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Changes in Polypeptide Composition of *Synechocystis* sp. Strain 6308 Phycobilisomes Induced by Nitrogen Starvation

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Phycobilisomes isolated from actively growing *Synechocystis* sp. strain 6308 (ATCC 27150) consist of 12 polypeptides ranging in molecular mass from 11.5 to 95 kilodaltons. The phycobilisome anchor and linker polypeptides are glycosylated. Nitrogen starvation causes the progressive loss of phycocyanin and allophycocyanin subunits with molecular masses between 16 and 20 kilodaltons and of two linker polypeptides with molecular masses of 27 and 33 kilodaltons. Nitrogen starvation also leads to enrichment of four additional polypeptides with molecular masses of 46, 53, 57, and 61 kilodaltons and a transient enrichment of 35- and 41-kilodalton polypeptides in isolated phycobilisomes. The 57-kilodalton additional polypeptide was identified by immunoblotting as the large subunit of ribulosebisphosphate carboxylase/oxygenase. Proteins with the same molecular weights as the additional polypeptides were also coisolated with the 12 phycobilisome polypeptides in the supernatant of nitrogen-replete *Synechocystis* thylakoid membranes extracted in high-ionic-strength buffer and washed with deionized water. These observations suggest that the additional polypeptides in phycobilisomes from nitrogen-starved cells may be soluble or loosely bound membrane proteins which associate with phycobilisomes. The composition and degree of association of phycobilisomes with soluble and adjacent membrane polypeptides appear to be highly dynamic and specifically regulated by nitrogen availability. Possible mechanisms for variation in the strength of association between phycobilisomes and other polypeptides are suggested.

Phycobilisomes (PBsomes) of both cyanobacteria and eucaryotic red algae typically contain 4 to 10 polypeptides ranging in molecular mass from 12 to 120 kilodaltons (kDa) in different organisms (13, 22, 26). Levels of the pigmented polypeptides phycocyanin and allophycocyanin and of their associated linker polypeptides with molecular masses of 27 to 35 kDa are affected by light (5), temperature (5), and the presence or absence of nitrogen (25). Nitrogen starvation leads to the rapid and specific degradation of phycobiliproteins in *Synechocystis* sp. strain 6308 (2). Nitrogen starvation of *Synechococcus* sp. strain 6301 causes decreases in phycocyanin, decreases in 75- and 33-kDa polypeptides, and the complete loss of a 30-kDa linker polypeptide (25). Degradation of phycocyanin is under the control of a specific protease in *Anabaena* spp. (24) and is reversible upon replenishing of nitrogen in *Synechocystis* strain 6308 (2). PBsomes thus appear to serve as a nitrogen reserve as well as a light-harvesting apparatus.

A recent report by Riethman et al. (20) that anchor and linker polypeptides of *Anacystis* sp. strain R2 are glycosylated suggests further that a dynamic interaction exists between PBsome structure and cell carbohydrate pools. Carbohydrate contents of cells increase dramatically in nitrogen-starved cells (4; C. S. Duke and M. M. Allen, unpublished data). If parallel changes occur in the glycosylation level of PBsome polypeptides, nitrogen starvation could affect PBsome structure indirectly, as well as directly affecting phycobiliprotein levels.

The intent of this research was to characterize the polypeptide composition of PBsomes from nitrogen-replete and nitrogen-starved *Synechocystis* strain 6308 as part of a continuing study of cellular nitrogen dynamics in this species (1–4). PBsomes isolated from nitrogen-replete *Synechocystis* strain 6308 were shown to contain 12 polypeptides ranging in molecular mass from 11.5 to 95 kDa. PBsomes from nitrogen-starved cells contain up to six additional polypeptides ranging in molecular mass from 35 to 61 kDa. It is suggested that these six polypeptides may be soluble or loosely bound membrane proteins which become associated with PBsomes under nitrogen-depleted conditions.

MATERIALS AND METHODS

Culture conditions. The unicellular cyanobacterium *Synechocystis* strain 6308 (ATCC 27150) was grown in BG-11 (1) supplemented with 2.4 g of Na_2CO_3 per liter at 35°C under cool white fluorescent lights at 100 $\mu E/m^2$ per s in Roux flasks bubbled with 5% CO_2 in air. Growth rates were routinely monitored by A_{750} readings on a Gilford spectrophotometer (optical density at 750 nm). Cells for membrane and PBsome preparations were harvested by centrifugation at 10,410 × *g* for 15 min.

