Crystal Structure of NblA from *Anabaena* sp. PCC 7120, a Small Protein Playing a Key Role in Phycobilisome Degradation

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Cyanobacterial light-harvesting complexes, the phycobilisomes, are proteolytically degraded when the organisms are starved for combined nitrogen, a process referred to as chlorosis or bleaching. Gene *nblA*, present in all phycobilisome-containing organisms, encodes a protein of about 7 kDa that plays a key role in phycobilisome degradation. The mode of action of NblA in this degradation process is poorly understood. Here we presented the 1.8-Å crystal structure of NblA from *Anabaena* sp. PCC 7120. In the crystal, NblA is present as a four-helix bundle formed by dimers, the basic structural units. By using pull-down assays with immobilized NblA and peptide scanning, we showed that NblA specifically binds to the α-subunits of phycocyanin and phycoerythrocyanin, the main building blocks of the phycobilisome rod structure. By site-directed mutagenesis, we identified amino acid residues in NblA that are involved in phycobilisome binding. The results provided evidence that NblA is directly involved in phycobilisome degradation, and the results allowed us to present a model that gives insight into the interaction of this small protein with the phycobilisomes.

Cyanobacteria are photosynthetic prokaryotes, which perform plant-type oxygenic photosynthesis. Light harvesting in these organisms is mainly mediated by phycobilisomes, large water-soluble multiprotein complexes that are attached to the cytoplasmic surface of the photosynthetic membrane. Phycobilisomes are composed of brilliantly colored phycobiliproteins, to which open chain tetrapyrrole chromophores are covalently attached, and of linker proteins, which, with one exception, do not contain chromophores (1). A typical cyanobacterial phycobilisome is assembled by two domains, a central triangular core from which six or more peripheral rods radiate in a hemidiscoidal array. The most abundant phycobiliproteins in a phycobilisome are allophycocyanin, which is present in the core, and phycocyanin, which is present in the rods. Some species also contain phycoerythrin or, like *Anabaena*, phycoerythrocyanin in their rods. All phycobiliproteins have a common subunit organization that consists of α- and β-subunits forming a heterodimer (see Fig. 6). These so-called αβ monomers form disk-shaped trimers, (αβ)3. By face-to-face aggregation of two trimers, hexamers (αβ)6, are formed that are assembled into the rod and core structures through interaction with linker proteins (1, 2). In cell extracts, the phycobilisomes dissociate into subcomplexes like hexamers and trimers unless special buffers of high ionic strength, like 0.8 M phosphate plus 0.3 M citrate, stabilize them.

Phycobiliproteins can constitute up to 50% of the total soluble protein of a cyanobacterial cell. Together with chlorophyll, they are causal for the typical blue-green color of cyanobacteria. Upon changes in environmental conditions like nitrogen starvation, cyanobacteria rapidly degrade their phycobilisomes, which helps to prevent photodamage under stress and provides a large pool of nitrogen-containing substances for adaptation. Phycobilisome degradation is an ordered proteolytic process visible by a color change of the cyanobacterial cell from blue-green to yellow-green, referred to as bleaching or chlorosis (3). A key role in phycobilisome degradation is played by a small protein of about 7 kDa, NblA (Nbl stands for non-bleaching). Homologs of the *nblA* gene are found in all phycobilisome-containing organisms, including red algae. Mutations of *nblA* lead to a nonbleaching phenotype under nitrogen-limiting conditions in non-diazotrophic strains like *Synechococcus 7942* (4) or *Synechocystis 6803* (5–7). In diazotrophic, filamentous cyanobacteria such as *Anaabaena*, this nonbleaching phenotype is most obvious in heterocysts (8), differentiated cells specialized for fixing $N_2$. Heterocysts are usually devoid of phycobiliproteins.

