The mechanism of down-regulation of photosystem I content under high-light conditions in the cyanobacterium *Synechocystis* sp. PCC 6803.

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Abbreviations: HL, high light; LL, low light; PS, photosystem; LA, levulinic acid; ALA, 5-aminolevulinic acid; WT, wild type.

Summary

Down-regulation 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, all the levels of PSI content, chlorophyll and transcripts of the *psaAB* genes encoding reaction center subunits of PSI could not be maintained low during HL incubation, although 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, levulinic acid, in *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-aminolevulinic acid synthesis. We conclude that, upon the shift to HL conditions, activities of *psaAB* transcription and of 5-aminolevulinic acid synthesis are strictly down-regulated 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 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 damages, photosynthetic organisms must acclimate 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 CO_2 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; Neal & 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 were reported so far (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 defect in keeping PSI content at 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 (ca. 24 h) to HL, 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 PSI reducing side (Sonoike *et al*., 2001). Apparently, 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.

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 defect in the transcriptional regulation of *psaAB* genes, encoding the reaction center subunits of photosystem I, 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 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 were also down-regulated cooperatively. Secondly, we found that the *pmgA* mutant has 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 for the down-regulation 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. Moreover, the role of PmgA in the regulation of these activities is discussed in terms of 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 *psaAB*-OX strain (see below) were grown under the same condition, except that spectinomycin (20 μg ml^{-1}) and chloramphenicol (25 μg ml^{-1}) were added to the medium, respectively. Cell density was estimated by optical density at 730 nm using a spectrophotometer (model UV-160A; Shimadzu). Cultures containing 1.0 x 10^8 cells ml^{-1} give OD_{730} of 1.0 with this spectrophotometer. HL shift experiments were performed by transferring cells at the exponential growth phase (OD_{730}=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**

An *E. coli* strain, XL1-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 in 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 dideoxy-chain termination method using dye terminator cycle sequencing ready reaction kit (ABI PRISM; Applied Biosystems).

Generation of the *psaAB* overexpressing strain (*psaAB*-OX)

The full intergenic region between sll1730 and *psaA* (slr1834) genes (415 bp in length) was amplified by PCR using the primer pair, PpsaA-F (5'-AACGTACGTGTCAAAATCCGCTTCTT-3') and XhoI-PpsaA-R (5'-AACTCGAGCAGGGTTCTCCTCGCT-3'). The underlined sequences correspond to the restriction site of *Bsi*WI and *Xho*I, respectively. The amplified PCR products were cloned into the TA cloning site of pT7Blue vector (Novagen) and cut out by digestion with *Sph*I (a restriction site within the multiple cloning site of pT7Blue vector) and *Xho*I. A fragment including the chloramphenicol resistance cassette and the promoter region of *psbA2* gene (slr1311) was cut out from pTCP2031V by digestion with *Xho*I and *Nde*I. pTCP2031V vector is a kind gift from Prof. M. Ikeuchi (The University of Tokyo, Japan). The coding region, 501 bp fragment from the start codon of *psaA*

gene, was amplified by PCR using the primer pair, NdeI-psaAcod-F (5'-AAGTTCCTCAGGCATATGACAATTAGTCCACCC-3') and psaAcod-R (5'-AACCAAACCGCCAATGGCG-3'). The underlined sequence corresponds to the restriction site of *Nde*I. The amplified PCR products were cloned into the TA cloning site of pT7Blue vector (Novagen) and the resultant plasmid was digested with *Sph*I (a restriction site within the multiple cloning site of pT7Blue vector) and *Nde*I. Then, the *Sph*I/*Xho*I fragment containing the *psaA* upstream region and the *Xho*I/*Nde*I fragment containing the chloramphenicol resistance cassette and the *psbA2* promoter were cloned with specific restriction sites into the *Sph*I/ *Nde*I-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 ml^{-1} chloramphenicol.

RNA isolation and Northern blot analysis

RNA isolation and Northern blot analysis were performed as described previously (Muramatsu & Hihara, 2003).

Immunoblot Analysis

LL or HL exposed *Synechocystis* cells at the exponential growth phase (OD_{730}=0.2) were harvested by centrifugation. The pellet was resuspended in