Starvation experiments. Cells were grown in BG-11 to an optical density at 750 nm of ~2.0 and the contents of a flask (750 ml) were concentrated by centrifugation. The pellet was washed three times in BG-11 lacking N (−NBG-11), suspended in 10 ml of −NBG-11, and added to a fresh flask of −NBG-11. Cells were allowed to starve for 5 to 6 days, during which time they typically doubled once in the first 24 h and then stopped growing, turning yellow-green as phycocyanin and allophycocyanin were depleted. Cells were then harvested and either frozen at −20°C for 24 to 72 h or used immediately for PBsome extraction and purification. In the time series starvation experiment, cells growing in BG-11 were labeled with 2 mCi of $H_2{}^{35}SO_4$ (2.7 μCi/ml). Cells were harvested, washed with −NBG-11 lacking ^{35}S, and inoculated into a flask of −NBG-11. Samples (100 ml, except 200 ml at 141 h) were removed for PBsome extraction at 0, 21, 45, 69, and 141 h of starvation. The remaining 100 ml was

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transferred to a small flask, and $NaNO_3$ was added to a final concentration (1.5 g/liter) equal to that of BG-11. These cells were allowed to grow for 24 h and then harvested as described above.

PBsome isolation. Cell pellets were suspended in 5 to 10 ml of 0.75 M potassium phosphate (KP_i) buffer (pH 6.8), and the protease inhibitors phenylmethylsulfonylfluoride, benzamidine, and ε-aminocaproic acid were added to final concentrations of 1 mM. Sodium azide was added to a final concentration of 0.02% (wt/vol). PBsome extracts in the time series starvation experiment lacked protease inhibitors and sodium azide. Suspended cells were broken in a French pressure cell at 1,406 kg/cm^2, and Triton X-100 was added to the extract to a final concentration of 1% (vol/vol). The extract was allowed to stand at room temperature for 30 min, with stirring by occasional vortexing, and then centrifuged at 30,000 × *g* for 30 min. The clarified extract (crude PBsome extract) was layered on 0.25 to 2.0 M sucrose step gradients (five steps consisting of 0.25, 0.5, 0.75, 1.0, and 2.0 M) prepared with 0.75 M KP_i buffer. Gradients were centrifuged at 100,000 × *g* for 16 h at 4°C (time series experiment) or 18°C (subsequent experiments). PBsomes were collected from the 0.75 M layer (N-replete cells) or the 0.50 M layer (N-starved cells) with a sterile Pasteur pipette.

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), PBsomes were either precipitated in 10% (wt/vol) trichloroacetic acid (TCA), washed with 80% (vol/vol) ethanol and then with ether, and suspended in electrophoresis sample buffer (15), or diluted four to five times with 0.75 M KP_i buffer, pelleted at 130,000 × *g* for 2 h, suspended in 1 ml of buffer, and mixed with an equal volume of 2× electrophoresis buffer. Crude PBsome extract and the green fraction (F1) remaining at the top of sucrose gradients were also prepared for SDS-PAGE by treatment with 10% (wt/vol) TCA, followed by washing with 80% (vol/vol) ethanol and then with ether. The pellet was then suspended in electrophoresis sample buffer as described above. TCA treatment had no effect on the polypeptide compositions observed.

Trypsin digestion. PBsomes were isolated as above and washed with 0.75 M KP_i buffer lacking protease inhibitors. One milliliter of PBsomes (2.4 mg/ml of protein) was treated with trypsin (1 μg/100 μg of protein) as described in reference 26, with the omission of sucrose. Samples (200 μl) were withdrawn at 0, 5, 15, 30, 60, and 120 min after trypsin addition and precipitated with TCA as described above. The pellet was solubilized in 50 μl of 1× electrophoresis buffer for SDS-PAGE.

