Rod Substructure in Cyanobacterial Phycobilisomes: Analysis of *Synechocystis* 6701 Mutants Low in Phycoerythrin

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ABSTRACT *Synechocystis* 6701 phycobilisomes contain phycoerythrin, phycocyanin, and allophycocyanin in a molar ratio of ~2:2:1, and other polypeptides of 99-, 46-, 33.5-, 31.5-, 30.5-, and 27-kdaltons. Wild-type phycobilisomes consist of a core of three cylindrical elements in an equilateral array surrounded by a fanlike array of six rods each made up of 3–4 stacked disks. Twelve nitrosoguanidine-induced mutants were isolated which produced phycobilisomes containing between 0 and 53% of the wild-type level of phycoerythrin and grossly altered levels of the 30.5- and 31.5-kdalton polypeptides. Assembly defects in these mutant particles were shown to be limited to the phycoerythrin portions of the rod substructures of the phycobilisome. Quantitative analysis of phycobilisomes from wild-type and mutant cells, grown either in white light or chromatically adapted to red light, indicated a molar ratio of the 30.5- and 31.5-kdalton polypeptides to phycoerythrin of 1:6, i.e., one 30.5- or one 31.5-kdaltons polypeptide per $(\alpha\beta)_6$ phycoerythrin hexamer. Presence of the phycoerythrin-31.5-kdalton complex in phycobilisomes did not require the presence of the 30.5-kdalton polypeptide. The converse situation was not observed. These and earlier studies (R. C. Williams, J. C. Gingrich, and A. N. Glazer. 1980. *J. Cell Biol.* 85:558–566) show that the average rod in wild type *Synechocystis* 6701 phycobilisomes consists of four stacked disk-shaped complexes: phycocyanin $(\alpha\beta)_6$-27 kdalton, phycocyanin $(\alpha\beta)_6$-33.5 kdalton, phycoerythrin $(\alpha\beta)_6$-31.5 kdalton, and phycoerythrin-30.5 kdalton, listed in order starting with the disk proximal to the core.

On a quantitative basis, biliproteins are the major light-harvesting components of the photosynthetic apparatus of cyanobacteria and red algae (1, 2). These chromoproteins are organized into multiprotein complexes, phycobilisomes (3), which are arrayed on the outer surface of the thylakoid membranes. In cyanobacteria, phycobilisomes range in size from ~5 to 8 $\times 10^6$ daltons while those in the majority of red algae are much larger, of the order of 30–40 $\times 10^6$ daltons. The major biliproteins, phycoerythrin (λ_{max} ~565 nm), phycocyanin (λ_{max} ~620 nm), and allophycocyanin (λ_{max} ~650 nm), together account for ~85% of the weight of these particles. The gross localization of these major components was readily established from kinetic studies of partial dissociation coupled with immunoelectron microscopy (4–6). The majority of these studies were performed on the large hemispherical phycobilisomes of the unicellular red alga *Porphyridium cruentum* and led to a model of the phycobilisome with a concentric arrangement of biliproteins in which phycoerythrin formed an outer shell upon a layer of phycocyanin which in turn surrounded a core of allophycocyanin (4, 5). This arrangement of biliproteins was consistent with spectroscopic measurements which unequivocally established a path of radiationless energy transfer within phycobilisomes, as well as in intact cells, of *P. cruentum*, from phycoerythrin → phycocyanin → allophycocyanin (7, 8).

Electron microscopy of phycobilisomes of the unicellular rhodophyte *Rhodella violacea* (9) and of numerous cyanobacteria (10–12) have revealed a common morphology of these structures in the majority of these organisms. These phycobilisomes consist of a core of three cylindrical units arranged equilaterally and surrounded by a hemispherical array of six rods each composed of three to five stacked disks (10–12). The phycobilisome of *Synechocystis* 6701 belongs to this category (10, 11, 13). In accord with the earlier observations on *P. cruentum* (3–5) and *Rhodella violacea* (14), biochemical and electron microscope studies of these structures have shown that phycoerythrin is in the distal portions of the rods, phycocyanin in the portions proximal to the core, and allophycocyanin is within the core (10, 13, 15).

While such studies revealed the gross features of the structure of the phycobilisome, other observations had shown that the

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phycobilisome contained a number of additional components which functioned in the assembly of the structure and the energy transduction process. In addition to the major biliproteins, another biliprotein, allophycocyanin B (λ_{max} 670 nm) is known to be a minor component of most (perhaps all) phycobilisomes and to function in the transfer of energy from the allophycocyanin to the reaction centers in the thylakoid membrane (16). Moreover, several other polypeptides, each quantitatively minor, have been shown to be present in phycobilisomes (17, 18) and to play an essential role in the assembly of the structure (19). It is now evident that phycobilisomes are structures of considerable complexity imposed by the combined requirements of assembly and optimization of energy transfer.