100 µl of TBS buffer (20 mM Tris-HCl, pH 7.5, 0.15 M NaCl). The cell suspension was mixed with approximately 50 µl volume of glass beads (diameter 0.1 mm; BioSpec Products) and cells were disrupted by three times of vigorous agitation for 2 min, each followed by cooling on ice for 1 min. After removal of unbroken cells and debris by centrifugation at 700 g for 3 min, cell extracts were treated with 5% (w/v) lithium dodecyl sulfate, 60 mM DTT, and 60 mM Tris-HCl (pH 8.0) for 2 h at room temperature. Then, samples corresponding to 5×10^5 cells per a lane for the detection of PsaAB, 1×10^7 cells for that of PedR (Nakamura & Hihara, 2006) and 2×10^7 cells for that of HemA were loaded onto SDS polyacrilamide gel. SDS gel electrophoresis was performed by the procedure of Laemmli (1970). After electroblotting onto PVDF membranes (Immobilon-P; Millipore), samples were probed with polyclonal antibodies. The antiserum against PsaAB from *Thermosynechococcus elongatus* (Kashino *et al*., 1990) was kindly provided by Prof. I. Enami (Science University of Tokyo, Japan). The antiserum against HemA from cucumber was a kind gift from Prof. A. Tanaka (Hokkaido University, Japan). Goat anti-rabbit IgG conjugated to alkaline phosphatase was used for secondary antibodies. PedR, a transcriptional regulator whose amount was reported to remain unchanged during HL acclimation (Nakamura & Hihara, 2006), was used for the loading control. The PedR gives 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 ALA synthesis

Activity of 5-aminolevulinic acid (ALA) synthesis was determined according to Goslings *et al.* (2004) with some modifications. Cells were incubated under LL or HL in growth medium with levulinic acid (LA) for 3 h to accumulate ALA. 3 mM of LA could completely inhibit chlorophyll synthesis both in WT and in the *pmgA* mutant. Exceptionally, 4 mM of LA was required for the complete inhibition of chlorophyll synthesis of the *pmgA* mutant after 9 h of HL incubation. 50 ml of LL-grown cultures (OD_{730}=0.4) or 100 ml of HL-grown cultures (OD_{730}=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 of 4% (w/v) TCA and cells were disrupted by four times of vigorous agitation 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 of supernatant was mixed with 1 ml of 50 mM NaH_2PO_4 (pH 7.5) for neutralization. 100 μl of ethylacetoacetate was added to the aliquot (500 μl) of reaction mixture and the sample was boiled at 100ºC for 10 min to yield porphobilinogen by condensation of ALA. After cooling on ice for 5 min, 600 μl of 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. The sample mixed with Ehrlich's reagent without *p*-dimethylaminobenzaldehyde was used as a control. The produced 2-methyl-3-acetyl-4-(3-propionic acid) pyrrole derivatives were spectroscopically quantified by the absorbance at 553 nm. The concentration was estimated using absorption coefficient of 74.5 mM^{-1} cm^{-1} (Harel & Klein, 1972). The activity of ALA synthesis was shown in terms of μmol of ALA accumulated (g fresh weight of cells)$^{-1}$ (3 h)$^{-1}$.

Results and Discussion

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

Previous observation told us that the decrease in the *psaAB* transcripts and of chlorophyll content in HL-illuminated *Synechocystis* cells is achieved by the 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 first 6 h of HL exposure), drastic decrease of the levels of *psaAB* transcripts and of chlorophyll occurs, 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 re-accumulation of the *psaAB* transcripts and of chlorophylls occurred in the *pmgA* mutant, while WT cells

maintained those at low levels.

To know if the levels of PsaAB proteins are regulated in the two phase manner, the change of their amount after the shift to HL conditions was followed by immunoblot analysis. Amount of PsaAB decreased similarly in WT and the *pmgA* mutant until 6 h of HL exposure while *pmgA*-mutant specific re-accumulation was observed after 6 h (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 in the amount of *psaAB* transcript and chlorophyll. Considering that phenotypic differences between the WT and the *pmgA* mutant appeared only in Phase 2, decrease of *psaAB* transcripts, chlorophyll and PSI complex observed in Phase 1should be achieved by some factors 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 *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 can be the determinant of PSI content.

Does the amount of *psaAB* transcripts determine PSI content under HL

conditions?

We overexpressed the endogenous *psaAB* genes by inserting the HL-inducible *psbA2* promoter to just upstream of the *psaA* coding region (*psaAB*-OX strain) and examined if PSI content under HL would be affected. Upon the shift to HL, *psaAB*, *psaA*, and *psaB* transcripts drastically decreased and were kept at low levels in WT cells, while they were highly accumulated in the *psaAB*-OX strain as expected (Fig. 2a). However, the amount of PsaAB proteins on a per cell basis in the *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 *psaAB*-OX strain than in WT upon the shift to HL condition (Fig. 2g). Since the total chlorophyll content remains unchanged during the first 9 h after the shift to HL both in WT and in the *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 *psaAB*-OX strain in the decrease 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 *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 *psaAB*-OX strain did not show the increase of chlorophyll synthesis

even after 9 h (Fig. 2f). It took more than 24 h for the *psaAB*-OX strain to decrease the chlorophyll content to the steady state levels for HL-acclimated cells (Fig. 2g).