Membrane preparation. Membranes containing intact PBsomes were prepared by extracting log-phase cells in 0.75 M KP_i buffer as described above but omitting the Triton X-100 treatment. The extract was centrifuged at 11,950 × *g* for 10 min to remove cellular debris, and the supernatant was centrifuged at 130,000 × *g* for 2 h. The supernatant was removed, and the membrane pellet was suspended in deionized water and centrifuged again at 130,000 × *g* for 2 h. The supernatant from this centrifugation was removed, and a sample was precipitated with TCA as described above. The precipitated protein was suspended in 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) sodium hydroxide buffer (pH 8.0) containing 5% (vol/vol) β-mercaptoethanol and mixed with 2× electrophoresis buffer. The membrane pellet was washed twice more, and the supernatants were discarded. The pellet was suspended in 0.75 M KP_i buffer and mixed with an equal volume of 2× electrophoresis buffer.

SDS-PAGE. Samples in electrophoresis buffer were assayed for protein after precipitation with sodium deoxycholate and TCA by the improved Lowry technique of Peterson (19). SDS-PAGE was carried out by using the discontinuous buffer system of Laemmli (15). Samples were solubilized in buffer at room temperature for 30 min or at 100°C for 2 min and applied to a 12% resolving gel with a 5% stacking gel. Gels were run at room temperature for approximately 4 h at 30 mA and then stained with Coomassie blue (0.1%) or silver (17). Gels containing ^{35}S-labeled proteins were stained in Coomassie blue, photographed, soaked in En^{3}Hance (Dupont, NEN), and dried. Dried gels were exposed to AR X-Omat film (Eastman Kodak Co.) for 5 days to 2 weeks with intensifying screens at −70°C.

Immunoblotting. Antibody to the 95-kDa anchor polypeptide of *Synechocystis* strain 6714 PBsomes was a gift from L. Sherman and G. Bullerjahn. Immunoglobulin G purified from antisera to the large subunit of ribulosebisphosphate carboxylase/oxygenase (Rubisco) of *Nostoc* sp. was a gift from J. Meeks. For immunoblotting, protein was transferred to nitrocellulose paper from gels by using the buffer system of Towbin et al. (23). Gels to be transferred were typically run with prestained standards (Bio-Rad Laboratories) as a check on transfer efficiency. Following transfer, the nitrocellulose was rinsed twice in deionized water and dried in the dark between filter papers. Binding sites were blocked with 3% gelatin and probed with antibody diluted 200× to 5,000× in 1% gelatin in Tris-buffered saline (20 mM Tris, 500 mM NaCl [pH 7.5]). Proteins were visualized by using an Immuno-Blot assay kit (Bio-Rad). Blots were rinsed in deionized water and dried before photography.

ConA binding. Proteins were separated by SDS-PAGE and transferred to nitrocellulose as described above. Glycoproteins were visualized by concanavalin A (ConA) binding following the procedure of Clegg (11), with the substitution of Tween 20 for Triton X-100 and the use of *o*-dianisidine as the chromogen (20). Specificity of ConA binding to sugar residues was confirmed by treating blots with ConA in the presence of 100 mM α-methyl mannoside.

RESULTS

Nitrogen-replete cells. The polypeptide profile of PBsomes from nitrogen-replete cells is shown in Fig. 1 (lane 3), next to the crude PBsome extract (lane 1) and the green F1 fraction (lane 2) to show the purification achieved by sucrose gradient centrifugation. Twelve polypeptides, ranging in molecular mass from 11.5 to 95 kDa, were consistently observed (Table 1). Pigmented polypeptides are indicated in the table. The 95-kDa polypeptide of cyanobacterial PBsomes is reported to contain a chromophore (27), but it was not visibly pigmented in our gels. The 95-kDa polypeptide reacted strongly and the 84-kDa polypeptide reacted weakly with antibody to the 95-kDa polypeptide of *Synechocystis* strain 6714 PBsomes (Fig. 1, lane 5). ConA bound to the 95-, 84-, 74-, 50-, 33-, and 27-kDa polypeptides at pH 7.8 (Fig. 1, lane 4) and pH 7.2. α-Methyl mannoside partially inhibited ConA binding to these PBsome polypeptides, except to the 50-kDa polypeptide, at pH 7.8 but not at pH 7.2 (data not shown). α-Methyl mannoside totally blocked binding to an ovalbumin standard at pH 7.8 (data not shown). No staining of PBsome polypeptides was observed when ConA or horseradish peroxidase was omitted from the staining procedure (data not shown).

