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NATIVE AND IN VITRO-ASSOCIATED PHYCOBILISOMES OF *NOSTOC* sp.

COMPOSITION, ENERGY TRANSFER, AND EFFECT OF ANTIBODIES

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The relationship of the structure and function of the light-harvesting antennae in the blue-green alga *Nostoc* sp. was further elucidated by reconstitution experiments. Separated phycoerythrin-phycocyanin complexes and allophycocyanin fractions were reassociated as described earlier (Canaani, O., Lipschultz, C.A. and Gantt, E. (1980) FEBS Lett. 115, 225–229) into functional phycobilisomes with a 70% yield. Native and reassociated phycobilisomes had molar ratios of about 1.4:1.1:1.0 of phycoerythrin:phycocyanin:allophycocyanin. Energy transfer was demonstrated by their fluorescence emission maximum at approx. 675 nm (20°C), and their excitation spectra (emission wavelength 680 nm) which reflected the contribution of the three constitutive phycobiliproteins. Scans of Coomassie blue-stained SDS-polyacrylamide gels showed that the polypeptide composition of native and reassociated phycobilisomes was virtually indistinguishable. Reassociation of phycobilisomes was dependent on the interaction of allophycocyanin and phycocyanin, because it could be blocked with antisera to phycocyanin and allophycocyanin, but not to phycoerythrin. In addition, reassociation did not occur when a 31 000 Da polypeptide, which is part of the phycoerythrin-phycocyanin complex, was reduced in size (by 4000 Da). These results suggest that at least two domains are required for functional reassociation of phycobilisomes involving phycocyanin and allophycocyanin.

Introduction

The light-harvesting proteins of blue-green algae (phycobiliproteins) are assembled into complex structures called phycobilisomes, that are attached to the outer surface of the thylakoid membranes. The light energy absorbed by the phycobiliproteins migrates with high efficiency to chlorophyll *a* by inductive resonance [1,2] and drives photosynthesis. The sequence of energy transfer in the isolated phycobilisome, as well as in the intact cell, is: phycoerythrin → phycocyanin → allophycocyanin [3,4]. Electron microscopy and immunological studies of phycobilisomes isolated from several blue-green and red algae have shown that in many species these particles consist of an allophycocyanin core and phycoerythrin-phycocyanin peripheral rods [5–7]. The general structure of phycobilisomes, as first described in the red alga *Rhodella violacea* [6], contains a triangular core, from which radiate up to six rods composed of three to five stacked discs. This type of phycobilisome is present in several cyanobacteria, including *Nostoc* sp. [7–10]. In addition to

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phycobiliproteins, phycobilisomes contain several polypeptides which have been named 'linker' polypeptides [11]. These polypeptides were first observed by Tandeau de Marsac and Cohen-Bazire [12] and were suggested to be essential for the assembly of phycobiliproteins into disc-shaped aggregates and for the interaction between these discs in the formation of the phycobilisome structure [11–15].

The main criteria by which phycobilisomes are defined, are their absorption and fluorescence spectra [16], their morphology as observed by electron microscopy and their position on the isolation gradient [16–18]. Intact phycobilisomes recovered from a 1 M sucrose layer of a 0.75 M potassium phosphate-buffered sucrose step gradient have a fluorescence emission at about 675 nm (20°C), independent of the excited phycobiliprotein [16]. The first in vitro reassociation of phycobilisomes from isolated phycobiliprotein fractions was reported for *Nostoc* sp. [18]. Phycobilisomes had been dissociated and separated into two fractions. One fraction consisted of the entire allophycocyanin complement [19], while another fraction consisted of phycoerythrin and phycocyanin in a functional complex [18]. From these fractions phycobilisomes were reassembled in 0.75 M potassium phosphate/2 M sucrose essentially by reversing the dissociation conditions. Moreover, hybrid phycobilisomes were obtained from two blue-green algae by reassociation of allophycocyanin of *Nostoc* sp. with the phycoerythrin-phycocyanin complex of *Fremyella diplosiphon* and vice versa [9].

Recently, in vitro reassociation of phycobilisomes from *Nostoc* sp. was also reported by Glick and Zilinskas [20], using a modified method whereby the phycobiliproteins were separated on brushite columns and reassociated in 0.75 M potassium phosphate in the absence of sucrose. However, *Nostoc* sp. phycobilisomes obtained from the two laboratories, using the same algal strain, were different in their absorption and emission spectra. The native and reassociated phycobilisomes in our laboratory had a higher phycoerythrin content and, more importantly, they were energetically more tightly coupled.

This manuscript provides evidence that native and reassociated phycobilisomes were fully intact by their fluorescence excitation and emission spectra, and that they have a highly similar polypeptide composition. Additionally, it was shown that reassociation was prevented by antisera specific for phycocyanin and allophycocyanin, and the degradation of a 31 000 Da polypeptide.