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

Unexpectedly, growth of the *psaAB*-OX strain was strongly inhibited under HL conditions (Fig. 2d), while that under LL condition was normal (Fig. 2e). Although we cannot exclude the possibility that growth inhibition of the *psaAB*-OX strain is due to the polar effect originated from the interruption of 5'-UTR region of the *psaAB* genes, keeping the *psaAB* transcripts at low level may be essential for the 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 levulinic acid (LA), an inhibitor of 5-amino levulinic acid 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 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. For 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 of 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 on the PSI content that showed 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 down-regulation of *psaAB* transcript level (Fig. 3d). Moreover, we observed that the higher rate of *psaAB* transcription in the *psaAB*-OX strain could not bring about over-accumulation of chlorophyll (Fig. 2f). Thus, there seems to be no causal relationship between the synthesis of chlorophyll and *psaAB* transcripts. During Phase 2 of HL acclimation, levels of chlorophyll and of *psaAB* transcripts should be independently repressed by the PmgA-mediated regulatory mechanism.

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 5-aminolevulinic acid (ALA). It is widely accepted that chlorophyll synthesis is regulated at three steps, that is, ALA synthesis, branching point of heme and chlorophyll, and reduction step of

protochlorophylide to chlorophylide. 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 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 block of ALA synthesis. The regulation of tetrapyrrole biosynthesis at a step preceding the formation of ALA seems reasonable, since accumulation of any tetrapyrrole intermediates having visible light absorption could cause photodamage to cells under HL conditions.

Unlike WT, ALA synthesis in the *pmgA* mutant started again at Phase 2, although ALA synthesis decreased both in 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 the repression of ALA synthesis.

ALA is synthesized from glutamate in three steps catalyzed 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 catalyzed 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 the measurement 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 are comparable, i.e. 9.2 and 9.7 $\mu mol \cdot (g\ fresh\ weight)^{-1}$, respectively. Thus, we assume that HemA activity is under posttranslational regulation under HL conditions.

In higher plants and algae, HemA activity is known to be subject to feedback inhibition by intermediates of the tetrapyrrole biosynthesis pathway (Reinbothe & Reinbothe, 1996; Vavilin & Vermaas, 2002). In *Synechocystis* sp. PCC 6803, HemA activity is inhibited by the addition of (proto)heme *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 proteinous 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 the point that it regulates the activity of ALA synthesis independently of the levels of tetrapyrrole intermediates. The mechanism of such regulation, directly or indirectly, 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 down-regulated by mechanisms independent of PmgA. Repression of chlorophyll synthesis is mainly achieved at the level of ALA synthesis, which is crucial for the down-regulation 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 report, we recently revealed that an AT-rich light-responsive element located just upstream of the basal promoter region is responsible for the coordinated and rapid down-regulation of PSI genes upon the shift to HL conditions during Phase 1 (Muramatsu & Hihara, 2006, 2007). The stability of *psaAB* and *psaA* mRNA was lower under HL than under LL, suggesting mRNA degradation also contributes to the dramatic loss of *psaAB* transcript following

HL shift (Muramatus and 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 down-regulation 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 photooxidative 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 a regulatory mechanism.

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Figure legends

Fig. 1. The 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 5×10^5 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 were quantified

densitometrically by Scion Image software. Values are presented relative to that in LL-grown WT cells.

Fig. 2. Characterization of the *psaAB*-OX strain.

(a) The change in the levels of the *psaA*, *psaB* and *psaAB* transcripts after the shift to HL conditions in WT and the *psaAB*-OX strain. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. The *psaA*, *psaB* and *psaAB* transcripts were analyzed by Northern blot using gene specific RNA probes of *psaA* (the upper panel) or *psaB* (the lower panel). An aliquot of total RNA (2 μg) was loaded in each lane. rRNA was visualized with methylene blue staining (lower panel).

(b) Immunoblot analysis of PsaAB in WT and the *psaAB*-OX strain grown under LL or HL conditions for 6 h and 24 h. Total proteins corresponding to 5×10^5 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) Growth curves of the WT (open circle) and the *psaAB*- OX strain (closed circle) under HL and (e) those under LL conditions. LL-grown cells were shifted to HL conditions at time 0. The data represent the mean ± SD of three independent experiments.

(f) The changes in chlorophyll content on a per liquid culture and (g) those on a

per cell basis after the shift to HL conditions in WT (open circle) and the *psaAB*-OX strain (closed circle). LL-grown cells were shifted to HL conditions at time 0. The data represent the mean ± SD of three independent experiments.

Fig. 3. Effect of the inhibition of chlorophyll synthesis.

2 mM of LA 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 (gray bars), WT with LA (hatched bars) and the *pmgA* mutant with LA (gray bars with hatches) 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 5×10^5 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.