Although the phycobilisomes of *Synechocystis* 6701, examined in this report, are among the simpler representatives of phycoerythrin-containing particles, they show considerable complexity of composition. These phycobilisomes contain phycoerythrin, phycocyanin, and allophycocyanin in a molar ratio of ~2:2:1, allophycocyanin B, and polypeptides of 99, 46, 33.5, 31.5, 30.5, and 27 kdaltons (13, 15). Examination of mutants has been essential to the determination of the details of organization and assembly of complex structures such as those of phages. Pigment mutants of cyanobacteria are easily detected, and we have accumulated a large number of such *Synechocystis* 6701 mutants defective in phycobilisome assembly. Since phycoerythrin is on the periphery of the phycobilisome, we have initiated a detailed analysis of phycobilisome structure in *Synechocystis* 6701 by examining those mutants with assembly defects confined to this biliprotein.

MATERIALS AND METHODS

Organisms and Culture Conditions

Synechocystis 6701 used in this study is maintained in the American Type Culture Collection (ATCC 27170), and in the culture collection of the Unité de Physiologie Microbienne, Institut Pasteur, Paris (PCC 6701; [20]). Mutants of *Synechocystis* 6701 were obtained by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis essentially as described by Sherman and Cunningham (21). After mutagenesis, cells were plated onto 1% agar plates containing medium BG11 (20). Colonies appearing various shades of green to greenish black, as compared to the black color of wild-type colonies, were picked and purified by repeated replating. All pigment mutants described in this report show no reversion after continued replating (reversion frequency <1 in 10^9), as judged by appearance of colonies and constancy of composition of phycobilisomes isolated from mass cultures. Single colonies from plates were transferred to liquid medium BG11 containing double the usual amount of carbonate and were grown to a density of 0.5–1.0 g wet weight/l under warm white fluorescent light at 30°C. For chromatic adaptation experiments, cells were grown in red or green light obtained through the acetate filters described by Tandeau de Marsac (22).

Preparation of Phycobilisomes

Phycobilisomes were prepared as previously described (13) with Triton X-100 as the membrane solubilization reagent.

Spectroscopic Measurements

Absorption spectra were measured on a Beckman Model 25 recording spectrophotometer. Corrected fluorescence emission spectra were obtained with a Perkin-Elmer MPF-44B recording spectrofluorometer, equipped with a DCSU-2 differential corrected spectra unit and a Hamamatsu R926 phototube. Sample absorbance ranged from 0.05 to 0.1 cm^{-1} at 620 nm in all cases. Circular dichroism (C. D.) spectra were determined on the spectrometer described by Sutherland et al. (23) at a sample absorbance of ~0.7 cm^{-1} at 620 nm.

SDS PAGE

Slab gel electrophoresis was carried out on 0.9 mm thick 14% polyacrylamide gels (30:0.8 wt/wt acrylamide:bisacrylamide) as previously described (24). Gels stained with Coomassie Brilliant Blue were scanned with a Helena Laboratories Quick Scan densitometer (Helena Laboratories, Beaumont, Texas) operated at slow speed with narrow beam. Peak weights were determined and divided by the molecular weight of the appropriate polypeptide in each instance to obtain the molar ratios of the separated components.

RESULTS

Mutagenesis of *Synechocystis* 6701 with nitrosoguanidine led to the appearance of strains which produce abnormally low amounts of phycoerythrin, or of phycocyanin, or of both of these major constituents of the phycobilisome, as well as of a mutant with a defect in core assembly. Since there is abundant evidence that phycoerythrin occupies a peripheral location in phycobilisomes, it would be expected that mutants in which the abnormality in biliprotein production was confined to phycoerythrin would include those that produce phycobilisomes normal in all other respects. Such mutants would be valuable in the analysis of the phycoerythrin-containing portion of the phycobilisome structure. Mutants in which the ratio of phycocyanin:allophycocyanin was similar to that found in wild-type cells were found to belong to this class. We examined twelve such mutants. The phycoerythrin content of the phycobilisomes of these mutants ranged from 0 to 53% of the wild type level (Table I).

Compositions of Wild-type and Mutant Phycobilisomes

Phycobilisomes from wild type and from the mutants described here all sedimented on sucrose gradients as single bands violet to blue in color depending on their phycoerythrin content. The sedimentation rate correlated with the amount of phycoerythrin present in the particle. For example, phycobilisomes devoid of phycoerythrin, from mutant CM70, sedimented 90% as far as those of wild type, whereas those of CM1, containing ~50% of wild-type level of phycoerythrin, sedimented ~95% as far as wild-type phycobilisomes on the sucrose gradients.

Examples of absorption spectra of phycobilisomes from wild type and three of the mutants are shown in Fig. 1. Above 610 nm, where phycoerythrin does not contribute to the absorption of phycobilisomes, all four preparations have identical absorption spectra. Large differences are evident between 500 and 600 nm, where phycoerythrin absorbance is large. These spectra demonstrate that the phycocyanin:allophycocyanin ratio in these mutants is identical to that of the wild type, whereas there are large differences in phycoerythrin content. Absorption ratios of 620 nm/650 nm (phycocyanin absorption peak to allophycocyanin absorption peak) and 570 nm/650 nm (phycoerythrin absorption peak to allophycocyanin absorption peak) are given in the left-hand part of Table I for six wild-type phycobilisome preparations, obtained from independent cultures, and for twelve different mutant phycobilisome preparations, all from cells grown under warm white fluorescent light. For the wild-type phycobilisomes, the 620 nm/650 nm absorbance ratio varies from 1.48 to 1.54. Nine of the twelve mutant phycobilisomes have 620 nm/650 nm absorbance ratios ranging from 1.49 to 1.56, and hence are equivalent to wild type in their phycocyanin:allophycocyanin ratio. Three of the mutants, CM36, CM44, and CM82, have 620 nm:650 nm absorption ratios of 1.63, 1.66, and 1.64, respectively. These represent a subclass of mutants whose phycobilisomes contain ~15% more phycocyanin than do those of wild type.