Materials and Methods

Culture conditions and isolation of phycobilisomes. Nostoc cells were grown for 10–14 days at 37°C in a liquid medium [21] supplied with 5% CO_2 and 95% air with continuous shaking. The cultures were continuously irradiated with daylight/fluorescent light (about 1500 $\mu W/cm^2$). Some cultures were also grown in red light (above 600 nm, at approx. 1000 $\mu W/cm^2$) by using a red plastic filter (P-14, Gelatin Products, Glen Cove, NY) over cool white deluxe lamps (Sylvania F48T12/CWX/VHO) which had an increased output in the red wavelength region. Phycobilisomes were isolated by the procedure of Gantt et al. [16], except for some modification. Instead of breaking the cells in a French pressure cell, the cell suspension was mixed with Triton X-100 to a final concentration of 3% and incubated for 2 h with stirring as described in Ref. 22. The isolation procedure routinely used in our laboratory was then followed.

Separation of phycobiliproteins. Phycobilisomes were suspended (2–4 mg protein/ml) in 0.4 M potassium phosphate buffer (pH 7.0) and dialyzed for 2 h at 22°C against 0.1 M potassium phosphate buffer/0.1 M NaCl/pH 7.0. A 2 ml sample was layered on a linear gradient (20 ml), 0.25–1.0 M sucrose/0.4 M potassium phosphate/pH 7.0, over 2 M sucrose (4 ml) and sedimented at 136 000 $\times g$, for 4 h at 20°C. An allophycocyanin-rich fraction was collected by syringe from the upper portion of the gradient, and the fraction of phycoerythrin-phycocyanin existing as a complex from the middle [18].

Since the allophycocyanin fraction contained 5% phycoerythrin it was further purified by chromatography on a brushite column (2.0 × 16 cm). Following a 0.01 M potassium phosphate step allophycocyanin was eluted with 0.4 M potassium phosphate, pH 7.0, and was phycoerythrin free as ascertained by fluorescence. Alternatively, the allophycocyanin fraction was separated from resid-

ual phycoerythrin by re-centrifugation on the separation gradient. Phycobilisomes from red-light-grown cells were isolated as described above. Upon dissociation and separation of phycobilisomes on the linear sucrose gradient buffered with 0.4 M potassium phosphate, pH 7.0, a major band of phycocyanin complex was obtained near the middle of the gradient and only a minor band below it contained a small amount of phycoerythrin. The phycocyanin complex was removed from the gradient for further analysis.

In vitro association. The fractions isolated as above were mixed as described in Table I and dialyzed at room temperature (approx. 20°C) for 6–8 h in 0.75 M potassium phosphate, 2.0 M sucrose, pH 7.0. After further dialysis for 3 h in 0.75 M potassium phosphate, 2-ml samples were layered on a sucrose gradient as used for phycobilisome isolation. Phycobilisomes were recovered in the 1 M sucrose layer.

Spectroscopic measurements. Spectra were measured at room temperature under conditions previously described [4]. For fluorescence spectra samples were diluted in potassium phosphate (pH 7.0) to a protein concentration of approx. 35 μg/ml (absorbance approx. 0.1 at maximum of predominant phycobiliprotein). Phycobiliprotein concentrations were always determined by absorbance of samples in low ionic conditions (0.1 M potassium phosphate, pH 7.0) using the following molar extinction coefficients: C-phycoerythrin, $4.88 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$, 562 nm [23]; C-phycocyanin $2.81 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$, 620 nm [24]; allophycocyanin $2.3 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$, 650 nm [25]. The relative absorbance of phycocyanin at 650 nm/620 nm = 0.2 and of allophycocyanin at 620 nm/650 nm = 0.47. Application of the extinction coefficients determined by Bennett and Bogorad [26] yielded essentially the same phycobiliprotein ratios.

SDS-polyacrylamide gel electrophoresis. Separation of polypeptides was essentially carried out according to the method of Laemmli [27] on 1.5 or 3 mm thick slab gels (15 × 30 cm) with a gradient of 8–15% acrylamide (30 : 0.8 acrylamide : bisacrylamide) containing 0.1% SDS. For electrophoresis, samples were dissolved in 1% SDS (w/v)/1% β-mercaptoethanol (v/v)/50 mM Tris-HCl/ 0.002% bromophenol blue (w/v)/10% glycerol (v/v) and heated (100°C) for 3 min. Following electrophoresis (12 h, 20°C at constant 10 mA) gels were stained with Coomassie blue R-250 and destained in 10% acetic acid. To determine the apparent molecular weight of the polypeptides, marker proteins in the molecular weight range 14400–200000 (Bio-Rad, Richmond, CA) were used.