(d) The 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 analyzed by Northern blot using *psaA* gene specific RNA probe. An aliquot of total RNA (2 μg) was

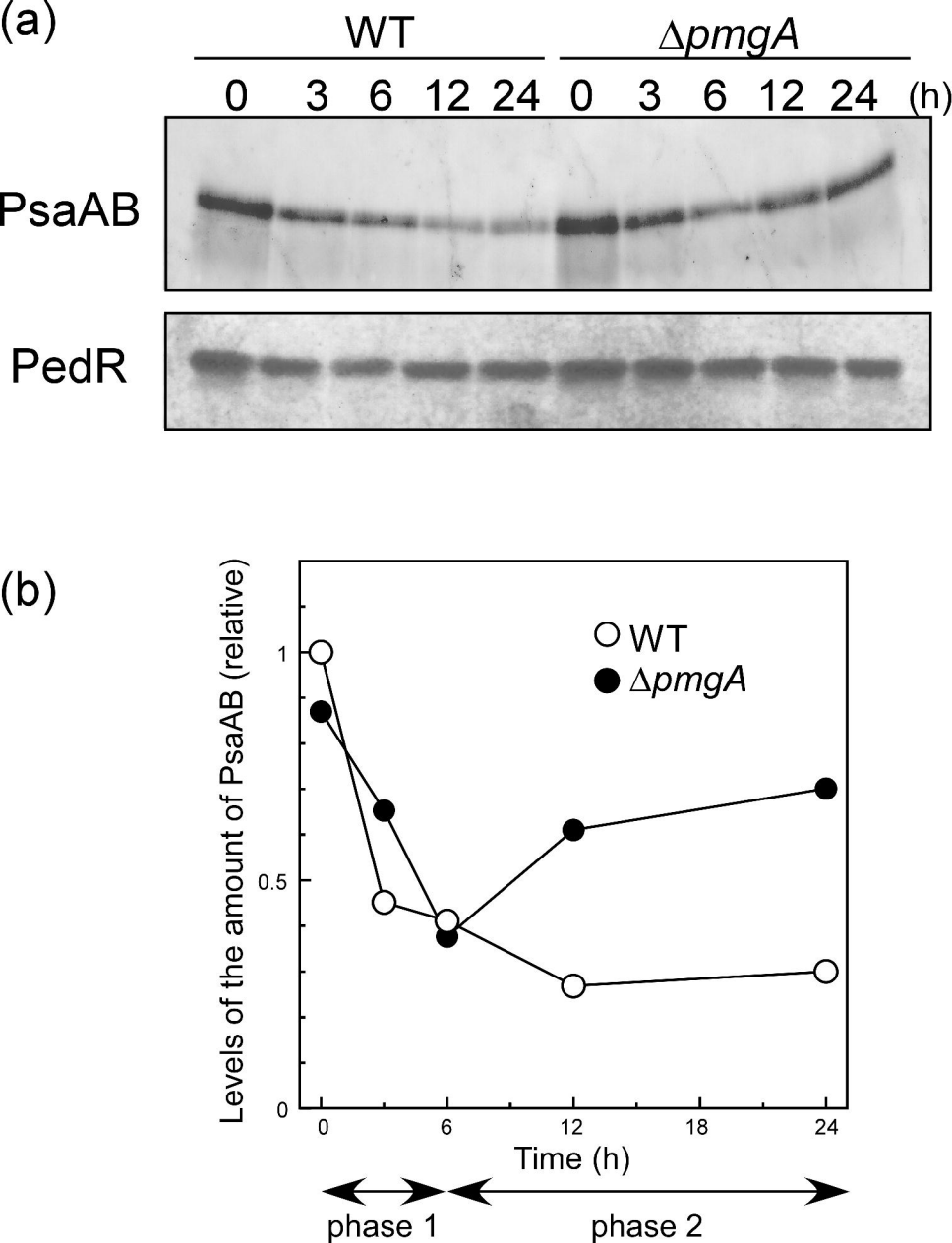
loaded in each lane. rRNA was visualized with methylene blue staining (lower panel).

