Arch. Microbiol., 124, 39–47 (1980)

Archives of
Microbiology
© by Springer-Verlag 1980

Dynamic Aspects of Phycobilisome Structure

Phycobilisome Turnover During Nitrogen Starvation in *Synechococcus* sp.

Gregory Yamanaka and Alexander N. Glazer*

Department of Bacteriology and Immunology, Life Sciences Building, University of California, Berkeley, California 94720, USA

Abstract. In exponentially growing cells of *Synechococcus* sp. 6301, over 95% of the phycobiliproteins are located in phycobilisomes, and the remainder is present in the form of low molecular weight aggregates. In addition to the subunits of the phycobiliproteins (C-phycocyanin, allophycocyanin, allophycocyanin B), the phycobilisomes of this unicellular cyanobacterium contain five non-pigmented polypeptides. During the initial phase of starvation (24 h after removal of combined nitrogen from the growth medium), the phycobiliproteins in the low molecular weight fraction largely disappeared. Phycocyanin was lost more rapidly from this fraction than allophycocyanin. Simultaneous changes in the phycobilisome were (1) a decrease in sedimentation coefficient, (2) a decrease in phycocyanin: allophycocyanin ratio, (3) a shift in the fluorescence emission maximum from 673 to 676 nm, and (4) a selective complete loss of a 30,000 dalton non-pigmented polypeptide. Upon extensive nitrogen starvation (72 h), the intracellular level of phycocyanin decreased by over 30-fold. These results indicate that in the early stage of nitrogen starvation, the free phycobiliproteins of the cell are degraded, as well as a significant proportion of the phycocyanin from the periphery of the phycobilisome. However, the structures partially depleted of phycocyanin still function efficiently in energy transfer. On extended starvation, total degradation of residual phycobilisomes takes place, possibly in conjunction with the detachment of these structures from the thylakoids.

None of the effects of the absence of combined nitrogen were seen when cells were starved in the presence of chloramphenicol, or in a methionine auxotroph starved for methionine.

Key words: Cyanobacteria – *Synechococcus* – Phycobilisomes – Phycobiliproteins – Phycocyanin – Turnover – Nitrogen starvation.

A consideration relevant to all studies of phycobilisome structure and function is that these particles are a heterogeneous population of structures rather than many copies of an identical structure. This is a consequence of the fact that the phycobilisome content of a cell, and the relative amounts of the various light-harvesting components depend on light quality and intensity, the age of the culture, and its nutritional state. Phycobiliproteins are the most abundant proteins of the cyanobacterial cell. However, they are not indispensable to the cell. In cells depleted of phycobiliproteins, sufficient chlorophyll *a* remains associated with photosystem II to allow continued function of the photosynthetic apparatus (Lemasson et al., 1973). Consequently, the phycobiliproteins represent a potential pool of amino acids to be utilized under conditions of nitrogen starvation with no irretrievable damage to the cell. Indeed, when cyanobacteria are transferred to a medium lacking in combined nitrogen, the cells degrade phycobiliproteins, and upon prolonged starvation, cultures change in color from blue-green to yellow-green (Allen and Smith, 1969).

We have examined the changes in phycobilisome structure during the course of nitrogen starvation to answer the following question. Does the disappearance of phycobilisomes during starvation represent a decrease in the cell content of these structures, or does it represent a step-wise destruction of these particles, such

* To whom offprint requests should be sent

Abbreviations Used. NaK-PO_4 = NaH_2PO_4 titrated with K_2HPO_4 to a given pH; SDS = sodium dodecyl sulfate; Tris = Tris(hydroxymethyl)aminomethane

0302-8933/80/0124/0039/$01.80

that the residual structures remain functional in energy transfer until a late stage in nitrogen deprivation? We also report some preliminary studies of the events which trigger phycobilisome turnover.

Materials and Methods

Chemicals. L-Methionine and chloramphenicol were obtained from Sigma Chemical Co., St. Louis, Missouri, Triton X-100 and 2-mercaptoethanol from Eastman Organic Chemicals, Rochester, New York, and density gradient sucrose from Calbiochem, La Jolla, California. N-Methyl-N′-nitro-N-nitrosoguanidine was from Aldrich Chemical Co., Milwaukee, Wisconsin. Acrylamide, N,N′-methylenebisacrylamide, N,N,N′N′-tetramethylethylenediamine, and ammonium persulfate were purchased from BioRad Laboratories, Richmond, California. Sodium dodecyl sulfate (specially pure) was from BDH Chemicals Ltd., Poole, England. All other chemicals were of reagent grade.

Strains and Culture Conditions. Synechococcus sp. 6301 (ATCC 27144) (Stanier et al., 1971) has been maintained in this laboratory since 1971. A methionine auxotroph, strain AN1, was isolated from the parent strain 6301 through penicillin selection (Stevens et al., 1975) following mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine (Sherman and Cunningham, 1977). This strain grows as well as the wild type when supplemented with 20 µg/ml of L-methionine, and produces phycobilisomes of identical polypeptide composition and spectroscopic properties to those of the parent strain.

Synechococcus sp. 6301 was grown in 2.8 l Fernbach flasks containing 1 l of medium BG-11 (Stanier et al., 1971) at 30° C with continuous stirring. Illumination was provided by one 15 Watt Deluxe warm white fluorescent tube, 12 – 15 cm from the culture surface. Strain AN1 was grown in the same manner, but supplemented with 20 µg/ml of filter-sterilized L-methionine.

Experiments involving starvation of cells for combined nitrogen were performed on the wild type. Culture aliquots of 400 ml were harvested aseptically at specified intervals, washed once in BG-11 lacking $NaNO_3$, and resuspended in the same medium. Where indicated, filter-sterilized chloramphenicol was added to a final concentration of 25 µg/ml. Methionine starvation of strain AN1 was performed in an identical manner, except that the starvation medium contained unmodified ($NaNO_3$ – containing) BG-11.

