Molecular Architecture of a Light-harvesting Antenna

STRUCTURE OF THE 18 S CORE-ROD SUBASSEMBLY OF THE SYNECHOCOCUS 6301 PHYCobilISOME*

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The 18 S subassembly particles obtained by partial dissociation of phycobilisomes from Synechococcus 6301 (Anacystis nidulans) strain AN112 contain approximately one-half of the mass of the phycobilisome and include core-rod junctions (Yamanaka, G., Lundell, D. J., and Glazer, A. N. (1982) J. Biol. Chem. 257, 4077-4086). The polypeptide composition of 18 S complexes, determined by analysis of uniformly 14C-labeled phycobilisomes, gave the following stoichiometry: 75K:27K:18.3K:40K:11K allophycocyanin monomer:48 β phycocyanin monomer of 1:2:1:5:6; where 75K, 27K, etc. represent polypeptides of 75, 27 kilodaltons, etc. The 18.3K polypeptide is a hitherto undescribed biliprotein bearing a single phycocyanobilin. The NH2-terminal sequence of this subunit was determined to be homologous to that of the β subunit of allophycocyanin.

Chromatography of products resulting from limited trypsin treatment of the 18 S complex led to the isolation of three subcomplexes: a mixture of (αβ)2, 22K and (αβ)3, 24K phycocyanin complexes, an (αβ)1 allophycocyanin trimer, and an (αβ)1, 18.3K, 40K, 11K allophycocyanin-containing complex. The 22K and 24K components were products of the degradation of the 27K polypeptide, whereas the 40K and 11K components were derived from the 75K polypeptide. The subcomplexes accounted for the composition of the 18 S complex. Determination of the composition, stoichiometry, and spectroscopic properties of the subcomplexes has led to a model of the polypeptide arrangement within the 18 S complex and of the pathway of energy transfer among these polypeptides.

The phycobilisome is a complex multiprotein particle that functions as an important accessory light-harvesting component of the photosynthetic apparatus of cyanobacteria and red algae (1, 2). Phycobilisomes are attached in regular arrays to the outer surface of the photosynthetic lamellae. The particles vary in size from 6–7 × 105 daltons in cyanobacteria to about 20 × 106 daltons in some red algae, although in all cases their major components are various brightly colored phycobiliproteins (3). Under appropriate conditions phycobilisomes can be detached from the membrane and purified in a water-soluble form, apparently without alteration in their structure or impairment of their function. Consequently, phycobilisomes are particularly attractive objects for the study of the detailed molecular organization of a light-harvesting system.

In this laboratory a comparatively simple phycobilisome, that of Synechococcus 6301 (Anacystis nidulans), has been chosen for detailed study. The polypeptide composition of this phycobilisome is shown in Table I. Electron micrographs show these phycobilisomes, when seen in "face view," to consist of two major structural elements—a core of two circular units, each ~11 nm in diameter, from which radiate from five or six rods in a hemisidical array (e.g. see Ref. 7, Fig. 4). The rods are 11.5 nm in diameter and consist of stacks of discs (2–6 per rod) 6 nm thick. Studies utilizing a variety of approaches have demonstrated that the rod substructures are made up of phycocyanin (λmax, 620 nm) and uncolored linker polypeptides of 27,000, 30,000, and 33,000 daltons that serve to assemble the phycocyanin into hexameric discs, (αβ)6-X, where X represents a linker polypeptide (5). In addition, the rods contain a 12,000-dalton polypeptide of unknown function. The 27K1 polypeptide also functions in the attachment of the rods to the core.

Less is known about the core substructure. It consists mainly of allophycocyanin (λmax, 650 nm) as well as three minor biliproteins—allophycocyanin B (λmax, 670 nm) and 18,300- and 75,000-dalton polypeptides—and as a 10,500-dalton polypeptide (8, 12, 15). Study of the core has been facilitated by the isolation of a Synechococcus 6301 mutant, strain AN112 (7). The phycobilisomes from this mutant have normal cores but the rods are only one disc long and are made up exclusively of phycocyanin (αβ)3, 27K complexes (see Table I). Electron micrographs of these phycobilisomes have provided views of the core unobtainable with wild type phycobilisomes (see Ref. 7, Fig. 4). Each cylinder of the core is seen to consist of a stack of four discs, 11 nm across and ~3 nm thick, dimensions approximating those reported for an allophycocyanin trimer (17, 18).