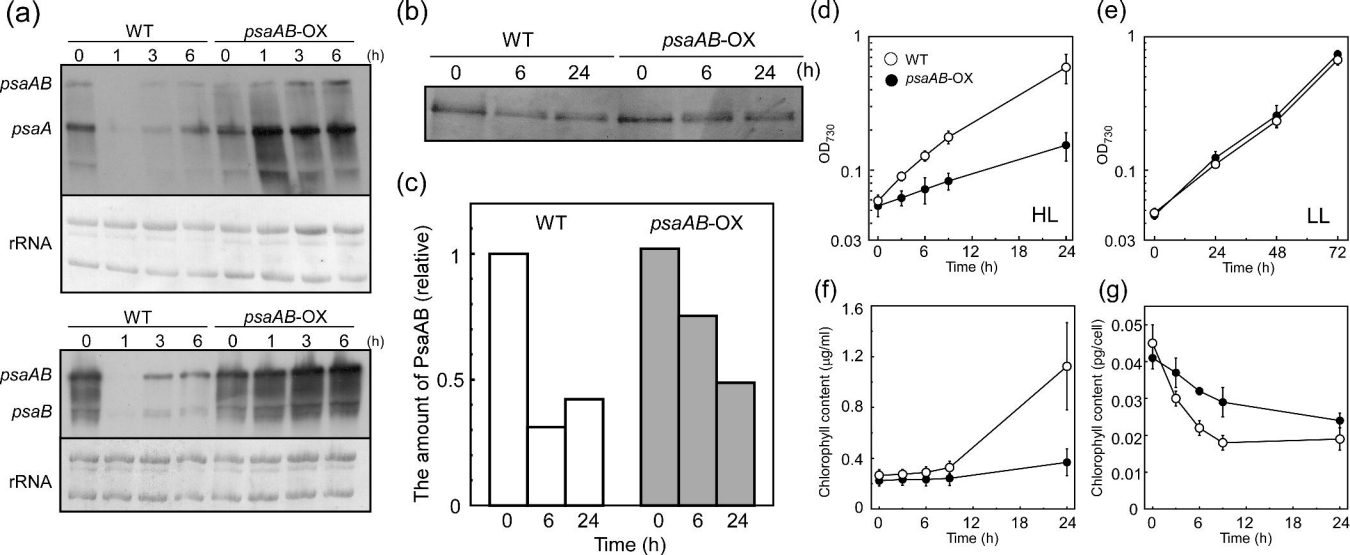
Fig. 4. The changes in the activity of ALA synthesis in WT (open circle) and the *pmgA* mutant cells (closed circle) after the shift to HL conditions. 3 h prior to the harvesting of cells at the indicated time point, LA was added to the culture and the amount of the accumulated ALA during the 3 h period was regareded as the activity of ALA synthesis. The data represent the mean ± SD of three independent experiments.

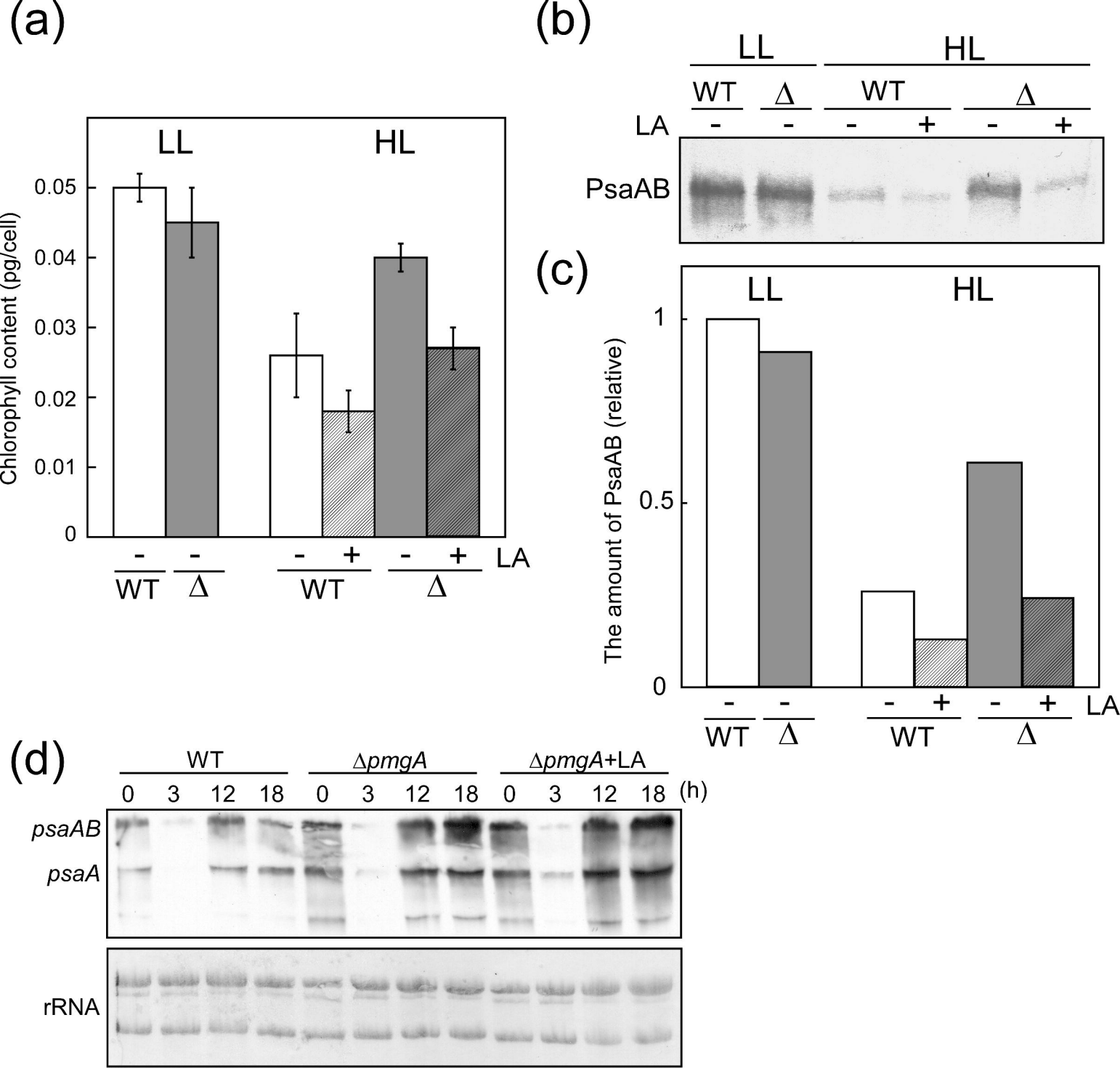
Fig. 5. The changes in HemA levels in WT and the *pmgA* mutant after the shift to HL conditions.