Preparation of Phycobilisomes. Phycobilisomes were isolated as described previously (Yamanaka et al., 1978) with slight modifications. All buffers contained 10^{-3} M 2-mercaptoethanol. Cells were harvested, washed twice in 0.65 M NaH_2PO_4/K_2HPO_4 (NaK-PO_4), pH 8.0, then resuspended to a concentration of 0.12 g/ml (wet weight) in the same buffer. The cell suspension was broken by passage through a French pressure cell, incubated for 30 min in the presence of 1.2 % Triton X-100, then clarified by centrifugation at 31,000 × *g* for 30 min at 18° C. The supernatant fraction was layered onto sucrose step gradients containing 2.3 ml steps of 1.0, 0.8, 0.6, 0.4 and 0.2 M sucrose, all in 0.75 M NaK-PO_4, pH 8.0, containing 10^{-3} M NaN_3. Centrifugation was performed in a Spinco SW41 rotor at 24,000 rev/min^{-1} (98,000 × *g*) for 13 h at 18° C.

Polyacrylamide Gel Electrophoresis. Slab gel electrophoresis was carried out in 12 % acrylamide (0.32 % bisacrylamide) gels containing 0.1 % SDS in the discontinous Tris-glycine buffer system of Laemmli (1970). Samples were prepared by precipitating 100 to 200 µg of protein in an excess volume of 10 % trichloroacetic acid, followed by resuspension in an SDS solubilization mixture, and heating to 80° C for 2 min. The solubilized sample contained 0.2 M Tris-Cl, pH 8.8, 2 % SDS, 1 M 2-mercaptoethanol, 15 % glycerol, and 0.01 % bromphenol blue. Ten microliter samples (= 7 µg protein) were applied to the gel and electrophoresis was performed at a constant current of 30 mA. Gels were stained and quantitated by densitometer scans as described (Yamanaka et al., 1978).

Spectroscopic Measurements. Absorption spectra were obtained on a Beckman 25 recording spectrophotometer (Beckman Instruments, Inc., Palo Alto, California). Fluorescence emission spectra, corrected for the sensitivity of the detection system, were determined on a Spex Fluorolog recording spectrofluorimeter (Spex Industries, Inc., Metuchen, N.J.) at a sample absorbance of 0.1/cm at λ_{max} (Yamanaka et al., 1978). Relative amounts of phycocyanin and allophycocyanin were estimated by determining the ratio of absorbances at the extinction maxima of these proteins, 624 and 654 nm, respectively. Phycobilisome protein concentration was determined spectroscopically as described previously (Yamanaka et al., 1978). A sample of *Synechococcus* sp. 6301 phycobilisomes having an A_{624}:A_{654} ratio of 2.6 and containing 0.14 mg of protein per ml has an absorbance of 1.0/cm at 624 nm.

Results

Starvation of Cells for Combined Nitrogen

The aim of the initial experiment was to obtain cells starved for nitrogen to varying extent, which could be harvested at the same time so as to permit isolation of their phycobilisomes under identical conditions. Two 1 l cultures were grown to mid-log phase and on three successive days 400 ml aliquots harvested aseptically. The cells from each aliquot were washed once, resuspended in 500 ml of starvation medium, and incubated again under normal conditions. A slight yellowing of the cultures was apparent after 1 day of nitrogen starvation (i.e., for cells incubated in medium BG-11 lacking $NaNO_3$), and became very extensive after 3 days. The amounts of phycobiliproteins in whole cell extracts decreased both relative to chlorophyll *a* and the cell mass (wet weight). The degree of loss was slightly variable from one experiment to another, depending upon the state of the cells prior to nitrogen starvation, and the temperature and light intensity of the incubation. In this experiment, the absorbance at 624 nm of supernatant fractions obtained by breakage of cells starved for fixed nitrogen for 0, 1, 2, and 3 days was 192, 73, 72, and 14 units per cm per gram wet weight of cells per ml.

After nitrogen starvation, cultures were harvested in parallel and used for phycobilisome isolation. Figure 1 A, tube *a* shows a sucrose density gradient profile of phycobilisomes prepared from control cells (i.e., cells incubated continuously in normal medium BG-11) as it appeared following the last step of phycobilisome purification (see "*Materials and Methods*"). In such a gradient the chlorophyll *a*, solubilized membrane components, and detergent remained at the top, followed by a light blue band of free phycobiliproteins just below. Phycobilisomes were pre-

