Involvement of the SppA1 Peptidase in Acclimation to Saturating Light Intensities in *Synechocystis* sp. Strain PCC 6803

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The *sll1703* gene, encoding an *Arabidopsis* homologue of the thylakoid membrane-associated SppA peptidase, was inactivated by interposon mutagenesis in *Synechocystis* sp. strain PCC 6803. Upon acclimation from a light intensity of 50 to 150 μE m⁻² s⁻¹, the mutant preserved most of its phycobilisome content, whereas the wild-type strain developed a bleaching phenotype due to the loss of about 40% of its phycobiliproteins. Using in vivo and in vitro experiments, we demonstrate that the Δ*psspA1* strain does not undergo the cleavage of the L₉³₃ and L₉₉₉ linker proteins that develops in the wild type exposed to increasing light intensities. We conclude that a major contribution to light acclimation under a moderate light regime in cyanobacteria originates from an SppA1-mediated cleavage of phycobilisome linker proteins. Together with changes in gene expression of the major phycobiliproteins, it contributes an additional mechanism aimed at reducing the content in phycobilisome antennae upon acclimation to a higher light intensity.

Light is the principal energy source for all photosynthetic organisms. Pigment-protein complexes, which act as light-harvesting antennae, transfer absorbed light to photochemical reaction centers that convert it into chemical energy utilized by living cells. However, despite its high physiological importance, light energy can also become harmful for cell viability when absorbed in excess, due to the production of radical species that, combined with molecular oxygen, alter macromolecular structures by destroying chemical bonds. Thus, photosynthetic organisms have evolved specific protective and acclimative mechanisms in order to cope with unfavorable environmental conditions. In cyanobacteria the major targets for light access in the cell are the phycobilisome (PBS) antennae. PBS are multicompartmental membrane structures that comprise pigmented phycobiliproteins and nonpigmented linker polypeptides. In *Synechocystis* sp. strain PCC 6803, the major phycobiliproteins, allophycocyanin (APC) and phycocyanin (PC), are retained in a multicompartmental structure by several types of linker proteins (23). The core-membrane linker, L_CM subunit, is responsible for the energy transfer from PBS to photosystem II (PSII) (12, 25); the rod-core linkers, L_RC, attach the peripheral rods to the core of PBS. In addition, rod linkers, L_R, and core linkers, L_C, are involved in the assembly of rods and core domains of PBS, respectively (35). The major mechanisms underlying light acclimation involve the modulation in size and structure of the PBS, although other changes have also been reported either in the stoichiometry or in the composition of photochemical reaction centers (26, 27) with respect to other components of the electron transfer chain or in the concentration of enzymes for CO₂ fixation (1).

The synthesis, assembly, and membrane binding of PBS structures are controlled by a variety of regulatory mechanisms, operating under different environmental conditions. The amount and composition of PBS are modified with light conditions and nutrient availability (2, 6, 16, 17). Acclimation to higher light intensity occurs primarily through changes in gene expression (4, 18, 22) that result in a decreased number of PBS per cell and in a shortening of PBS rods (18, 30). Other well-studied examples of acclimation are the degradation of PBS during nitrogen, phosphor, and sulfur starvations (29, 41). Screening of cyanobacterial mutants that retained their PBS during sulfur starvation led to the identification of several genes that control PBS degradation in cyanobacteria (9, 34). Among these are *nlbS*, encoding a sensor histidine protein kinase, and *nlbR*, which encodes a response regulator. These represent a two-component regulatory system controlling the expression of a series of factors critical for the acclimation of the photosynthetic apparatus in response to both light and nutrient stresses (34, 39), including *nlbA*, which encodes a protein triggering PBS degradation under nutrient deprivation (7, 21). These last two studies and others (6, 9, 21, 41) showed that changes in the rates of PBS degradation also contribute to a cyanobacterial response to changes in light intensity or nutrient availability. Other studies concurred with the conclusion that peptidases also participate in the posttranslational modification of the PBS antenna (40, 41). However, the identification of proteases involved in PBS degradation or remodeling has not been achieved, with the exception of two peptidase activities from *Anabaena* that could be involved in heterocyst formation during nitrogen limitation (11). However, the study of an *Anabaena* gene, encoding a Ca-dependent peptidase, could not demonstrate its actual involvement in PBS degradation (24).