One of the first responses to nitrogen deprivation in all examined cyanobacterial species is the expression of the *nblA* gene. However, it is poorly understood how the NblA protein leads to phycobilisome degradation. NblA shows no sequence similarity to proteins with known function and has no detectable proteolytic activity. Among several hypotheses, Collier and Grossman (4) suggested that NblA could tag or disrupt the phycobilisome, rendering it susceptible to degradation. In support of this suggestion, Luque et al. (9) found that NblA binds to phycobiliproteins from the filamentous cyanobacterium *Tolyphothrix PCC 7601*, preferentially to the α-subunits of phycocyanin and phycoerythrin.

Here we present the crystal structure of NblA from *Anabaena* sp. 3The abbreviations used are: *Synechococcus 7942, Synechococcus elongatus* PCC 7942; *Anabaena 7120, Anabaena* sp. PCC 7120; GST, glutathione S-transferase; Mse, sel-enomethionine; PBS, phosphate-buffered saline; PC, phycocyanin; PCC, Pasteur Culture Collection; *Synechocystis 6803, Synechocystis* sp. PCC 6803; TBS, Tris-buffered saline; r.m.s.d., root mean square deviation.
PCC 7120 together with biochemical data that map the sites of interaction of NblA with phycobiliproteins.

**EXPERIMENTAL PROCEDURES**

**NblA Overexpression and Purification**—The chromosomal gene asr4517 coding for NblA (kazusa.or.jp/cyano/Anabaena/index.html) from *Anabaena* 7120 was cloned into plasmid pET11a (Novagen) as described earlier (10). A selenomethionine (Mse) NblA derivative was produced in *Escherichia coli* B834 (DE3), which is auxotrophic for methionine, according to a protocol from Budisa et al. (11). NblA was purified via ammonium sulfate precipitation, followed by size exclusion chromatography using the hanging-drop vapor-diffusion method over a reservoir solution containing 100 mM Tris/HCl (pH 8.5), 100 mM MgCl₂, 10% (w/v) poly(ethylene glycol)2000, and 15% ethylene glycol at 20 °C. NblA crystals grew as long but thin rods (1.0 × 0.182 × 0.217 Å, and 95.9° × 96.9° × 96.9°). A second data set with higher resolution (1.8 Å) was collected at the European Synchrotron Radiation Facility (Grenoble, France) beam line ID14-4 from flash-frozen crystals. The positions of 24 of 36 possible selenium sites were found using the program SOLVE (14). The program RESOLVE (15, 16) was used to improve the initial phases and to build a starting model of NblA. About 60% of the residues (12–50 of each monomer) were included automatically by the program. To complete the structure determination, multiple cycles of manual model building in O (17) and TLS groups two and three were formed by residues 26–50 of each monomer. The residues at the termini were not included in TLS refinement because of their high flexibility and resulting high individual atomic displacement factors. All molecular drawings were produced with Molmol (21) and Pov-Ray. The atomic coordinates and the structure factors of NblA have been deposited in the Protein Data Bank with the ID code 1OJH.

**Expression, Purification, and Site-directed Mutagenesis of GST-tagged NblA**—The chromosomal *nblA* gene (asr4517) was PCR-amplified from total *Anabaena* 7120 DNA using the following primers: 5′-GGT TTT TAG GGA TCC GTT ATG AAC C-3′, incorporating a BamHI site, and 5′-G CGA CAC ATG GGA ATT GTA TGC CCG-3′, incorporating an EcoRI site. The PCR fragment was ligated into plasmid pGEX-2TK (Amersham Biosciences) to yield plasmid pBB10. By using plasmid pBB10 as template, primers were designed to produce site-specific mutations, and mutagenesis was performed using the QuikChange® site-directed mutagenesis kit (Stratagene, La Jolla, CA). All of the DNA constructs were confirmed by DNA sequencing. The GST-NblA fusion protein and its variants were expressed and purified according to the manufacturer’s directions for GST expression and purification systems (Amersham Biosciences).

