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Core Substructure in Cyanobacterial Phycobilisomes

Jeffrey C. Gingrich, Daniel J. Lundell, and Alexander N. Glazer

Department of Microbiology and Immunology, University of California, Berkeley, California 94720

The tricylindrical core of Synechocystis 6701 phycobilisomes is made up of four types of allophycocyanin-containing complexes: A, $(\alpha^{AP}\ \beta^{AP})_3$; B, $(\alpha^{AP}\ \beta^{AP})_3$ .10K; C, $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K; D, $(\alpha^{AP}\ \beta^{AP})_2$.18.5K.99K; where AP is allophycocyanin, APB is allophycocyanin B, and 10K, 18.5K, and 99K are polypeptides of 10,000, 18,500, and 99,000 daltons, respectively. The 18.5K polypeptide is a hitherto unrecognized biliprotein subunit with a single phycocyanobilin prosthetic group. The tricylindrical core is made up of 12 subcomplexes in the molar ratio of A:B:C:D: of 4:4:2:2. Complexes C and D act as terminal energy acceptors. From these results and previous analysis of the bicylindrical core of Synechococcus 6301 phycobilisomes [14,15] it is proposed that the two cylinders of the Synechocystis 6701 core, proximal to the thylakoid membrane, each have the composition ABCD, and that the distal cylinder has the composition A_2B_2.

Key words: cyanobacteria, phycobilisome substructure, allophycocyanin complexes, biliproteins, energy transfer

In cyanobacteria and red algae, phycobiliproteins are the major light-harvesting components of photosystem II. These intensely colored chromoproteins are a family of conjugated proteins with open-chain tetrapyrrole prosthetic groups. The spectroscopic properties of a phycobiliprotein result from the interplay of several factors: the structure of its tetrapyrrole prosthetic groups, the modulation of the properties of these groups by the conformation of the protein subunit to which they are bound, and the interactions which take place upon incorporation of the subunit into higher aggregates [1,2]. In vivo, phycobiliproteins are constituents of high molecular weight particles—phycobilisomes that exist as peripheral complexes on the cytoplasmic surfaces of thylakoid membranes. Simple procedures permit isolation of intact phycobilisomes [3,4]. Such isolated particles exhibit efficient energy transfer from the major constituent phycobiliproteins to those that function as terminal energy acceptors and are present in minor amounts. For example, the phycobilisome of Synechocystis 6701 contains phycoerythrin (λ^F_{max} 580 nm), phycocyanin (λ^F_{max} 650 nm), and allophycocyanin (λ^F_{max} 660 nm), as major biliproteins [5]. However, excitation at 500–600 nm results in emission with a maximum at 680 nm [5]. This emission maximum

Abbreviations used: λ^F_{max}, fluorescence emission maximum; AP, allophycocyanin; APB, allophycocyanin B; α^{AP} and β^{AP}, α and β subunits of allophycocyanin; α^{APB}, α subunit of allophycocyanin B; 10K, 18.5K, etc, polypeptides of 10,000, 18,500 daltons, etc.

Jeffrey C. Gingrich is now at Department of Chemistry, University of Colorado, Boulder, CO 80309.

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is characteristic of the minor phycobiliproteins, allophycocyanin B [6], and the 99K polypeptide [7] present in this phycobilisome. The 680-nm emission is largely lost upon dissociation of the particle [5].

Electron microscopy has revealed that phycobilisomes from different organisms vary in morphology [3]. Most frequently, phycobilisomes of cyanobacteria [8,9], as well as those of the red alga Rhodella violacea [10], are hemidiscoidal objects of ~5–10 million daltons, consisting of two distinct morphological domains—rods and a core. Two examples of such hemidiscoidal particles, the phycobilisomes of Synechococcus 6301 and Synechocystis 6701, are shown schematically in Figure 1. The bi- or tricylindrical core substructure (each cylinder being 11 nm across and 14 nm tall) contains the minor biliproteins that act as terminal energy acceptors and presumably interact with the membrane-bound photosystem II. The phycobiliprotein allophycocyanin (λ^{F}_{max}660 nm) is a major constituent of the core and functions as a bridge for the transfer of energy from the large number of chromophores in the rods to the terminal energy acceptors. The rod substructure has been extensively studied in Synechococcus 6301 [see 2 for a review]. Up to six rods radiate from the core. Each rod is a stack of hexameric, $(\alpha^{PC}\beta^{PC})_6$, phycocyanin discs (each ~250,000 daltons). Each disc contains a specific linker polypeptide of either 27 or 33 or 30K daltons, lacking prosthetic groups. Discs proximal to the core contain the 27K linker polypeptide, followed by those containing the 33K polypeptide, with discs containing the 30K polypeptide at the periphery of the rods (Fig. 1A). Each linker polypeptide modulates the spectroscopic properties of phycocyanin in a manner such as to bias the energy transfer within the rods towards the core [11]. Synechocystis 6701 phycobilisomes show the same type of rod organization (Fig. 1B). The rods of these phycobilisomes contain both phycoerythrin and phycocyanin, with the phycoerythrin-containing discs occupying the distal position in the rods [5,8,12]. The rod structures of several other hemidiscoidal phycobilisomes show the same pattern of organization [8,13].

