CYANOBACTERIAL PHYCOBILISOMES

Particles from *Synechocystis* 6701 and Two Pigment Mutants

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ABSTRACT

The phycobilisomes of the unicellular cyanobacterium *Synechocystis* 6701, grown in white light, contain C-phycoerythrin, C-phycocyanin, and allophycocyanin in a molar ratio of ~2:2:1, and in addition, polypeptides of 99, 46, 33.5, 31.5, 30.5, and 27 × 10^3 daltons, as well as a trace of a ~9 × 10^3-dalton component. Two nitrosoguanidine-induced mutants of this organism produce aberrant phycobilisomes. Crude cell extracts of these mutants, 6701-NTG25 and NTG31, contain phycoerythrin, phycocyanin, and allophycocyanin in a molar ratio of 1.5:1:1 and 0.55:0.3:1.0, respectively. The phycobilisomes from both mutants lack the 33.5 × 10^3-dalton polypeptide.

Wild-type phycobilisomes consist of a core composed of an equilateral array of three cylindrical elements surrounded by six rods in a fanlike arrangement. The rods are made up of stacked disks, 11 nm in diameter and 6 nm thick. In phycobilisomes of mutant 6701-NTG25, numerous particles with fewer than six rods are seen. Mutant 6701-NTG31 produces incomplete structures that extend from triangular core particles, through cores with one or two attached rods, to cores with as many as five rods. The structure of the core appears unaltered throughout. The amount of phycocyanin (relative to allophycocyanin) appears to determine the number of rods per core.

A common assembly form seen in 6701-NTG31 is the core with two rods attached at opposite sides. From observations of this form, it is concluded that the core elements are cylindrical, with a height of 14 nm and a diameter of 11 nm. No consistently recognizable structural details are evident within the core elements.

All cyanobacteria contain biliproteins as major photosynthetic accessory pigments. Two representatives of this class of proteins, C-phycocyanin (λ_{max}, ~620 nm) and allophycocyanin (λ_{max}, ~650 nm), are invariably present, and allophycocyanin B (λ_{max}, ~671 nm) may also be a general constituent. Some cyanobacteria contain in addition a red protein, phycoerythrin (λ_{max}, ~565 nm), as a major "antenna" pigment (7). The biliproteins are organized into particles, phycobilisomes, which are attached to the outer surface of the thylakoids (4). Virtually all of the biliprotein of cyanobacterial cells is contained within these particles. Phycobilisomes contain several polypeptides in addition to the bilin-bearing subunits of the biliproteins (21, 23). Ultrastructural studies of cyanobac-

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terial phycobilisomes have shown that these hemispherical particles consist of a morphologically distinct core from which radiate rods made up of stacked disks (2, 11, 15). There is strong evidence that phycoerythrin, when present, is in the distal part of the rods and phycocyanin in the part proximal to the core (2, 5, 6, 10, 12). Allophycocyanin is contained within the core (2, 5, 6, 12).

To gain further insight into the structure and assembly of phycobilisomes, we have chosen those of *Synechocystis* 6701 for closer study. The phycobilisomes of this organism are particularly attractive subjects for ultrastructural examination. As extracted from wild-type cells, they possess a prominent core of three cylindrical elements arranged equilaterally and surrounded by a hemispherical array of six rods, each composed of three to five stacked disks (2, 11). Their phycoerythrin content is virtually zero when the cells are grown in red light, thus permitting a comparison to be made of structures containing and lacking this biliprotein. This report describes the properties of phycobilisomes produced by *Synechocystis* 6701 and of the aberrant particles produced by two nitrosoguanidine-induced mutants of this organism.

MATERIALS AND METHODS

Organisms and Culture Conditions

Synechocystis 6701 used in this study is maintained in the culture collection of the Unité de Physiologie Microbienne, Institut Pasteur, Paris (PCC 6701; [16]), and in the American Type Culture Collection (ATCC 27170). Mutants 6701-NTG25 and 6701-NTG31 were isolated after nitrosoguanidine mutagenesis (1). Colonies of these mutants, grown on agar plates in warm white fluorescent light in medium BG-11 (18), appeared brownish, whereas wild-type colonies appeared almost black. No revertants of these mutants were detected even after prolonged growth in liquid medium. Cells were grown in stirred 1-liter cultures at ~28°C in white light at ~50 fc in medium BG-11 containing double the usual amount of carbonate (18) to a density of 0.5 to 1.0 g wet weight per liter and were used immediately after harvest. One culture of mutant 6701-NTG25 was grown in red light obtained through the red acetate filter described by Tandeau de Marsac (20).

Detergents

Triton X-100 was obtained from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.. Deriphat 160 (disodium-*N*-lauryl-iminodipropionate; lot no. 8G570) was a gift of General Mills Chemical Division, Kankakee, Ill., and Miranol S2M-SF (1-H-imidazolium, 1-[2-(carboxy-methoxy)-ethyl]-1-(carboxymethyl)-2-nonyl-, 4,5-dihydro, hydroxide, disodium salt; lot no. 4829D79) was a gift of the Miranol Chemical Corp., Irvington, N. J.