Wild-type and mutant phycobilisomes vary in their phycoerythrin content. The 570 nm/650 nm absorbance ratio

TABLE I

Absorbance Ratios and Phycoerythrin Content of Phycobilisomes from Synechocystis 6701 Wild-type and Mutant Cells

Cells grown in white light				Red and green light-adapted cells			
Phycobilisome preparation	$\frac{A_{620}}{A_{650}}$	$\frac{A_{570}}{A_{650}}$	Phycoerythrin content*	Phycobilisome preparation	$\frac{A_{620}}{A_{650}}$	$\frac{A_{570}}{A_{650}}$	Phycoerythrin content*
Wild type (Wt)	1.48	1.80	79	Wild type			
Wt	1.52	1.87	84	Green light	1.51	2.51	125
Wt	1.54	2.08	97	Green light	1.55	2.69	137
Wt	1.52	2.13	101	Green light	1.45	2.77	142
Wt	1.54	2.27	110	Wt			
Wt	1.52	2.56	129	Red light	1.47	0.83	16
				Red light	1.56	1.17	38
				Mutant			
Mutant				Red light			
CM 70	1.54	0.59	<0.01	CM 70	Not determined (ND)		
CM 65	1.49	0.60	1	CM 65	ND		
CM 3	1.44	0.64	3	CM 3	ND		
CM 36	1.63	0.77	12	CM 36	1.67	0.74	10
CM 82	1.64	0.82	15	CM 82	1.66	0.73	9
CM 31	1.50	0.88	19	CM 31	1.54	0.69	6
CM 44	1.66	0.89	20	CM 44	1.64	0.84	16
CM 30	1.56	1.00	27	CM 30	1.63	0.82	15
CM 23	1.52	1.07	31	CM 23	1.59	0.78	12
CM 35	1.53	1.10	33	CM 35	1.53	1.57	64
CM 4	1.54	1.33	48	CM 4	1.55	0.76	11
CM 1	1.51	1.40	53	CM 1	1.51	0.78	12

* Expressed as the percent of average white-light wild-type phycoerythrin content as determined by the equation $\left(\frac{A_{570}/A_{650} - 0.59}{1.53}\right) \times 100$.

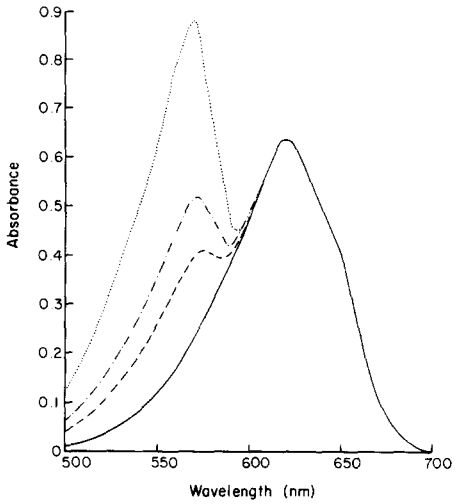

FIGURE 1 Absorption spectra of phycobilisomes from *Synechocystis* 6701 wild type (....), and mutants CM4 (-···-), CM30 (----), and CM70 (——). Absorption spectra were determined in 0.75 M NaK-phosphate, pH 8.0, of samples adjusted to equal absorbance at 620 nm. Light pathlength: 1 cm.

provides a measure of the amount of phycoerythrin present in the phycobilisomes. To determine this amount, the absorbance at 570 nm is corrected by subtraction of the absorbance at that wavelength due to phycocyanin and allophycocyanin. The 570 nm/650 nm absorbance ratio of CM70 phycobilisomes was used to provide the zero baseline for phycoerythrin content; any increase above this value is due to the presence of phycoerythrin. CM70 phycobilisomes were shown to contain <0.01% of the average wild-type phycobilisome phycoerythrin content by dissociating CM70 and wild-type phycobilisomes at $A_{620\,nm}$ of 0.05 in 0.05 M Na-phosphate buffer at pH 8.0. No fluorescence from phycoerythrin was detected (λ_{exc} = 550 nm; λ_{em} = 580 nm) in CM70 phycobilisomes even with the fluorimeter set at the highest sensitivity. Consequently, the value of 570 nm/650 nm above that of 0.59 for CM70 phycobilisomes is directly proportional to the phycoerythrin content of the phycobilisomes (assuming constant contributions to the absorbance from phycocyanin and allophycocyanin). The average of the phycoerythrin contents of the six wild-type phycobilisome preparations was selected to represent the 100% phycoerythrin content reference value. The phycoerythrin content of the various phycobilisome preparations, calculated as a percentage of this value, is given in Table I. Whereas the ratio of phycocyanin:allophycocyanin appears to be tightly regulated (see above), the phycoerythrin content of wild-type phycobilisomes from cells grown under white light varied from 79% to 129% of the average value.[1] Wild-type cells grown under green light to maximize phycoerythrin synthesis contain from 125% to 142% of the average level of phycoerythrin found in cells grown in white light. The green light culture with the 142% level of phycoerythrin had been grown under green light, with