The systematic inactivation of putative genes for peptidase components in *Synechocystis* sp. strain PCC 6803 revealed four enzymes that could be involved in light acclimation and one in response to nutrient deprivation (36). One of these components, the SppA1 peptidase, is an integral membrane endopeptidase that initiates the degradation of signal peptides in bacteria (5, 28). It was recently identified as a thylakoid mem-
brane-associated protein in Arabidopsis that showed light induction at the transcriptional, translational, and possibly post-translational levels (20). As do all other bacterial organisms, cyanobacteria express two SppA homologues, SppA1 and SppA2 (36), one of which is described in the present study. We provide evidence for involvement of the SppA1 peptidase in light acclimation of the PBS antenna. Although transfer from light close to saturation for growth (50 μE m⁻² s⁻¹) to saturating light (150 μE m⁻² s⁻¹) caused similar decreases in the rate of synthesis of PBS proteins in the wild-type and mutant strains, the latter retained much more phycobiliprotein than the former. We show that this difference results from a defect in cleavage of membrane and rod linkers in the PBS structure from the mutant. These observations support the view that light acclimation involves changes in PBS antennae that are not exclusively due to a decreased expression of those genes that encode PBS components. It also results from a truncation of antenna rods by cleavage of distinct linker polypeptides.

MATERIALS AND METHODS

Strains and growth conditions. A wild-type, nonmotile Synechocystis sp. strain, PCC 6803, was obtained from the culture collection of the Department of Genetics at Moscow State University. Wild-type and mutant strains were cultivated in standard BG11 medium (32) under white light (LL) of 50 μE m⁻² s⁻¹ at 30°C under constant agitation. For some experiments, cell cultures were bubbled with 3% CO₂. Cell concentrations were routinely measured by determining the optical densities of the cultures at 750 nm (OD₇₅₀) (6). There was no significant change in cell size between the wild type and mutant grown under LL and medium light (ML) conditions (data not shown). In all instances, an OD₇₅₀ of 1.0 corresponded to 1.6 × 10⁶ cells/ml. For light acclimation experiments, cells were grown up to an OD₇₅₀ of 1.0 and then diluted with BG11 to an OD₇₅₀ of 0.5 and transferred to ML (150 μE m⁻² s⁻¹) for 3 days, except when otherwise indicated. For nitrogen depletion experiments, cells were grown in BG11 medium and then washed and subcultured in nitrate-free medium. Growth media for ΔsppA1 and pVZsppA1-complemented strains were supplemented with 40 μg of kanamycin/ml and 10 μg of chloramphenicol/ml, respectively.

Construction of ΔsppA1 mutant strains. The gene encoding the SppA1 peptidase (sll1703) was amplified from genomic DNA of Synechocystis sp. strain PCC 6803 with primers PrF (5'-GGTTTGCGCTAGGACATG-3') and PrR (5'-GCCATGCAATAATTGC-3'). The resulting PCR product of 890 bp was cloned into pGEM-TEasy vector (Promega, Mannheim, Germany). A kanamycin resistance cassette from plasmid pUC4K (Pharmacia) was inserted into the EcoRI site of sl1703. This plasmid construct was used for transformation of Synechocystis sp. strain PCC 6803 as described previously (15). Complete segregation was verified by PCR analysis, using primers PrF and PrR and Southern analysis.

The ΔsppA1 gene is a member of a gene cluster, sl1702-sll1703-sl1704, where sl1702 and sl1703 contain four overlapping nucleotides in their termination and start codons. sl1704 is located downstream of sl1703. We ruled out any pleiotropic or polar effect of sl1703 disruption on upstream sl1702 and downstream sl1704 gene expression by performing Northern analysis of RNA transcripts for sl1704, sl1703, and sl1702 in the wild-type and mutant strains (experiments not shown). Furthermore, in order to assess the specificity of our gene-targeted inactivation, we generated a pVZsppA1-complemented strain of the ΔsppAI mutant by introducing an autonomously replicating plasmid, pVZ321, carrying the sl1703 gene. The DNA fragment containing the entire coding region of sl1703 was amplified by PCR with primers PrAF (5'-GGTTTGCGCTAGGACATGTTGGCGCTAGG-3') and PrAR (5'-GAAGGCGATGAAATCCCGAACCACA-3') and cloned into the pGEM-TEasy vector. This fragment was excised with PvuII and recloned into the Smal site of a cyanobacterial autonomous replication vector, pVZ321 (43). The resulting plasmid, pVZsppA1, was transferred from Escherichia coli into Synechocystis sp. strain PCC 6803 cells by triparental mating (43). Transconjugants were selected on BG11-containing plates with 40 μg of chloramphenicol/ml and 10 μg of kanamycin/ml.