Antisera. Conditions for preparation of antisera to phycoerythrin, R-phycocyanin and allophycocyanin were previously described in Ref. 5. An antiserum specific against C-phycocyanin from *Phormidium calothricoides* was a gift from Dr. D. Berns. The antisera were specific for the phycobiliprotein class, i.e., anti-phycoerythrin reacted only against phycoerythrin, anti-phycocyanin reacted only against phycocyanin, and anti-allophycocyanin reacted only against allophycocyanin, whether the phycobiliprotein was derived from red or blue-green algae. Preliminary to precipitation reactions reported in this study, serial dilutions of the phycoerythrin-phycocyanin complex and allophycocyanin fraction were made separately to determine the proper concentration of antisera and antigen needed to reach the equivalence zone. Protein concentrations of phycoerythrin and phycocyanin in the phycoerythrin-phycocyanin complex were in the range of 110 and 90 μg/ml, respectively, and were sufficient to form a colored precipitate with the antisera used usually within 1–2 h. Protein concentration of reassociable allophycocyanin fraction was in the range of 90 μg/ml and formed a blue precipitate with anti-allophycocyanin within 1 h. The appropriate antisera in 0.75 M potassium phosphate buffer, pH 7.0, were incubated at 20°C for 2 h in an equal volume of (a) phycoerythrin-phycocyanin plus anti-phycoerythrin; (b) phycoerythrin-phycocyanin plus anti-phycocyanin. Reassociable allophycocyanin fraction was added to the reaction mixture followed by overnight dialysis against 0.75 M potassium phosphate buffer in 2 M sucrose. In a similar manner, reassociable allophycocyanin fraction was incubated for 2 h with an equal volume of anti-allophycocyanin in 0.75 M potassium phosphate, pH 7.0, at 22°C followed by the addition of phycoerythrin-phycocyanin complex and overnight dialysis against the same buffer in 2 M sucrose. Reassociation was assayed by scans of the fluorescence emission (550–700 nm) at approx. 20°C.

Results

The yield of reassociation of total allophycocyanin and the phycoerythrin-phycocyanin complex into functional phycobilisomes was dependent on the concentration of the interacting phycobiliproteins (Table I). Association was enhanced by high protein concentration, reaching a maximal 70% yield (Expt. 3). Lowering protein concentration by about 5-fold resulted in substantial decrease in the yield to approx. 20% (Expt. 1). Large aggregates equivalent to phycobilisomes were not obtained with allophycocyanin fractions (Expt. 4), or with phycoerythrin-phycocyanin fractions (Expt. 5) alone even at high protein concentrations. These results indicate that phycobilisome reassociation involved specific interactions between the phycoerythrin-phycocyanin complex with the allophycocyanin fraction. The molar ratio of phycoerythrin : phycocyanin : allophycocyanin of approx. 1.4 : 1.1 : 1.0 was virtually the same in reassociated and native phycobilisomes. Performing the reassociation in the presence of a 2-fold excess of allophycocyanin relative to phycoerythrin-phycocyanin did not affect the molar ratio of the phycobilisomes formed in vitro. Similarly, a 2-fold excess of phycoerythrin-phycocyanin relative to allophycocyanin in the reassociation mixture did not change the molar ratio of the phycobilisomes.

It was already reported that the phycoerythrin-phycocyanin fraction by itself was a functional complex, transferring energy from phycoerythrin to phycocyanin as shown by absorption and fluorescence spectra [19]. But it should be noted that when the phycoerythrin-phycocyanin complex was completely dissociated and the constituent phycoerythrin and phycocyanin were separated by either sucrose gradient centrifugation or brushite chromatography, the components retained the ability to recombine. When phycoerythrin was mixed with phycocyanin in 0.4–0.75 M potassium phosphate, pH 7.0, reassociation into a functionally transferring complex quickly occurred with 90% recovery of the phycobiliproteins in the phycoerythrin-phycocyanin complex. Similar reassociation also occurred with other phycoerythrin-phycocyanin complexes [14], in addition to that from *Nostoc* [20].

The similarity in the absorption spectra of native and in vitro-associated phycobilisomes, as illustrated in Fig. 1A, shows the phycoerythrin maxima at 553 and 573 nm, phycocyanin at 620 nm and allophycocyanin at 650 nm. The fluorescence excitation spectra of native and reassociated phycobilisomes (Fig. 1B) exhibited a shoulder at approx. 553 nm and a peak at 573 nm, another shoulder at 620 nm and a peak at 650 nm indicating that energy absorbed primarily by phycoerythrin and phycocyanin was effectively transferred to allophycocyanin-675 * and all the pigments were contributing to the emission at approx. 675 nm in both native and reassociated phycobilisomes. As previously noted [9], the quantum contribution of phycoerythrin, particularly in the 553 nm region, was lower than would have been expected from the absorption spectra. The fluorescence emission maximum appeared at 670–680 nm at 20°C (Fig. 1C) in native and reassociated phycobilisomes. At −196°C (Fig. 1C), most of the emission of reassociated phycobilisomes was located at 688 nm with minor peaks at 665 and 580 nm (from phycocyanin and phycoerythrin, respectively). Fluorescence, excitation and emission spectra clearly demonstrate that the reassociated phycobilisomes were functionally active and capable of transferring energy between phycobiliproteins. Emission at 20 and −196°C from allophycocyanin at approx. 670–680 and 688 nm, respectively, was high. At the same time it was very low from phycoerythrin (approx. 580 nm), the predominant phycobiliprotein. The separated allophycocyanin fraction had an absorption maximum at approx. 650 nm and fluorescence maximum at 665 nm which was resolved at −196°C into 665 and 688 nm peaks (not shown), and corresponded to the short- and long-wavelength * emitting allophycocyanin forms in *Nostoc* phycobilisomes. The allophycocyanin fraction contained some phycoerythrin (approx. 5%) which was not

* Two allophycocyanins (allophycocyanin-B and -I) with fluorescence emission at approx. 675–680 nm (20°C) in the light of new evidence [28,29] can no longer be considered as distinct phycobiliproteins as previously characterized [30,31]. To avoid confusion we propose the designation allophycocyanin-675 for the long-wavelength emitting form.