Nitrogen-starved cells. PBsomes isolated from *Synechocystis* strain 6308 starved for nitrogen were typically

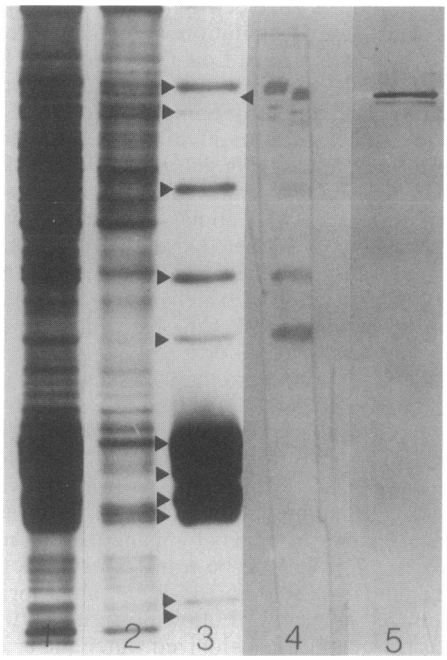

FIG. 1. Polypeptide composition of PBsomes isolated from nitrogen-replete *Synechocystis* strain 6308. Lanes: 1, autoradiogram of SDS-PAGE gel of crude PBsome extract, 478,000 dpm; 2, green fraction from top of sucrose gradient following PBsome isolation, 126,000 dpm; 3, purified PBsomes, 165,000 dpm (▶, 12 polypeptides with molecular masses of 11.5, 12.5, 16, 17, 19, 20, 27, 33, 50, 74, 84, and 95 kDa); 4, purified PBsomes separated by SDS-PAGE, transferred to nitrocellulose, and stained with ConA; 5, immunoblot of PBsomes reacted with antibody against the 95-kDa polypeptide from *Synechocystis* strain 6714.

less dense than those from nitrogen-replete cells, as illustrated by their recovery from the 0.50 M sucrose layer rather than from the 0.75 M layer. The time series starvation experiment showed decreases in both staining intensity (Fig. 2A) and amount of label present (Fig. 2B) in the 16- to 20-kDa pigmented polypeptides and in the 27- and 33-kDa colorless polypeptides. Labeled polypeptides of 35 and 41 kDa were present at 69 and 141 h of starvation (Fig. 2B, lanes 4 and 5) but disappeared after 24 h of regreening (Fig. 2B, lane 6). The amount of label in polypeptides of 46, 53, 57, and 61 kDa increased with time of starvation and did not decrease during the regreening period (Fig. 2B). The amount of label in the 13.5-kDa polypeptide also increased with starvation, while the amount of label in the 11.5- and 12.5-kDa polypeptides decreased during the experiment (Fig. 2B). These changes are summarized in Table 1. Polypeptides with the same molecular masses as those which increased in amount of label with starvation in purified PBsomes (Fig. 2B) were present in the crude PBsome extracts throughout starvation (Fig. 2C). This suggests that these polypeptides were present in nitrogen-replete cells but that they were not strongly attached to the isolated PBsomes from these cells. Turnover of pigmented polypeptides and of the 27- and 33-kDa linker polypeptides was demonstrated by their increase in protein staining after 24 h of regreening (Fig. 2A, lane 6), despite their continued loss of ^{35}S-labeling intensity (Fig. 2B, lane 6).

Two polypeptides in PBsomes from nitrogen-starved cells reacted with antibody to the 95-kDa polypeptide of *Synechocystis* strain 6714 (Fig. 2D), one at 95 kDa and the other at a slightly lower molecular mass which overlapped with several polypeptides immediately below the 95-kDa polypeptide. The 57-kDa polypeptide in PBsomes isolated from nitrogen-starved cells cross-reacted with antibody to the large subunit of Rubisco from *Nostoc* sp. (Fig. 3), but no PBsome polypeptides from nitrogen-replete cells cross-reacted with this antibody (data not shown).

To test the hypothesis that the additional polypeptides might be proteolytic breakdown products of higher-molecular-weight proteins, starvation experiments were run with protease inhibitors added to PBsome extracts. No major differences in the polypeptide composition of nitrogen-starved PBsomes (Fig. 2B, lane 5) were observed as a result of the addition of protease inhibitors (Fig. 2D, lane 2).