We have recently reported on the dissociation of AN112 phycobilisomes into three biliprotein fractions of approximate S values of 18, 11, and 6 (12). The 18 S fraction was shown to consist of a single complex of ~550,000 daltons. This complex was postulated to have two trimeric phycocyanin- and two trimeric allophycocyanin-containing elements. Since the 18 S complex is present in two copies per phycobilisome, it accounts for one-half (four of eight) of the allophycocyanin-containing trimeric units of the core.

Understanding the structure of the 18 S particle is critical because it exhibits fluorescence emission properties indistinguishable from those of the intact phycobilisome (λmax, 680 nm), yet completely lacks allophycocyanin B (λmax, 680 nm).

The abbreviations used are: 75K, 27K, etc., designations used to specify polypeptides of 75,000 and 27,000 daltons, etc., respectively; AP, allophycocyanin; AP-B, allophycocyanin B; PC, phycocyanin; SDS, sodium dodecyl sulfate.

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**Phycobilisome Core-Rod Subassembly**

| M, x 10^{-3} | Number of Copies | Phycocyanobilin monomers per polypeptide | Function | Ref. |
|--------------|-----------------|---------------------------------------|----------|-----|
| Rod substructure | Wild type | AN112 | | | |
| 19.0 PC-β | ~100 | 36 | 2 | Light-harvesting and transfer | 4 |
| 17.7 PC-α | ~100 | 36 | 1 | Light-harvesting | 4 |
| 33 | -6 | 0 | 0 | Rod linker | 5, 6 |
| 30 | 3-6 | 0 | 0 | Rod linker | 5, 6 |
| 27 | 6-6 | 0 | 0 | Core-rod junction | 6, 7 |
| 12 | 4-6 | 0 | 0 | Unknown | 6 |

| Core substructure | | | | | |
| 18.2 AP-α | 20 | 20 | 1 | Light-harvesting and transfer | 5 |
| 17.6 AP-β | 22 | 22 | 1 | Light-harvesting and transfer | 9 |
| 17.7 AP B-α | 2 | 2 | 1 | Terminal energy acceptor | 10, 11 |
| 18.3 | 2 | 2 | 1 | Unknown | 12 |
| 75 | 2 | 2 | 1 | Terminal energy acceptor | 13-15 |
| 10.5 | 4 | 4 | 0 | Unknown | 6, 12 |

Location undetermined: 45 & 1 & 1 & 0 & Unknown & 16 |

* Abbreviations used are: PC, phycocyanin; AP, allophycocyanin, AP B, allophycocyanin B.

26% of phycocyanobilin complexes in the rod substructures have λ\text{max} = 620-625 nm and fluorescence emission maxima at 643-652 nm (5).

26% of phycocyanobilin trimers have a λ\text{max} = 650 nm and λ\text{max} = 660 nm (8).

4% AP B-a subunit, or 75K polypeptide-containing complexes have λ\text{max} = 680 nm (12, 13).

previously believed to be the terminal energy acceptor (10, 11). Two newly discovered biliproteins of 75,000 and 18,300 daltons are present in this particle (12, 15). Based on its properties, we have postulated that the 75K polypeptide acts as the terminal energy acceptor in the 18 S particle; preliminary evidence indicated it was bound tightly to an allophycocyanin-containing complex (15). The function of the 18.3K polypeptide is unknown.

In this report, we present a model of the 18 S particle based on (a) an accurate quantitation of its component polypeptides; (b) isolation and characterization of the three sub-complexes that make up this particle; and (c) characterization of the 18.3K polypeptide. This model represents one-half of the structure of the core.

**Experimental Procedures**

**Strains and Culture Conditions—**All of the studies reported here used strain AN112, a previously described (6, 7) nitrosoguanidine-induced mutant of *Synechococcus 6301* (ATCC 27144). Cultures were grown as previously described (16). For preparation of radiolabeled cells, strain AN112 was grown on 14C. A dilute cell suspension (200 ml, about 25 mg of cells, wet weight) in medium BG11 (19), containing 4 times the normal phosphate concentration but lacking carbonate, was placed in a 1-liter round-bottom flask. Four-ml aliquots of a 0.15 M solution of [14C]Na2CO3, 1 mCi/mmol, were added at 4 times over a period of 3% days to the tightly sealed flask. The culture was grown with shaking at 37 °C in warm white light. The cells were harvested after 4 days. The yield was approximately 300 mg weight, indicating that the cells had gone through 3-4 doublings.