TABLE I

YIELD OF PHYCOBILISOMES ASSOCIATED IN VITRO

The phycobilisome yield represents recovery of phycobiliproteins, in 1 M sucrose gradient layer, of total layered on the gradient. The phycoerythrin: phycocyanin molar ratio was approx. 1.3 for all experiments.

Expt. No.	Phycobiliprotein reassociation mixture				Phycobilisome yield	
	Allophycocyanin		Phycoerythrin-phycocyanin		Protein (mg)	%
	concentration (mg/ml)	protein (mg)	concentration (mg/ml)	protein (mg)		
1	0.045	0.144	0.07	0.35	0.1	20
2	0.144	0.75	0.35	1.92	1.64	61
3	0.264	1.5	0.53	4.0	3.85	70
4	0.244	1.5	0	0	0	0
5	0	0	0.35	1.92	0	0

energetically coupled to allophycocyanin according to the emission spectra [18] and was easily removable on a brushite column. It is noteworthy that purified allophycocyanin from brushite reassociated with phycoerythrin-phycocyanin complex with equally high recovery of phycobilisomes. Similarly, some large molecular weight allophycocyanin (approx. 3%) which sometimes co-isolated with the phycoerythrin-phycocyanin fraction also was not energetically coupled because excitation of phycoerythrin did not produce emission from allophycocyanin (660 or 688 nm, −196°C).

The interaction between phycoerythrin-phycocyanin and allophycocyanin was examined by using

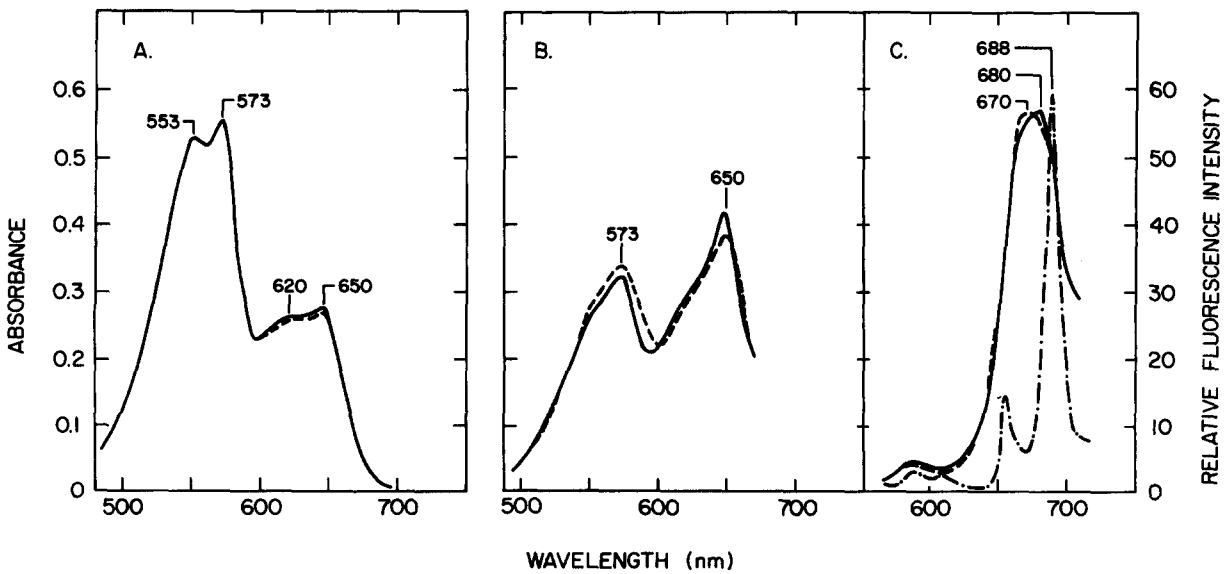

Fig. 1. Spectral characteristics of native (———) and reassociated (- - - - -) phycobilisomes at approx. 20°C in 0.75 M potassium phosphate buffer, pH 7.0. (A) Absorption spectra. (B) Excitation spectra, $\lambda_{em} = 675$ nm. (C) Emission spectra, $\lambda_{exc} = 545$ nm. Phycobilisomes at −196°C (– · – · –) had a main emission maximum at 688 nm, and smaller peaks at 580 nm (phycoerythrin) and 655 nm (phycocyanin). For excitation and emission spectra the absorbance at 570 nm ≈ 0.08. Excitation and emission spectra made in quantum-corrected mode were normalized at 550 nm.

