; HL, high light; LA, laevulinic acid; LL, low light; PS, photosystem; WT, wild-type.
In the course of characterization of the pmgA mutant, we noticed two mutant phenotypes that may lead to the elucidation of the regulatory mechanism of PSI content. First, we realized that the pmgA mutant has a defect in the transcriptional regulation of the psaAB genes, encoding the reaction-centre subunits of PSI, under HL conditions. In both WT cells and the pmgA mutant, the transcript levels of psaAB genes rapidly decreased upon the shift to HL conditions. After 6 h of HL exposure, psaAB transcripts were maintained at a low level in WT cells, whereas they began to accumulate enormously in the pmgA mutant (Muramatsu & Hihara, 2003). As for the transcription of other PSI genes, the pmgA mutant did not show such an obvious defect. Apparently, the regulation of the psaAB transcription is the key factor for the HL acclimation, even though the transcription of other PSI genes was also downregulated cooperatively. Secondly, we found that the pmgA mutant has a higher amount of chlorophyll on a per cell basis than WT cells after 6 h of HL exposure (Hihara et al., 1998). Although this may be a consequence of the increased PSI content, there is a possibility that enhanced chlorophyll synthesis is the cause of the increased PSI content in the mutant, not the result of it. These observations imply that the regulation of psaAB transcription or of chlorophyll synthesis might have a crucial role in the downregulation of PSI content under HL conditions. In this study, we estimated the contribution of psaAB transcription and chlorophyll synthesis to the regulation of PSI content by modulating these activities during HL acclimation. Also, the role of PmgA in the regulation of these activities is discussed in terms of a two-phase mechanism of the repression of PSI content under HL conditions.

METHODS

Strains and culture conditions. A glucose-tolerant WT strain of Synechocystis sp. PCC 6803 was grown at 31 °C in BG-11 liquid medium (Stanier et al., 1971) with 20 mM HEPES/NaOH, pH 7.0. Cells were grown in test tubes (3 cm in diameter) and bubbled with air. Unless otherwise stated, cultures were grown under continuous illumination at 20 μmol photons m⁻² s⁻¹ provided by fluorescent lamps. The pmgA (sll1968) disrupted mutant, which was made by insertion of the spectinomycin-resistance cassette (Hihara et al., 1998), and the psaB-OX strain (see below) were grown under the same conditions, except that spectinomycin (20 μg ml⁻¹) and chloramphenicol (25 μg ml⁻¹), respectively, were added to the medium. Cell density was estimated by optical density at 730 nm using a spectrophotometer (model UV-160A; Shimadzu). Cultures containing 1.0 × 10⁸ cells ml⁻¹ give an OD₇₃₀ of 1.0 with this spectrophotometer. HL shift experiments were performed by transferring cells in the exponential growth phase (OD₇₃₀ 0.1–0.2) from low-light (LL) (20 μmol photons m⁻² s⁻¹) to HL conditions (250 μmol photons m⁻² s⁻¹).

Determination of chlorophyll content. In vivo absorption spectra of whole cells suspended in BG-11 medium were measured at room temperature using a spectrophotometer (model 557; Hitachi) with an end-on photomultiplier. Chlorophyll content was calculated from the peak height of absorption spectra using the equations of Arnon et al. (1974).

Escherichia coli and DNA manipulation. E. coli strain XL-1-Blue MRF’ (Stratagene) was the host for all plasmids constructed in this study. Procedures for the growth of E. coli strains and for the manipulation of DNA were carried out as described by Sambrook et al. (1989). When required, ampicillin (100 μg ml⁻¹), spectinomycin (20 μg ml⁻¹) or chloramphenicol (25 μg ml⁻¹) was added to Terrific Broth medium for selection of plasmids in E. coli. Plasmids were sequenced by the dideoxy-chain-termination method using the dye terminator cycle sequencing ready reaction kit (ABI PRISM; Applied Biosystems).