**Preparation of 18 S Particles—**Pelleted AN112 phycobilisomes were obtained by a procedure described previously for phycobilisomes of wild type cells (5). The preparation of 18 S particles followed the procedure of Yamanaka et al. (12). The pelleted material from about 5 g of cells, wet weight, was dissolved at about 2 mg of protein/ml with shaking in 10% v/v glycerol-5 mM CaCl2-50 mM N-β-hydroxy-1,1-bis(hydroxymethyl)ethylglycine (titrated to pH 7.8 with NaOH), and was fractionated on sucrose step gradients (5 equal steps from 0 to 1.0 M sucrose) in a Sorvall TV3000 vertical rotor at 4°C at 46,000 rpm (170,000 g at average radius) and 18 °C. Approximately 8 mg of protein (4 ml) were applied to each 30-ml gradient. The fastest sedimenting band, containing the 18 S particles, was collected. These particles contained on the average 2-3 more copies of phycocyanin per 18 S particle than those previously reported (12). To ascertain the dilution of the 18 S particle preparation, and repetition of the sucrose density gradient centrifugation, led to the recovery of 18 S particles of phycocyanin composition identical with that of preparations described previously (12); a zone of free phycocyanin was seen on the repeat gradient. The presence of additional phycocyanin in the 18 S particle band may result both from the higher initial concentration of protein loaded on the gradient (2 versus 1 mg/ml (12)), and from the use of pelleted phycobilisomes as starting material. Since the extra phycocyanin behaved as free phycocyanin during subsequent subfractionated procedures (see below), the 18 S particles were routinely used without further purification. Phycobilisomes from 14C-labeled cells were prepared by density gradient centrifugation. The 18 S particles obtained from these phycobilisomes were identical with those previously described (12).

**Isolation of Components of 18 S Particles—**All steps were performed at room temperature unless otherwise specified. The 18 S particles (~20 mg of protein), from 5 g of cells, wet weight, were concentrated by adsorption to hydroxyapatite and step eluted with 0.2 M Na-phosphate-10% v/v glycerol at pH 7.0. The sample was dialyzed at 4 °C overnight against ~40 volumes of 10% v/v glycerol. The dialyzed sample, typically at 1-2 mg of protein/ml, was brought to 0.5 M NaCl by the addition of 4 M salt solution, and trypsin was added at an enzyme/substrate ratio of 1% by weight. The digestion was stopped after 30 min with the addition of soybean trypsin inhibitor in a 5-fold weight excess over trypsin. The sample was allowed to stand for 2-4 h, and was then applied to a column (2 × 3 cm) of hydroxyapatite pre-equilibrated with 1 mM Na-phosphate-100 mM NaCl-10% (v/v) glycerol, pH 7.0. The column was developed with step increases of Na-phosphate concentration in 100 mM NaCl-10% glycerol, pH 7.0. The elution profile is shown in Fig. 1. The molecular concentrations of the biliproteins in the various fractions (Fig. 1) were calculated in terms of αβ equivalents as follows: Fractions I and III had significant absorbance at 670 nm, the concentration of fraction II, allophycocyanin, was determined using an ε660 of 190 m-M cm⁻¹. Fraction 1 phycocyanin had an εmax of 250 m-M cm⁻¹, and fraction III phycocyanin of 310 m-M cm⁻¹. Fraction IV allophycocyanin had an ε660 of 290 m-M cm⁻¹; the average coefficient between fractions III and IV were examined by SDS-polycrylamide gel electrophoresis. Further purification was often necessary to remove residual phycocyanin from fraction II (undissociated 18 S particles co-eluted with this fraction). Fraction II material was dialyzed against 1 mM Na-phosphate-100 mM NaCl-10% glycerol, pH 7.0, and further digested with trypsin (1% w/w) for 30 min. The digest was then rechromatographed on hydroxyapatite. As a final step in the purification, the peak fractions were subjected to sedimentation on linear sucrose gradients, 5-15%, in 50 mM Na-phosphate-10% glycerol, pH 7.0, in a Spinc So 421 rotor at 39,000 rpm (180,000 x g at average radius) for 16-20 h at 18 °C.