RESULTS

Phenotypic and spectroscopic characterization of the ΔsppA1 mutant. We have previously reported that the ΔsppA1 mutant of Synechocystis displays a pattern of light sensitivity distinct from that of the wild-type strain (36). Figure 1A illustrates the phenotypical differences of the ΔsppA1 mutant versus the wild type and the pVZsppA1-complemented strain grown under two light regimes, either 50 μE m⁻² s⁻¹, hereafter referred to as LL, or 150 μE m⁻² s⁻¹, referred to as ML. In these particular experiments, cell batches grown under LL conditions to the end of exponential phase were diluted to an OD₇₅₀ of 0.5 and either transferred to ML or kept under LL

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for another 3 days. As previously reported (34), the transfer of wild-type cells to ML caused a bleaching phenotype (Fig. 1A). In marked contrast, the ΔsppA1 mutant showed no evidence for bleaching under ML. When the mutant strain was complemented with the sppA1 gene, yielding the pVZsppA1 strain (see Materials and Methods for details), the wild-type behavior was restored, i.e., bleaching was again observed under ML. Typical absorption spectra of wild-type cells grown under LL and ML are shown in Fig. 1B (bold line). The blue region (400 to 500 nm) displays a broad absorption peak, with several shoulders that correspond to the Soret region of the Chl a absorption spectrum (440 nm) and to the absorption of various carotenoid bands (above 450 nm). The red region shows two distinct peaks, one centered on 620 nm due to phycobilin-containing proteins, PC and APC, the other around 680 nm corresponding to Chl a. Consistent with the bleaching of the wild-type cultures in ML, their absorption spectrum changed from LL to ML with a significant decrease of both the Chl a and PC/APC absorption bands relative to that of the carotenoids (as can be seen in Fig. 1B, middle panel, by the higher contribution to the overall spectrum of the shoulder at 480 nm). However, the decrease in PC/APC was more pronounced than that of Chl a, leading to a marked change in the ratio of the two absorption peaks in the red region of the spectrum. The absorbance spectrum of the ΔsppA1 mutant was similar to that of the wild type under LL but became markedly different from that of the wild type under ML (Fig. 1B, middle panel). Although a decrease in Chl a absorption relative to that of carotenoids was also observed, the PC/APC absorption peak at 620 nm remained higher for the ΔsppA1 mutant. Consequently, the ratio between the Chl a and PC/APC absorption peaks in the mutant cells under ML remained similar to that under LL. These observations suggest that the wild type adapts to ML by losing more of its PC/APC-containing phycobiliproteins than does the ΔsppA1 mutant. The loss of phycobiliproteins in the wild type of Synechocystis is well documented in another instance, when cells are deprived of nitrogen sources. To check whether SppA1 inactivation also resulted in the pres-

FIG. 1. Growth phenotype (A) and absorbance spectra (B) of the wild type (WT) and the ΔsppA1 mutant during acclimation to the ML regime and nitrogen deprivation. *Synechocystis* cultures were grown under LL to an OD at 750 of 1.0. Cells were then transferred to ML or kept at LL for the next 3 days. For nitrogen limitations, cells were grown in BG11 medium and then transferred to nitrogen-free BG11 (-N) medium for 3 days as described in Materials and Methods. The spectra were measured on whole cyanobacterial cells. Bold line, wild type; dashed line, ΔsppA1 mutant.

FIG. 2. Cell growth and pigment analysis of the wild type and the ΔsppA1 mutant strain under the ML regime. (A) *Synechocystis* cells were grown first under LL till exponential phase, diluted with BG11 medium, and transferred to ML for 72 h. The cell growth (A) and Chl (B) and PC (C) concentrations were measured as percentages per OD at 750. Bold line, wild type; dashed line, ΔsppA1 mutant.
The wild type and the ΔsppA1 mutant were grown in nitrogen-depleted medium and the loss of PBS was visualized by the loss of the PC/APC absorbance peak in the 620-nm region of the spectrum (Fig. 1B, right panel). The same decrease in the PC/APC absorbance was observed with the two strains, showing that SppA1 plays no part in the loss of PBS under nitrogen starvation conditions.