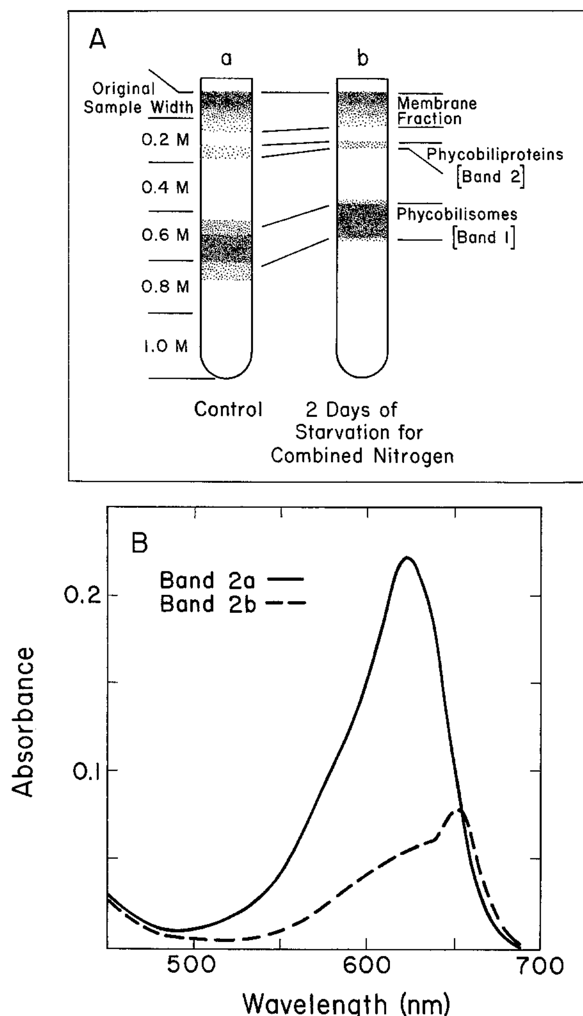


Fig. 1. A Sucrose step gradient profiles after centrifugation of the soluble supernatant fractions from control cells and cells that were starved 2 days for combined nitrogen (see "*Materials and Methods*"). Numbers on the left represent the initial molar sucrose concentrations. **B** Absorption spectra of phycobiliprotein regions *(Bands 2)* of the sucrose gradients shown in part A. Absorption maxima are at 624 nm for the control *(Band 2a)* and 654 nm for the nitrogen starvation sample *(Band 2b)*

sent about two-thirds of the distance down the gradient and were seen as a heterogeneous, deep blue band of protein, free of non-phycobilisome proteins of the cell (Yamanaka et al., 1978).

When phycobilisomes were isolated from nitrogen-starved cells, the sucrose density gradient profile (Fig. 1A, tube *b*) revealed two major differences from the control gradient shown in tube *a*. First, a decrease in sedimentation rate and a decrease in polydispersity was seen in the phycobilisome region (compare bands 1 in Fig. 1A), and second, band 2 showed a decrease in sedimentation rate and a nearly total loss of free phycobiliprotein material. Spectroscopic analysis of the material from these gradients showed that both changes were related to the selective loss of phycocyanin. As shown in Fig. 1B, the phycobiliprotein region (band 2) of the control sucrose gradient had an absorption spectrum with a maximum at 624 nm, with only a slight long wavelength shoulder. Such a spectrum is characteristic of phycocyanin with a minor contribution from allophycocyanin. In sucrose gradients with material prepared from cells starved for combined nitrogen for 1 or 2 days prior to harvest, the spectrum of the small amount of biliprotein present in this region of the gradient was completely different, exhibiting an absorption maximum at 654 nm and a shape characteristic of allophycocyanin (Fig. 1B). The material in the phycobilisome region likewise displayed a shift in the absorption peak, from 624 nm in control phycobilisomes to 626 nm in phycobilisomes from cells starved for 2 days (Table 1). Here, however, there is no gross difference in the shape of the curve, but the quantitative change is most apparent in the 624:654 nm absorption ratios. Control phycobilisomes have a 624:654 nm absorption ratio of about 2.6, while phycobilisomes from nitrogen-starved cells display ratios of about 2.0 after 1 or 2 days and 1.7 after 3 days of nitrogen starvation (Table 1). This clearly demonstrates the enrichment for a long wavelength component and suggests that a selective turnover of phycocyanin relative to allophycocyanin takes place during nitrogen starvation, resulting in a smaller and slower sedimenting phycobilisome particle. The conclusions from spectroscopic analysis are supported by densitometer scans (not shown) of polypeptide patterns on SDS-gels obtained from phycobilisomes from nitrogen-starved and control cells. Such scans show a relative increase in the area of the peaks corresponding to the allophycocyanin subunits as compared to those corresponding to the phycocyanin subunits in phycobilisomes from nitrogen-starved cells.

Figure 2 compares the fluorescence emission spectra of phycobilisomes prepared from control cells and those from cells starved for 2 days for combined nitrogen. When samples were excited with light of 580 nm, a wavelength absorbed primarily by phycocyanin, both phycobilisome samples exhibited spectra characteristic of emission from allophycocyanin and allophycocyanin B. These latter phycobiliproteins have emission maxima of 660 and 680 nm, respectively (Gantt and Lipschultz, 1973; Glazer and Bryant, 1975). Relative to the control, the phycobilisomes from nitrogen-starved cells showed decreased short wavelength fluorescence emission with a consequent shift in the emission maximum from 673 to 676 nm. Thus, the partial loss of phycocyanin upon nitrogen starvation has little effect on the efficiency of energy transfer within the residual phycobilisome structure.

Table 1. Spectroscopic properties of phycobilisomes from *Synechococcus* sp. 6301 after varying periods of combined nitrogen starvation

Sample	Duration of combined nitrogen starvation	Duration of incubation with chloramphenicol	Absorption		Fluorescence emission
			λ_{max}	A_{624}:A_{654}	λ_{max}
Experiment 1					
Control	–	–	624	2.6	673
1	1 day	–	626	1.9	676
2	2 days	–	626	2.0	676
3	3 days	–	627	1.7	673
Experiment 2					
Control	–	–	624	2.6	673
+ Cm	–	2 days	624	2.6	673
$-NO_3$	2 days	–	626	2.0	676
+ Cm } $-NO_3$	2 days	2 days	624	2.5	673
Experiment 3[a]					
Control	–	–	623	2.7	675
$-NO_3$	2 days	–	626	2.2	678
+ Cm } $-NO_3$	2 days	1 day	624	2.4	675

[a] Cells used in this experiment were grown for 3 days at 39°C and gassed with 1% CO_2 : 99% N_2, then for 2 days at 30°C with air prior to the onset of combined nitrogen starvation