[1] It is interesting to note the parallels between these observations and those reported for the photosynthetic accessory pigments of green and purple bacteria. In green bacteria, the bacteriochlorophyll *a*-protein complex serves as a structural and an energy transfer bridge between the chlorosomes, which contain complexes of the major light-harvesting pigment, bacteriochlorophyll *c*, and the reaction centers in the cytoplasmic membrane (25, 26). The levels of bacteriophyll *c* have been found to vary over as much as an order of magnitude upon variation of light intensity and temperature, whereas the levels of the bacteriochlorophyll *a*-protein complex were virtually constant (27, 28). Schmidt et al. (28) note in their study on *Chloroflexus aurantiacus* pigmentation "... slight changes in culture conditions result in significant changes in pigmentation of the cells." Similar results have been reported for the purple bacteria (see Drews [29] for a review and references) with respect to the ratios of the various light harvesting complexes and reaction center components.

transfers at two-month intervals for a year. This would appear therefore to represent the maximum lervel of phycoerythrin production in green light.

The phycoerythrin content of mutant phycobilisomes from cells grown in white light ranged from 0 to 53% of average wild-type level. In contrast to the observations made with wild-type cells, the 570 nm/650 nm ratio in the mutant phycobilisomes fluctuated by <5% between independent preparations. Mutants which contained >53% of wild-type level of phycoerythrin were not detected. This could well be because the color of colonies of such mutants is similar to that of the wild type. Colonies of CM1 and CM4, which contain 53% and 43% of wild-type level of phycoerythrin, appear dark green, approaching the almost black color of wild-type colonies on agar plates.

Organization of Biliproteins in Mutant Phycobilisomes

The near constancy of the 620 nm/650 nm ratio in the wild-type and mutant phycobilisomes indicates that the molar ratio of phycocyanin to allophycocyanin is similar in all cases, but it does not establish that the content and mode of assembly of these biliproteins in the mutant phycobilisomes is normal. This has been established by spectroscopic observations and electron microscopy. For excitation at 620 nm, the mutant and wild-type phycobilisomes show similar fluorescent emission spectra and quantum yields (data not shown). The portions of circular dichroism spectra, covering the red absorption bands of phycocyanin, allophycocyanin and allophycocyanin B, are superimposable for wild-type and mutant particles (see Fig. 2). Electron microscope examination of many of the mutant phycobilisomes show them all to contain wild-type triangular cores with the usual number of rods radiating from the core (see, for example, micrographs of red light–adapted mutant NTG 25, which contain <10% of wild-type level of phycoerythrin, in reference 13). These data, taken together, indicate that the structural defects in these mutant phycobilisomes are restricted to the distal phycoerythrin-containing portions of the rods.

Polypeptide Composition of Phycobilisomes

In addition to the four phycobiliproteins, *Synechocystis* 6701 phycobilisomes also contain ~12% by weight of the six major polypeptides whose molecular weights range from 27,000 to 99,000 (13, 15) (see Fig. 3). A 91-kdalton polypeptide is a variable constitutent of *Synechocystis* 6701 phycobilisomes. It is probably derived from the 99-kdalton polypeptide by proteolysis. Its presence is more evident in preparations from older cultures and in phycobilisome solutions which had been stored for extended periods of time. The relative levels of the 46-, 33.5-, and 27-kdalton polypeptides are similar in all wild-type and mutant phycobilisomes. In earlier studies, we had shown that the 31.5- and 30.5-kdalton polypeptides were associated with phycoerythrin (15) and that the 33.5-kdalton polypeptide was associated with phycocyanin (13).

The amounts of the 30.5- and 31.5-kdalton polypeptides in wild-type and mutant phycobilisomes vary in parallel with the phycoerythrin content. As illustrated in Fig. 3, the amount of 30.5 kdalton decreases with decrease in phycoerythrin content and it is absent when phycoerythrin is at 20% of average wild-type level (see CM44 in Fig. 3); however, 31.5 kdalton is present until no phycoerythrin remains. These observations are presented in a quantitative manner in Fig. 4. The mode of presentation of data in Fig. 4 was chosen on the basis of the

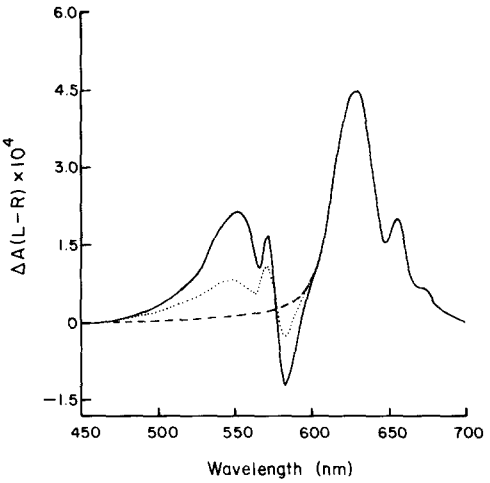

FIGURE 2 Circular dichroism spectra of phycobilisomes from *Synechocystis* 6701 wild type grown in white light (——), grown in red light (....), and from mutant CM70, lacking detectable phycoerythrin (----). Spectra were measured in 0.75 M NaK-phosphate, pH 8.0, at sample absorbance of 0.65 cm^{-1} at 620 nm.