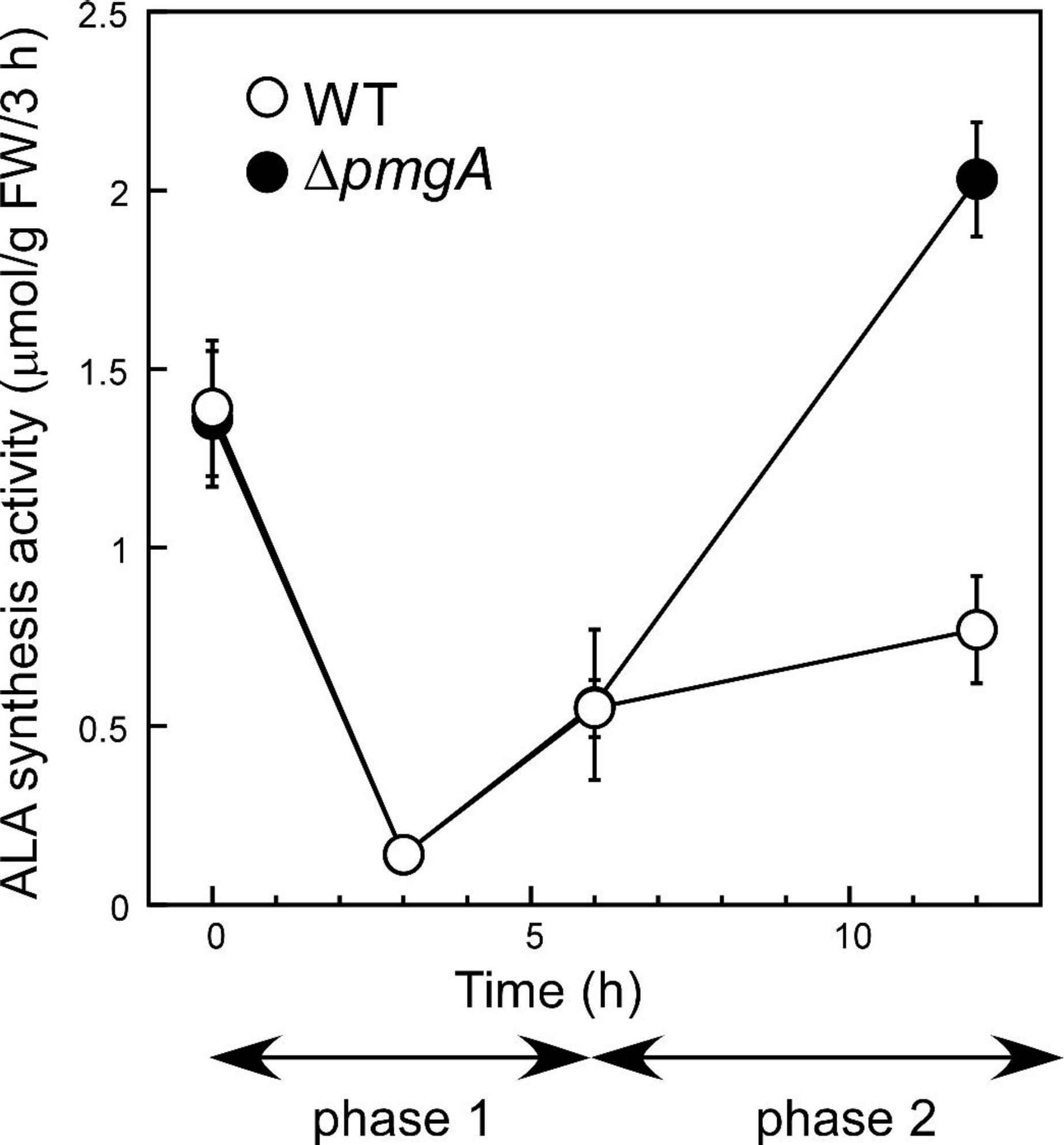
(a) Immunoblot analysis of HemA in WT and the *pmgA* mutant. Cells grown under LL conditions were transferred to HL conditions and sampled at the indicated time. Total protein corresponding to 2 x 10^7 cells for HemA detection and 1 x 10^7 cells for PedR detection were loaded in each lane.