**Electrophoresis and Isoelectric Focusing—**Samples were precipitated in 10% trichloroacetic acid prior to electrophoretic analyses. The SDS-polycrylamide slab gel electrophoresis procedure, staining, and densitometric scanning have been previously described (6, 16). All gels contained 14% acrylamide and 0.37% methylene bisacrylamide. Approximate pH ranges for each fraction were applied per gel. The running conditions were as previously described (12). At the completion of focusing, densitometric scans were performed on unstained gels.

**Polypeptide Stoichiometry in 14C-labeled 18 S Particles—**The 18 S particles from cells grown with 14C were prepared for electrophoresis in the same manner as described above. Samples (~100 μg of protein) were diluted about 1:20 with unlabeled strain AN112 phycobilisomes before trichloroacetic precipitation, 20,000-40,000 cpm were applied per gel lane. Replicate samples were run on 12% acryl-
with a scalpel and placed in 10-m! glass scintillation vials. Water (100
envelope. The counts/min values were corrected for background,
~1) and NCS tissue solubilizer (Amersham; 900 ~1) were added; the
polypeptide 53.6%. A value of 53% was assumed for the carbon content
52.7%, B-subunit 53.9%; 27 kilodalton polypeptide 53.9%; 75 kilodalton
particles.

Formic acid
to l-2 h. The Coomassie brilliant blue-stained bands were excised
...bly stained gel slices from
isolectric focusing were prepared essentially as described by Cleve-
land et al. (21). A gel slice with 10-15 µg of protein was placed in a
slab gel well and was overlayed with a solution containing 2, 4, or 6
µg of Staphylococcus aureus V8 protease.

For cyanogen bromide cleavage, gel slices containing 20-30 µg of
protein were suspended in 150 µl of 88% formic acid containing 0.5 mg
of CNBr. After an overnight incubation at room temperature, the gel
slices were dried, rehydrated in electrophoresis buffer, and placed in
slab gel wells. Slab gels for peptide mapping were 18% acrylamide-0.48%
bisacylamide and 2-mm thick. All other conditions were as
described above.

Sequence Determination.—Determination of the NH2-terminal se-
quence of the 18.3K polypeptide was performed in a Beckman Se-
quence, Model 860C, equipped with a microprocessor controlled
programmer. Beckman dilute Quadrol buffer program #031281 was
used. The sequence run was performed on ~10 nmol of the 18.3K
polypeptide in the presence of 3 mg of Polybrene. One microcycle with
no phenylisothiocyanate was run. Phenylthiohydantoin derivatives
were identified as previously described (14). The initial percentage of
the 18.3K polypeptide coupled was ~70% and the repetitive yield was
97%.

RESULTS

Polypeptide Stoichiometry as Determined with 14C-labeled 18 S Particles.—We have previously reported the composition of the 18 S particle isolated from AN112 phycobilisomes to be: 75K polypeptide, 1 copy; 18.3K, 1-2; phycocyanin mono-
mer (ap), 6; allophycocyanin monomer (α/β), 6 (12). This was
based on densitometric scanning of SDS-polyacrylamide gels and
was only approximate given the close spacing of the biliprotein subunits (12). In particular the 18.3K polypeptide,
unique to the 18 S particle and of unknown function, was
poorly quantitated. Consequently, a better method to deter-
mine the polypeptide stoichiometry was sought.

Table II

Composition of uniformly 14C-labeled 18 S complex

| Polypeptide | Number per 18 S particle |
|-------------|-------------------------|
| AP-β        | 4.9 ± 0.4               |
| PC-α        | 6.1 ± 0.3               |
| AP-α + 18.3K| 6.2 ± 0.2               |
| PC-β        | 5.9 ± 0.1               |
| 27K         | 2.0                     |
| 75K         | 0.94 ± 0.02             |

*Abbreviations are AP-β, β subunit of allophycocyanin; PC-α, α
subunit of phycocyanin; etc.; 18.3K, polypeptide of 18,300 daltons, etc.