**In Vitro Binding Assay Using GST Fusion Protein**—GST-NblA or its variants were bound to glutathione-Sepharose 4B columns in PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄) and incubated with soluble crude extracts from *Anabaena*. Crude extracts were prepared as described earlier (8). After extensive washing with PBS, proteins bound to the resins were eluted with 20 mM glutathione in 50 mM Tris/HCl (pH 8.0) and analyzed by SDS-PAGE and Western blotting.

SDS-PAGE was performed in slab gels containing 15% (w/v) acrylamide:bisacrylamide (29:1) in the buffer system of Laemmli (23). For Western blots, proteins were transferred electrophoretically to nitrocellulose membranes. Phycocyanin subunits and GST were detected by specific antisera. Antigen-antibody complexes were visualized using alkaline phosphatase-conjugated secondary antibodies and developed with 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium as substrate.

**Peptide Blot Assay**—Four peptide arrays were synthesized on a modified cellulose membrane using the SPOT synthesis technique (24). On each array, a set of overlapping 15-mer peptides was synthesized. The peptides represented the complete amino acid sequences of the entire α-chains and β-chains of phycocyanin and phycoerythrocyanin. A three-amino acid shift between neighboring peptides was used.

The in vitro binding of NblA to the synthetic peptide arrays was analyzed as follows. The membrane was washed with ethanol and Tris-buffered saline (TBS) and was then blocked with blocking buffer as described (25). GST-fused NblA in blocking buffer was added to a final concentration of 10 μg/ml followed by overnight incubation at 4 °C. After three washings with TBS, an anti-GST monoclonal antibody (G1160, Sigma) was added to a concentration of 1 μg ml⁻¹ in blocking buffer. After 2 h at room temperature, the membrane was washed with TBS, and a POD-labeled anti-mouse monoclonal antibody (1 μg ml⁻¹ in blocking buffer) was applied for 1.5 h at room temperature followed by washing with TBS. Binding of NblA was quantified using chemiluminescence substrate and the Lumi-Imager™ instrument (Roche Diagnostics). For quantification, the spot-signal intensities were measured in Boehringer light units. We used the intensity of background signal plus false-positive results in the NblA incubation experiment, the membrane

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*POV-ray, version 3.6, Persistence of Vision Pty. Ltd.*
**RESULTS**

**Crystal Structure of NblA from Anabaena sp. PCC 7120**—Because Collier and Grossman (4) reported that the increased expression of gene nblA is responsible for phycobilisome degradation in *Synechococcus* 7942, homologous genes have been identified in other phycobilisome-containing species of cyanobacteria and red algae (Fig. 1). Although the sequence identities are not very high among the members of the NblA containing species of cyanobacteria and red algae (Fig. 1). Although the sequence identities are not very high among the members of the NblA containing species of cyanobacteria and red algae (Fig. 1). Although the sequence identities are not very high among the members of the NblA containing species of cyanobacteria and red algae (Fig. 1).

Because Collier and Grossman (4) reported that the increased expression of gene nblA is responsible for phycobilisome degradation in *Synechococcus* 7942, homologous genes have been identified in other phycobilisome-containing species of cyanobacteria and red algae (Fig. 1). Although the sequence identities are not very high among the members of the NblA family (about 30% sequence identity on average), they seem to share structural similarity. Both CD spectroscopy (10) and PHD (28, 29) secondary structure predictions (data not shown) suggest that they are mostly α-helical proteins.

In order to get more insights into the function of NblA, we determined the crystal structure of the protein from *Anabaena* 7120. The structure was determined at 1.8-Å resolution with the single-wavelength anomalous dispersion technique using a selenomethionine derivative of the protein. The quality check of the structure, using the programs PROCHECK (30) and WHATCHECK (31), indicated good agreement between stereochemical parameters of the experimental structure and expected ones (32). In the Ramachandran diagram (33), all residues were in the energetically preferred areas with the exception of one residue located in the generously allowed region and 8 of 584 which residues were in the additional allowed region, as defined by PROCHECK (for further statistics see Table 1).