The core substructure has been analyzed in detail only for the phycobilisomes of Synechococcus 6301. We have recently characterized four distinct trimeric complexes derived from the core: $(\alpha^{AP}\beta^{AP})_3$.10.5K, $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10.5K, $(\alpha^{AP}\beta^{AP})_2$.18.3K.75K, and $(\alpha^{AP}\beta^{AP})_3$ [14,15]. Each complex is present at two copies per phycobilisome; together these complexes account for the entire core structure. Whereas all complexes contain allophycocyanin subunits (α^{AP} and β^{AP}), each is structurally unique. Two complexes $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10.5K [15] and $(a^{AP}\beta^{AP})$.18.3K.75K [14], show emission maxima near 680 nm and presumably function as terminal energy acceptors. The chromophores responsible for the 680-nm emission are on the α^{APB} subunit and the 75K polypeptide [16,17]. A unique biliprotein of 18.3K was found within the 75K-containing complex. This biliprotein was shown to be homologous to the β^{AP} subunit by sequence analysis. A nonpigmented polypeptide of ~10.5K was present in two of the complexes (see above).

Among hemidiscoidal phycobilisomes thus far examined, only those of Synechococcus 6301 possess a bicylindrical core. The information on the tricylindrical core is limited. In the most complete examination to date of core components from a tricylindrical core-containing Nostoc sp phycobilisome, Zilinskas et al [18] isolated three allophycocyanin components, designated API, II, and III, as well as a very small amount of allophycocyanin B. APII and APIII appeared similar to previously described trimers of pure allophycocyanin in that they had fluorescence emission

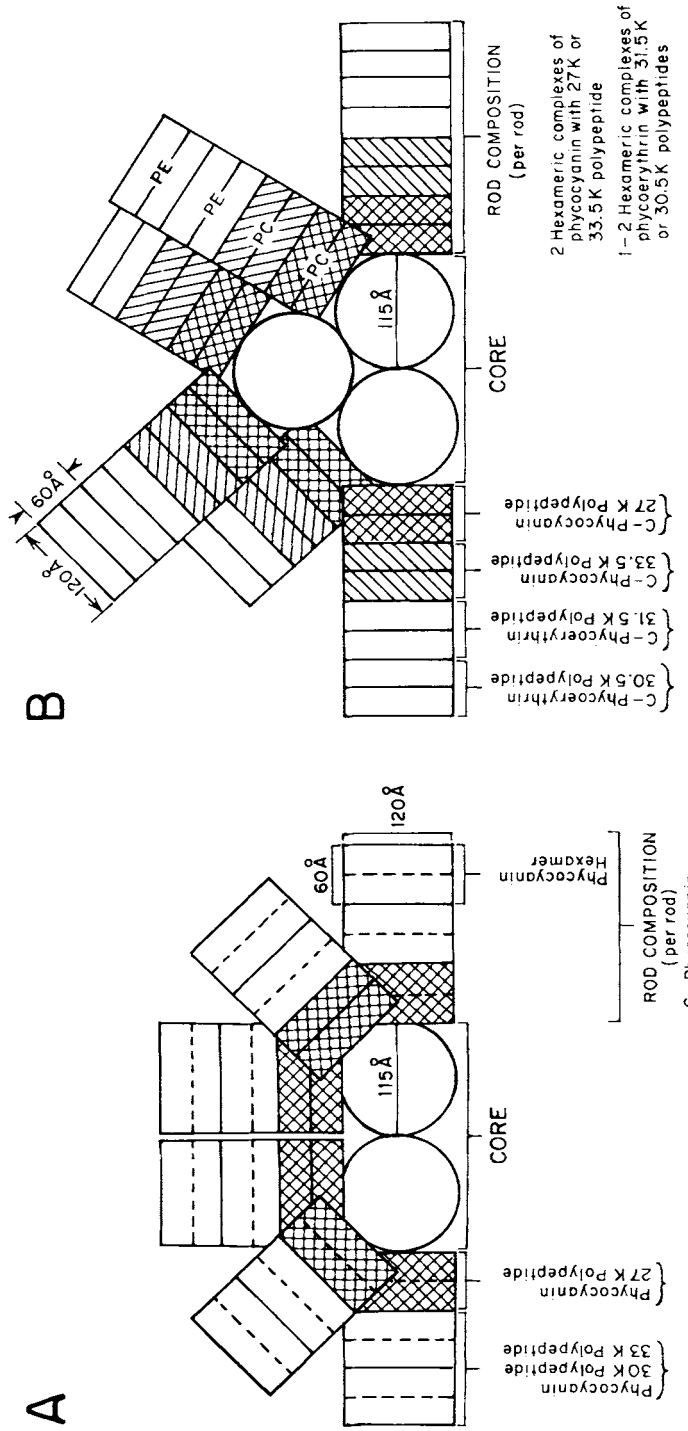


Fig. 1. Schematic representation of the phycobilisomes from Synechococcus 6301 (A) and Synechocystis 6701 (B). Composition of the rod substructure is indicated. Abbreviations used are PE, phycoerythrin, PC, phycocyanin.

maxima at ~660 nm. API was a unique complex with an emission maximum at 680 nm [18].

In this report, we describe the isolation and characterization of four types of complexes that make up the entire tricylindrical core of the Synechocystis 6701 phycobilisomes [9]. Indeed, the core complexes of the two phycobilisomes were found to be closely analogous. Consequently, the results allow us to present a plausible proposal for the structure of the tricylindrical core. A comparison of these data with those from Nostoc sp is discussed.