Figure 2 shows the generation times of the two strains at three different light intensities, 20 \( \mu \text{E m}^{-2} \text{s}^{-1} \), 108 \( \mu \text{E m}^{-2} \text{s}^{-1} \), and 108 \( \mu \text{E m}^{-2} \text{s}^{-1} \). Doubling times and cell concentrations were means (± standard deviations) from at least three experiments.

### Table 1. Doubling time of the wild type and the ΔsppA1 mutant strain of *Synechocystis* sp. strain PCC 6083 at different light intensities

<table>
<thead>
<tr>
<th>Genotype (growth condition[s])</th>
<th>Doubling time (h)</th>
<th>Cell concen. (no./ml) at T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (20 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>36.3 ± 3.0</td>
<td>8.0 ( \times 10^6 ) ± 0.3 ( \times 10^6 )</td>
</tr>
<tr>
<td>ΔsppA1 (20 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>35.6 ± 1.5</td>
<td>8.4 ( \times 10^6 ) ± 0.4 ( \times 10^6 )</td>
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<tr>
<td>Wild type (50 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>11.4 ± 0.9</td>
<td>26.0 ( \times 10^6 ) ± 0.2 ( \times 10^6 )</td>
</tr>
<tr>
<td>ΔsppA1 (50 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>10.5 ± 1.8</td>
<td>29.5 ( \times 10^6 ) ± 0.3 ( \times 10^6 )</td>
</tr>
<tr>
<td>Wild type (150 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>10.0 ± 1.7</td>
<td>40.1 ( \times 10^6 ) ± 0.1 ( \times 10^6 )</td>
</tr>
<tr>
<td>ΔsppA1 (150 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>9.8 ± 0.8</td>
<td>44.5 ( \times 10^6 ) ± 0.3 ( \times 10^6 )</td>
</tr>
<tr>
<td>Wild type (3% CO2; 150 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>12.0 ± 0.3</td>
<td>21.7 ( \times 10^6 ) ± 0.4 ( \times 10^6 )</td>
</tr>
<tr>
<td>ΔsppA1 (3% CO2; 150 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>11.1 ± 0.8</td>
<td>26.1 ( \times 10^6 ) ± 0.7 ( \times 10^6 )</td>
</tr>
<tr>
<td>Wild type (3% CO2; 150 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>10.3 ± 1.2</td>
<td>38.7 ( \times 10^6 ) ± 0.2 ( \times 10^6 )</td>
</tr>
<tr>
<td>ΔsppA1 (3% CO2; 150 ( \mu \text{E m}^{-2} \text{s}^{-1} ))</td>
<td>9.1 ± 1.1</td>
<td>44.0 ( \times 10^6 ) ± 0.1 ( \times 10^6 )</td>
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</table>

* \( T_2 \), time of cell growth under indicated conditions. Doubling times and cell concentrations are means (± standard deviations) from at least three experiments.

# Figure 3

**A.** LL

**B.** ML

**FIG. 3.** 77K excitation spectra of the wild type and the ΔsppA1 strain with LL and ML regimes. *Synechocystis* cells of the wild type and the ΔsppA1 mutant were grown in LL and adapted to ML for 36 h. The excitation spectra were recorded for PSII emission at 695 nm. Bold line, wild type; dashed line, ΔsppA1 mutant.
No significant differences in the content of ATP synthase and that of the cytochrome b/f complex were observed with various light regimes between the two strains. In contrast, the content in Chl a-containing reaction center proteins of PSI, the D1 protein of PSII, the Rieske FeS protein of cytochrome b/f complex, and major bilin-containing proteins of PBS antennae. The proteins were normalized to the content in the β subunit of ATP synthase. WT, wild type.