Preparation of Phycobilisomes

All buffers contained 1 mM 2-mercaptoethanol and 1 mM sodium azide. All procedures were performed at room temperature unless otherwise specified. Cells were harvested by centrifugation, washed once with 0.65 M Na_2HPO_4-KH_2PO_4 at pH 7.0, and resuspended at 0.1 to 0.25 g wet weight per milliliter of this buffer. The cell suspension was passed twice through an Aminco French pressure cell at 20,000 lb/in^2. The broken cells were incubated with 1% Triton X-100, or with Miranol or Deriphat 160, for 30 min. Whole cells and membrane particles were then removed by centrifugation at 30,000 *g* for 30 min. The biliprotein-containing supernatant layer was carefully withdrawn from beneath a top chlorophyll/detergent layer, and transferred to the top of sucrose step gradients consisting of 3.5, 3.5, 2.2, and 2.0 ml of 1.0, 0.75, 0.5, and 0.25 M sucrose, respectively, in 0.75 M NaH_2PO_4-K_2HPO_4 at pH 8.0. For extracts originally prepared with Miranol or Deriphat, 0.1% of the appropriate detergent was included throughout the gradient. Centrifugation was performed at 83,000 *g* for 18 h (22 h for 6701-NTG31) at 18°C in a Spinco SW41 rotor (Beckman Instruments Co., Spinco Div., Palo Alto, Calif.). Gradient fractions were collected through a needle inserted through the bottom of the centrifuge tube. Spectroscopic and electron microscope studies were performed within 24 h of the preparation of the material. Samples for SDS-polyacrylamide gel electrophoresis were removed immediately after fractionation of gradients.

SDS-Polyacrylamide Gel Electrophoresis

Electrophoresis on polyacrylamide slab gels (0.8-mm thick, 20-cm long) was performed with the discontinuous buffer of Laemmli (13) and the apparatus of Studier (19). Sample preparation and densitometer scans were performed as described previously (23).

Spectroscopic Measurements

Fluorescence emission spectra were obtained at room temperature with a Fluorolog recording spectrofluorimeter (Spex Industries, Inc., Metuchen, N. J.) at a sample absorbance of 0.1 at λ_{max}. The emission spectra were corrected for the variation with wavelength in the sensitivity of the detection system (23). Absorbance spectra were determined with a model 25 recording spectrophotometer (Beckman Instruments, Inc.).

The relative content of C-phycoerythrin, C-phycocyanin, and allophycocyanin in phycobilisomes was determined by the following procedure. A phycobilisome sample (5.0 ml; ~300 μg protein/ml) from the sucrose density gradient was passed through a Sephadex G-25 (fine) column (1.5 × 20 cm) equilibrated with 0.1 M NaCl–0.001 M K-phosphate, pH 7.0. The entire colored eluate was applied to a column of hydroxylapatite (1.0 × 2.0 cm) (17) equilibrated with the same buffer. The biliproteins were quantitatively adsorbed as a narrow band to the top of this column. Elution with phosphate in 0.1 M NaCl, in steps of 10 mM, resulted in consecutive elution of a phycocyanin fraction, a phycoerythrin fraction containing some phycocyanin, a phycocyanin fraction containing some phycoerythrin, and finally a fraction containing solely allophycocyanin. The amounts of the biliproteins were calculated from extinction coefficients of 4.88 × 10^5 M^{-1} cm^{-1} at 562 nm for C-phycoerythrin (8), 2.81 × 10^5 M^{-1} cm^{-1} at 620 nm for C-phycocyanin (9), and 2.3 × 10^5 M^{-1} cm^{-1} at 650 nm for allophycocyanin (3), and from the relative absorbance of phycocyanin at 620 and 565 nm. A_{565}/A_{620} for

Synechocystis 6701 C-phycocyanin is 0.42. C-Phycoerythrin makes a negligible contribution to the absorbance at 620 nm (9).

Electron Microscopy

Preparations of phycobilisomes were examined in the electron microscope as described previously (11). Small drops of ~5 μl of the samples, diluted to 10–20 μg protein/ml in the buffers used for isolation, were placed upon the surface of glowed carbon-filmed grids. The residual material left on the films after suction removal of most of the applied drop was fixed for 5 min in glutaraldehyde diluted to 0.3% in the same buffer, washed twice in ammonium acetate (100 mM and 10 mM), and then negatively stained with 1% (wt/vol) uranyl formate. Electron micrographs were obtained under conditions of minimal beam exposure (22).

RESULTS

Composition and Spectroscopic Properties of Phycobilisomes

SYNECHOCYSTIS 6701 WILD TYPE: The absorption and fluorescence emission spectra of phycobilisomes from wild-type 6701 are shown in Fig. 1*A*. Excitation at 545 nm led to emission at 673 nm with little fluorescence from phycoerythrin and phycocyanin. Comparison of relative fluorescence emission at 580 nm of intact phycobilisomes in 0.75 M Na-K-phosphate at pH 8.0, with that of phycobilisomes after 30 min dissociation in 0.05 M phosphate at pH 8.0, showed 98–99% transfer of the energy absorbed by phycoerythrin in the intact particle to the other chromoproteins. These phycobilisomes contained phycoerythrin, phycocyanin, and allophycocyanin in a molar ratio of 1.8:2.2:1.