antibodies specific to the phycobiliproteins. Antisera were reacted with the fractions prior to being placed under reassociation conditions and analyzed by fluorescence emission (Fig. 2). The fluorescence emission, at approx. 670–675 nm with a major shoulder at 680 nm, indicated that the association was not inhibited by anti-phycoerythrin. In contrast, preincubation of phycoerythrin-phycocyanin with anti-phycocyanin prevented the reassociation. Similarly, reaction of allophycocyanin with anti-allophycocyanin and subsequent incubation with phycoerythrin-phycocyanin also prevented reassociation into phycobilisomes. The fluorescence emission in the latter two experiments was at 655 nm, from phycocyanin, whereas the allophycocyanin was present in the mixture and was not functionally coupled. These results clearly indicate that association within the phycobilisome occurs

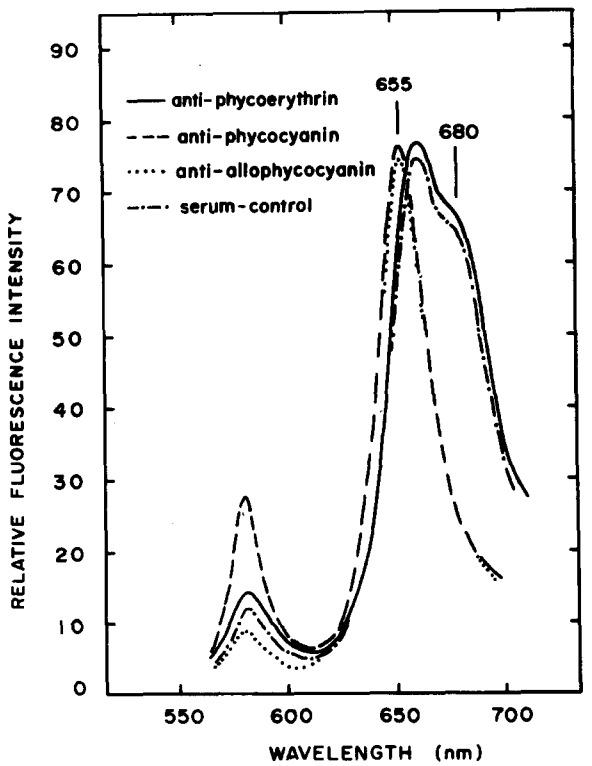

Fig. 2. Fluorescence emission spectra of phycobiliprotein mixtures incubated under reassociation conditions (see Materials and Methods). Reassociation was inhibited with antisera to phycocyanin and allophycocyanin. Reassociation occurred, as indicated by the transfer to 675–685 nm, with rabbit control serum and with antiserum to phycoerythrin.

between allophycocyanin and phycocyanin, and that the sites of interaction can be blocked by specific antibodies. In the presence of large excess of anti-phycocyanin, the equilibrium of phycoerythrin-phycocyanin complex was sometimes shifted because the antigen-antibody reaction caused partial dissociation of phycoerythrin-phycocyanin complex as indicated by increased emission from phycoerythrin (approx. 580 nm) (Fig. 2). Probably, the binding domains on the phycocyanin molecules became exposed during the precipitation with the excess antiserum.

Native and reassociated phycobilisomes were similar in polypeptide composition (Fig. 3) and morphology (Fig. 4). The polypeptides of native and reassociated (Fig. 3, lanes I and II, respectively) phycobilisomes were composed of the α- and β-subunits of allophycocyanin of 15 500–17 500 Da, as well as the α- and β-polypeptides of phycocyanin, 17 500–18 500, and of phycoerythrin, 18 500–20 000 Da. These colored polypeptides were not individually resolved on gels shown here due to overloading required to show clearly the uncolored polypeptides, but they were determined separately from isolated phycobiliproteins. Three distinct polypeptides were present with molecular masses of 31 000, 34 000 and 38 000 Da, respectively (Fig. 3, I and II). These polypeptides appeared colorless on SDS gels and were always present in the same proportion (Table II) in intact phycobilisomes. They did not show any significant degradation as long as the phycobilisomes were not in the dissociated state. By their molecular weight ranges and their isoelectric points (p*I* 6–7, unpublished results) which were more basic than those of the colored polypeptides, they were similar to polypeptides reported in other blue-green algae [11,12,15]. The three polypeptides definitely seemed to be part of the phycoerythrin-phycocyanin complex (Fig. 3, IV). Only the 31 000 Da polypeptide was sometimes detected in the allophycocyanin fraction (Fig. 3, III), and then only in minor amounts.