FIG. 4. Biochemical analysis of major photosynthetic complexes. Thylakoid membrane proteins isolated from cyanobacterial cells adapted for 3 days to LL and ML were separated by 12% PAGE. The major photosynthetic proteins were visualized by immunodetection with antisera raised against the β subunit of ATP synthase, PsaA/B reaction center proteins of PSI, the D1 protein of PSII, the Rieske FeS protein of cytochrome b/f complex, and major bilin-containing proteins of PBS antennae. The proteins were visualized by immunodetection with antisera raised against the β subunit of ATP synthase. WT, wild type.

FIG. 5. Evolution of whole-cell content in PC in vivo. Wild-type (WT) and ΔsppA1 strains were grown in LL till mid-log phase and then diluted with BG11 to an OD750 of 0.5 (point 0) and transferred to ML or kept in LL for 36 h. Cells were taken after 12, 24, or 36 h in ML and after 36 h in LL. Total proteins were extracted from cells at the same OD750 and separated by 12% SDS–PAGE. Proteins were visualized by Coomassie blue staining. Coomassie-stained cell proteins from the wild type (A) and visualization of PCα and PCβ bands during acclimation to ML (B) are shown. The upper part of the panel with α- and β-ATPase subunits was used as a loading control.
light conditions for 3 days was then analyzed by an immuno-
logical assay (Fig. 7A). The amount of L Run 35 was stable in the
wild type under two light conditions, while those of L Run 99 and
LR run 33 strongly decreased upon acclimation to ML. In marked
contrast, these two linker proteins remained stable in the mu-
tant strain under ML. These observations are consistent with
the loss of membrane-bound APC and PC observed in the wild
type but not in the ΔsppA1 mutant and reflect an SppA1-driven
loss in phycobiliproteins in Synechocystis during light acclima-
tion.

The fact that SppA1 controls acclimation of antennae to
increased light intensity could be due to a light-dependent
expression of the sppA1 gene. This prompted us to probe the
transcript pattern for sppA1 in wild-type cells grown under LL
before being acclimated (or not) for 36 h to ML (Fig. 7B). We
found that the sppA1 transcript was equally expressed under
LL and ML regimes. These data indicated that SppA1, if light
regulated, is controlled at a (post)translational level.

Early studies with isolated PBS have mentioned that they
were subjected to rapid degradation in vitro (33). In an attempt
to link these early observations with the present findings, PBS
from the wild-type, pVZsppA1-complemented, and ΔsppA1
cells adapted to LL conditions were isolated and subsequently
incubated at 4°C in the dark and at 37°C for 3 h under ML
conditions (Fig. 7C). A degradation of the membrane (L Cm run 99)
and rod (L R run 33) linkers was observed exclusively with PBS prep-
arations from the wild-type and pVZsppA1 strains. L Cm run 99 and
L R run 33 linker proteins were stable in the ΔsppA1 mutant. This
experiment demonstrates that at least two types of linker pep-
tides, L Cm run 99 and L R run 33, are degraded in isolated PBS fractions.
In addition, it shows that PBS isolated from the ΔsppA1 mutant
lack the peptidase activity responsible for linker degradation in
the wild type. Taken together, the in vitro and in vivo experi-
mental data demonstrate the proteolytic resistance of two ma-
jor PBS linker polypeptides, L Cm run 99 and L R run 33, in the absence of
the SppA1 peptidase.

**DISCUSSION**

Under our experimental conditions, the wild-type strain of
Synechocystis has a doubling time of about 11 h when grown at
a light intensity of 50 μE m⁻² s⁻¹ under white light. This light
intensity is close to saturation, since a switch to 150 μE m⁻²

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**FIG. 6.** Analysis of protein translation rate in the ΔsppA1 mutant and the wild type under various light regimes by pulse labeling with L-[³⁵S]methionine. Cells were adapted to LL and ML for 12, 24, or 36 h. Cells were labeled with L-[³⁵S]methionine as described in Materials and Methods, after immediate transfer to various light regimes for 30 min (lane 0.5) and after each time point of incubation under ML. The whole-cell proteins were separated by 12% SDS-PAGE, and the gel was fluorographed in a Fuji phosphorimager.