In the current model of the NblA structure, between 1 and 6 N-terminal residues and between 7 and 11 C-terminal residues are disordered and not visible in the electron density (see Table 2) in the 12 different NblA chains present in the asymmetric unit of the crystal. This observation is also manifested in the increase of individual atomic displacement factors at the ends of the polypeptide chains that are not stabilized was pre-examined with GST/anti-GST and anti-mouse monoclonal antibodies.

**TABLE 1**

| TABLE 1 Data collection and refinement statistics | Peak | Remote |
|---|---|---|
| Beam line | ESRF ID14-4 | ESRF ID14-4 |
| Wavelength (Å) | 0.9794 | 0.9393 |
| Space group | P2₁ | P2₁ |
| Unit cell dimension (Å, °) | 43.1, 96.5, 105.2, 96.9 | 43.2, 95.9, 104.8, 97.0 |
| Resolution (Å) | 28 to 2.12 (2.25 to 2.12)* | 20 to 1.80 (1.97 to 1.80) |
| Measured reflections* | 251,109 | 292,512 |
| Unique reflections | 47,683 | 77,921 |
| Completeness (%) | 96.5 (69.0) | 99.8 (100) |
| Rmerge (%) | 5.4 (19.8) | 6.9 (47.3) |
| Rfree (last shell) | 11.9 (3.8) | 9.4 (2.8) |
| Figure of merit after density modification | 0.56 | |
| Refinement parameters | | |
| R (%) | 18.2/21.7 |
| Rmerge (%) | 20 to 1.80 (1.97 to 1.80) |
| Rfree was calculated for 95% randomly selected reflections. The Rmerge was calculated for the other 5% of the data. |
| Total non-hydrogen atoms | 5445 |
| No. ethylene glycol molecules | 254 |
| Mean B factors for main chain/side chain (Å²) | 30.7/38.0 |
| Root mean square deviation | | |
| Bond lengths (Å) | 0.015 |
| Bond angle (°) | 1.48 |
| Torsion angles (°) | 4.6 |
| Planarity (Å) | 0.006 |
| B factors for main chain/side chain (Å²) | 2.173/5.372 |
| Ramachandran diagram allowed/additional/disallowed (%) | 99.8 / 0.2 / 0.0 |

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* Values in parentheses are for the highest resolution shell.
* Values are for Rmerge = 3. For calculations no cut off was used.
* For definition of Rmerge, see Ref. 48.
by secondary structure. In addition to the 12 polypeptide chains, 254 water molecules and 2 ethylene glycol molecules were built into the electron density. The conformation of the 12 molecules is similar with a mean r.m.s.d. for all 66 possible pairwise superpositions (using C⃗ atoms from residue 7–54) of 1.14 Å as calculated by LSQKAB (34), with small structural differences in chains C, F, I, and L. In the asymmetric unit, six dimers were found assembling to two compact hexamers (chains A, B; C, D; K, L, G, H; I; J; E, F; see supplemental Fig. 1), which are related by a noncrystallographic dyad axis defined by the polar angles ω = 94.2°, φ = −90.4°, and κ = 179.1°. The angle ω = 94.2° indicates a tilt of 42° relative to the b axis of the unit cell. Therefore, this (pseudo-) dyad cannot be a crystallographic axis. The dimers differ by a mean r.m.s.d. value of 1.28 Å, and the hexamers are similar to each other with an r.m.s.d. of 0.63 Å.