Trypsin digestion of PBsomes from nitrogen-replete cells. Trypsin digestion caused the progressive loss of the 95-kDa polypeptide with time and a large increase in the amount of the 84-kDa polypeptide within 5 min (Fig. 4). Further digestion resulted in the appearance of minor bands at 32 and 25 kDa, probably as a result of breakdown of the 33- and 27-kDa linker polypeptides, respectively. Trypsin digestion also produced minor bands at 13.5, 22, and 23 kDa. The pigmented polypeptides were unaffected by trypsin digestion. Polypeptides of 38, 40, 43, and 52 kDa were present in the initial sample and were unaffected by trypsin. These are likely minor contaminants visualized as a result of the large amount of protein loaded in each lane for maximum resolution of breakdown products.

Membrane washing experiment. The supernatant of washed membranes not treated with Triton X-100 contained polypeptides with molecular weights corresponding to those present in PBsomes from nitrogen-starved cells, with differences in the relative amounts of the polypeptides (Fig. 5). There were also four to five additional polypeptides in the 14- to 29-kDa region. The washed membranes were depleted in polypeptides with the same molecular masses as those enriched in the supernatant. Three chlorophyll-containing bands with molecular masses greater than 100 kDa were visible in unstained gels of membranes and stained very darkly with silver (Fig. 5).

TABLE 1. Molecular masses of PBsome proteins in nitrogen-replete and nitrogen-starved *Synechocystis* strain 6308

Band	Mol mass (kDa) in *Synechocystis* strain 6308	
	Nitrogen replete	Nitrogen starved
1	95[a]	
2	84[a]	
3	74[a]	
3a		61
3b		57
3c		53
4	50	
4a		46
4b		41
4c		35
5	33[a]	
6	27[a]	
7	20[b]	
8	19[b]	
9	17[b]	
10	16[b]	
10a		13.5
11	12.5	
12	11.5	

[a] Glycosylated polypeptides.
[b] Pigmented polypeptides.