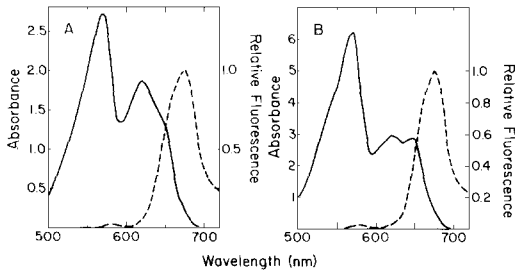

FIGURE 1 Absorption spectra (——) and fluorescence emission spectra (-----) of phycobilisomes prepared from (*A*) *Synechocystis* 6701 wild-type cells, and (*B*) mutant 6701-NTG25. Absorption spectra were determined in 0.75 M Na-K-phosphate–0.75 M sucrose, pH 8.0, immediately after removal of phycobilisome fractions from sucrose density gradients. For these spectra, and those presented in Fig. 4, the absorption was determined in short path-length cells so as to avoid possible changes in absorbance as a result of dilution-induced changes in the state of aggregation of the particles. The ordinate shows the absorbance calculated for a path length of 1 cm. Corrected fluorescence emission spectra were obtained on phycobilisome solutions of 0.1 absorbance at 565 nm in 0.75 M Na-K-phosphate, pH 8.0. Excitation was at 545 nm and excitation and emission slits were set at a 4-nm band pass.

The SDS-polyacrylamide gel electrophoresis pattern obtained with wild-type phycobilisomes showed the presence of several polypeptides (labeled *1–6* in Fig. 2) in addition to the bilin-carrying subunits of the biliproteins. A trace amount of a polypeptide of ~9,000 daltons, which moves with the dye front in this gel system, is also present. Phycobilisomes prepared with Triton X-100 and with Deriphat 160 had the same polypeptide compositions qualitatively and quantitatively; those prepared in Miranol showed an additional component of 91,000 daltons and a decreased amount of the 99,000-dalton polypeptide *1* (Fig. 1). Because polypeptides *1* to *6* co-sediment with phycobilisomes through sucrose density gradients in the presence and absence of detergents, they are bona fide components of these phycobilisomes (21).

MUTANT 6701-NTG25: On sucrose density gradients, the phycobilisomes of mutant 6701-NTG25 exhibited lower sedimentation coefficients and sedimented in a broader zone than those from wild-type cells. Qualitatively similar absorption spectra were obtained from the leading edge, center, and trailing edge of the phycobilisome zone. Consequently, the phycobilisome zone was collected as a single fraction. The absorption and fluorescence emission spectra of this material are shown in Fig. 1*B*. A comparison of the fluorescence emission spectra in Fig. 1*A* and *B* showed that energy transfer from phycoerythrin to allophycocyanin and allophycocyanin B was equally efficient in mutant and wild-type particles. This is particularly noteworthy because the composition of the mutant particles was very different from that of wild-type phycobilisomes. The relative molar proportions of phycoerythrin, phycocyanin, and allophycocyanin in 6701-NTG25 were 1.5:1.0:1.0. Moreover, the phycobilisomes from this mutant were almost completely lacking polypeptide *3* (Fig. 2).

MUTANT 6701-NTG31: Upon sedimentation on sucrose density gradients, a continuum of biliprotein-containing particles was seen in extracts from this mutant (Fig. 3). The distribution of material on the gradient was very similar in the presence and absence of zwitterionic detergents,

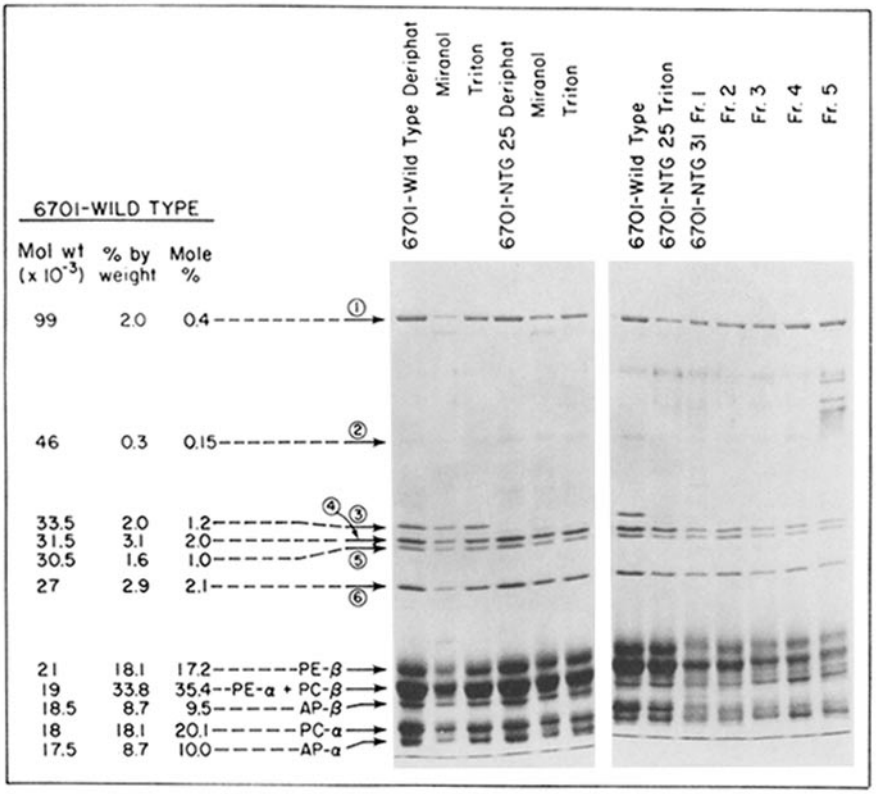

FIGURE 2 SDS-polyacrylamide gel electrophoresis of phycobilisomes from *Synechocystis* 6701 wild type and from mutants 6701-NTG25 and 6701-NTG31. Lanes *1–3* and *4–6* represent samples of 6701 wild type and 6701-NTG25 phycobilisomes, respectively, prepared with Triton X-100 or two zwitterionic detergents as indicated (see Materials and Methods for details). Samples of 6701 wild type and 6701-NTG25 phycobilisomes (lanes *7* and *8*) are compared with 6701-NTG31 phycobilisome fractions obtained from sucrose density gradients (Fig. 3). All samples in lanes *7–13* were obtained by the Triton procedure. The values for percent by weight and mole percent of the components of wild-type phycobilisomes were calculated from densitometer scans of gels stained with Coomassie brilliant blue R-250.