Generation of the psaAB overexpressing strain (psaAB-OX). The full intergenic region between the sll1730 and psaA (sll1834) genes (415 bp in length) was amplified by PCR using the primer pair PpsaA-F (5’-AACGTACGGTGTCATAAAATCCGCTTTCTT-3’) and Xhol-PpsaA-R (5’-AAGTTCTCAAGGTTCTCTCTCG-3’). The underlined sequences correspond to the restriction site of BstWI and Xhol, respectively. The amplified PCR products were cloned into the TA cloning site of pT7Blue vector (Novagen) and cut out by digestion with Sphi (a restriction site within the multiple cloning site of pT7Blue vector) and Xhol. A fragment including the chloramphenicol-resistance cassette and the promoter region of the psbA2 gene (sll1311) was cut out from pTCP2031V by digestion with Xhol and Ndel. pTCP2031V vector was a kind gift from Professor M. Ikeuchi (The University of Tokyo, Japan). The coding region, a 501 bp fragment from the start codon of psaA, was amplified by PCR using the primer pair, Ndel-psaAcod-F (5’-AACAACTCAGGTAATGTTTACCA- TGCAATTAGTCACCC-3’) and psaAcod-R (5’-AACAACTCCGG- CCATGGCC-3’). The underlined sequence corresponds to the restriction site of Ndel. The amplified PCR products were cloned into the TA cloning site of pT7Blue vector (Novagen) and the resultant plasmid was digested with Sphi (a restriction site within the multiple cloning site of pT7Blue vector) and Ndel. Then, the Sphi/Xhol fragment containing the psaA upstream region and the Xhol/Ndel fragment containing the chloramphenicol-resistance cassette and the psbA2 promoter were cloned with specific restriction sites into Sphi/Ndel-digested pT7Blue vector containing the psaA coding region to yield an overexpression plasmid for psaAB. The WT strain of Synechocystis sp. PCC 6803 was transformed with this construct, and transformants were selected on plates containing 25 μg chloramphenicol ml⁻¹.

RNA isolation and Northern blot analysis. These were performed as described previously (Muramatsu & Hihara, 2003).
antibody. PedR, a transcriptional regulator whose amount was reported to remain unchanged during HL acclimation (Nakamura & Hihara, 2006), was used for the loading control. PedR gives a single monomer band at around 12 kDa when reduced. The results of immunoblot analysis were digitized by a scanner and the band intensity was quantified by using Scion Image software (Scion Corporation).

**Determination of the activity of 5-aminolaevulinic acid (ALA) synthesis.** Activity of ALA synthesis was determined according to Goslings et al. (2004) with some modifications. Cells were incubated under LL or HL in growth medium with laevulinic acid (LA) for 3 h to accumulate ALA; 3 mM LA could completely inhibit chlorophyll synthesis both in WT and in the pmgA mutant. Exceptionally, 4 mM LA was required for the complete inhibition of chlorophyll synthesis of the pmgA mutant after 9 h of HL incubation. Samples (50 ml) of LL-grown cultures (OD730 0.4) or 100 ml of HL-grown cultures (OD730 0.2) were used for the analysis. After 3 h of LA treatment, cells were harvested by centrifugation. The pellet was resuspended in 150 μl 4 % (w/v) TCA and cells were disrupted by vigorous agitation four times with glass beads (diameter 0.1 mm; BioSpec Products) for 2 min with intervals of 2 min. After cell debris and glass beads were removed by centrifugation, 100 μl supernatant was mixed with 1 ml 50 mM NaH2PO4 (pH 7.5) for neutralization. Then 100 μl ethyl acetocetate was added to an aliquot (500 μl) of reaction mixture and the sample was boiled at 100 °C for 10 min to yield porphobilinogen by condensation of ALA. After cooling on ice for 5 min, 600 μl freshly made Ehrlich’s reagent (consisting of 0.2 g p-dimethylaminobenzaldehyde; 8.4 ml acetic acid; 1.6 ml 70 %, v/v, perchloric acid) was added and incubated for 15 min. A sample mixed with Ehrlich’s reagent without p-dimethylaminobenzaldehyde was used as a control. The 2-methyl-3-acetyl-4-(3-propionic acid) pyrrole derivatives produced were spectrophotometrically quantified by absorbance at 553 nm. The concentration was estimated using an absorption coefficient of

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\text{Absorption coefficient} = 2.2 \times 10^{-4} \text{ mol cm}^{-1} \text{ cm}^{-1}
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RESULTS AND DISCUSSION