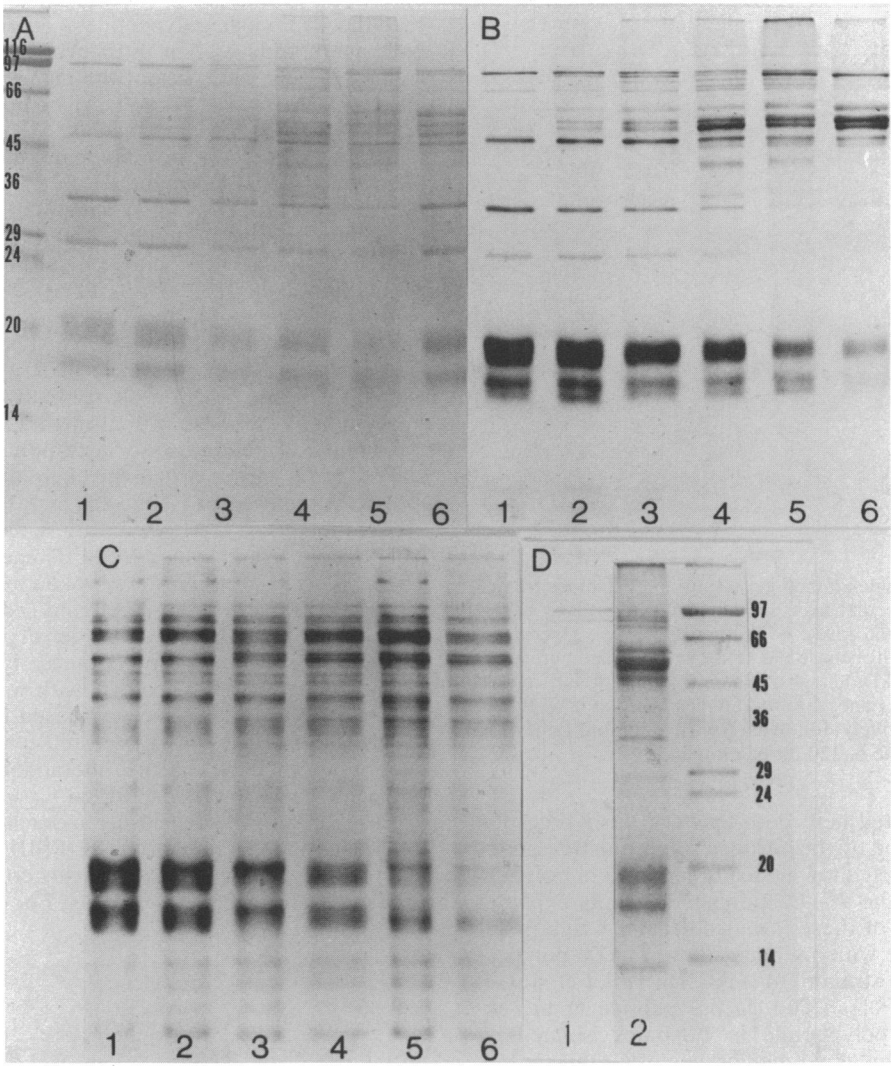

FIG. 2. Polypeptide composition of PBsome preparations from nitrogen-starved *Synechocystis* strain 6308. (A to C) Time series starvation experiment. (D) Separate experiment with cells harvested after 6 days of nitrogen starvation. (A) Coomassie blue-stained SDS-PAGE gel of purified PBsomes with 30 μg of protein loaded in each lane. Left, molecular mass markers: β-galactosidase (116 kDa), phosphorylase *b* (97.4 kDa), bovine albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.2 kDa). Lanes: 1 to 5, PBsomes extracted after 0, 21, 45, 69, and 141 h of nitrogen starvation; 6, PBsomes extracted 24 h after addition of nitrogen to starved cells. (B) Autoradiogram of SDS-PAGE gel with 15,000 dpm of purified PBsomes loaded in each lane. Lanes are as described for Fig. 2A. (C) Autoradiogram of crude PBsome extract from nitrogen-starved *Synechocystis* strain 6308; 25,000 dpm were loaded in each lane. Lanes are as described for Fig. 2A. (D) Lanes: 1, purified PBsomes from nitrogen-starved (6 days) *Synechocystis* strain 6308, separated by SDS-PAGE, transferred to nitrocellulose, and reacted with antibody against the 95-kDa polypeptide from *Synechocystis* strain 6714; 2, purified PBsomes as in lane 1, stained with Coomassie blue (36 μg of protein loaded in each lane). Right, molecular mass standards as described for Fig. 2A, with the omission of β-galactosidase (116 kDa).

DISCUSSION

The polypeptide composition of *Synechocystis* strain 6308 PBsomes is similar to that reported for a number of cyanobacteria and eucaryotic red algae (13, 22, 26). This composition appears to be highly conserved, as illustrated by the antigenic similarity of the 95-kDa polypeptide from a variety of sources (27) and confirmed here for two *Synechocystis* strains. In parallel with the results of Zilinskas and Howell (27), antibody to the 95-kDa polypeptide of *Synechocystis* sp. strain 6714 reacted both with the 95-kDa polypeptide and with a lower-molecular-mass polypeptide at 84 kDa of *Synechocystis* strain 6308. This result was attributed by Zilinskas and Howell to proteolysis of the 95-kDa protein. This

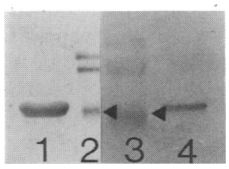

FIG. 3. Immunoblot of purified PBsomes from nitrogen-starved *Synechocystis* strain 6308, reacted with antibody to the large subunit of Rubisco from *Nostoc* sp. Lanes: 1, purified spinach Rubisco; 4, PBsomes isolated from nitrogen-starved cells; 2 and 3, prestained standards (Bio-Rad) (◀, ovalbumin with a molecular mass of 50 kDa). Lanes 1 and 2 were run on a different gel from lanes 3 and 4; thus, direct alignment is impossible.