MATERIALS AND METHODS

Cells, Culture Conditions, and Phycobilisome Preparation

A previously described mutant CM25 [19] of Synechocystis 6701 (ATCC 27170) was used for this study. This mutant produced phycobilisomes which lacked the phycoerythrin disc components of the rod substructures, but were otherwise equivalent to the wild-type particle. Cells were grown in 10 l carboys of medium BG-11 as previously described [5]. CM25 phycobilisomes were prepared by the procedure described earlier for those from wild-type cells [5].

Spectroscopic Measurements

Absorption spectra were recorded on a Beckman Model 25 recording spectrophotometer. Corrected fluorescence emission spectra were obtained with a Perkin-Elmer MPF-44B recording spectrofluorimeter equipped with a DCSU-2 differential corrected spectra unit.

Electrophoresis and Isoelectric Focusing

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue R-250 were performed as previously described [20]. All slab gels contained 14% acrylamide-0.36% methylenebisacrylamide. Isoelectric focusing gels in 9.2 M urea and two-dimensional gels were prepared as previously described [14]. The isoelectric focusing range was pH 4–6 (4:1 v/v ratio of L.K.B. pH 4–6 ampholines and Bio-Rad pH 3–10 ampholytes).

Isolation of Core Complexes of CM25 Phycobilisomes

CM25 phycobilisomes were exchanged into 1 mM Na phosphate-100 mM NaCl-10% (v/v) glycerol, pH 7, by rapid gel filtration on Sephadex G-25 and were then applied to a hydroxylapatite column, equilibrated with the same buffer, at a load of ~ 100 mg of protein per 10 ml of hydroxylapatite. The column was eluted with 50 mM Na phosphate-100 mM NaCl-10% (v/v) glycerol, pH 7, to remove phycocyanin and its associated linker polypeptides. The allophycocyanin-containing complexes (allophycocyanin pool) were then frontally eluted with 200 mM Na phosphate, pH 7. The above steps were carried out rapidly at room temperature; all subsequent steps were performed at 5°C. The allophycocyanin pool was dialyzed against 5 mM Na phosphate-10% (v/v) glycerol, pH 7, and applied to a diethylaminoethyl (DEAE) cellulose column, equilibrated in the same buffer, at a load of ~25 mg protein per 10-ml settled column bed volume. The column was eluted with a 10-column volume linear gradient of 5 mM to 200 mM Na phosphate, pH 7, in 10% (v/v) glycerol, at a flow rate of 20 ml/hr. Absorbance of the fractions was determined at 360 nm.

Absorbance at 360 nm is proportional to bilin concentration [15] with a small correction for allophycocyanin B-containing fractions. The molar extinction coefficient at 360 nm for α^{APB} in Synechococcus 6301 is 36 mM^{-1} cm^{-1} versus 18 mM^{-1} cm^{-1} for the other phycobiliprotein subunits. Linear sucrose density gradients (5–20% w/v), used for further fractionation, were run in the buffers indicated below, in a Spinco SW41 rotor at 38,000 rpm, at 5°C, for 16–18 h.

Molecular Weight Determination

Estimates of the molecular weights for the core complexes were based on their sedimentation behavior in linear (0.2 – 1.0 M) sucrose density gradients calibrated with allophycocyanin and B-phycoerythrin [1] as molecular weight markers.

RESULTS

Preliminary Data on the Composition of the Synechocystis 6701 Phycobilisome Core

Partial data on the composition of the core substructure of Synechocystis 6701 wild-type [5] and strain CM25 phycobilisomes were obtained from densitometric scans of one-dimensional SDS-polyacrylamide gels. Per particle, assuming 72 $\alpha\beta$ phycocyanin monomers [12], these phycobilisomes contained ~ 36 $\alpha\beta$ allophycocyanin monomers, 2.0 $\pm$ 0.4 copies of the 99K polypeptide, and 6.5 $\pm$ 0.8 copies of a 10K polypeptide (presumably a counterpart to the 10.5K polypeptide present in the core of Synechococcus 6301 phycobilisomes [15]).

The 99K polypeptide, when isolated by the procedure described for the 75K polypeptide of the Synechococcus 6301 phycobilisome [17], was shown to carry a covalently bound phycocyanobilin. The 99K polypeptide is very susceptible to endogenous proteolytic degradation during isolation and storage and gives rise to a 91K fragment [12, see also 21]. Allophycocyanin B, a core component in Synechococcus 6301 phycobilisomes [15], has been previously isolated from Synechocystis 6701 [6], but its amount could not be determined adequately by densitometry of stained SDS-gels of the phycobilisome components due to its close migration to other biliprotein subunits.

Comparison of the above data with that determined earlier for the two cylinder core of Synechococcus 6301 phycobilisomes [14,15] showed that the Synechocystis 6701 phycobilisomes contained approximately 1.5 times as much allophycocyanin as do those of the former organism, and six copies (as opposed to four in Synechococcus 6301) of a low molecular weight, 10K, polypeptide. This comparison suggested that the third core cylinder of Synechocystis 6701 phycobilisomes was made up of four allophycocyanin trimers and contained two copies of the 10K polypeptide.

Isolation and Quantitation of Core Subcomplexes

To simplify the isolation of the core subcomplexes, we chose to use the phycobilisomes of Synechocystis 6701 mutant strain CM25. The phycobilisomes of CM25 differ from those of the wild type in that they lack phycoerythrin and its associated linker polypeptides of 30.5K and 31.5K; in all other respects they are equivalent to those of wild type [19].