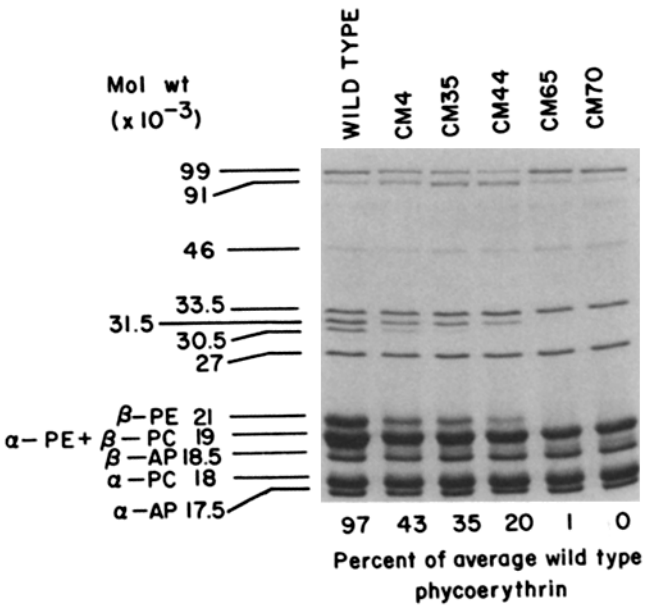

FIGURE 3 SDS PAGE of phycobilisomes from *Synechocystis* 6701 wild type and from five mutants grown under white light. For identification of components and determination of molecular weights, see reference 13. Phycoerythrin content was determined spectroscopically (see text). A decrease in the 30,500 and 31,500 dalton polypeptides is seen with decrease in phycoerythrin content. The level of the 33,500- and 27,000-dalton polypeptides remains constant. The abbreviations used are: α-AP and β-AP, α-PC, and β-PC, α-PE and β-PE, α and β subunits of allophycocyanin, phycocyanin, and phycoerythrin, respectively.

following considerations. The polypeptides in the 27- to 33.5-kdalton range are present in much lower amounts than the subunits of the biliproteins. If sample loads are increased to compensate for the decrease in the levels of 31.5- and 30.5-kdalton polypeptides, overlap of the biliprotein subunit bands on SDS polyacrylamide gels is significant. Moreover, the large difference in absolute amounts of the biliprotein subunits and of the polypeptides in the 27- to 33.5-kdalton range renders quantitation by densitometric gel scan inaccurate. In separate

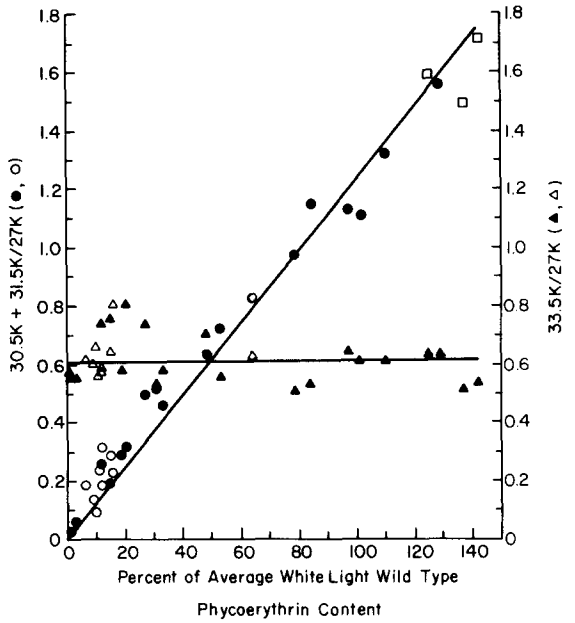

FIGURE 4 Relationship of polypeptide composition of phycobilisomes to phycoerythrin content. Each point compares, for a single phycobilisome preparation, the phycoerythrin content with the level of a given polypeptide or polypeptides (expressed as a ratio to the amount of the 27,000-dalton polypeptide). ●, ○, and □ represent the molar ratio of (30,500 + 31,500)/27,000 vs. phycoerythrin content for phycobilisomes from cells grown in white, red, and green light, respectively. ▲ and △, represent the molar ratio of 33,500/27,000 for phycobilisomes from cells grown in white, and red light, respectively.

experiments, we have established that relative to the α-subunits of allophycocyanin and phycocyanin, the level of the 27-kdalton polypeptide was constant to within ±10%. Consequently, in Fig. 4, we have compared the levels of the 33.5-, 31.5-, and 30.5-kdalton polypeptides to that of the 27-kdalton polypeptide. It is evident that the level of the 33.5-kdalton is relatively constant in phycobilisomes of wild type and mutants. The values average 0.61 mol 33.5 kdalton/mol 27 kdalton with a standard deviation of 0.08 mol. When the molar ratio of (30.5 kdalton + 31.5 kdalton)/27 kdalton is plotted vs. phycoerythrin content (determined by spectrophotometry), a linear relationship is observed. When phycoerythrin is absent, as in CM70 (see Table I, and Fig. 3), both 30.5 kdalton and 31.5 kdalton are absent. These observations indicate a stoichiometric relationship between the 30.5 kdalton + 31.5 kdalton and phycoerythrin content of *Synechocystis* 6701 phycobilisomes. This relationship was maintained for all phycobilisomes, whether obtained from cells grown in white, red, or green light (see Fig. 4).