The small NblA polypeptide of 65 amino acids consists of two α-helices that are assembled at an ~37° angle in an antiparallel, V-shaped arrangement. The first helix, α1, extends from Ser26 to Lys53, His54, or Gln56, and the second helix, α2, from Ser26 to Lys53, His54, or Gln56, depending on the monomer that is considered. Internal stabilization of the molecules is provided by a backbone hydrogen pattern that is typical for α-helices. The short helix α1 is capped N-terminally by Ser26 that is hydrogen-bonded to the amide nitrogen of Gln14. For further stabilization of this part of the helix, there is a hydrogen bond between the carbonyl oxygen of the side chain of Gln14 and the amide nitrogen of Ser26. A very similar arrangement is found at the N terminus of the long helix α2, with a serine at position 26 that forms a hydrogen bond to the amide nitrogen of Gln14 via its Oe, and a hydrogen bond between Oe of Gln29 and the amide nitrogen of Ser29. The bend between the two helices is formed by residues 24–26, whereas the residues Met25 and Ser26 adopt ϕ and ψ torsion angles similar to those found in polyproline type II or polyglycine helices (ϕ ~ −80° and ψ ~ 158°). In most molecules the bend and/or the end of helix α1 is stabilized by a hydrogen bond between the amide nitrogen of Asn24 and the carbonyl oxygen of Thr25.

Two NblA monomers form the basic structural unit of NblA, a four-helix bundle with the tips of the V superimposing on the same side of a dimer (Figs. 2 and 3). The dimer is formed by two molecules related by a noncrystallographic dyad axis, and the contacts of the two monomers shield on average 1553 Å2 per monomer from the solvent, corresponding to 30% of the total solvent-accessible surface per monomer. The two α2-helices are in contact with each other via Ala30, Leu34, Leu37, and Mse41 of each chain forming a prolate hydrophobic surface that is flanked on both sides by Ile38, Phe18, and Val22 from the two α1-helices to build up the hydrophobic core, including Val22 from the second helix, α2, and the hydroxyl group of Tyr48 from the partner molecule and between the side chain nitrogen atoms of Gln49 and the hydroxyl oxygen of Tyr48 from the partner molecule and between the side chain nitrogen atoms of Gln49 and the carboxylate oxygen of Gln56 within molecule A (Fig. 3, box B).

In Vitro Binding of Phycobiliproteins to GST-tagged NblA—For in vitro binding assays, we expressed the NblA protein from Anabaena 7120 with an N-terminal GST tag, and we immobilized the fusion protein on glutathione-Sepharose. The affinity resin was incubated with total soluble proteins from Anabaena as described under "Experimental Procedures." After extensive washing, the resins retained an intensive blue color, indicating a strong association of phycobiliproteins. Control resins to which only GST was immobilized remained colorless. After elution with glutathione, proteins were separated by SDS-PAGE (Fig. 4a). The eluted phycobiliproteins showed the typical zinc-enhanced fluorescence of biliproteins under ultraviolet illumination (Fig. 4a, lane 2). The polypeptide bands indicated on Fig. 4a, lane 1, were identified by peptide mass mapping as the α- and β-subunits of phycoerythrocyanin and phycocyanin, respectively (data not shown). A weak band migrating

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**TABLE 2**

**Defined residues in the structure**

| Chain ID | A | B | C | D | E | F | G | H | I | J | K | L |
|----------|---|---|---|---|---|---|---|---|---|---|---|---|
| Residue range | 5–56 | 5–54 | 6–58 | 2–57 | 5–54 | 5–54 | 4–56 | 5–54 | 4–57 | 7–57 | 7–56 | 5–54 |

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**FIGURE 2. Stereo representation of an NblA dimer.** Dimer CD is depicted. Selected residues are labeled.
slightly faster than α-phycocyanin also showed zinc-enhanced fluorescence (Fig. 4a, lane 2). This band represents allophycocyanin, as proven by immunodetection with an anti-allophycocyanin antiserum and by binding of purified allophycocyanin subunits to the NbIA affinity resins (data not shown).