Chromatography of dissociated phycobilisomes on hydroxylapatite (see Materials and Methods) permitted the separation of a fraction containing all of the

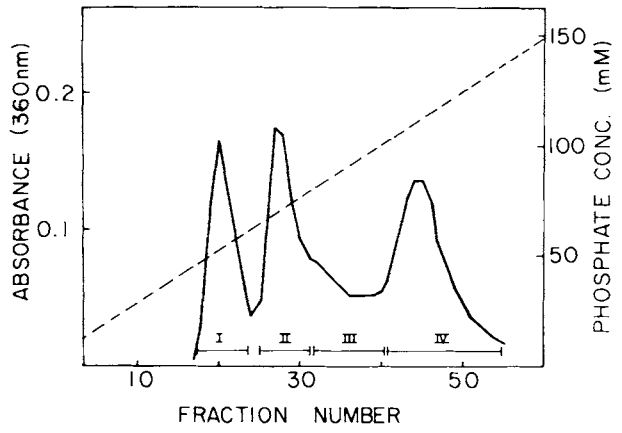


Fig. 2. DEAE cellulose chromatography of the allophycocyanin-containing components of Synechocystis 6701 strain 25 phycobilisomes. For experimental conditions, see Materials and Methods. Fractions were pooled as indicated.

allophycocyanin of the phycobilisome from phycocyanin and its linker polypeptides. The allophycocyanin fraction was then chromatographed on DEAE cellulose (Fig. 2). Three well-resolved peaks, I, II, and IV, and a broad peak, III, were obtained. The profile shown in Figure 2 reflects a quantitative recovery of all components since no colored material remained on the column at the end of the elution. Since the absorbance at 360 nm is directly related to bilin concentration [15], the data of Figure 2 can be used to determine the stoichiometry of the core components. The implicit assumption that each peak represents an individual component is documented to be valid below. Based on 12 trimeric complexes per core, the ratio of I:II:III:IV was determined to be 2.2:3.6:2.1:4.1.

Characterization of the Core Subcomplexes of Synechocystis 6701 Phycobilisome

Characterization of the complexes present in pools I, II, and IV (Fig. 2) is presented in this section. Characterization of the complex in pool III presented special difficulties and is discussed in the next section. Figure 3 shows an SDS-polyacrylamide gel pattern for all four complexes, while Figure 4 shows a two-dimensional gel revealing the phycobiliprotein composition of each of the complexes in greater detail. Figure 4 also shows the pattern given by the entire allophycocyanin pool used as the starting material for the DEAE cellulose chromatography.

The complex in pool IV (Fig. 2) contained only α^{AP} and β^{AP} subunits in equal amounts (Figs. 3E, 4, AP). On sucrose density gradient centrifugation, this complex sedimented as a single component of ~ 100,000 daltons. It had an absorption maximum at 646 nm and a fluorescence emission maximum at 657 nm (Fig. 5). We conclude that this complex is the well-characterized trimer of allophycocyanin $(\alpha^{AP}\beta^{AP})_3$ [1] and is present at four copies per phycobilisome (see above).

The complex in pool II (Fig. 2) contains α^{AP} and β^{AP} subunits and an uncolored 10K polypeptide in a molar ratio at 3:3:1 (Figs. 3D, 4, AP-10K). Its apparent

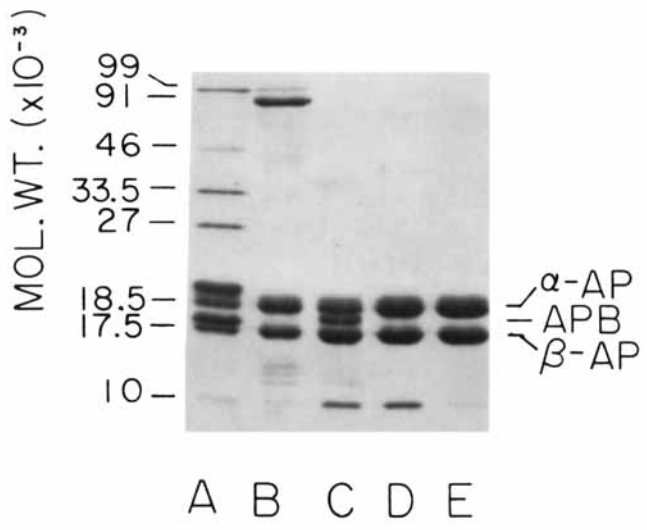


Fig. 3. SDS-polyacrylamide gel electrophoresis of allophycocyanin-containing complexes. Lane A, strain CM25 phycobilisomes; B, "allophycocyanin 99K" hexamer (see text); C, $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K complex (pool I, Fig. 2); D, $(\alpha^{AP}\beta^{AP})_3$.10K complex (pool II, Fig. 2); E, $(\alpha^{AP}\beta^{AP})_3$ (pool IV, Fig. 2). Molecular weights are given on the left-hand margin. The abbreviations used are AP, allophycocyanin; APB, allophycocyanin B α subunit.

molecular weight on sucrose density gradients is ~ 100,000; therefore, this complex has the composition $(\alpha^{AP}\beta^{AP})_3$.10K. As shown in Figure 5, the $(\alpha^{AP}\beta^{AP})_3$.10K complex has a red-shifted absorption maximum and an enhanced absorbance relative to the $(\alpha^{AP}\beta^{AP})_3$ trimer (649 nm versus 646 nm), as well as a slightly red-shifted fluorescence emission maximum (660 nm versus 657 nm). Four copies are present per phycobilisome.