**Time-course of the change in PSI content after the transfer to HL conditions**

Previous observations told us that the decrease in the psaAB transcripts and of chlorophyll content in HL-illuminated *Synechocystis* cells is achieved by a two-phase mechanism in terms of the involvement of PmgA (Hihara et al., 1998; Muramatsu & Hihara, 2003). In the initial phase, designated phase 1 (i.e. during the first 6 h of HL exposure), a drastic decrease of the levels of psaAB transcripts and of chlorophyll occurred, owing to the strong repression of their *de novo* synthesis. In contrast, in the second phase, designated phase 2 (i.e. from 6 h onward following HL shift), significant reaccumulation of the psaAB transcripts and of chlorophylls occurred in the pmgA mutant, while WT cells maintained those at low levels.

To determine if the levels of PsaAB proteins are regulated in a two-phase manner, the change of their amount after the shift to HL conditions was followed by immunoblot analysis. The amount of PsaAB decreased similarly in WT and the pmgA mutant until 6 h of HL exposure, while reaccumulation was observed after 6 h for the pmgA-mutant only (Fig. 1a, b). Equal loading of samples was verified by detection of PedR (Fig. 1a), a transcriptional regulator whose amount was not affected by the changes in environmental photon flux densities (Nakamura & Hihara, 2006). These data clearly indicate that the decrease of PSI content under HL is also accomplished in two phases as for the amount of psaAB transcript and chlorophyll. Considering that phenotypic differences between the WT and the pmgA mutant appeared only in phase 2, the decrease of psaAB transcripts, chlorophyll and PSI complex observed in phase 1 should be achieved by some factor(s) other than PmgA, while the regulation of their amount in phase 2 should be under the control of PmgA. Since the amount of PsaAB proteins (Fig. 1) changes in parallel with that of the psaAB transcript (Muramatsu & Hihara, 2003) and of chlorophyll (Hihara et al., 1998) through both phase 1 and phase 2, either the level of psaAB transcription or that of chlorophyll synthesis could be the determinant of PSI content.

**Fig. 1.** Change in the level of PsaAB after the shift to HL conditions. (a) Immunoblot analysis of PsaAB and PedR in WT and the pmgA mutant. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. Total proteins corresponding to 5×10^{6} cells for PsaAB detection and 1×10^{7} cells for PedR detection were loaded in each lane. (b) The intensity of PsaAB bands in the immunoblot analysis was quantified densitometrically by Scion Image software. Values are presented relative to that in LL-grown WT cells.
Does the amount of \textit{psaAB} transcripts determine PSI content under HL conditions?

We overexpressed the endogenous \textit{psaAB} genes by inserting the HL-inducible \textit{psbA2} promoter to just upstream of the \textit{psaA} coding region (\textit{psaAB-OX} strain) and examined if PSI content under HL was affected. Upon the shift to HL, \textit{psaAB}, \textit{psaA} and \textit{psaB} transcripts drastically decreased and were kept at low levels in WT cells, while they were highly accumulated in the \textit{psaAB-OX} strain, as expected (Fig. 2a). However, the amount of PsaAB proteins on a per cell basis in the \textit{psaAB-OX} strain still decreased under such conditions, albeit at a slower rate compared with WT (Fig. 2b, c). Concomitantly, chlorophyll content on a per cell basis decreased more slowly in the \textit{psaAB-OX} strain than in the WT upon the shift to HL (Fig. 2g). Since the total chlorophyll content remains unchanged during the first 9 h after the shift to HL both in the WT and in the \textit{psaAB-OX} strain (Fig. 2f), the apparent decrease of chlorophyll content on a per cell basis should be the consequence of the dilution effect during cell proliferation. Thus, the difference between WT and the \textit{psaAB-OX} strain in the decreased rate of the apparent chlorophyll content (Fig. 2g), as well as of the PsaAB protein content (Fig. 2b, c), could be ascribed to the slower growth of the \textit{psaAB-OX} strain than that of WT (Fig. 2d; also see below). After 9 h of HL exposure, WT cells resume chlorophyll synthesis (Fig. 2f) to maintain a certain level of cellular chlorophyll concentration (Fig. 2g). In contrast, the \textit{psaAB-OX} strain did not show an increase of chlorophyll synthesis even after 9 h (Fig. 2f). It took more than 24 h for the \textit{psaAB-OX} strain to decrease its chlorophyll content to the steady-state levels for HL-acclimated cells (Fig. 2g).