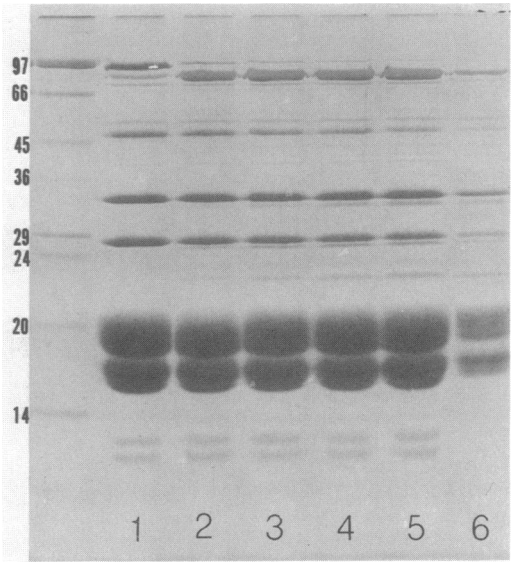

FIG. 4. Coomassie blue-stained gel of purified PBsomes treated with trypsin. Left, molecular mass markers: phosphorylase *b* (97.4 kDa), bovine albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.2 kDa). Lanes 1 to 6, Samples taken at 0, 5, 15, 30, 60, and 120 min, respectively, following trypsin addition. Lanes 1 to 5, 240 μg of protein; lane 6, 120 μg of protein.

hypothesis is supported here both by the trypsin digestion experiment (Fig. 4) and by the nitrogen starvation time series (Fig. 2A and B), which show a steady increase in polypeptides directly below the 95-kDa polypeptide at its expense. Further, at least one of these lower-molecular-weight polypeptides was reactive with antibody to the 95-kDa polypeptide of *Synechocystis* strain 6714 (Fig. 2D). Hiller et al. (14) reported enrichment of an 80-kDa polypeptide at the expense of a 94-kDa polypeptide in PBsomes isolated by trypsin digestion of thylakoid membranes of *Griffithsia monilis*.

Nitrogen starvation of cyanobacteria results in the degradation of phycocyanin and allophycocyanin (4). Associated changes occur in the polypeptide composition of the PBsome, for example, the loss of the 30-kDa linker polypeptide and decreases in amounts of 33- and 75-kDa polypeptides in a *Synechococcus* sp. (25). *Synechocystis* strain 6308 PBsomes showed comparable decreases in the pigmented polypeptides and in the 27- and 33-kDa colorless polypeptides but also showed increases in a number of polypeptides with molecular masses between 35 and 61 kDa. Several lines of evidence suggest that these increases are not intrinsic changes in PBsome composition but are rather changes in the strength of association between PBsome polypeptides and soluble or extrinsic membrane polypeptides.

First, polypeptides with molecular masses of 35 to 61 kDa were present in crude PBsome extracts at the beginning of the nitrogen starvation experiment (Fig. 2C), suggesting that they are present in actively growing cells. This suggestion is strengthened by the observation that polypeptides with similar molecular masses can be readily extracted from *Synechocystis* strain 6308 membranes by washing with water (Fig. 5). The resulting supernatant contains the full complement of PBsome and additional polypeptides, plus several others. Experiments with protease inhibitors and trypsin digestion of PBsomes (Fig. 4) argue against the hypothesis that the additional polypeptides, other than the 13.5-kDa one, are products of in vitro proteolysis. While constant loading of gels, with loss of the pigmented proteins, would magnify any remaining proteins, a comparison of Fig. 1, lane 3, with Fig. 2B, lane 1, indicates that increasing the gel loading by an order of magnitude did not allow visualization of any additional polypeptides in PBsomes isolated from nitrogen-replete cells (Fig. 1, lane 3).

Second, immunoblotting showed that the 57-kDa polypeptide in PBsomes from nitrogen-starved cells is the large subunit of Rubisco (Fig. 3). In the presence of 20 mM $MgCl_2$, Rubisco and several Calvin cycle enzymes from pea chloroplasts can form complexes which can be isolated on sucrose gradients (21). In the presence of 50 mM $MgCl_2$ or NaCl, Rubisco will also bind loosely to thylakoid membranes (16). Formation of such associations might explain the increase in the number of PBsome-associated polypeptides in nitrogen-starved cells, particularly in the high salt concentrations (750 mM KP_i) required to isolate intact PBsomes. An alternate explanation of the presence of Rubisco in PBsome extracts is isolation of carboxysomes with PBsomes. Carboxysomes of *Thiobacillus neapolitanus* are broken by passage of the cells through a French pressure cell and sediment at about 25% sucrose, or 0.73 M, the same density at which PBsomes sediment (8). However, no PBsome polypeptides from nitrogen-replete cells cross-reacted with antibody to the large subunit of *Nostoc* Rubisco. Increased Rubisco in PBsomes of nitrogen-starved cells therefore seems more likely to be due to an increase in the interaction between PBsome polypeptides and soluble Rubisco.