Pool I (Fig. 2) contains a complex very similar to that in pool II except for an additional polypeptide, clearly seen in Figure 3, which migrates between α^{AP} and β^{AP} subunits. We believe that this polypeptide is the α subunit of allophycocyanin B, and thus that the composition of this complex is $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K. This conclusion is based on the similarities of this complex to the $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10.5K complex isolated from Synechococcus 6301 phycobilisomes and extensively characterized [15]. The absorption spectrum of this complex shows a shoulder centered near 670 nm and the fluorescence emission spectrum has a maximum near 680 nm (Fig. 6). These spectroscopic features are characteristic of the α^{APB} subunit, as established earlier for the Synechococcus 6301 complex [15]. The two-dimensional gel of this complex (Fig. 4, APB) shows α^{AP} and β^{AP} subunits identical to those in the $(\alpha^{AP}\beta^{AP})_3$ and $(\alpha^{AP}\beta^{AP})_3$.10K complexes; and the α^{APB} subunit is split into two spots of the same molecular weight but different isoelectric point. Both spots are seen in the allophycocyanin pool prior to DEAE cellulose chromatography. However, we believe that there is only a single α^{APB} subunit and that the splitting is an artifact of handling. This conclusion is based on two observations. When intact phycobilisomes are analyzed on two-dimensional gels only the more acidic α^{APB} spot is seen. Second,

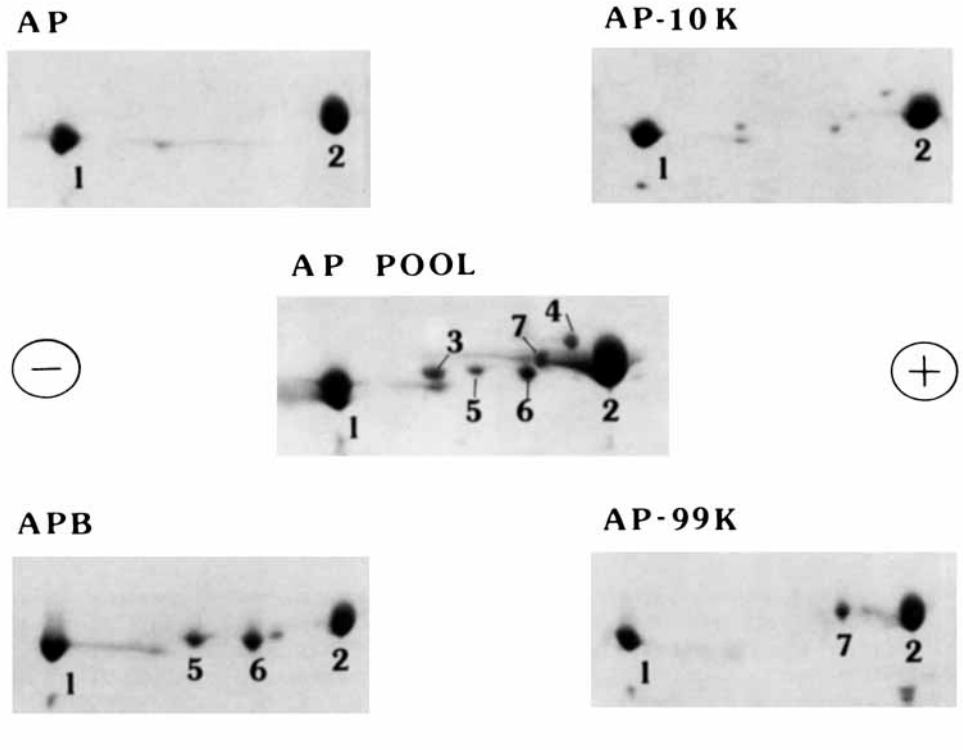


Fig. 4. Two-dimensional maps of the biliprotein components of allophycocyanin-containing complexes from Synechocystis 6701 strain CM25 phycobilisomes. AP, $(\alpha^{AP}\beta^{AP})_3$; AP-10K, $(\alpha^{AP}\beta^{AP})_3$.10K; APB, $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K; AP-99K, $(\alpha^{AP}\beta^{AP})_3$.$(\alpha^{AP}\beta^{AP})_2$.18.5K.99K (see text); AP POOL, sample applied to DEAE cellulose column (Fig. 2). The identity of the spots is as follows: 1, β^{AP}; 2, α^{AP}; 3, α^{PC}; 4, β^{PC}; 5 and 6, α^{APB}; 7, 18.5K polypeptide.

peptide maps of α^{APB} subunits from each of the spots of Figure 4 APB after either CNBr cleavage or S aureus V8 protease digestion by the method of Cleveland et al [22] are identical. The complex has an apparent molecular weight on sucrose density gradients of ~100,000, consistent with the proposed structure. Two copies of this complex are present per phycobilisome.

Allophycocyanin—99K Complex

Material in pool III (Fig. 2) was shown by SDS-polyacrylamide gel electrophoresis to contain the 99K polypeptide (or a fragment of 91K derived from it) as well as α^{AP} and β^{AP} subunits. However, pool III did not contain the 10K polypeptide or the α^{APB} subunit. The fluorescence emission of pool III had a peak at 660 nm and a small shoulder at 680 nm. The $(\alpha^{AP}\beta^{AP})_2$.18.3K.75K complex from Synechococcus 6301 phycobilisomes has a composition analogous to that observed for pool III and an emission maximum at 680 nm [14]. These observations suggest that the Synechocystis 6701 complex in pool III was probably unstable under the conditions used for ion-exchange chromatography and another isolation method was sought.