Degradation of the 31 000 Da polypeptide to 27 000 Da (Fig. 3,V) occurred on dialysis of the phycoerythrin-phycocyanin fraction for 6 h at 0.01 M potassium phosphate. The high susceptibility to degradation of the 31 000 Da polypeptide in the isolated phycoerythrin-phycocyanin fraction indi-

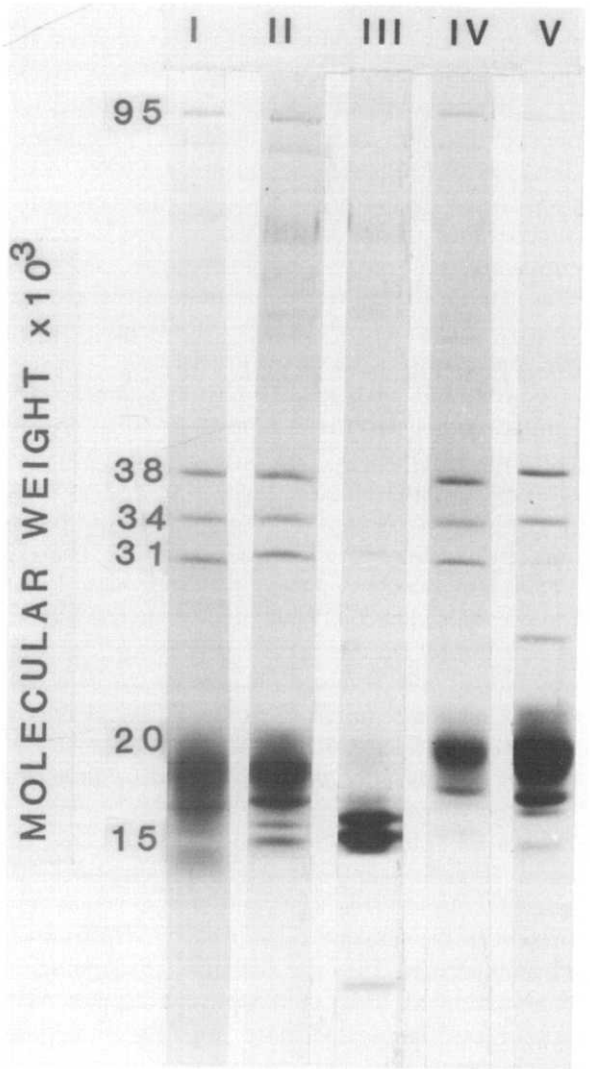

Fig. 3. Polypeptide bands of phycobilisomes and phycobilisome fractions after SDS-polyacrylamide gel electrophoresis and staining with Coomassie blue. (I) Native phycobilisomes; (II) reassociated phycobilisomes; (III) allophycocyanin fraction; (IV) phycoerythrin-phycocyanin fraction capable of reassociating with allophycocyanin in III, resulting in II; (V) phycoerythrin-phycocyanin fraction (after dialysis) not capable of reassociation with III. (Details in Materials and Methods and Results.)

cates that it is probably exposed in the phycoerythrin-phycocyanin complex. Prolonged dialysis concomitantly resulted in a phycoerythrin-phycocyanin complex which did not reassociate with allophycocyanin to form phycobilisomes. The phycoerythrin-phycocyanin fraction, with the degraded 31 000 Da polypeptide, although unable to recombine with allophycocyanin into phycobilisomes, was fully functional in energy transfer from phycoerythrin to phycocyanin, as evidenced by fluorescence excitation and emission spectra which were identical to those of phycoerythrin-phycocyanin complex capable of reassociating with allophycocyanin into phycobilisomes [18]. These results indicate that the binding between phycoerythrin and phycocyanin was unaffected by the absence of a fully intact 31 000 Da polypeptide. Glick and Zilinskas [20] have independently drawn the same conclusion, except under their conditions they obtained an apparent molecular mass of 29 000 Da, which we assume corresponds to the 31 000 Da polypeptide under our conditions. Other colorless polypeptides, present in minor amounts in native and reassociated phycobilisomes, were 58 000 and 86 000 Da, as well as a more prominent polypeptide of 95 000 Da (sometimes faintly bluish) (Fig. 3, I and II).

The relative amounts of the polypeptides in native and reassociated phycobilisomes were estimated from scans of Coomassie blue-stained gels (Table II). In both the native and reassociated phycobilisomes about 85% of the protein was contributed by the α- and β-polypeptides of the phycobiliproteins. The colorless polypeptides (uncolored prior to staining) comprised the remaining 15%. Polypeptide distribution was very similar except for a somewhat lower percentage of the 95 000 Da polypeptide in reassociated phycobilisomes. The 95 000 Da polypeptide was found to be susceptible to proteolysis in the phycobilisomes of several blue-green [20,32] and red algae [33], indicating that it is probably exposed and probably on the surface of the phycobilosome facing the thylakoid.