**FIG. 7.** Degradation of linker polypeptides of PBS antennae. (A) Wild-type and ΔsppA1 mutant cells were grown under LL and then transferred to the ML regime for the next 72 h. Thylakoid membrane proteins were separated, transferred onto nitrocellulose membranes, and immunodetected with antisera against the various linker proteins: membrane linker L Cm run 99 and rod linkers L R run 35 and L R run 33. Protein loading was normalized to the β-ATPase subunit as shown in Fig. 4. (B) Gene expression of sppA1 under LL and ML. RNA was extracted from the wild-type and mutant strains grown under LL and then acclimated to ML for 36 h. SppA1 transcripts were identified by hybridization analysis with a gene-specific probe. (C) PBS were isolated from wild-type, pVZsppA1-complemented, and ΔsppA1 cells grown in LL. Isolated PBS were incubated at 4°C in the dark (lanes 1, 3, and 5) and at 37°C under ML (lanes 2, 4, and 6) for 3 h. The reaction was stopped by placing the samples on ice. Proteins were separated by 12% SDS-PAGE. For protein visualization, the gel was silver stained.
s\(^{-1}\) produces only a modest decrease in generation time, by about 10%. This generation time is not limited by CO\(_2\) availability, since bubbling CO\(_2\) in the culture does not change the rate of cell division under LL and ML regimes. However, this transition from close-to-saturating light to saturating light was accompanied by a marked bleaching of the cultures that became visible after 24 h of exposure to the new light regime and slowly developed over the next 48 h. This delay in bleaching was observed for initial cell OD\(_{750}\) of either 0.1 or 0.5 as well as in culture conditions that were kept at constant cell density by a daily dilution up to an OD\(_{750}\) of 1.0. The 40% decrease in phycobilins and Chl after 3 days under 150 \(\mu\)E m\(^{-2}\) s\(^{-1}\) was accounted for by the loss of a significant proportion of the PC and APC phycobiliproteins and of the PSI and PSII Chl-binding proteins. The former change reflects an acclimation of *Synechocystis* to increasing light intensities that has been previously attributed to changes in the expression of genes encoding phycobiliproteins (4, 8).

The \(\Delta\)sppA1 mutant grows at rates similar to those for the wild type—even a little faster—under the various conditions used in this study. In that respect it is similar to other genetically modified cyanobacterial strains that were previously reported to have higher growth rates than wild-type strains (10, 21). When cultures of the \(\Delta\)sppA1 mutant were exposed to a 50/150-\(\mu\)E m\(^{-2}\) s\(^{-1}\) light transition, we observed the same changes in Chl and photosystem contents and the same drop in synthesis of the major phycobiliproteins as in the wild type. However, the mere comparison of the cultures showed that the pronounced bleaching observed with the wild type was no longer seen with the mutant. Although a minor part of this difference can be ascribed to the higher pigment/cell content and higher cell density reached by the mutant cultures, the major contribution came from the better preservation of phycobiliproteins in \(\Delta\)sppA1 grown at 150 \(\mu\)E m\(^{-2}\) s\(^{-1}\). The higher content in peripheral antenna in the mutant was documented both at the protein level, relative to other photosynthetic proteins, and by a higher PBS sensitization of PSI fluorescence emission at 77K. Because the two strains showed a similar down-regulation of PBS expression at 150 \(\mu\)E m\(^{-2}\) s\(^{-1}\), we are bound to conclude that the greater loss of phycobiliproteins in the wild type is due to a degradation process that is hampered in the \(\Delta\)sppA1 mutant.

The participation of SppA1 in the degradation of phycobiliproteins when reaching light intensities immediately above saturation is specific to this acclimation process. The loss in phycobiliproteins and PBS due to other changes in environmental conditions, such as nitrogen deprivation (this study) or deprivation in S, P, Fe, and Cu (36), remained unaltered in the \(\Delta\)sppA1 mutant. PBS degradation responses are thus controlled by different proteases depending on the environmental stimulus. They differ in both amplitude and kinetics, with nitrogen deprivation leading to a rather rapid decrease of up to 90% of PBS (21), while the drop in intracellular phycobiliproteins reported here, upon acclimation to saturating light, is a delayed process and does not exceed 40%. During nitrogen deprivation, cell division and synthesis of phycobiliproteins stop, while preexisting phycobiliproteins are degraded. Therefore, there is a net loss in phycobiliproteins. During light acclimation, the synthesis of new phycobiliproteins is decreased, and the content in preexisting phycobiliproteins also declines due to a limited and specific degradation process. However, since the cell cultures continue to grow, there still is a relative increase in phycobiliproteins per unit volume although the content per cell decreases. We note that the degradation of LHCl antenna upon acclimation of higher plants to high light conditions is also a slow process, with 30% degradation only, observed after 3 days of acclimation to high light intensities (42).