The number of 30.5-kdalton + 31.5 kdalton–polypeptides per hexamer of phycoerythrin can be estimated from the molar ratio of these polypeptides to the β-subunit of phycoerythrin as determined from densitometry of SDS polyacrylamide gels. Data for mutants CM1, CM4, and numerous wild-type preparations lead to a value of 1.1 ± 0.1 copies of 30.5 kdalton + 31.5 kdalton per phycoerythrin hexamer, $(\alpha\beta)_6$. Bryant et al. (10) reported two disks of hexameric phycocyanin per rod in *Synechocystis* 6701 phycobilisomes obtained from cells grown in red light (see Table I, ref. 10). Since the phycocyanin content of *Synechocystis* 6701 phycobilisomes does not vary with light quality (see Table I, and references 10 and 13) and since these phycobilisomes have six rods each, there are twelve hexamers of phycocyanin per phycobilisome under all conditions of illumination. The ratio of the β-subunit of phycoerythrin to the α-subunit of phycocyanin can be determined by gel scans. From this ratio and the assumption that there are 12 hexamers of phycocyanin per phycobilisome, it can be calculated that every 9.5% of average wild-type phycoerythrin content represents one hexamer of phycoerythrin per phycobilisome, i.e., a wild-type phycobilisome, from cells grown in white light, contains ~10 hexamers of phycoerythrin and has rods of average length of 22/6 or 3.7 disks. Bryant et al. (10) reported a value of 3.6 for the average length of rods of phycobilisomes obtained from *Synechocystis* 6701 cells grown in green light.

The relationship between the number of copies of the 31.5- and 30.5-kdalton polypeptides and the number of phycoerythrin hexamers in the various phycobilisome preparations is shown in Fig. 5. When fewer than two hexamers of phycoerythrin are present per phycobilisome, only the 31.5-kdalton polypeptide is found. The slope of the line of best fit through the points up to two hexamers of phycoerythrin per phycobilisome indicates the presence of one copy of 31.5-kdalton per phycoerythrin hexamer. The 30.5-kdalton polypeptide appears when more than two hexamers of phycoerythrin are present in the phycobilisome. Above three hexamers of phycoerythrin per phycobilisome the slope of the line relating 31.5 kdalton to phycoerythrin decreases to less than one-half of the original slope. The slope of the line of best fit for 30.5 kdalton is constant and, within experimental error, consistent with the

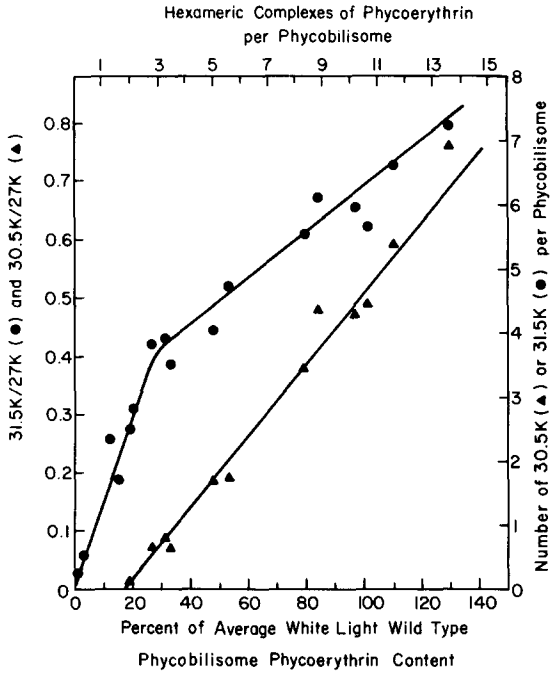

FIGURE 5 Relationship between the number of copies of the 30,500 and 31,500 dalton polypeptides and the number of hexamers of phycoerythrin in wild-type and mutant phycobilisomes from cells grown in white light (for details, see text). The number of copies of the 30,500- and 31,500-dalton polypeptides per phycobilisome was computed from densitometric scans of SDS polyacrylamide gel patterns, with the assumption that a phycobilisome contained twelve hexamers of phycocyanin (or 72 copies of the α subunit of phycocyanin). The ratio of 31,500/27,000 and 30,500/27,000 is a convenient measure of the relative content of the 31,500 and 30,500 polypeptides in different phycobilisomes, because all of the phycobilisomes examined here had a constant amount of the 27,000 polypeptide per phycobilisome.

addition of one copy of 30.5 kdalton for every two hexamers of phycoerythrin.