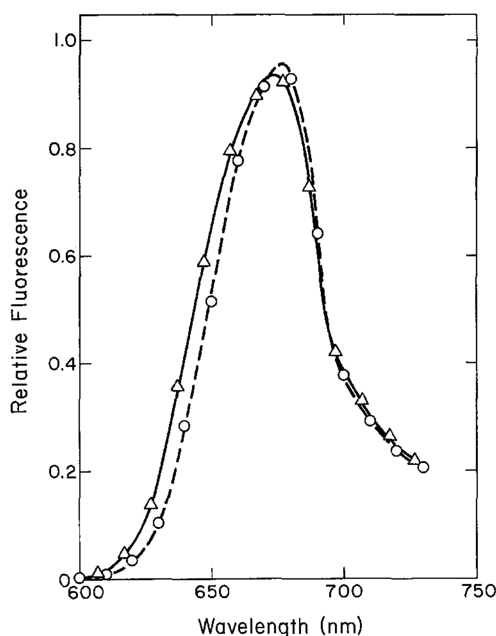
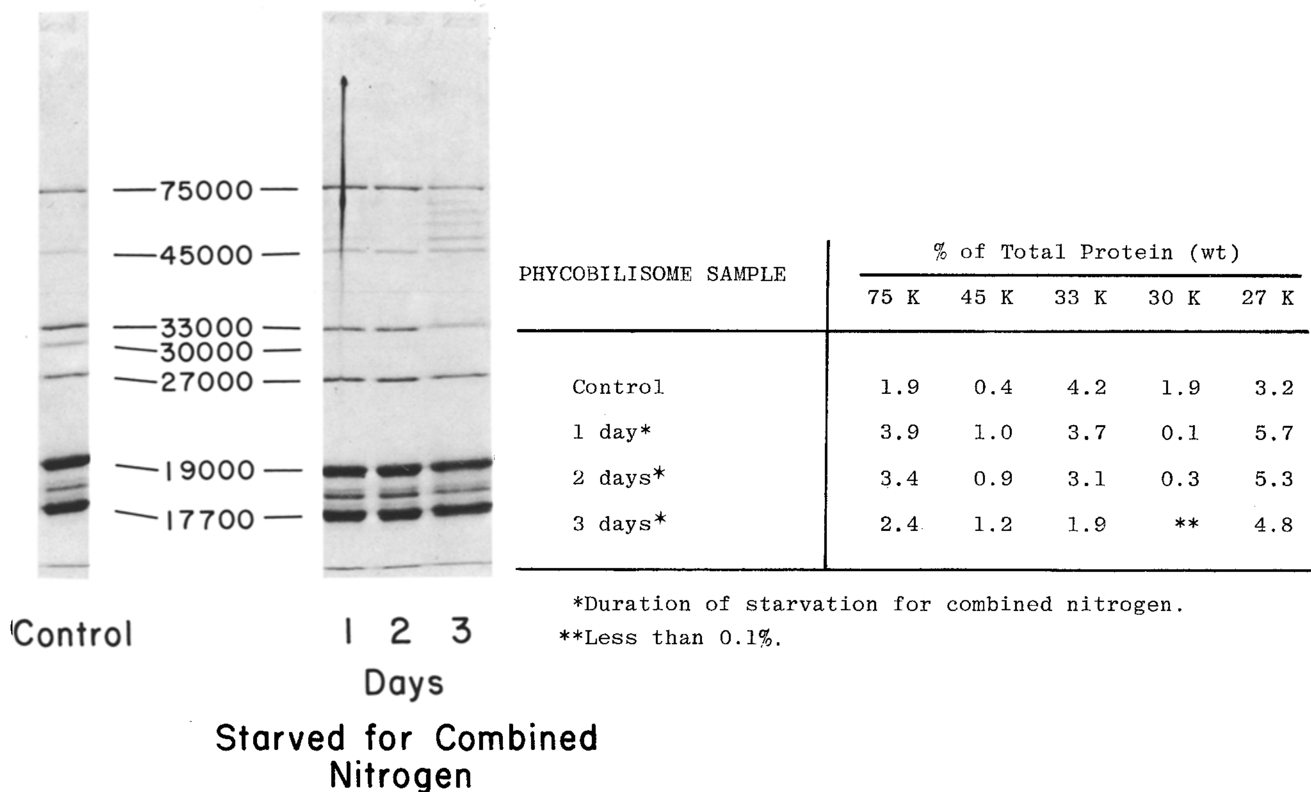


Fig. 2. Corrected fluorescence emission spectra of phycobilisomes from control cells (△——△), or cells starved 2 days for combined nitrogen prior to phycobilisome isolation (○----○). Samples were diluted into 0.75 M NaK-PO_4, pH 8.0, to a final protein concentration of 14 μg/ml. Excitation was at 580 nm and excitation and emission slits were set at 4.0 nm bandpass

To examine possible changes in the polypeptide composition of phycobilisomes induced by nitrogen starvation, samples were analyzed by SDS gel electrophoresis. As shown previously (Yamanaka et al., 1978), phycobilisomes from *Synechococcus* sp. contain five non-pigmented polypeptides in addition to the α and β subunits of the phycobiliproteins, phycocyanin, allophycocyanin, and allophycocyanin B, this last protein not being resolvable in our gel system. Figure 3 compares the SDS gel patterns of phycobilisomes prepared from nitrogen-starved cells with those of control cells. After 1 day of nitrogen starvation, there was a selective loss of a 30,000 dalton non-pigmented polypeptide from the phycobilisomes still present in the cell. As shown in the Table to Fig. 3, the loss of this component was by far the most significant change that occurred during days 1 and 2 of starvation. Other changes, such as the increase in intensity of the 75,000, 45,000 and 27,000 dalton bands reflect an enrichment relative to phycocyanin; the latter protein normally accounts for about 75% of the total phycobilisome protein in *Synechococcus* sp. (Yamanaka et al., 1978). By day 3 of nitrogen starvation, the amount of phycobilisome material was about 30-fold below normal levels, and a decrease in the amount of the 33,000 and 75,000 dalton polypeptides was also apparent (Fig. 3).