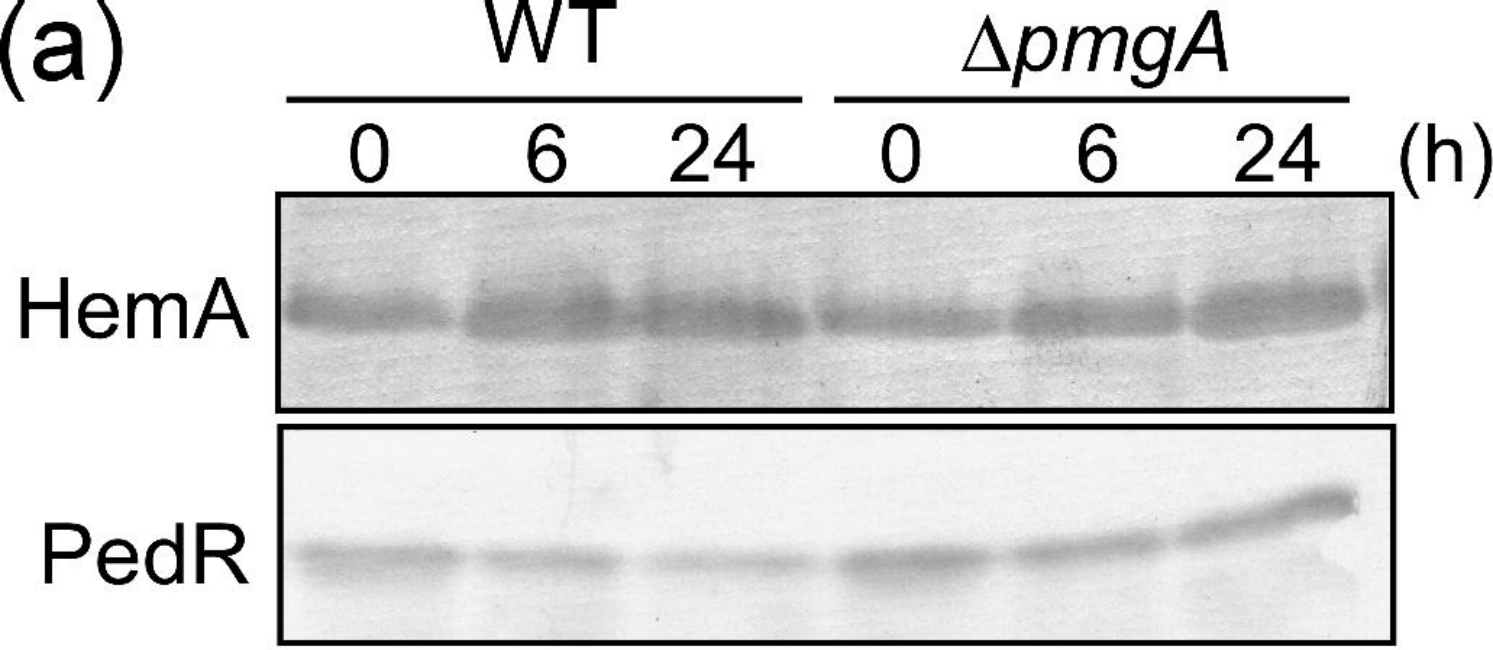
(b) Quantification of the bands of HemA in the immunoblot by Scion Image software. Values are presented relative to that in LL-grown WT cells.