We assume that the lack of the overaccumulation of PsaAB protein in the \textit{psaAB-OX} strain is due to the degradation of the newly synthesized PsaAB polypeptide without proper insertion of chlorophylls, rather than the suppressed translation of \textit{psaAB} messengers. Enhancement of chlorophyll synthesis as well as overexpression of \textit{psaAB} genes might be required for the high accumulation of PSI complexes. The results indicate that the high level of the \textit{psaAB} transcripts alone is not sufficient for the aberrant accumulation of PSI complex under continuous HL conditions.

Unexpectedly, growth of the \textit{psaAB-OX} strain was strongly inhibited under HL conditions (Fig. 2d), while that under LL conditions was normal (Fig. 2e). Although we cannot exclude the possibility that growth inhibition of the \textit{psaAB-OX} strain is due to a polar effect originating from the

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**Fig. 2.** Characterization of the \textit{psaAB-OX} strain. (a) Change in the levels of the \textit{psaA}, \textit{psaB} and \textit{psaAB} transcripts after the shift to HL conditions in WT and the \textit{psaAB-OX} strain. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. The \textit{psaA}, \textit{psaB} and \textit{psaAB} transcripts were analysed by Northern blotting using gene-specific RNA probes for \textit{psaA} (upper panel) or \textit{psaB} (lower panel). An aliquot of total RNA (2 \textmu g) was loaded in each lane. rRNA was visualized with methylene blue staining (lower half of each panel). (b) Immunoblot analysis of PsaAB in WT and the \textit{psaAB-OX} strain. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. Total proteins corresponding to 5\times10^5 cells were loaded in each lane. (c) Quantification of the band intensity of PsaAB in the immunoblot by Scion Image software. Values are presented relative to that in LL-grown WT cells. (d, e) Growth curves of the WT (○) and the \textit{psaAB-OX} strain (●) under HL (d) and LL (e) conditions. For (d), LL-grown cells were shifted to HL conditions at time 0. The data represent the mean ± SD of three independent experiments. (f, g) Changes in chlorophyll content on a per liquid culture (f) and on a per cell (g) basis after the shift to HL conditions in WT (○) and the \textit{psaAB-OX} strain (●). LL-grown cells were shifted to HL conditions at time 0. The data represent the mean ± SD of three independent experiments.
interruption of the 5′-UTR region of the psaAB genes, keeping the psaAB transcripts at a low level may be essential for normal cell growth under HL conditions. Upon the shift to HL conditions, many stress-responsive genes were induced to support cell growth in WT cells (Hihara et al., 2001). Repression of psaAB transcription under HL may be important to recruit the transcriptional machinery for such stress-inducible genes.

**Does the amount of chlorophyll a determine PSI content under HL conditions?**

The possibility that chlorophyll content determines PSI content under HL conditions could be tested through the inhibition of chlorophyll synthesis by the addition of LA, an inhibitor of ALA dehydratase. When 2 mM LA was added 6 h after HL shift, cellular chlorophyll content of both WT and the pmgA mutant after 24 h of HL incubation was suppressed to 70% compared with the respective cultures without LA addition. Chlorophyll content of the LA-treated mutant decreased to the levels of the untreated WT cells (Fig. 3a). Concomitantly, the amount of PsaAB in the LA-treated mutant decreased nearly to the level of the untreated WT after 24 h of HL exposure (Fig. 3b, c), although psaAB transcripts remained at a high level (Fig. 3d). This clearly indicates that the regulation of chlorophyll content plays a critical role in the modulation of PSI content under HL conditions. To date, the influence of chlorophyll availability on photosystem content has been studied under LL or in darkness.