SDS-polyacrylamide gel analyses (not shown) of the material from the band 2 regions of sucrose gradients (Fig. 1A) showed a selective loss of phycocyanin subunits, which paralleled the changes in the absorption spectra (Fig. 1B). In comparison with the samples from control cells, the band 2 regions from 1- or 2-day starved cells showed a large decrease in the intensity of the components corresponding to the α and β subunits of phycocyanin relative to those derived from allophycocyanin.



PHYCOBILISOME SAMPLE	% of Total Protein (wt)				
	75 K	45 K	33 K	30 K	27 K
Control	1.9	0.4	4.2	1.9	3.2
1 day*	3.9	1.0	3.7	0.1	5.7
2 days*	3.4	0.9	3.1	0.3	5.3
3 days*	2.4	1.2	1.9	**	4.8

*Duration of starvation for combined nitrogen.
**Less than 0.1%.

Fig. 3. Polyacrylamide gel electrophoresis in the presence of SDS of phycobilisomes from control and nitrogen starved cells. The α and β subunits of *Synechococcus* 6301 phycocyanin have molecular weights of 17,000 and 19,000, respectively. The remaining numbers represent the molecular weights of the five non-pigmented phycobilisome polypeptides. The table on the right shows the percent protein concentration by weight of the five non-pigmented polypeptides relative to the total phycobilisome protein. Values were obtained by densitometer scans of the Coomassie Blue – stained gels, shown on the left, in which the areas under each peak were cut out and weighed

Effects of Chloramphenicol on Phycobilisome Turnover

To examine the possible interrelationship between protein synthesis and phycobilisome turnover, cells were incubated with chloramphenicol for 2 days prior to phycobilisome isolation. When such treatment was carried out either in the presence or absence of combined nitrogen, no effect on phycobilisomes comparable to simple starvation was seen. Phycobilisomes from chloramphenicol-treated cells displayed sedimentation properties, absorption and fluorescence emission maxima, and 624:654 nm absorption ratios characteristic of control samples (Table 1, experiment 2).

The SDS gel profiles of phycobilisomes described above are shown in Fig. 4, lanes 1–4. In this analysis only the sample from nitrogen-starved cells (lane 3) differed noticeably from the control. Lane 4 shows that the presence of chloramphenicol prevented the loss of the 30,000 dalton polypeptide from phycobilisomes, normally seen upon nitrogen starvation. This observation is consistent with the spectroscopic data which show that phycobilisome turnover brought about by nitrogen starvation is not induced by chloramphenicol, but on the contrary is prevented (Table 1).

A further relevant question was to consider whether continuous protein synthesis is required for phycobilisome turnover or whether the effects of nitrogen starvation may proceed independently following an induction event. To answer this question, duplicate cultures were incubated in nitrogen starvation medium; chloramphenicol was added to one culture 1 day after the onset of nitrogen starvation, and both cultures (along with a control) were harvested on day 2 and used for phycobilisome isolation. As shown in Table 1, experiment 3, the phycobilisome prepared from chloramphenicol-treated, nitrogen-starved cells displayed spectroscopic properties intermediate between those of control and nitrogen-starved samples. It should be noted at this point that this experiment was performed on cells which were cultured at 39°C in an atmosphere of 1 % CO_2 – 99 % N_2 until 2 days prior to the initiation of nitrogen starvation. As a consequence, these cells contained a higher than normal concentration of phycocyanin (see Goedheer, 1976), and

displayed spectroscopic properties slightly different from those seen in experiments 1 and 2 (Table 1). This high phycocyanin concentration, however, did not prevent the nitrogen starvation-induced disappearance of the 30,000 dalton polypeptide band seen by SDS gel electrophoresis (Fig. 4, lane 6). It is apparent from this figure that the sample from chloramphenicol-treated, nitrogen-starved cells (lane 7) displayed a behavior intermediate between that of the control (lane 5) and nitrogen starvation sample (lane 6).

Methionine Starvation

Since the inhibition of protein synthesis by chloramphenicol occurs at the level of polypeptide synthesis (Pestka, 1971) and does not involve depletion of amino acids (a condition which might be predicted for nitrogen starvation), an alternative approach was employed to investigate the effects of decreased protein synthesis on phycobilisome turnover. This approach involved the use of a methionine requiring auxotroph of *Synechococcus* sp. 6301, strain AN1, which produces phycobilisomes identical in spectroscopic properties and polypeptide composition to those of the parent organism. This mutant was used in a methionine starvation experiment similar to the initial nitrogen starvation study. Cells were grown to mid-log phase in methionine-supplemented BG-11, washed, and starved for methionine for 2, 3, and 4 days, then harvested and used for phycobilisome isolation. In no case was chlorosis of cultures observed, nor were there significant variations in spectroscopic properties or sedimentation rates of phycobilisomes on sucrose gradients. Figure 5, experiment 4 shows that the 30,000 dalton polypeptide did not disappear in phycobilisomes from methionine-starved cultures. Indeed, its level was similar to that in phycobilisomes from chloramphenicol-treated cultures. The fluorescence emission spectra of phycobilisomes also show that methionine starvation does not promote the long wavelength shift seen in the particles from nitrogen-starved cells. The fluorescence emission spectra of phycobilisomes of control and methionine-starved (2 days) cells were qualitatively identical.