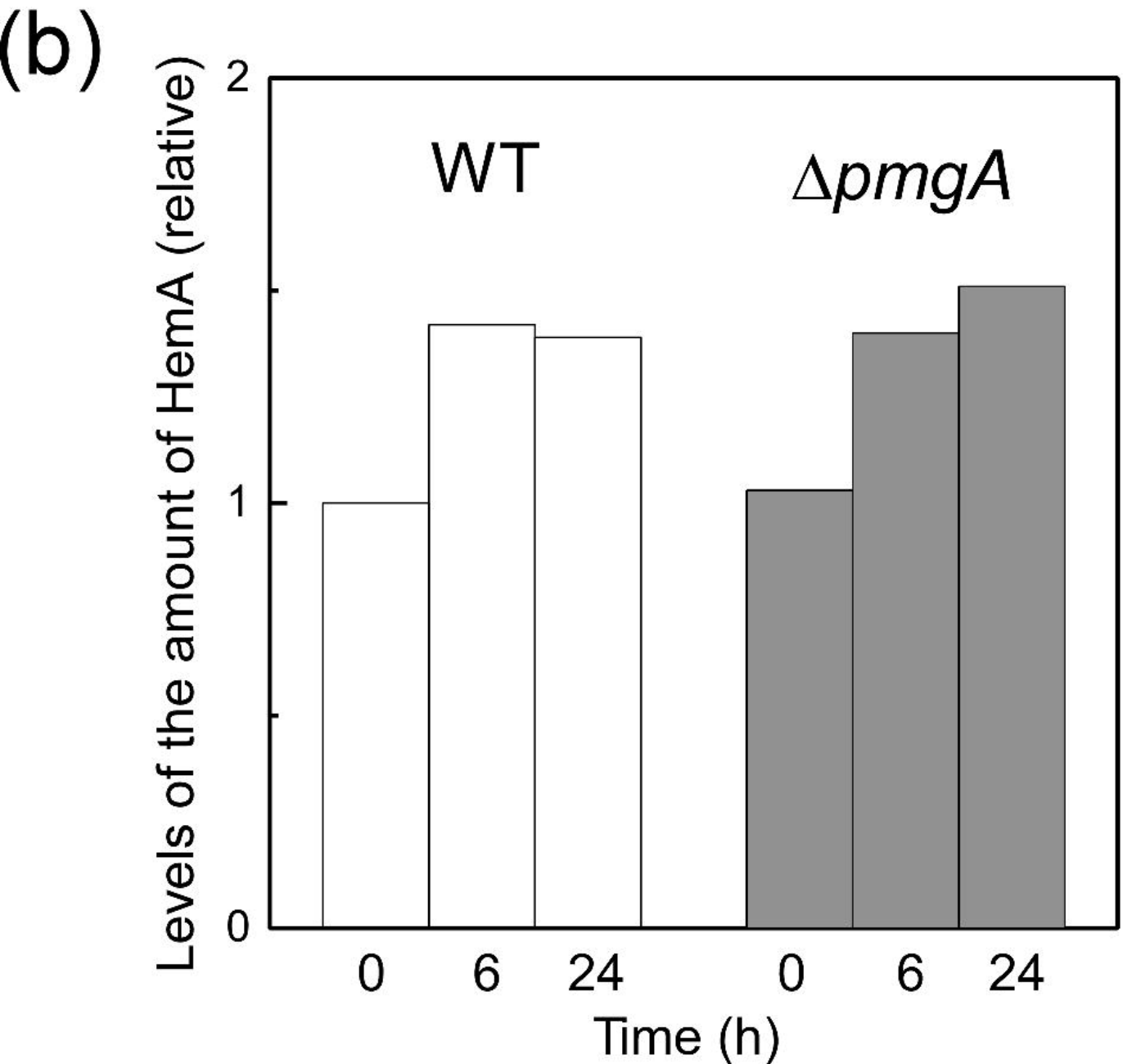

(a)
WT
ΔpmgA
0 3 6 12 24 0 3 6 12 24 (h)
PsaAB
PedR
(b)
Levels of the amount of PsaAB (relative)
1
0.5
0
WT
ΔpmgA
0
6
12
18
24
Time (h)
phase 1
phase 2


(a)
WT
psaAB-OX
0 1 3 6 0 1 3 6 (h)
psaAB
psaA
rRNA
WT
psaAB-OX
0 1 3 6 0 1 3 6 (h)
psaAB
psaB
rRNA
(b)
WT
psaAB-OX
0 6 24 0 6 24 (h)
(c)
WT
psaAB-OX
The amount of PsaAB (relative)
1
0.5
0
0 6 24 0 6 24
Time (h)
(d)
WT
psaAB-OX
OD730
1
0.1
0.03
HL
0 6 12 18 24
Time (h)
(e)
OD730
1
0.1
0.03
LL
0 24 48 72
Time (h)
(f)
Chlorophyll content (μg/ml)
1.6
1.2
0.8
0.4
0
0 6 12 18 24
Time (h)
(g)
Chlorophyll content (pg/cell)
0.05
0.04
0.03
0.02
0.01
0
0 6 12 18 24
Time (h)


(a)
LL
HL
Chlorophyll content (pg/cell)
0.05
0.04
0.03
0.02
0.01
0
- - - + - + LA
WT Δ WT Δ
(b)
LL
HL
WT Δ WT Δ
LA - - - + - +
PsaAB
(c)
LL
HL
The amount of PsaAB (relative)
1
0.5
0
- - - + - + LA
WT Δ WT Δ
(d)
WT
ΔpmgA
ΔpmgA+LA
0 3 12 18 0 3 12 18 0 3 12 18 (h)
psaAB
psaA
rRNA


WT
ΔpmgA
ALA synthesis activity (μmol/g FW/3 h)
2.5
2
1.5
1
0.5
0
0
5
10
Time (h)
phase 1
phase 2


(a)
WT
ΔpmgA
0
6
24
0
6
24
(h)
HemA
PedR


(b)
WT
ΔpmgA
Levels of the amount of HemA (relative)
2
1
0
0
6
24
0
6
24
Time (h)