Identification of a Phycobiliprotein-binding Site on NbIA—To identify the amino acid residues of NbIA that are involved in its interaction with the phycobiliproteins, a series of site-directed mutants was generated. Selection of the positions of the amino acid residues that were replaced was guided by the conserved stretches of amino acid residues in NbIA (Fig. 1), by the here-described structure of the protein, and by geometrical considerations that took into account the modeled three-dimensional structures of the binding partners of NbIA, phycocyanin, and phycoerythrocyanin. The following NbIA variants were generated: L7A, E10A/Q11A, and Q12A/F13A with mutations located in the N-terminal helix; E50A, L51A, and K53A in the C-terminal helix; and Q23S/N24S/H27A/D28A with four changed amino acids located in the loop between the two α-helices. Like the wild-type protein, the mutated variants of NbIA were expressed as GST fusion proteins, and pull-down experiments were performed as described above. Fig. 4b shows a typical Western blot from five such experiments. Only two of the NbIA variants showed a significantly changed affinity to phycobiliproteins. The NbIA variant K53A did not bind phycobiliproteins, and variant L51A showed drastically lower binding. Amino acid residues Leu51 and Lys53 are located close to the C terminus, indicating that this region of NbIA is responsible for binding to the phycobilisome. NbIA variant K53A migrated somewhat faster in SDS-PAGE than wild-type NbIA and the other mutated variants (Fig. 4b). Determination of the molecular mass of the K53A variant by matrix-assisted laser desorption-mass spectrometry gave a m/z value of 8443.7, which is very close to its calculated mass of 8442.4 Da. Hence, the change in electrophoretic mobility of the K53A mutant may be due to the loss of a positive charge.

Identification of an NbIA-binding Site on Phycobiliproteins—Based on the amino acid sequences of phycocyanin and phycoerythrocyanin, a peptide scan assay was performed. A series of 15-mer peptides representing the α- and β-chains of phycocyanin and phycoerythrocyanin was incubated with GST-tagged NbIA, as described under "Experimen-
tal Procedures.” No binding of GST-NblA was found on blots carrying the peptides representing the β-chains of the two phycobiliproteins (data not shown). In contrast, the peptide blots representing the α-chains of phycocyanin and phycoerythrocyanin showed clear signals when incubated with GST-NblA (Fig. 5a). The peptides that interacted with NblA represent similar regions in the α-chains of both phycocyanin (Glu16–Leu39) and phycoerythrocyanin (Leu19–Ser45), covering the so-called Y-helix as well as the A-helix of the respective α-subunits (Fig. 5c).

**DISCUSSION**

It is well documented for several cyanobacterial species that NblA is essential for phycobilisome degradation under conditions of nitrogen limitation (4–7). According to the results of Collier and Grossman (4), the strongly increased expression of the nblA gene suffices to initiate this massive intracellular proteolysis. Although it has already been shown that NblA binds to phycobiliproteins (9), it is still not understood how the protein causes degradation of the complex phycobilisome structure. To gain more insight into the molecular mechanism of NblA function, we determined the crystal structure of the protein from *Anabaena* 7120, and we attempted to identify the sites of interaction between NblA and the phycobiliproteins in detail.

In the crystal, there are 12 NblA molecules in the asymmetric unit. Only 619 of the 780 residues of the asymmetric unit could be modeled into the electron density because of the absence of secondary structure elements and a high flexibility of the N and C termini. This observation is in agreement with predictions by bioinformatics sequence analysis tools, such as the PHD (28, 29) or PONDR (35) programs.

The x-ray structure of an NblA monomer consists of two helical elements that are connected by a turn. Two of these molecules form a dimer, which is probably the biologically active form (see below). One of the first questions that arose during structure determination concerned the native oligomerization state of NblA. Previous characterization of NblA by analytical ultracentrifugation indicated an association reaction of folded monomers to form trimers (10). Furthermore, analytical size exclusion chromatography yielded apparent molecular masses of 18.3–20.6 kDa, indicating a dimer or trimer (data not shown). In the crystal structure, however, six dimers but no trimers of NblA were found. To clarify this discrepancy, the analytical ultracentrifugation experiments were repeated. The new data could be fitted clearly to a dimer-tetramer model.5 Furthermore, in a Western blot analysis of NblA (referred to in this paper as NblAI) of the cyanobacterium *Tolyphothrix* sp. PCC 7601, a band at 15 kDa was found that could indicate the presence of NblA as a dimer (9).