Effect of Chromatic Adaptation

Synechocystis 6701 is a Group II chromatic adapter (22); in red light the synthesis of phycoerythrin is substantially decreased with no change in phycocyanin level (see Table I). Moreover, in red light the synthesis of other polypeptide components of the phycobilisome is also affected (2, 17); the 30.5-kdalton polypeptide is no longer present and the 31.5 kdalton is reduced in amount (Fig. 6). The 30.5-kdalton polypeptide was absent from phycobilisomes obtained from red-adapted cells of the mutants as well, with the exception of those of CM35, which does not adapt chromatically and actually doubles its phycoerythrin level in red light (see below, and Table I). In red light-adapted cells of wild type and mutants, the amount of phycoerythrin remaining varies depending on the light intensity, temperature, and age of the culture. It is of particular interest that a wild-type culture grown in red light at low intensity at 23°C retained 35% of the normal white-light level of this biliprotein but no trace of the 30.5-kdalton polypeptide. The phycobilisomes obtained from these cells contained four hexamers of phycoerythrin per phycobilisome. Overall, the results obtained with the red light-adapted cells reinforce the indications obtained from the results of studies on cells grown on white light that the attachment of phycoerythrin hexamers containing the 31.5-kdalton polypeptide proceeds independent of the presence of 30.5-kdalton polypeptide, but that the converse situation is not observed.

The unusual mutant, CM35, merits special attention. The phycoerythrin content of CM35 increases from 32% of the wild-type level in white light to 65% of the white-light level in cells grown under red light. In CM35 white-light phycobilisomes, the ratio of 30.5 and 31.5 kdalton to phycoerythrin follows the general trend (Fig. 5). However, in red-light phycobilisomes, 31.5 kdalton is at a higher level than that usually observed, and the 30.5-kdalton polypeptide at a lower level. Thus, there are six copies of 31.5 kdalton and one of 30.5 kdalton for every seven hexamers of phycoerythrin compared to an expected value (Fig. 5) of five copies of 31.5 kdalton and two to three copies of 30.5 kdalton for every seven hexamers of phycoerythrin per phycobilisome.

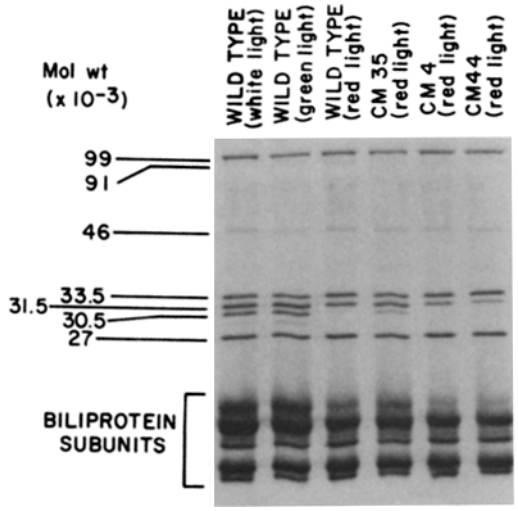

FIGURE 6 SDS PAGE of phycobilisomes from *Synechocystis* 6701 wild type grown in white, green and red light, and from three mutants grown in red light. Note disappearance of 30,500-dalton polypeptide from phycobilisomes obtained from red light-adapted cultures, except for mutant CM35 which does not adapt chromatically (see text).

Circular Dichroism Studies

The spectra of all wild-type and mutant phycobilisome preparations we examined showed a peak at 670 nm (contributed to by allophycocyanin B [16]), a peak at 645 nm attributed to allophycocyanin and a peak at 630 nm arising from phycocyanin. When phycobilisome solutions are matched for absorbance at 620 nm, these three peaks are identical in magnitude, except for the mutants with ratios of 620 nm/650 nm higher than wild type. In these mutants, the 630 nm C.D. peak is proportionally higher with no change in the relative magnitude of the 645- and 670-nm components. CM70 phycobilisomes which totally lack phycoerythrin show no C.D. peaks between 450 and 600 nm (Fig. 2). Wild-type phycobilisomes, and those from all mutants containing phycoerythrin, have three additional C.D. peaks at 582, 572, and ~550 nm (Fig. 2). The amplitude of the sharp negative 582-nm peak is directly proportional to the amount of phycoerythrin present in the phycobilisome.

DISCUSSION

A number of pigment mutants of *Synechocystis* 6701 have phycobilisomes low in phycoerythrin but normal with respect to the relative amounts and organization of the other biliproteins. The phycobilisomes of the mutants we examined have absorption and circular dichroism spectra >610 nm indistinguishable from those of the wild type, show the same qualitative and quantitative fluorescence emission upon 620-nm excitation, and have wild-type level of the phycocyanin-associated 27-kdalton polypeptide. By electron microscopy, the assembly of the core components of the mutant particles appears the same as that of wild-type phycobilisomes. Hence, the significant differences in the structure of the mutant and wild-type particles are confined to the distal portions of the rods, made up in the main of phycoerythrin.

All of the mutants examined here, which produce phycoerythrin, appear to produce a protein indistinguishable from its counterpart in the wild type as judged by spectroscopic properties, SDS polyacrylamide gels, and isoelectric focusing in the presence of urea. Consequently, it is likely that the mutations in these strains affect the regulation of phycoerythrin production. We have no evidence that any of these mutants carry mutations in the structural genes for phycoerythrin.