indicating that the polydispersity was not the consequence of aggregation (11). The biliprotein-containing portion of the gradient was arbitrarily divided into six fractions (Fig. 3) whose absorption spectra are shown in Fig. 4. The average composition obtained for a pool of fractions *1–6* was phycoerythrin, phycocyanin, and allophycocyanin in a molar ratio of 0.55:0.3:1.0. However, the proportions of the biliproteins differed significantly in the individual fractions. For example, fraction *4* had a ratio of phycoerythrin to phycocyanin to allophycocyanin of 1:0.5:1.0 (see Fig. 4). SDS-polyacrylamide gel electrophoresis of pooled fractions *1–6* showed that polypeptide *3* was absent (Fig. 2). Fractions *1–4* had qualitatively equivalent polypeptide compositions; additional polypeptides were seen in fractions *5* (Fig. 2) and *6*. Macromolecules of 150–300 × 10^3 daltons sediment to this region of these sucrose density gradients and the additional polypeptides are undoubtedly derived from proteins not associated with phycobilisomes.

Fluorescence emission spectra of fractions *1–4*, obtained upon excitation of phycoerythrin, showed energy transfer quantitatively similar to that obtained with wild-type particles. For example, Fig. 5 shows the fluorescence emission spectrum of fraction *4* particles compared with that of wild-type phycobilisomes. The efficiency of energy transfer from phycoerythrin in the wild-type preparation was 98.5%, and that in the mutant was 97.0%. This observation shows that the biliproteins present in fractions *1–4* are indeed organized into particles whose energy transfer properties are little impaired in spite of the gross variation in the

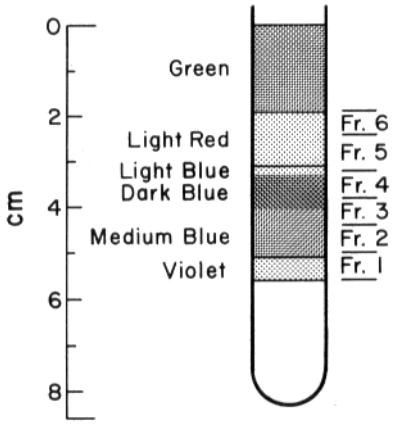

FIGURE 3 Distribution of 6701-NTG31 phycobiliprotein-containing components prepared by the Triton procedure after centrifugation on a sucrose step gradient containing 0.75 M Na-K-phosphate, pH 8.0. The colors indicated at left are a composite of the residual transmission from the incident white light through the tube (which depends at each point on the concentration and relative amounts of the biliproteins) and the emitted fluorescence. Fractions *1–6* were withdrawn by needle puncture. The spectra of these fractions are shown in Fig. 4.

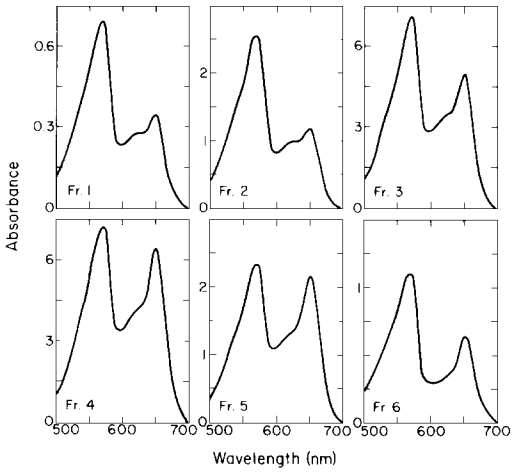

FIGURE 4 Absorption spectra of phycobiliprotein-containing components from 6701-NTG31 after fractionation by centrifugation on a sucrose step gradient (see Fig. 3).

proportions of the constituent biliproteins and the absence of polypeptide *3*. The fluorescence emission spectra of fractions *5* and *6*, obtained upon excitation at the appropriate wavelengths, were dominated by the emissions of the individual biliproteins, indicating that, in accord with the observed sedimentation velocity, much of the biliprotein in these fractions was not organized into complex particles.

Ultrastructure of Phycobilisomes

SYNECHOCYSTIS 6701 WILD TYPE: Ultrastructural information concerning 6701 wild-type phycobilisomes has been reported previously by Bryant et al. (2) and by us (11). The particles contain a triangular "core" partially surrounded, in a fanlike array, by six rods of variable length (Fig. 6). The core is an equilateral array of three elements that most commonly appear like disks. Occasionally an element looks more like a spokeless wheel, with a "hub" and a "rim" (Fig. 6, arrow). The rods radiating from the core consist of stacks of disks, seen here in edge view. The disks are slightly more than 11 nm in diameter and are 6.0-nm thick, with each disk having a midline septum. The most frequently observed number of rods is six per phycobilisome, a number never exceeded but occasionally reduced to five or four, particularly when the entire particle shows signs of severe distortion. The triangular array of the core is invariably placed such that two of its elements lie along the edge of the hemidiscoid particle, be the edge straight or bent. The third element lies interior to the other two.