When the entire allophycocyanin pool from the hydroxylapatite column was fractionated on a sucrose gradient in 0.1 M Na phosphate, pH 7, two colored zones

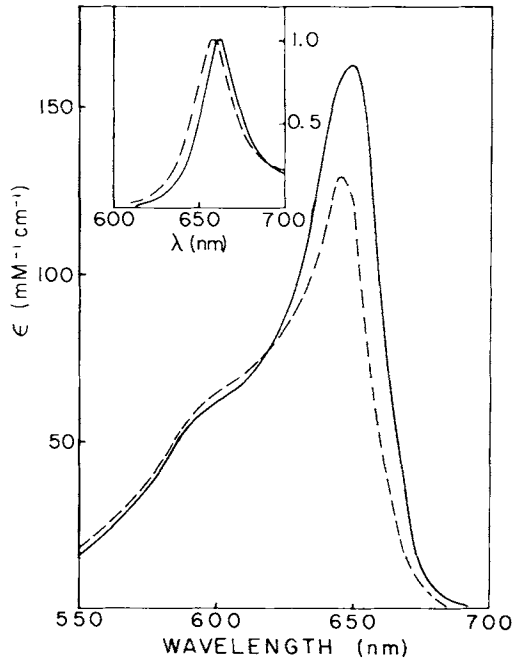


Fig. 5. Absorption and fluorescence emission spectra (inset) of the $(\alpha^{AP}\beta^{AP})_3$.10K (——) and $(\alpha^{AP}\beta^{AP})_3$ (-----) complexes. Extinction coefficients are given per bilin chromophore. There are six phycocyanobilin chromophores in each complex. Fluorescence emission of the two complexes was recorded for solutions of equal absorbance at the excitation wavelength, 580 nm. Excitation and emission band pass were set at 6 and 4 nm, respectively.

were resolved. The faster sedimenting zone contained α^{AP}, β^{AP}, and the 99K polypeptide, whereas the slower zone contained the other complexes described above. However, the material sedimenting in the leading zone trailed back into the slow sedimenting zone, indicating that it was dissociating in the course of the experiment. As the phosphate concentration in the gradient was increased, the faster-sedimenting zone was found to be more compact. Figure 7 shows a sucrose density gradient in 0.4 M Na phosphate, pH 7, as well as the SDS-poly-acrylamide gel pattern of fractions sampled across the colored zones. The 99K polypeptide is clearly seen to be concentrated in the faster sedimenting zone, but even at this high phosphate concentration, a small amount of the 99K polypeptide still trailed into the slower zone. Under optimum conditions, about 25% of the total bilin chromophore applied to the gradient was recovered in the fast zone. The composition of fraction 1 (Fig. 7), determined by densitometry is one copy of 99K per ~6 $\alpha\beta$ monomers of allophycocyanin. Thus the 99K polypeptide is in a complex containing two "trimetric" units. The slow dissociation of this complex presumably accounts for its behavior on sedimentation. It is evident that complete dissociation occurs on DEAE cellulose chromatography. What are the two trimeric complexes constituting the complex of fraction 1 (Fig. 7)? We infer that one of these must be $(\alpha^{AP}\beta^{AP})_3$ since fraction 1 does not contain the 10K polypeptide that is present in the other two complexes whose characterization is described above.

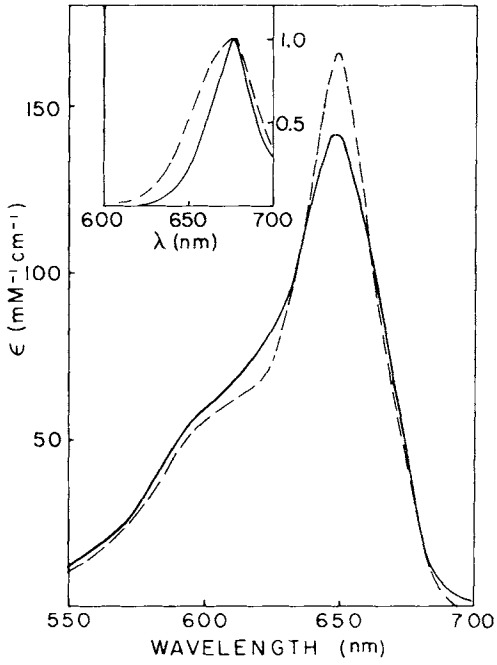


Fig. 6. Absorption and fluorescence emission spectra (inset) of the $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K (——) and the $(\alpha^{AP}\beta^{AP})_3.(\alpha^{AP}\beta^{AP}{}_2)$.18.5K.99K (-----) complexes. For other details, see legend to Figure 5.