![Fig. 3. Effect of the inhibition of chlorophyll synthesis. LA (2 mM) was added to the culture 6 h after the shift to HL conditions to keep the chlorophyll content of the mutant to the level of WT cells. (a) Chlorophyll content on a per cell basis in WT (white bars), the pmgA mutant (grey bars), WT with LA (hatched bars) and the pmgA mutant with LA (grey bars with hatching) grown under LL or HL conditions for 24 h. The data represent the mean ± SD of four independent experiments. (b) Immunoblot analysis of PsaAB in WT, WT with LA, the pmgA mutant (Δ) and the pmgA mutant with LA, grown under LL or HL conditions for 24 h. Total proteins corresponding to 5×10⁵ cells were loaded in each lane. (c) Quantification of the bands of PsaAB in the immunoblot by Scion Image software. Values are presented relative to that in LL-grown WT cells; bar designations are the same as in (a). (d) Changes in the level of the psaA and psaAB transcripts after the shift to HL conditions in WT, the pmgA mutant and the pmgA mutant with LA. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. The psaA and psaAB transcripts were analysed by Northern blotting using psaA gene-specific RNA probe. An aliquot of total RNA (2 μg) was loaded in each lane. rRNA was visualized by methylene blue staining (lower panel).](http://mic.sgmjournals.org)
example, Xu et al. (2004) reported a decrease in the PSI/PSII ratio following the addition of gabaculin, an inhibitor of chlorophyll synthesis, to WT cells of *Synechocystis* sp. PCC 6803. Kada et al. (2003) reported a decrease in PSI subunits (PsaAB, PsaC) but not in PSII subunits (D1 and CP47) in a *chll* mutant of *Plectonema boryanum* incubated in the dark, where chlorophyll synthesis in the mutant was arrested. All these data suggest that biogenesis of PSI is more closely linked to chlorophyll availability compared to that of PSII under LL conditions or in darkness. The same seems to be true under HL conditions, judging from the observation that the PSI content showed a more drastic decrease than PSII content (Hihara et al., 1998).

We observed that the suppression of chlorophyll content in the *pmgA* mutant by the addition of 2 mM LA could not result in the downregulation of the *psaAB* transcript level (Fig. 3d). Moreover, we observed that the higher rate of *pmgA* decrease than PSII content (Hihara et al., 2004) reported a decrease in PSI/PSII ratio following the addition of gabaculin, an inhibitor of chlorophyll synthesis, to WT cells of *Synechocystis* sp. PCC 6803. Kada et al. (2003) reported a decrease in PSI subunits (PsaAB, PsaC) but not in PSII subunits (D1 and CP47) in a *chll* mutant of *Plectonema boryanum* incubated in the dark, where chlorophyll synthesis in the mutant was arrested. All these data suggest that biogenesis of PSI is more closely linked to chlorophyll availability compared to that of PSII under LL conditions or in darkness. The same seems to be true under HL conditions, judging from the observation that the PSI content showed a more drastic decrease than PSII content (Hihara et al., 1998).

What is the cause of the high chlorophyll content in the *pmgA* mutant under HL conditions?

In plants and cyanobacteria, the tetrapyrrole biosynthesis pathway starts from glutamate, which is converted to ALA. It is widely accepted that chlorophyll synthesis is regulated at three steps, that is, ALA synthesis, the branching point of haem and chlorophyll, and the reduction step of protochlorophyllide to chlorophyllide. Among them, ALA synthesis is the major regulatory step (Vavilin & Vermaas, 2002). We found that the activity of ALA synthesis drastically decreased in WT cells upon the shift from LL to HL conditions during phase 1 and the activity was maintained at a relatively low level after 6 h (phase 2) (Fig. 4). The time-course change of the activity in ALA synthesis upon the shift to HL is similar to that in chlorophyll content, showing that decrease of the chlorophyll content under HL conditions is mainly achieved by the blockage of ALA synthesis. The regulation of tetrapyrole biosynthesis at a step preceding the formation of ALA seems reasonable, since accumulation of any tetrapyrole intermediates having visible light absorption could cause photodamage to cells under HL conditions.

Unlike the WT, ALA synthesis in the *pmgA* mutant started again at phase 2, although ALA synthesis decreased in both WT and the *pmgA* mutant at phase 1. This result strongly suggests that the increase of the chlorophyll content in the *pmgA* mutant at phase 2 was due to the loss of repression of ALA synthesis.