MUTANT 6701-NTG25: The core and rod

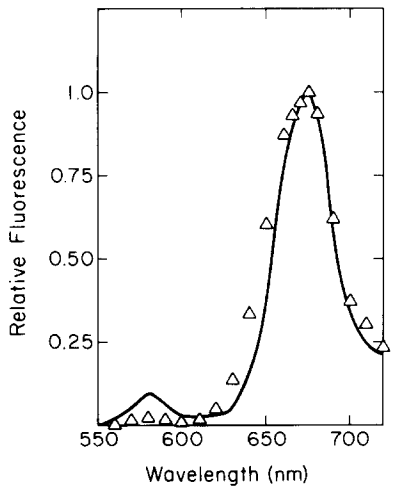

FIGURE 5 Corrected fluorescence emission spectra of 6701 wild-type phycobilisomes (△) and of 6701-NTG31 fraction *4* (Fig. 3). Solutions were adjusted to 0.1 A at 565 nm in 0.75 M Na-K-phosphate at pH 7.0. Excitation was at 545 nm and excitation and emission slits were set at 4-nm band pass.

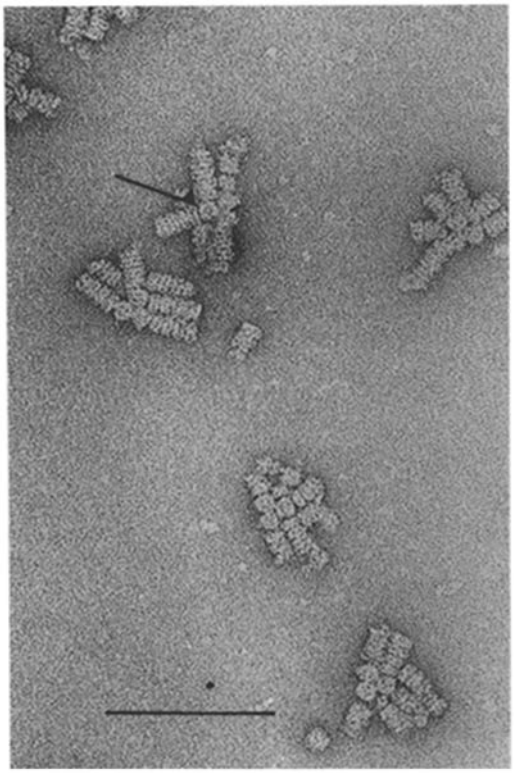

FIGURE 6 Phycobilisomes from wild-type *Synechocystis* 6701. Structure consists of rods of stacked disks and a core of three circular objects in triangular array. Uranyl formate negative stain. For explanation of arrow, see Results. Bar, 0.1 μm. × 225,000.

substructures seen in wild-type phycobilisomes persist in the particles from mutant 6701-NTG25 (Fig. 7), although a few differences are evident. The core and its organization appear unaltered in the mutant particle but there is larger variation in the number of rods associated with each core than there is in the wild-type. The number ranges from two (in rare instances) to the six encountered in the wild type; particles with only four or five rods are seen frequently. The mutant particles appear to be less stable under the conditions used here for the preparation of specimens for electron microscopy, as indicated by the presence of separate rods and occasional isolated core assemblies.

Phycobilisomes from 6701-NTG25 cells grown in red light, with a ratio of phycoerythrin to phycocyanin to allophycocyanin of <0.2:1:1, show no change in the organization of the core, but the rods in such particles are much shorter, in some cases consisting of a single disk (Fig. 8).

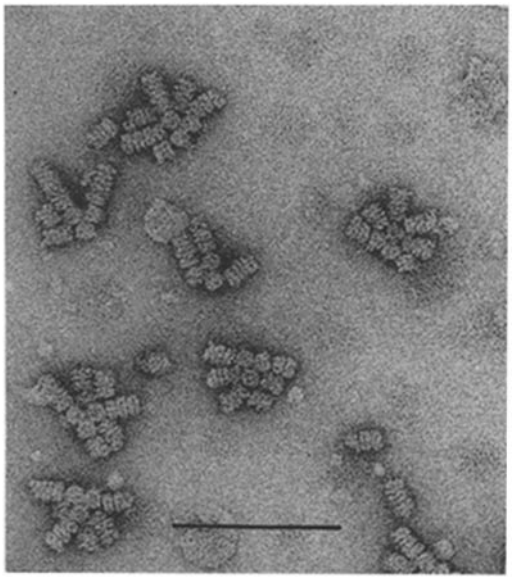

FIGURE 7 Phycobilisomes from *Synechocystis* 6701-NTG25. Structure generally similar to that of the wild type. Bar, 0.1 μm. × 225,000.

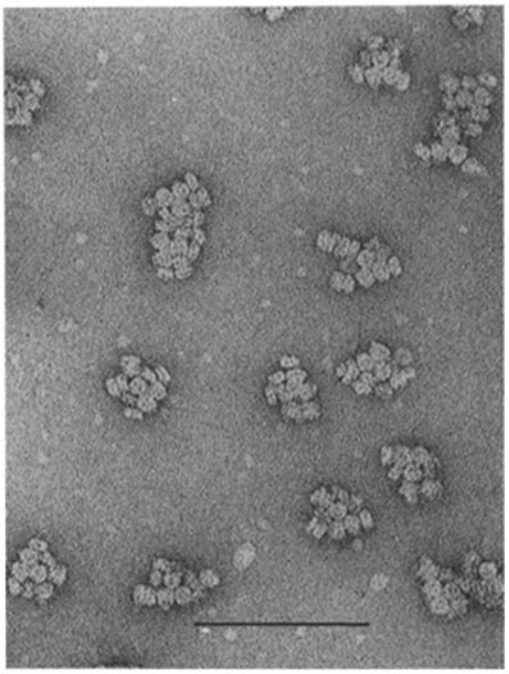

FIGURE 8 Phycobilisomes from *Synechocystis* 6701-NTG25, grown in red light. Core remains intact, but rods are greatly shortened compared to wild type. Bar, 0.1 μm. × 225,000.