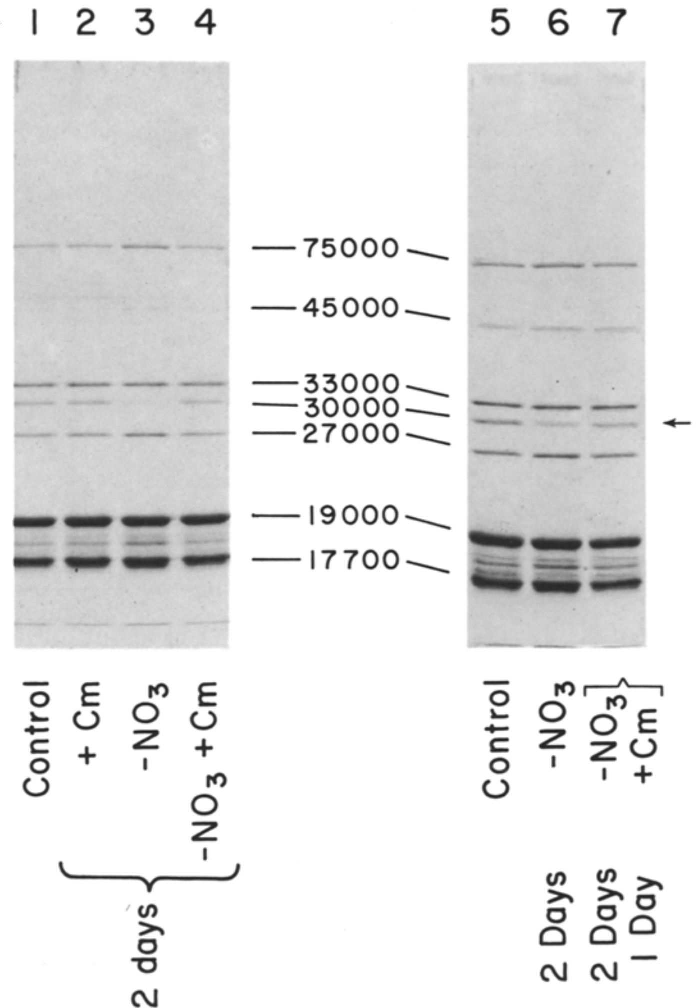


Fig. 4. The effect of combined nitrogen starvation and chloramphenicol treatment on phycobilisome polypeptide composition. SDS polyacrylamide gel electrophoresis was performed on phycobilisomes from control cells or cells incubated under the conditions shown. Where indicated, chloramphenicol was added to a final concentration of 25 µg/ml. The arrow points to the polypeptide ($M_r = 30{,}000$) which most rapidly disappears upon nitrogen starvation

Discussion

Phycobiliproteins make up a major portion of the soluble cell protein in cyanobacteria, up to 40% under optimal growth conditions (Myers and Kratz, 1955), but are not required in the photochemical reactions of photosynthesis (Arnon et al., 1974). In 1969, Allen and Smith showed that cellular levels of phycocyanin in the unicellular cyanobacterium *Synechococcus* sp. 6301 *(Anacystis nidulans)* decreased markedly when cells were deprived of a source of fixed nitrogen. Recently, Lau et al. (1977) showed that the loss in phycocyanin absorbance (usually used as an assay for phycocyanin degradation) is accompanied by a loss of the polypeptide components on SDS-polyacrylamide gels which correspond to the α and β subunits of this protein. On pulse labelling with ^{14}C-leucine under nitrogen starvation conditions, active incorporation of the amino acid into cell protein was demonstrated, but no incorporation of radioactivity into phycocyanin could be shown (Lau et al., 1977). It appears, therefore, that during "nitrogen chlorosis" pre-existing phycocyanin

Gel Slot	Strain*	Duration of Combined Nitrogen Starvation	Duration of Methionine Starvation	Duration of Incubation with Chloramphenicol	30 K Band Present
Experiment 1					
Control	6301	-	-	-	+
1	"	1 day	-	-	-
2	"	2 days	-	-	-
3	"	3 days	-	-	-
Experiment 2					
5	6301	-	-	-	+
6	"	-	-	2 days	+
7	"	2 days	-	-	-
8	"	2 days	-	2 days	+
Experiment 3					
9	6301	-	-	-	+
10	"	2 days	-	-	-
11	"	2 days	-	1 day	+
Experiment 4					
12	AN1*	-	-	-	+
13	"	-	2 days	-	+
14	"	-	3 days	-	+
15	"	-	4 days	-	+

*AN1 is a methionine-requiring, nitrosoguanidine-induced mutant of the strain 6301. The control growth medium for strain AN1 was BG-11 supplemented with 20 μg/ml of L-methionine.

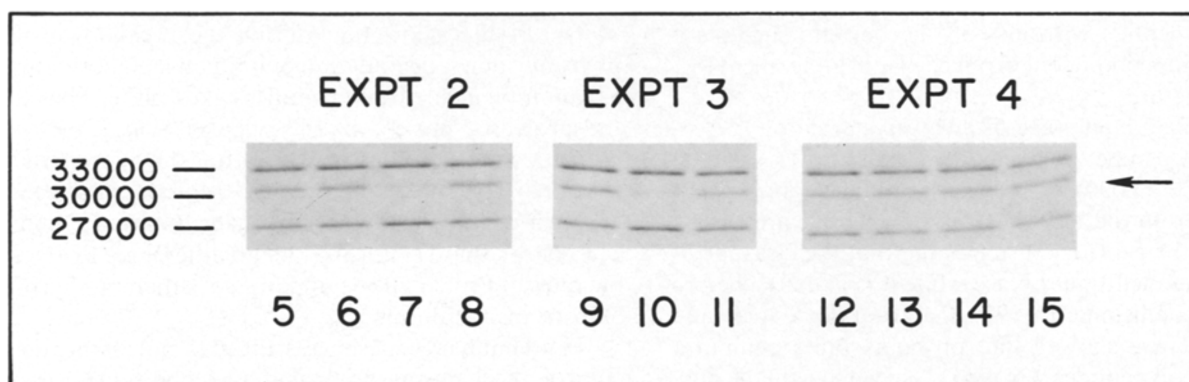


Fig. 5. A summary of experiments revealing the presence or absence of the 30,000 dalton phycobilisome polypeptide when cells are grown under the conditions described. The segments of SDS gels in the lower half of the figure refer to the experiments shown above. The arrow points to the 30,000 dalton polypeptide, absent in lanes 7 and 10. See footnote to Table 1 for exact conditions employed in expt. 3

is degraded and the synthesis of new phycocyanin is repressed.