ALA is synthesized from glutamate in three steps catalysed by GltX (GTS, glutamyl-tRNA synthetase), HemA (GTR, glutamyl-tRNA reductase) and HemL (GSA, glutamate-1-semialdehyde aminotransferase). ALA synthesis is suggested to be regulated mainly at the step catalysed by HemA (Reinbothe & Reinbothe, 1996; Vavilin & Vermaas, 2002). When we examined the amount of HemA protein under HL conditions by immunoblot analysis, an increase in HemA levels after the shift to HL conditions was observed both in WT and in the *pmgA* mutant (Fig. 5a, b). The extent of the increase in HemA was almost the same between WT and *pmgA* mutant, which is apparently inconsistent with the observed change in the activity of ALA synthesis after the HL shift (Fig. 4). Availability of the substrate is also not the cause of the difference in ALA synthetic activity between the two strains, since measurements using capillary electrophoresis mass spectrometry (CE/MS) revealed that the contents of glutamate, the substrate of ALA synthesis, in WT and the *pmgA* mutant after 12 h of HL incubation were comparable, i.e. 9.2 and 9.7 μmol (g fresh weight)$^{-1}$, respectively. Thus, we assume that HemA activity is under post-translational regulation under HL conditions.

In higher plants and algae, HemA activity is known to be subject to feedback inhibition by intermediates of the tetrapyrole biosynthesis pathway (Reinbothe & Reinbothe, 1996; Vavilin & Vermaas, 2002). In *Synechocystis* sp. PCC 6803, HemA activity is inhibited by the addition of (proto)haem in *vitro* (Rieble & Beale, 1991). However, the contribution of feedback inhibition can be excluded in this case because we measured the activity of ALA synthesis in the presence of LA, i.e. without accumulation of
intermediates in tetrapyrrole biosynthesis. There have been several studies reporting the isolation of regulatory factors for ALA synthesis. These proteinaceous factors, such as FLU in higher plants (Meskauskiene et al., 2001; Goslings et al., 2004), FLP in green algae (Falciatore et al., 2005) and SCPs in cyanobacteria (Xu et al., 2002), are all assumed to work in response to the availability of chlorophylls or its intermediates. PmgA seems to be an unusual factor in that it regulates the activity of ALA synthesis independently of the levels of tetrapyrrole intermediates. The mechanism of such regulation, direct or indirect, is yet to be elucidated.

Conclusion

During the first 6 h following the shift to HL (phase 1), levels of chlorophyll and psaAB transcripts are strictly downregulated by mechanisms independent of PmgA. Repression of chlorophyll synthesis is mainly achieved at the level of ALA synthesis, which is crucial for the downregulation of PSI content under HL conditions. Selective repression of PSI content is observed since PSI is more sensitive to chlorophyll availability than PSII. Although the mechanism of the repression of psaAB transcription during phase 1 was not assessed in this study, we recently found that an AT-rich light-responsive element located just upstream of the basal promoter region is responsible for the coordinated and rapid downregulation of PSI genes upon the shift to HL conditions during phase 1 (Muramatu & Hihara, 2006, 2007). The stability of psaAB and psaA mRNA was lower under HL than under LL, suggesting that mRNA degradation also contributes to the dramatic loss of psaAB transcript following HL shift (Muramatu & Hihara, 2003). Normal PSI content in the psaAB-OX strain suggests that psaAB transcription is not a rate-limiting step of PSI content under HL conditions, though its downregulation appeared to be indispensable for growth under HL conditions.

After 6 h of HL incubation (phase 2), the PmgA-mediated regulatory mechanism becomes active to maintain the amounts of chlorophyll and psaAB transcripts at a low level. Lack of this regulation causes aberrant accumulation of PSI under HL conditions and finally results in cell death (Hihara et al., 1998; Sonoike et al., 2001). It is likely that ALA synthesis and psaAB transcription are independent targets for the PmgA-mediated regulatory mechanism. Coordinated synthesis of chlorophyll and chlorophyll-binding protein is essential for photosynthetic organisms, since accumulation of free chlorophylls or chlorophyll intermediates causes severe photo-oxidative damage to cellular components. However, to date, the regulatory mechanism common to the synthesis of chlorophyll and chlorophyll-binding protein has not been identified. PmgA should be a key for the elucidation of such regulatory mechanisms.

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REFERENCES


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