MUTANT 6701-NTG31: Mutant 6701-NTG31 produces a heterogeneous collection of incomplete phycobilisome particles that are partially separable by density gradient centrifugation. The most rapidly sedimenting particles (fractions *1* and *2*; Fig. 3) appear similar to those obtained from mutant 6701-NTG25 described above. Fractions *4* and *5* contain distinctive, more slowly sedimenting particles that are enriched for allophycocyanin relative to phycocyanin and phycoerythrin (Fig. 4). Under the electron microscope, numerous particles are found that exhibit a face view of the three core elements joined to two rods, with two of the elements, and the rods, forming a straight line (Fig. 9). Frequently, only one rod is present, with one of its ends extended by the attachment of two core elements in the same line. Rarely, three rods are attached to the core, one from each unit.

Careful inspection of our micrographs has yielded some instances in which a particle (consisting of two rods and the attached core) seems to have been rotated (with the rod as axis) some 90° from its most usual orientation. The core triangle may then be viewed along its plane and its elements observed along a line perpendicular to their axes. Fig. 10 is a gallery of such objects. The stacked disks of the rods are readily identified, inasmuch as they appear the same as they do in, for example, Figs. 6 and 7. Between the two rod segments are two rectangular objects. The average width and length of ~20 rectangles measured are 11 nm and 14 nm, respectively. The former dimension is almost twice the thickness of a rod-disk but is the same as the diameter of the core elements as seen in face view (Figs. 6, 7, and 10). The average length of the rectangles is distinctly larger than the diameter of the rod-disks. Because of their number, their location between two rod segments, and the identity of their widths with the diameter of core elements seen end-on, we believe the rectangles represent core elements seen on their sides. There is no sign of a third element; it may have dissociated, but more probably the distribution of the negative stain is such as to obscure its presence. As can be seen from Fig. 10, no consistently recognizable structural detail is evident within the core elements. The core element thus appears to be a solid, right cylinder with a height of 14 nm and a diameter of 11 nm.

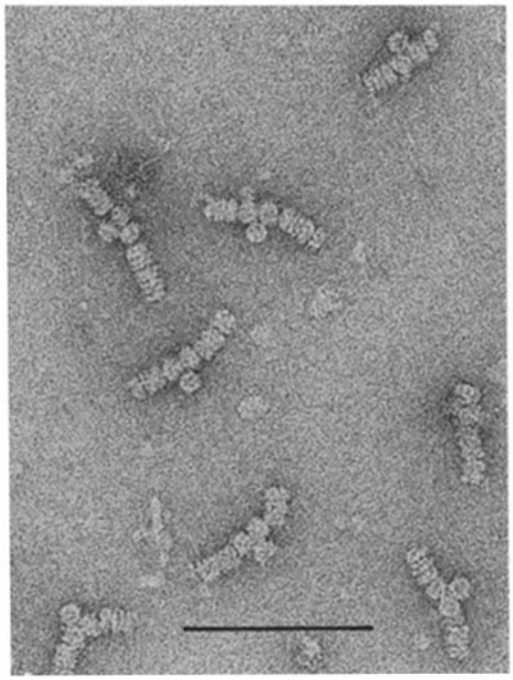

FIGURE 9 Phycobilisomes from *Synechocystis* 6701-NTG31. Core structures with only two rods are frequently seen. Bar, 0.1 μm. × 250,000.

Fraction *5* from the sucrose gradient (Fig. 3) contained a large proportion of incomplete phycobilisome particles. Particularly striking are the isolated, intact core units (Fig. 11). Their frequent presence suggests an interelement binding far stronger than the binding between core elements and rods. Incomplete cores are not seen. Whether single core elements are present in the preparations cannot be established with certainty, because such a structure, seen end-on, would have the same diameter as, and a thickness similar to, two stacked rod-disks.

DISCUSSION

The unicellular cyanobacterium, *Synechocystis* 6701, grown in white light, produces C-phycoerythrin, C-phycocyanin, and allophycocyanin, in a molar ratio of ~2:2:1 (14, and the present study). This organism is a member of Group II chromatic adapters (20), in which phycoerythrin synthesis is affected by light quality, but phycocyanin and allophycocyanin synthesis is unchanged; i.e., *Synechocystis* 6701 grown in red light produces C-phycocyanin and allophycocyanin in a molar ratio of 2:1, but phycoerythrin is almost absent. As shown in Fig. 2, the phycobilisomes of *Synechocystis* 6701 grown in white light contain, in addition to the subunits of the biliproteins, polypeptides of 99, 46, 33.5, 31.5, 30.5, and 27 × 10^3