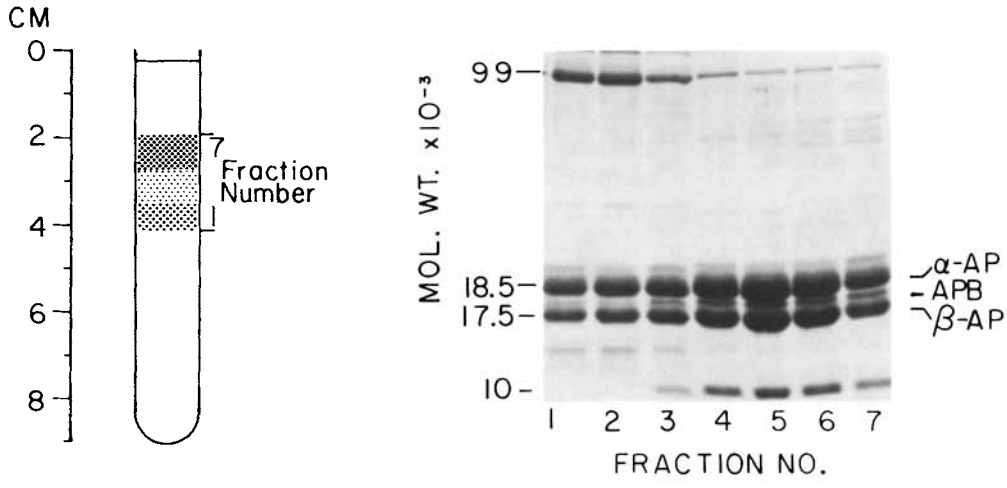


Fig. 7. Linear sucrose density gradient (0.2–1.0 M) in 0.4 M Na phosphate, pH 7.0, of the allophycocyanin-containing pool (see text). The colored zone was fractionated from the bottom in seven equal fractions. An SDS-polyacrylamide gel of each fraction is shown on the right.

An 18S complex related to that present in fraction 1 (Fig. 7), derived from the phycobilisomes of Synechococcus 6301 strain AN112, has been fully characterized [14]. This complex contains the subcomplexes $(\alpha^{AP}\beta^{AP})_3$, $(\alpha^{AP}\beta^{AP})_2$.18.3K.75K, as well as two $(\alpha^{PC}\beta^{PC})_3$.27K phycocyanin complexes [14]. The 18.3K polypeptide is a biliprotein subunit homologous in amino acid sequence to β^{AP}[14].

Densitometry of the SDS-gel of fraction 1 (Fig. 7) shows that the band migrating in the position of α^{AP} contains polypeptide in ~20% molar excess over that migrating in the position of the β^{AP} band. Two-dimensional gel analysis of this fraction (Fig. 4, AP—99K) shows the presence of an additional phycobiliprotein subunit with a molecular weight equal to that of α^{AP}. This polypeptide is also seen in the total allophycocyanin pool (Fig. 4) but is absent from the other three complexes. The properties of this polypeptide are consistent with the view that it is analogous to the 18.3K polypeptide of Synechococcus 6301 phycobilisomes.

When the conditions used to isolate the 18S complex from the phycobilisomes of Synechococcus 6301 are applied to the phycobilisomes of Synechocystis 6701 strain CM25, we can isolate a similar particle containing ~6 $\alpha\beta$ allophycocyanin monomers per 99K polypeptide as well as one $(\alpha^{PC}\beta^{PC})_3$.27K trimer. This complex does not contain either α^{APB} or 10K polypeptide.

From the data presented above, and by analogy with the corresponding Synechococcus 6301 complexes, we assign the structure $(\alpha^{AP}\beta^{AP})_3.(\alpha^{AP}\beta^{AP})_2$.18.5K.99K to the complex in fraction 1 (Fig. 7).

DISCUSSION

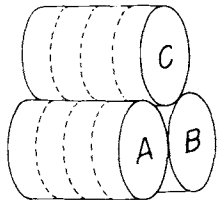
The results presented here lead to a composition for the core of Synechocystis 6701 phycobilisomes. In Table I, this composition is compared to that of the core of Synechococcus 6301 phycobilisomes. It is evident that the subcomplexes constituting those two types of core are qualitatively equivalent, the sole striking difference being in the size of the high molecular weight polypeptide, 99K as opposed to 75K. The equivalence of the complexes making up the bi- and tricylindrical cores is not surprising in view of the structural similarities between the rod elements of diverse phycobilisomes [2,4,13] and the highly conserved amino acid sequences of phycobiliproteins of both cyanobacterial and rhodophytan origin [23,24]. There is a large quantitative difference in the compositions of the bi- and tricylindrical cores. The Synechocystis 6701 tricylindrical core contains two additional copies of each of the $(\alpha^{AP}\beta^{AP})_3$.10K and $(\alpha^{AP}\beta^{AP})_3$ complexes.

In Figure 8, we propose a model for the tricylindrical core. This model has the following features. The contact of the phycobilisome with the thylakoid membrane is mediated through cylinders A and B. Each of these cylinders consists of each of the four distinct complexes listed in Table I in analogy with the bicylindrical core of Synechococcus 6301 phycobilisome [14,15]. The terminal energy acceptor bilins on the 99K polypeptide and/or the α^{APB} subunit occupy positions proximal to chlorophyll acceptor species in the membrane. The 99K polypeptide also functions as a membrane anchor. Cylinder C has a unique composition: two $(\alpha^{AP}\beta^{AP})_3$ and two $(\alpha^{AP}\beta^{AP})_3$.10K complexes. The absence of the terminal energy acceptors from this cylinder is reasonable since it is at least 110 Å away from the membrane at the point of the closest approach. Cylinder C functions solely as an energy transfer bridge between

TABLE I. Phycobilisome Core Components*

Synechococcus 6301 complex [14,15]	Copy no.	λ^F_{max} nm	Synechocystis 6701 complex	Copy no.	λ^F_{max} nm
$(\alpha^{AP}\beta^{AP})_3$	2	660	$(\alpha^{AP}\beta^{AP})_3$	4	657
$(\alpha^{AP}\beta^{AP})_3$.10.5K	2	662	$(\alpha^{AP}\beta^{AP})_3$.10K	4	660
$(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K	2	678	$(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K	2	678
$(\alpha^{AP}\beta^{AP})_2$.18.3K.75K	2	678	$(\alpha^{AP}\beta^{AP})_2$.18.5K.99K	2	678

*Abbreviations used are λ^F_{max}, fluorescence emission maximum; AP, allophycocyanin; APB, allophycocyanin B; 10.5K, 10K, etc., polypeptides of 10,500 daltons, 10,000 daltons, etc.