In phycobilisomes of *Nostoc* cells grown in red light, the phycoerythrin concentration was greatly reduced and the phycocyanin concentration doubled relative to allophycocyanin, resulting in a molar ratio of phycoerythrin : phycocyanin : allophycocyanin equal to 0.5 : 2.0 : 1.0. When phycoerythrin was substantially reduced in red-light-grown *Nostoc*, the 34 000 Da polypeptide almost disappeared from the phycobilisomes and was only visible on greatly overloaded gels (not

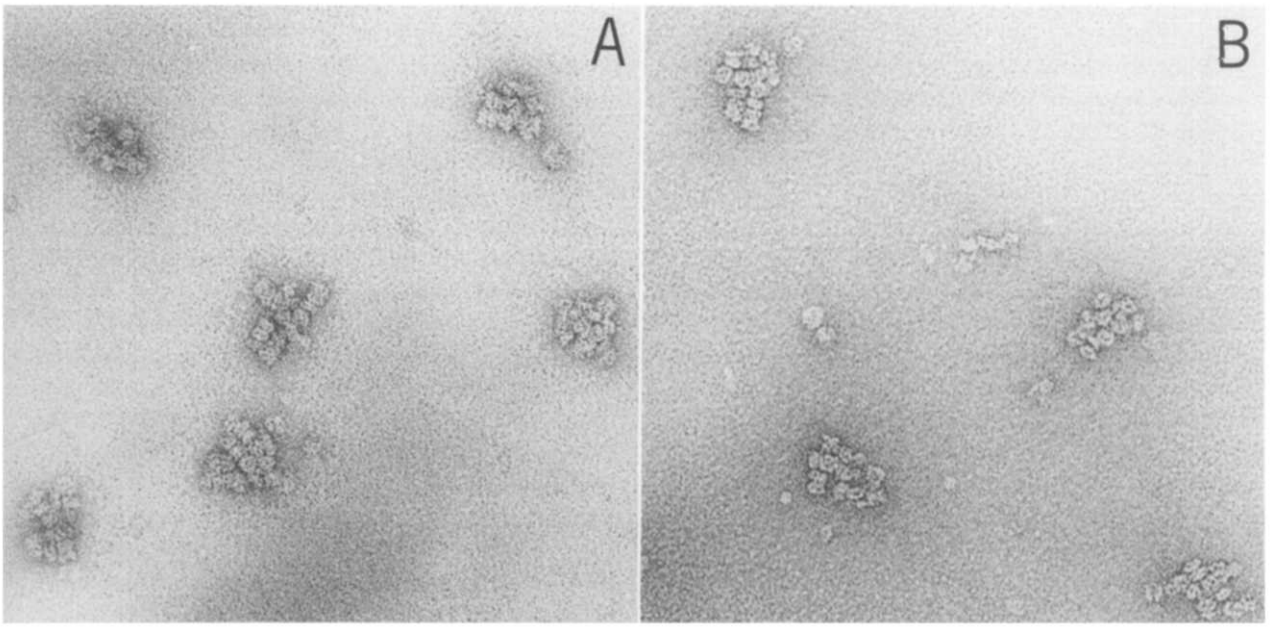

Fig. 4. Electron micrographs of native (A) and reassociated (B) phycobilisomes. Stained with 1% uranyl acetate after glutaraldehyde fixation [9]. Magnified 250000×.

shown). In addition, the 34000 Da polypeptide was absent from a phycocyanin-phycocyanin complex isolated from phycobilisomes of red-light-grown *Nostoc* cells. From these results it can be assumed that the 34000 Da polypeptide present in *Nostoc* phycobilisomes and in the isolated

TABLE II

POLYPEPTIDES OF NATIVE AND IN VITRO-ASSOCIATED PHYCOBILISOMES OF *NOSTOC*

APC, allophycocyanin; PC, phycocyanin; PE, phycoerythrin. Protein content was calculated from area under peaks of gel scans as described in Materials and Methods. These are average values from three gels each.

Band No.	Molecular weight	Native phycobilisome percent of protein (±S.D.)	Reassociated phycobilisomes percent of protein (±S.D.)	Presumed property
1	95000	1.4±0.2	0.94±0.2	phycobilisome-thylakoid anchor
2	86000 [a]	–	–	
3	58000	0.7±0.2	0.7±0.2	unknown
4	38000	3.9±0.2	3.9±0.2	PC linker
5	34000	3.0±0.2	3.3±0.2	PE linker [c]
6	31000	5.8±0.8	7.2±0.8	PC-APC linker
7	20000			β-PE; APC-core linker
8	18500			α-PE; β-PC
9	17400	85.0 [b]	85.0 [b]	α-PC; β-APC
10	16300			α-APC-675 [d]
11	15500			α-APC

[a] Assumed to be a partially degraded 95000 Da polypeptide.
[b] Molecular weights were independently determined from isolated phycobiliproteins.
[c] Absent in PC-PE complex.
[d] According to emission.

phycoerythrin-phycocyanin complex functions as a phycoerythrin linker (Table II). Since the 38 000 Da polypeptide was present in both phycoerythrin-phycocyanin and phycocyanin-phycocyanin complexes, it can be inferred that it is linked with phycocyanin.