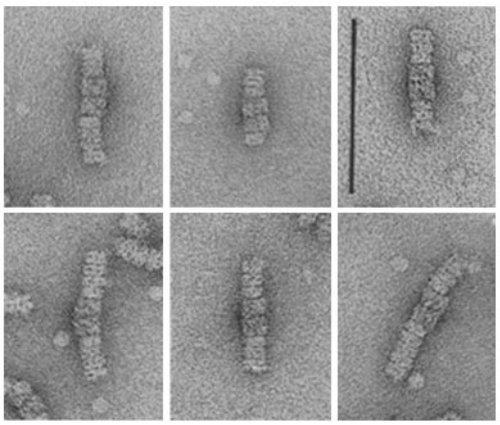

FIGURE 10 Particles selected from micrographs of *Synechocystis* 6701-NTG31 to show appearance of linear structures, like those in Fig. 9, but in an orientation rotated some 90° (with the rod segments as axes) from that usually seen (Fig. 9). (Note two contiguous objects with rectangular outlines near middle of each particle.) Bar, 0.1 μm. × 225,000.

daltons, as well as a trace of a ~9 × 10^3-dalton component. The functions of these polypeptides are largely unknown.

The two mutants examined in this study, 6701-NTG25 and -NTG31, lack the 33.5 × 10^3-dalton polypeptide *3*. Cells of these mutants also contain decreased amounts of phycoerythrin and phycocyanin relative to allophycocyanin. Mutant 6701-NTG25 produces ~85% of the wild-type level of phycoerythrin and 45% of that of phycocyanin, whereas the corresponding amounts for 6701-NTG31 are 30 and 15%. Both mutants produce aberrant phycobilisomes, but the range of incomplete structures from 6701-NTG31 is much broader, and extends from triangular core particles, through cores with one or two attached rods, to cores with as many as five rods. The most common assembly form seen in 6701-NTG31 is the core with rods attached at opposite sides. It is tempting to suggest that rods assemble preferentially at the point of contact of the core with the thylakoid membrane, with consequent predominance of these "linear" forms.

The core appears to be unaltered in the mutants investigated. This observation is consistent with the conclusions of earlier studies (2, 5, 6, 12) that allophycocyanin is contained within the core, whereas phycocyanin and phycoerythrin are confined to the rods. The major morphological difference between the phycobilisomes of 6701-NTG25 and -NTG31 is that the former have, on the average, twice as many rods extending from the core. The molar ratio of phycocyanin to allophycocyanin in the wild type, in 6701-NTG25, and in 6701-NTG31 is 2.2:1, 1:1, and 0.3:1, respectively. The corresponding average number of rods per core is 5–6, 4–5, and 1–2, respectively. This observation indicates that it is the amount of phycocyanin (relative to allophycocyanin) that determines the number of rods per core. Such an interpretation is supported by examination of phycobilisomes obtained from cells of 6701-NTG25 grown in red light. The molar ratio of phycoerythrin to allophycocyanin in these particles is reduced to <0.2:1, whereas the ratio of phycocyanin to allophycocyanin is unaltered. The rods in these particles are significantly shorter than those in the "white light" phycobilisomes from this mutant (compare Figs. 7 and 8), but the number of rods (mostly only one disk in length) per core is unchanged.

Polypeptide *5* is absent from phycobilisomes obtained from red-light-grown cells of *Synechocystis* 6701 and 6701-NTG25, and the amount of polypeptide *4* is greatly reduced (data not shown). Bryant et al. (2) have shown that the rods in phycobilisomes from *Synechocystis* 6701 grown in

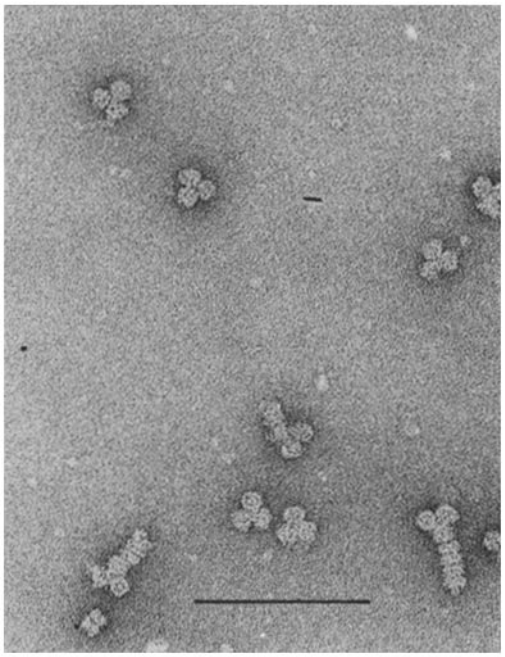

FIGURE 11 Free cores found in an upper fraction of sucrose gradient of a 6701-NTG31 phycobilisome preparation. Bar, 0.1 μm. × 225,000.

red light are approximately half as long as those in phycobilisomes grown in white light. As noted above, the phycobilisomes from 6701-NTG25 grown in red light also have shorter rods. From these observations, it may be inferred that polypeptides *4* and *5* are associated with phycoerythrin (see also reference 10) and that depletion of these components does not affect the assembly of the core or attachment of phycocyanin-containing rods. Because both mutants lack polypeptide *3* and yet are able to assemble rods on the core, it is clear that polypeptide *3* is not essential for formation of stacks of double disks or for the attachment of such stacked disks to the core. What then is the role of polypeptide *3*? The most plausible supposition is that polypeptide *3* stabilizes stacks of phycocyanin double disks and that the structure produced in its absence is much more labile. This view is prompted by the observed instability of the mutant structures and by the greater degree of stain penetration into these structures.