It was previously demonstrated (31, 33) that linker proteins could be degraded in vitro within isolated PBS by some coisolated proteolytic enzyme(s). Here we confirmed this observation: there was a selective loss of \(L_{r}^{33}\) and \(L_{cM}^{99}\) linker proteins in PBS isolated from the wild type when incubated at 37°C for 3 h. However, this linker degradation was no longer observed when using PBS isolated from \(\Delta\)sppA1. This points to an acclimation process with saturating light regimes that is caused by a linker-targeted and SppA1-mediated degradation process. That linker proteins were degraded in vitro under ML, whereas they remained stable in the dark, demonstrated that the protease can be activated by light, most likely by some conformational change. Indeed, at variance with the SppA1 homologue from *Arabidopsis*, which is light induced at the transcriptional level (20), we found that *sppA1* from *Synechocystis* is constitutively transcribed. Therefore, the light-dependent regulation of SppA1 proteolytic activity should rather involve some posttranslational modifications of the protease or some conformational changes of its protein substrates. We note that an extensive degradation of rod linker \(L_r^{33}\), but not of the membrane linker, was also reported during nitrogen starvation (21), a process that does not require SppA1. This means that PBS may undergo similar modifications of their structure during degradation through different regulatory mechanisms.

Proteolysis of PBS encompasses a diversity of phenomena from extensive degradation of all PBS subunits, as observed during nitrogen starvation, to some limited modifications in their supramolecular structure due to the selective action of endopeptidases. Our in vitro experiments suggest that such a fine regulation in antenna organization should start with the cleavage of the distal linker protein \(L_r^{33}\) and the membrane linker \(L_{cM}^{99}\). Decreased energy transfer from PBS to PSI during saturating light intensities can occur through shortening of the PBS rods via a detachment of the external rod segments or of the whole rods from PBS cores and/or through a decreased ratio of PBS to PSII per photosynthetic membrane due to their detachment from the membrane, leading ultimately to their degradation in the cytoplasm. Degradation of \(L_r^{33}\) and \(L_{cM}^{99}\) can account for both a shortening and a release of PBS from the membranes. The former process should be driven by the loss of \(L_r^{33}\)-PC, which represents the distal chains of the rods. We have shown that this loss is controlled by SppA1, an observation which is consistent with previous reports showing that the regulation of \(L_r^{33}\) accumulation is not primarily due to transcriptional changes but rather is due to a control at the translational or posttranslational level (8). The release of PBS from the membranes probably involves the other linker, \(L_{cM}^{99}\), which participates in assembling the PBS structure in an energy transfer-competent position towards PSII (12, 25). \(L_{cM}^{99}\) linker represents a chimeric protein with a heterogeneous domain structure. The C-terminal part of this protein contains
three repeat domains (REP1-3) which show high homology to conserved domains of the rod and rod-core linker polypeptides and provides the binding domains that interact with the APC trimmer. Sequencing of a 23-kDa peptide that was associated with an APC (αββ'ΔPAP) subcomplex showed that it originated from the C-terminal part of this membrane linker (14), which carried only the last REP domain. This crucial experiment demonstrates that there is a peptide able to cleave the C-terminal sequence of the LCM linker, which is tightly interacting with APC. Since there are two copies of LCM per PBS structure, each APC trimeric cylinder could potentially be detached, leading to a complete dissociation of PBS from the thylakoid membrane. Thus, one would expect that degradation of LCM should lead to a decrease in the whole-cell content in assembled PBS, an observation which was indeed reported for some cyanobacteria during light acclimation (30).

PBS linker proteins were protected from degradation in the ΔsppA1 strain under ML. This is in favor of SppA1 being the peptide coisolated with PBS that cleaves the linkers. However, we cannot exclude an indirect role of SppA. For instance, it could control the susceptibility of the PBS structure to another peptide or regulate the expression of this PBS-targeted peptide. The study of other intracellular targets for SppA1 should provide a better view of the regulatory function of thylakoid-bound peptides in cyanobacteria.

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