Incorporation of phycoerythrin into the rods of *Synechocystis* 6701 phycobilisomes involves first the addition of $(\alpha\beta)_6$-31.5-kdalton complexes of this protein onto the phycocyanin portion of the rod. Subsequently, addition to these phycoerythrin disks of $(\alpha\beta)_6$-30.5-kdalton phycoerythrin complexes completes the assembly of the rods. These conclusions are based on the data of Figs. 3, 5, and 6, which show that phycobilisomes can be obtained either from mutants or red light-adapted cells, which lack the 30.5-kdalton polypeptide but contain up to four hexamers of phycoerythrin per phycobilisome. The converse situation, i.e., presence of phycoerythrin and 30.5 kdalton in the absence of 31.5 kdalton polypeptide, is not observed in any instance. In all phycobilisomes, one copy of 30.5 + 31.5 kdalton per phycoerythrin hexamer was present. Evidently, one copy of either polypeptide suffices for the assembly of a phycoerythrin disk.

In wild-type phycobilisomes there are three to four disks per rod (10, 13). From studies of phycobilisomes from red light-adapted cells, it has been shown (10, 13) that the number of rods is unchanged, but that the rods are nearly all two disks long. From this observation, and the fact that the phycocyanin content of phycobilisomes is the same in red and white light, it can be inferred that the two disks proximal to the core contain phycocyanin. Hence, phycoerythrin is located in the distal one to two disks of each rod. Our study (see Fig. 5), as well as the earlier studies by Bryant et al. (10) and Williams et al. (13), makes it evident that the rods in wild-type phycobilisomes are very rarely longer than four disks. These various observations are consistent with the conclusion that each rod contains one disk of phycoerythrin–31.5-kdalton complex, and, in most cases, a second disk of phycoerythrin–30.5-kdalton complex. In vitro observations (unpublished data) are relevant to this conclusion. Purified phycoerythrin–31.5-kdalton complex forms long rods in 0.6 M phosphate, pH 8.0. However, when a mixture of phycoerythrin–31.5-kdalton and phycoerythrin–30.5-kdalton complexes is examined in this buffer, large aggregate formation is greatly reduced. These in vitro experiments suggest that the phycoerythrin–30.5-kdalton complex serves to terminate rod growth.

In earlier studies, we observed that a mutant, which had lost half of the normal phycocyanin content, had also lost the 33.5-kdalton polypeptide (13). However, this mutant still had the normal number of rods per phycobilisome and the normal content of the 27-kdalton polypeptide (13). From this observation, we conclude that the 27-kdalton polypeptide is associated with the phycocyanin disk proximal to the core and the 33.5-kdalton polypeptide with the second phycocyanin disk. In studies with *Synechococcus* 6301 phycobilisomes (which contain no phycoerythrin), we have established that a 27-kdalton polypeptide is associated with the phycocyanin hexamer proximal to the core and that a 33-kdalton polypeptide is a component of the subsequent disks in the rods (19, 30). These assignments are equivalent to those deduced above for the 33.5- and 27-kdalton polypeptides of *Synechocystis* 6701 phycobilisomes and suggest that the structure of these polypeptides in different cyanobacteria may be highly conserved.

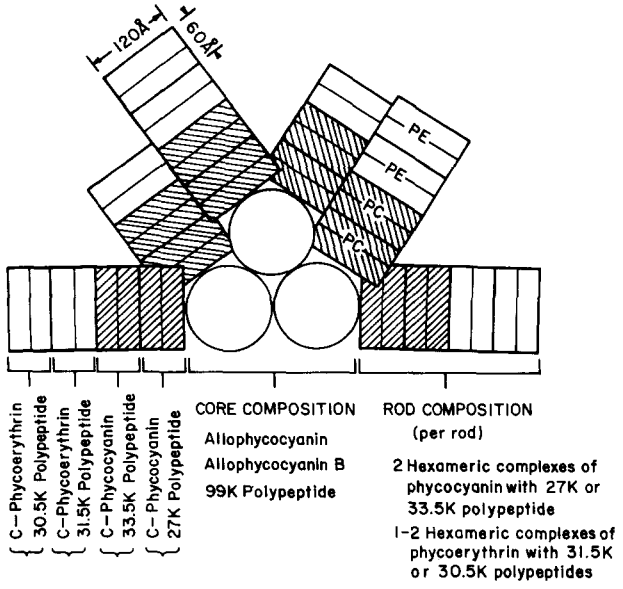

FIGURE 7 Diagrammatic representation of a *Synechocystis* 6701 wild-type phycobilisome. PC and PE designate hexameric assemblies of phycocyanin and phycoerythrin, respectively.

The combined data from the present and earlier studies (13, 15) lead to a model for the *Synechocystis* 6701 phycobilisome shown in Fig. 7. The role of the 27-, 33.5-, 31.5-, and 30.5-kdalton "linker" polypeptides (19) is to assemble phycocyanin and phycoerythrin into disks, and to ensure that the disks are added to the rods in proper number and sequence. From the difference in the levels of the 31.5- and 30.5-kdalton polypeptides in green and red light, it is clear that other factors, as yet undefined, play a role in the determination of phycobilisome size. From our studies of *Synechococcus* 6301 phycobilisomes, we have noted that the properties of the various complexes of phycocyanin with the linker polypeptides are such that nonradiative energy transfer takes place along the rods towards the core (14). It is likely that the same polarity of transfer between consecutive disks is established in the rods of *Synechocystis* 6701 phycobilisomes. The spectroscopic properties of the individual rod components will be examined in greater detail in the future.

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