Third, PBsomes are closely associated structurally and functionally with photosystem II (PSII) (12), which contains polypeptides with molecular weights comparable to those in PBsomes in nitrogen-starved cells. For example, the chloro-

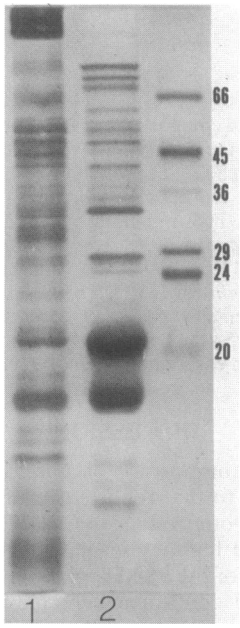

FIG. 5. Silver-stained gel of deionized water-washed *Synechocystis* thylakoid membranes (lane 1) and supernatant (lane 2); 28 μg of protein loaded in each lane. Right, molecular mass markers: bovine albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), and trypsin inhibitor (20.1 kDa).

phyll protein III (CPIII) complex isolated from PSII of *Synechocystis* strain 6714 contains polypeptides of 54 and 64 kDa (6, 7). This strain also contains a 52-kDa PBsome protein, identified as an extrinsic membrane protein, and intrinsic PSII proteins of 36, 40, and 45 kDa (6). The CPVI-1 complex of *Anacystis nidulans* R2 contains 42- and 71-kDa polypeptides, the latter identified as the PBsome anchor polypeptide of *A. nidulans* (18). This species also contains 52- and 45-kDa polypeptides in CPVI-2 and -3, respectively (18).

Finally, Gantt and co-workers have prepared PIIP particles containing both PBsomes and PSII activity from *Porphyridium cruentum* (9, 10, 12). PIIP particles, which are not readily distinguishable from PBsomes in electron micrographs (12), evolve oxygen and have polypeptide profiles similar to those of PBsomes, with enrichment of bands at 85 to 92, 42 to 44, and 25 to 27 kDa. *P. cruentum* PBsomes isolated at 5°C, but not at 20°C, can reduce dichlorophenol indophenol, indicating a partial PSII activity (9). Further, PBsomes from *P. cruentum* are contaminated with 46- and 50-kDa PSII polypeptides (10). The complexity of these interactions between PBsome and PSII polypeptides suggests that some of the polypeptides present in PBsomes of nitrogen-starved *Synechocystis* strain 6308 could be components of PSII.

It is also possible that the 50-kDa polypeptide consistently isolated from both nitrogen-starved and nitrogen-replete cells (Fig. 1 and 2) is a PSII protein. Its function in the PBsome is unknown, and trace amounts of free chlorophyll can be observed by fluorescence in PBsome gels illuminated with a UV lamp (Duke and Allen, unpublished data), although the 50-kDa polypeptide does not fluoresce.

The report of Reithman et al. (20) that the anchor and linker polypeptides of *Anacystis* R2 PBsomes are glycosylated was confirmed here for *Synechocystis* strain 6308 (Fig. 1, lane 4). This suggests an additional mechanism for the increase in non-PBsome polypeptides in PBsomes of nitrogen-starved cells. Carbohydrate levels of nitrogen-limited cells can be five times those of nitrogen-replete cells (4; Duke and Allen, unpublished data). If the level of glycosylation increases with the increasing carbohydrate content during nitrogen starvation, the PBsome might interact more with adjacent PSII proteins, in effect becoming stickier. Alternatively, the PBsome cores may be sticky and have their sticky surfaces exposed when the cores lose the pigmented proteins. Our ConA-binding data neither support nor refute these hypotheses, but they should be readily testable. Since α-methyl mannoside did not inhibit ConA binding to the 50-kDa polypeptide, we conclude that the binding observed is nonspecific and that the 50-kDa polypeptide is not glycosylated.

Nitrogen starvation of *Synechocystis* strain 6308 thus leads to a number of non-PBsome polypeptides, including Rubisco, being coisolated with PBsomes purified on sucrose gradients. This suggests that both the composition and the degree of association of PBsomes with soluble and adjacent membrane polypeptides are highly dynamic and specifically regulated by nitrogen availability.

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