The phycobilisome plays a dual role in photosynthesis and cellular nitrogen storage. Such a role is reasonable when one considers the abundance of cellular nitrogen contained in phycobilisomes, and also the low priority for light-harvesting by an accessory pigment system during nutrient starvation.

The changes brought about during the first stage of nitrogen starvation in the physical and spectroscopic properties of phycobilisomes show a preferential loss of phycocyanin from the structure. The phycobilisomes from partially chlorosed cells sediment at a slower velocity, have absorption and emission spectra shifted to the red (Table 1), and higher allophycocyanin:phycocyanin ratio. The suggested models for the location of the individual phycobiliproteins within membrane-bound phycobilisomes (Gantt, 1975; Gantt et al., 1976; Morschel et al., 1977) place phycocyanin in a position peripheral to allophycocyanin and allophycocyanin B. The known pathway for the transfer of absorbed light energy is phycocyanin → allophycocyanin → allophycocyanin B → chlorophyll *a* (Ley et al., 1977; Glazer, 1977). Consequently, partial loss of phycocyanin need not lead to a decrease in the effectiveness of the residual structure in energy transfer. This view is supported by the fluorescence emission data, which show efficient energy coupling between phycocyanin and allophycocyanin B in phycobilisomes from nitrogen-starved cells (Fig. 2).

Only one non-pigmented polypeptide (M_r = 30,000) is lost during the first 2 days of nitrogen starvation (see Fig. 3). This loss coincides with shifts in both absorption and fluorescence emission spectra to longer wavelengths and the decrease in phycobilisome sedimentation velocity. The data suggest that the 30,000 dalton polypeptide is closely associated with the peripheral phycocyanin in the phycobilisome, since the remaining particle from which it is absent shows unimpaired energy transfer. The concomitant loss of this polypeptide with loss of phycocyanin suggests a role for this component in phycocyanin attachment to a core structure.

During a later stage of nitrogen starvation (Fig. 3, day 3), two other polypeptides are lost (M_r = 75,000 and M_r = 33,000). Proteolysis products presumably derived from the 75,000 dalton polypeptide are clearly seen on the gel (Fig. 3) suggesting that the cleavage of this component takes place while it is still attached to the phycobilisome. In 1977, Tandeau de Marsac and Cohen-Bazire showed that phycobilisomes contain a class of polypeptides (Group I) associated with the photosynthetic membrane. In studies with several strains of cyanobacteria, these investigators broke cells in buffers of low ionic strength in the absence of detergents and found that under such conditions Group I polypeptides remained in the membrane fraction. Since in the case of *Synechococcus* phycobilisomes, the 75,000 dalton component belongs to this group, its proteolysis after day 3 of starvation (see Fig. 3) suggests that the phycobilisomes remaining at this late stage in nitrogen deprivation may be detached from the photosynthetic lamellae. Such an interpretation necessarily implies a termination in the light-harvesting function of these structures. It is at this stage that fluorescence emission of phycobilisomes shows a small but distinct shift to shorter wavelengths (Table 1), and the structures become less stable. In the samples shown in Table 1, experiment 1, the fluorescence emission maxima shifted after 15 days of storage at 4° C as follows: control and 1 day of nitrogen starvation, no shift; 2 days, 676 to 674 nm; 3 days, 673 to 670 nm. Allen and Smith (1969) had shown that the viable cell count of a nitrogen-starved culture of *Anacystis nidulans* reached a minimum around the time when phycocyanin was no longer detectable.

The enzymes responsible for phycobilisome turnover during nitrogen starvation have not been adequately identified, nor is the signal which triggers this turnover understood. Several groups have reported phycocyanin-degrading activities in cyanobacteria, but in no instance has the enzyme (or enzymes) responsible been isolated and characterized (Lau et al., 1977; Foulds and Carr, 1977; Wood and Haselkorn, 1977). Wood and Haselkorn (1977) have described two proteolytic activities in the nitrogen-fixing cyanobacterium *Anabaena* sp. 7120. One activity is induced upon heterocyst differentiation and is associated with the degradation of most of the vegetative cell proteins which turn over during heterocyst development with the exception of the phycobiliproteins. The other is present in the membranes of NH_4^+-grown cells, and was reported to be specific for phycobiliproteins when assayed in vitro following release from membranes by the nonionic detergent Nonidet P-40.

Our results show that during the breakdown of phycobilisomes, degradation of both phycobiliproteins and non-pigmented polypeptides takes place. Hence, the proteases present in the cells of *Synechococcus* sp. 6301 during nitrogen starvation show no strict specificity for phycobiliproteins. Moreover, phycocyanin is degraded more rapidly than allophycocyanin. This shows that significant kinetic differences exist in the rates of proteolytic degradation of different phycobiliproteins within the cell.