Our observations on the ultrastructure of the elements of the phycobilisome core differ from those of Bryant et al. (2) who concluded that a midline, transverse septum exists and that, consequently, the core consists of six elements arrayed in three stacked pairs. Their observations were made on structures believed to be the full, wild-type 6701 phycobilisome, so oriented that the plane of the core triangle was in the line of sight. The resultant images must have suffered from a considerable amount of superposition of electron-scattering material, inasmuch as six full rods remained attached to the cores. In our case, by use of 6701-NTG31, only two rods were attached to the cores and these were so placed that they could not have led to any superposition (Fig. 10). There is a very remote chance that both observations are correct: that the wild-type phycobilisome contains six disks in its core, whereas the NTG31 mutant contains three solid cylinders. It is unlikely that electron microscopy can settle this point.

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REFERENCES

1. ADELBERG, E. A., M. MANDEL, and G. C. C. CHEN. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. *Biochem. Biophys. Res. Commun.* **18**:788–795.
2. BRYANT, D. A., G. GUGLIELMI, N. TANDEAU DE MARSAC, A.-M. CASTETS, and G. COHEN-BAZIRE. 1979. The structure of cyanobacterial phycobilisomes. A model. *Arch. Microbiol.* **123**:113–127.
3. COHEN-BAZIRE, G., S. BÉGUIN, S. RIMON, A. N. GLAZER, and D. M. BROWN. 1977. Physico-chemical and immunological properties of allophycocyanins. *Arch. Microbiol.* **111**:225–238.
4. GANTT, E., and S. F. CONTI. 1967. Phycobiliprotein localization in algae. *Brookhaven Symp. Biol.* **19**:393–405.
5. GANTT, E., C. A. LIPSCHULTZ, and B. A. ZILINSKAS. 1976. Further evidence for a phycobilisome model from selective dissociation, fluorescence emission, immunoprecipitation and electron microscopy. *Biochim. Biophys. Acta.* **430**:375–388.
6. GANTT, E., C. A. LIPSCHULTZ, and B. A. ZILINSKAS. 1977. Phycobilisomes in relation to the thylakoid membranes. *Brookhaven Symp. Biol.* **28**:347–357.
7. GLAZER, A. N. 1977. Structure and molecular organization of the photosynthetic accessory pigments of cyanobacteria and red algae. *Mol. Cell. Biochem.* **18**:125–140.
8. GLAZER, A. N., and C. S. HIXSON. 1975. Characterization of R-phycocyanin. Chromophore content of R-phycocyanin and C-phycoerythrin. *J. Biol. Chem.* **250**:5487–5495.
9. GLAZER, A. N., S. FANG, and D. M. BROWN. 1973. Spectroscopic properties of C-phycocyanin and of its α and β subunits. *J. Biol. Chem.* **248**:5679–5685.
10. GLAZER, A. N., G. YAMANAKA, and J. C. GINGRICH. 1979. Phycobiliproteins: structure and organization. *Third International Symposium on Photosynthetic Prokaryotes.* Oxford, England. E4. (Abstr.).
11. GLAZER, A. N., R. C. WILLIAMS, G. YAMANAKA, and H. K. SCHACHMAN. 1979. Characterization of cyanobacterial phycobilisomes in zwitterionic detergents. *Proc. Natl. Acad. Sci. U. S. A.* **76**:6162–6166.
12. KOLLER, K.-P., W. WEHRMEYER, and E. MÖRSCHEL. 1978. Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea*. On the molecular composition of energy-transfering complexes (tripartite units) forming the periphery of the phycobilisome. *Eur. J. Biochem.* **91**: 57–63.
13. LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* **227**:680–685.
14. LEMASSON, C., N. TANDEAU DE MARSAC, and G. COHEN-BAZIRE. 1973. Role of allophycocyanin as a light-harvesting pigment in cyanobacteria. *Proc. Natl. Acad. Sci. U. S. A.* **70**:3130–3133.
15. MÖRSCHEL, E., K. P. KOLLER, W. WEHRMEYER, and H. SCHNEIDER. 1977. 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.
16. RIPPKA, R., J. DERUELLES, J. B. WATERBURY, M. HERDMAN, and R. Y. STANIER. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**:1–61.
17. SIEGELMAN, H. W., G. A. WIECZOREK, and B. C. TURNER. 1965. Preparation of calcium phosphate for protein chromatography. *Anal. Biochem.* **13**:402–404.
18. STANIER, R. Y., R. KUNISAWA, M. MANDEL, and G. COHEN-BAZIRE. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol. Rev.* **35**:171–205.
19. STUDIER, F. W. 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *J. Mol. Biol.* **79**:237–248.
20. TANDEAU DE MARSAC, N. 1977. Occurrence and nature of chromatic adaptation in cyanobacteria. *J. Bacteriol.* **130**:82–91.
21. TANDEAU DE MARSAC, N., and G. COHEN-BAZIRE. 1977. Molecular composition of cyanobacterial phycobilisomes. *Proc. Natl. Acad. Sci. U. S. A.* **74**:1635–1639.
22. WILLIAMS, R. C., and H. W. FISHER. 1970. Electron microscopy of tobacco mosaic virus under conditions of minimal beam exposure. *J. Mol. Biol.* **52**:121–123.
23. YAMANAKA, G., A. N. GLAZER, and R. C. WILLIAMS. 1978. Cyanobacterial phycobilisomes. Characterization of the phycobilisomes of *Synechococcus* sp. 6301. *J. Biol. Chem.* **253**:8303–8310.