In the same publication (9), it was demonstrated that NblA has an affinity for the α-subunits of both phycocyanin and phycoerythrin, suggesting a direct role for NblA in phycobilisome degradation. These data were confirmed for *Anabaena* 7120 with pull-down assays using a GST-tagged NblA protein (Fig. 4). Luque et al. (9) observed the interaction between NblAI of *Tolyphothrix* sp. PCC 7601 and phycocyanin and phycoerythrin of this cyanobacterium to be highly specific. Neither allophycocyanin from *Tolyphothrix* nor phycobiliproteins from other cyanobacterial species interacted with NblAI, although the sequence similarities between phycobiliprotein subunits are very high. In our binding studies, we observed different results. NblA from *Anabaena* 7120 bound to purified allophycocyanin subunits as well as to phycobiliproteins from the other cyanobacterial species tested, *Synechocystis* 6803 and *Synechococcus* 7942 (data not shown). It remains to be proven whether NblA can complement the nonbleaching phenotype of ΔnblA mutants from other cyanobacterial species.

The crystal structure of NblA allowed us to change amino acid residues of the protein that could be involved in the interaction with phycobiliproteins, as predicted from their position in the structure. From this mutational analysis (Fig. 4b), it became evident that residues at the end of the C-terminal helix, but neither the N-terminal helix nor the turn region of NblA, are involved in phycobilisome binding. Only the NblA variants L51A and K53A were affected in binding to phycobiliproteins (Fig. 4b). To what extent further residues in the highly flexible C-terminal end of NblA may be involved in PBS binding has not been tested. The very low sequence conservation of this part of NblA (Fig. 1) and the high degree of conservation of phycobiliproteins makes it unlikely that these residues are involved in binding at all.

Gene nblA was originally identified by complementation of a non-bleaching mutant of *Synechococcus* 7942 that was obtained by chemical mutagenesis (4). In this mutant, the nonconserved Ser6 (Fig. 1, line PCC 7942) was changed to phenylalanine, an amino acid with very different physicochemical properties. We have introduced several mutations in the conserved region immediately following Ser6, namely L7A, E10A, or Q11A, and Q12A/F13A (numbering according to the chromosomally encoded NblA protein studied here). None of these mutations had an influence on phycobiliprotein binding (Fig. 4b). This suggests that the original S9F mutation (4) may have caused unspecified changes in the properties of NblA, such as aggregation, or that this region of the protein may interact with another as yet unknown molecule(s) that could be required for phycobilisome degradation (see below).

To identify regions in the phycobilisome structure where NblA binds,
Crystal Structure of NblA

we performed peptide scans (Fig. 5). These data and the results of the pull-down studies using NblA variants allowed us to construct a model of NblA binding to phycocyanin (Fig. 6). In this model, the NblA dimer will get in contact via its residues Leu51 and Lys53 to the crossing points between the α-subunits of phycocyanin and, by inference, of phycerythrocyanin, respectively. Both the NblA dimer and the phycobilisomes show a 2-fold rotation axis at their binding sites, thus reinforcing the proposed model. Considering the fact that the structure of phycobilisomes requires their partial disassembly and contributes to their complete disassembly in vitro (49). Thus, a further interaction partner of NblA could be an as yet unknown protein phosphatase. Further studies may identify the interaction partner of NblA that is causal for initiation of phycobilisome degradation. This protein or cofactor could bind to the N-terminal helix of NblA, which carries the highest conserved stretch of amino acids among all NblA sequences (Fig. 1) and is accessible to a potential interaction partner according to the NblA structure presented here (Figs. 3 and 6).

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