C is made up of 2 copies each of —
$(\alpha^{AP}\beta^{AP})_3$
$(\alpha^{AP}\beta^{AP})_3 \cdot 10K$

A and B are each made up of —
$(\alpha^{AP}\beta^{AP})_3$
$(\alpha^{AP}\beta^{AP})_2 \cdot 18.5K \cdot 99K$
$(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP}) \cdot 10K$
$(\alpha^{AP}\beta^{AP})_3 \cdot 10K$

ENERGY TRANSFER PATHWAY WITHIN THE CORE

$(\alpha^{AP}\beta^{AP})_3$
$(\alpha^{AP}\beta^{AP})_3 \cdot 10K$
λ^F_{max} 660nm

$\longrightarrow$

$(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP}) \cdot 10K$
$(\alpha^{AP}\beta^{AP})_2 \cdot 18.5K \cdot 99K$
λ^F_{max} 678nm

Fig. 8. Proposed composition of the core cylinders of Synechocystis 6701 phycobilisomes and of the energy transfer pathway between the core subcomplexes.

the rods attached to it and cylinders A and B. It is noteworthy that electron micrographs show clearly that four of the six rods are attached to cylinder C [1,5,9].

How do the above data on core substructures compare with the information on the core of other phycobilisomes? Only one other tricylindrical core has been extensively studied—that of Nostoc sp phycobilisomes [18,25,26]. Zilinskas et al [18] separated three allophycocyanin-containing complexes, API, APII, and APIII, by brushite chromatography. Composition and spectroscopic properties indicate that APII is $(\alpha^{AP}\beta^{AP})_3$. APIII appears to be equivalent to the Synechocystis 6701 complex $(\alpha^{AP}\beta^{AP})$.10K, discussed above. The initial characterization of APIII showed the presence of a low molecular weight polypeptide [18]; however, the presence of this component was not mentioned in later publications [25,26]. Moreover, APIII has a circular dichroism (CD) spectrum very similar to that of the Synechococcus 6301 $(\alpha^{AP}\beta^{AP})_3$.10.5K complex [25]. API shows a 680-nm emission maximum. Its com-

position was reported to be $(\alpha_2^{AP}\beta_3^{AP})\gamma$ where γ is a 35K bilin-bearing polypeptide [18]. We have recently indicated reasons to conclude that API is analogous to the $(\alpha^{AP}\beta^{AP})_2$.18.3K.75K complex from Synechococcus 6301 [14]. In essence, we believe that the 35K polypeptide is a proteolytic degradation fragment of a 95K polypeptide. The 95K polypeptide is the Nostoc sp equivalent of the 75K and 99K polypeptides of Synechococcus 6301 and Synechocystis 6701, respectively. In support of this view, Rusckowski and Zilinskas [21] have recently shown that API from Nostoc sp can be isolated with an intact 95K polypeptide if the protease inhibitor phenylmethanesulfonyl fluoride is present during the isolation. Zilinskas [27] has also shown that at higher phosphate concentrations API is isolated as a larger aggregate with $(\alpha^{AP}\beta^{AP})_3$, very similar to the fraction 1 (Fig. 7) 99K-containing complex we have described in this report. The evidence for a complex in Nostoc sp phycobilisomes similar to the $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K complex has been less conclusive. Zilinskas et al [18] isolated a complex resembling it in spectroscopic properties in two of 20 preparations. Subsequently, Canaani and Gantt [25] reported the isolation of "allophycocyanin B" with a 670-nm absorption maximum; it represented less than 1% of the total allophycocyanin pool. We believe this latter complex to be equivalent to $(\alpha^{APB}\beta^{AP})_3$ isolated from Synechococcus 6301 [6,16]. $(\alpha^{APB}\beta^{AP})_3$ is formed from the $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10.5K complex by loss of the 10.5K polypeptide and subsequent monomer exchange, involving $\alpha^{APB}\beta^{AP}$ and $\alpha^{AP}\beta^{AP}$ monomers, to form a statistical mixture of new trimeric structures [16]. With Synechocystis 6701 phycobilisomes, care must be taken to work rapidly and at low temperatures to avoid dissociation of the $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP})$.10K complex. If this is also true in Nostoc sp, it may explain the difficulty in isolating the analogous complex from the phycobilisomes of this organism. From the foregoing consideration it is very likely that the core assemblies of Nostoc sp and Synechocystis 6701 phycobilisomes are equivalent.

Zilinskas [27] isolated a 10.3S component which contained small amounts of 46K and 44K polypeptides as well as allophycocyanin. From the polypeptide composition of this fraction and its absorption and emission spectra [27:Figs. 1, 2], it appears that this component may contain hexameric allophycocyanin and a ~10.5K polypeptide. No role for the 46K and 44K polypeptides was proposed, nor was the stoichiometry of the various components established. The Synechocystis 6701 phycobilisome does contain a 46K polypeptide in less than one copy per phycobilisome [5]. However, this polypeptide is not present in any of the core complexes obtained by the procedures described in this study.

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