Discussion

The integrity of native and reassociated phycobilisomes from *Nostoc* was supported by the equivalence of their absorption spectra, their fluorescence excitation and emission spectra, the positive correlation of the phycobiliprotein composition, and the similarity in their polypeptide patterns. The fact that anti-phycocyanin and anti-allophycocyanin, but not anti-phycoerythrin, prevented functional phycobilisome reassociation is direct evidence that phycocyanin and allophycocyanin must be directly linked for energy transfer to occur.

The yield of in vitro-associated phycobilisomes was favored by the high protein concentration and high phosphate level. These were also the critical factors in the dissociation and reassociation of *Anabaena variabilis* phycobilisomes [34]. It is of significance that under the same conditions particles equivalent to phycobilisomes were not obtained from either the phycoerythrin-phycocyanin fraction, or the allophycocyanin fractions alone.

The fluorescence emission maximum of native and reassociated phycobilisomes at approx. 675 nm (which shifted to 688 nm at $-196°C$) is similar to that of phycobilisomes isolated from numerous red and blue-green algae in many laboratories [7,13–17] and indicates that energy absorbed by the phycobiliproteins is transferred to allophycocyanin-675, the assumed terminal energy acceptor of the phycobilisomes. The one noteable exception to this is the report by Glick and Zilinskas [20], where *Nostoc* phycobilisomes, both native and reassociated, had a fluorescence emission maximum at 660 nm and only a small shoulder at 675 nm. Since the primary emission was due to short-wavelength emitting allophycocyanin, it appears that their phycobilisomes were not energetically fully coupled.

In native and reassociated *Nostoc* phycobilisomes about two copies of 31 000 Da polypeptide were present per copy of 34 000 Da polypeptide, and per copy of 38 000 Da polypeptide (Table II). The conclusion that the 31 000 Da polypeptide serves in linking phycocyanin to allophycocyanin is supported by the correlation of the disappearance of this polypeptide with the concomitant disappearance of the reassociation capacity of phycoerythrin-phycocyanin and allophycocyanin. It is assumed that the 31 000 Da polypeptide within the phycobilisome is protected from degradation, being exposed only upon dissociation of the phycobilisomes by dialysis in low phosphate buffer. The degradation of the 31 000 Da polypeptide, probably to a 27 000 Da polypeptide, did not affect the binding of phycoerythrin to phycocyanin in the complex, or the energy transfer from phycoerythrin to phycocyanin. It is suggested that the 31 000 Da linker polypeptide has two domains with distinct functions: a 27 000 Da portion necessary for stabilization of phycocyanin-phycocyanin and a 4000 Da portion necessary for the interaction between the peripheral phycocyanin and the allophycocyanin core. The 4000 Da portion was probably exposed in the phycoerythrin-phycocyanin complex and thus more readilly degraded. Yu et al. [15] had shown that linker polypeptides of 27 000 and 32 500 Da participate in the formation of the phycocyanin portions of the rod elements of *A. variabilis* phycobilisomes. In a further study, Yu and Glazer [35] concluded from tryptic digestion studies, that the 32 500 Da polypeptide has two domains: one of 28 000 Da which stabilizes trimeric and hexameric phycocyanin complexes and another of 4500 Da which is necessary for the interaction of phycocyanin hexamers. We support the interpretation made by Yu and Glazer [35] because it appears that with the 31 000 Da polypeptide we have an analogous system. The main difference being that the 31 000 Da polypeptide is involved in linking phycocyanin (in rods) to the allophycocyanin (in the core).

Substantial reduction of the phycoerythrin content, and decrease in the α- and β-polypeptide region occurred along with the reduction of the 34 000 Da polypeptide. These observations suggest that the 34 000 Da polypeptide is a phycoerythrin linker polypeptide, which perhaps was synthesized with phycoerythrin on the same gene, and then was separated by posttranslational cleavage.

Another possibility is that phycoerythrin and the 34000 Da polypeptide were regulated by different promoters, both of which may have been regulated by the spectral quality of light.

The 95000 Da polypeptide was present in reassociated phycobilisomes in somewhat reduced amounts compared to native *Nostoc* phycobilisomes. It was also observed that the 95000 Da polypeptide readily degraded apparently to a 86000 Da polypeptide with time. A 95000 Da polypeptide was found not only in *Nostoc* [20,37], but also in *Fremyella* [9], and in *Pseudoanabaena* [36]. Furthermore, a polypeptide of 95000 Da present in the core fraction of phycobilisomes [33] has been isolated from *P. cruentum* phycobilisomes and separately from thylakoid membrane fractions stripped of phycobilisomes. On the basis of immunological cross-reactivity and similar digest patterns, it has been suggested to serve as an anchor protein between the phycobilisome and thylakoids [38]. In *Nostoc* it may serve a similar function.

Phycobilisomes of *Nostoc* sp. have now been examined in two other laboratories [20,37] in addition to ours. Interestingly, all three used the same strain (MAC) but the results are not identical. There are variations in the phycoerythrin content relative to the phycocyanin and allophycocyanin content. Either cultures in the three laboratories have adapted differentially to different growth conditions, and/or have spontaneously mutated. This is cause for some concern, because it makes it difficult to compare results between laboratories working with the same organism.

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