In preliminary experiments aimed at elucidating the control mechanisms governing phycobilisome turnover, we examined the consequences of inhibiting protein synthesis in two different ways. Inhibition of the peptidyl transferase reaction in wild-type cells with

chloramphenicol did not trigger phycobilisome turnover. Treatment with chloramphenicol concomitantly with nitrogen starvation prevented phycobilisome degradation. This result suggests that when protein synthesis is halted, the major need for free amino acids is eliminated and, hence, the breakdown of phycobilisomes ceases. Starvation for a single amino acid does not initiate phycobilisome degradation as shown by our finding that starvation of a methionine auxotroph for methionine does not result in a decrease in phycobilisome content or change in phycobilisome composition. Wood and Haselkorn (1978) likewise did not detect phycobiliprotein degradation during methionine starvation of a methionine auxotroph of *Anabaena variabilis*. The level of the amino acid pool is related to the level of products of nitrogen metabolism, and it is possible that one of the latter metabolites (e.g., glutamine) controls the induction of phycobilisome degradation.

It is possible that nitrogen starvation results in a signal which induces the synthesis of a new protease (or proteases). To test this possibility, chloramphenicol was added to wild-type cells which had been starved for nitrogen for 24 h. Semi-quantitative analysis (Table 1 and Fig. 5) indicated that further turnover of phycobilisomes was stopped by the antibiotic. This implies that if a new proteolytic enzyme appears upon nitrogen starvation, its half-life must be relatively short.

Acknowledgements. This work was supported in part by grant PCM 76-15243A01 from the National Science Foundation. G.Y. was supported by a fellowship from U.S. Public Health Service Training Grant T32 GM07232-02.

References

Allen, M. M., Smith, A. J.: Nitrogen chlorosis in blue-green algae. Arch. Mikrobiol. **69**, 114–120 (1969)

Arnon, D. I., McSwain, B. D., Tsujimoto, H. Y., Wada, K.: Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll *a* and removal of phycocyanin. Biochim. biophys. Acta (Amst.) **357**, 231–245 (1974)

Foulds, I. J., Carr, N. G.: Proteolytic enzyme degrading phycocyanin in the cyanobacterium *Anabaena cylindrica*. FEMS Lett. **2**, 117–119 (1977)

Gantt, E.: Phycobilisomes: light-harvesting pigment complexes. Bioscience **25**, 781–788 (1975)

Gantt, E., Lipschultz, C. A.: Energy transfer in phycobilisomes from phycoerythrin to allophycocyanin. Biochim. biophys. Acta (Amst.) **292**, 858–861 (1973)

Gantt, E., Lipschultz, C. A., Zilinskas, B. A.: Phycobilisomes in relation to the thylakoid membranes. Brookhaven Symposia Biol. **28**, 347–357 (1976)

Glazer, A. N.: Structure and molecular organization of the photosynthetic accessory pigments of cyanobacteria and red algae. Mol. cell. Biochem. **18**, 125–140 (1977)

Glazer, A. N., Bryant, D. A.: Allophycocyanin B (λ_{max} 671, 618 nm). A new cyanobacterial phycobiliprotein. Arch Microbiol. **104**, 15–22 (1975)

Goedheer, J. C.: Spectral properties of the blue-green alga *Anacystis nidulans* grown under different environmental conditions. Photosynthetica **10**, 411–422 (1976)

Lau, R. H., MacKenzie, M. M., Doolittle, W. F.: Phycocyanin synthesis and degradation in the blue-green bacterium *Anacystis nidulans*. J. Bacteriol. **132**, 771–778 (1977)

Lemasson, C., Tandeau de Marsac, N., Cohen-Bazire, G.: Role of allophycocyanin as a light-harvesting pigment in cyanobacteria. Proc. nat. Acad. Sci. (Wash.) **70**, 3130–3133 (1973)

Ley, A. C., Butler, W. L., Bryant, D. A., Glazer, A. N.: Isolation and function of allophycocyanin B of *Porphyridium cruentum*. Plant Physiol. **59**, 974–980 (1977)

Morschel, E., Koller, K. P., Wehrmeyer, W., Schneider, H.: Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea*. I. Electron microscopy of phycobilisomes in situ and analysis of their architecture after isolation and negative staining. Cytobiologie **16**, 118–129 (1977)

Myers, J., Kratz, W.: Relations between pigment content and photosynthetic characteristics in a blue-green alga. J. gen. Physiol. **39**, 11–22 (1955)

Pestka, S.: Inhibition of ribosome function. Ann. Rev. Microbiol. **25**, 487–562 (1971)

Sherman, L. A., Cunningham, J.: Isolation and characterization of temperature-sensitive, high-fluorescence mutations of the blue-green alga, *Synechococcus cedrorum*. Pl. Sci. Lett. **8**, 319–326 (1977)

Stanier, R. Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G.: Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. **35**, 171–205 (1971)

Stevens, C. L. P., Stevens, S. E., Myers, J.: Isolation and initial characterization of a uracil auxotroph of the blue-green alga *Anacystis nidulans*. J. Bacteriol. **124**, 247–251 (1975)

Tandeau de Marsac, N., Cohen-Bazire, G.: Molecular composition of cyanobacterial phycobilisomes. Proc. nat. Acad. Sci. (Wash.) **74**, 1635–1639 (1977)

Wood, N. B., Haselkorn, R.: Protein degradation during herocyst differentiation in the blue-green alga *Anabaena* 7120. Abst. Fed. Proc. **36**, 886 (1977)

Wood, N. B., Haselkorn, R.: Control of phycobiliprotein degradation during nitrogen starvation in *Anabaena*. Abst. Proc. Steenbock-Kettering Intern. Symp. Nitrogen Fixation (Madison, Wisconsin) C-33 (1978)

Yamanaka, G., Glazer, A. N., Williams, R. C.: Cyanobacterial phycobilisomes. Characterization of the phycobilisomes of *Synechococcus* sp. 6301. J. biol. Chem. **253**, 8303–8310 (1978)

Received February 2, 1979