About 20-25% of the 75K polypeptide was present as a 60K
proteolytic degradation fragment.
Since all of the carbon in cyanobacteria can be derived from CO₂, metabolic labeling of the 18 S particle with ¹⁴C CO₂ was used to obtain more accurate analytical data. The details of labeling and quantitation are described under "Experimental Procedures." When labeled phycocyanin was used as a control of the quantitation methods, it gave an αβ molar ratio of 1.00:0.98 ± 0.04 (theory 1:1). The results of analyses of 14 replicate samples of the 18 S particle, normalized to 2.0 copies of the 27K polypeptide, are given in Table II. Phycocyanin α and allophycocyanin β subunits (17.7 and 17.6 kilodaltons, respectively) were sufficiently resolved to quantitate separately; the somewhat higher standard deviation of the values for these two subunits may be due to a small, variable cross-contamination during the excision of the gel slices prior to counting. The 18.3K polypeptide and the allophycocyanin α subunit were excised and counted together because of less satisfactory resolution. Isoelectric focusing of the labeled 18 S particle in urea gave a molar ratio of allophycocyanin αβ subunits of 1:0. The 18.3K polypeptide could not be quantitated by isoelectric focusing in a similar manner because of partial overlap with breakdown products of the phycocyanin components. These data in Table II coupled with the isoelectric focusing data showed that there are five αβ allophycocyanin monomers per 18 S particle and one copy of the 18.3K polypeptide. Allophycocyanin is normally isolated as an αβ₃ trimer (8), consequently the presence of five αβ monomers per 18 S particle was very surprising. Corroborating evidence was sought from analyses of components obtained by subfractionation of the 18 S particle.

Subfractionation of the 18 S Particle—Various approaches aimed at dissociation of the 18 S particle into smaller components were examined. One approach exploited the known high susceptibility of the 75K polypeptide to trypic digestion (16). Prolonged digestion of the 18 S particle causes a shift in the emission maximum to 655-660 nm, upon 580 nm excitation, but a prominent shoulder at 680 nm is still evident. SDS-polyacrylamide gel electrophoresis revealed that trypsin digested the 75K polypeptide to a series of similar sized fragments of ~40 kilodaltons and a component of ~11 kilodaltons; the 27K polypeptide was digested to a number of products of 20-25 kilodaltons (12). None of the biliprotein subunits, including the 18.3K polypeptide, were affected by trypsin. Intact 18 S particles do not dissociate upon chromatography on hydroxylapatite. However, when trypsin-treated 18 S particles were applied to hydroxylapatite, the constituent polypeptides no longer eluted together, rather a number of distinct complexes were separated. Conditions for digestion and fractionation are described under "Experimental Procedures." The results of the fractionation are shown in Fig. 1.

The four major components eluted from the hydroxylapatite column account for the entire 18 S particle. The compositions of these four fractions are shown in Fig. 1. The absorption and fluorescence emission spectra of fractions I, II, and IV are shown in Fig. 2. Phycocyanin was recovered in fractions I and III, and allophycocyanin in fractions II and IV. The phycocyanin fractions will be described first.

Fraction I contained the α and β subunits of phycocyanin and degradation products resulting from the tryptic cleavage of 27K polypeptide (Fig. 1C). The molar ratio of phycocyanin to 27K polypeptide fragments was 3:1. The spectroscopic properties of this complex, λmax 640 nm and λmax' 654 nm, are very similar to those of the (αβ₃) 27K phycocyanin complex isolated by Yu et al. (22) from dissociated Anabaena variabilis phycobilisomes. Fraction III contained only the α and β subunits of phycocyanin. The relative amount of phycocyanin in fractions I and III varied, the amount of III increasing with longer lapse of time between the end of digestion and the beginning of fractionation. This indicates that a slow dissociation of the phycocyanin complex in fraction I is occurring. In general, we have noted that the phycocyanin (αβ₃) 27K complex, from Synechococcus 6301, isolated by a method similar to that of Yu et al. (22), is less stable than the corresponding complex from A. variabilis. Part of the phycocyanin in fraction III also derives from "free" phycocyanin bound to the 18 S particle (see "Experimental Procedures").

Fractions II and IV were present in equimolar amount. Fraction IV contained only the α and β subunits of allophycocyanin, and exhibited absorption and fluorescence emission maxima at 650 and 660 nm, respectively, as reported for Synechococcus 6301 allophycocyanin trimer (8). Fraction II was very different, both with respect to spectroscopic properties and composition (Figs. 1 and 2). The long wavelength absorption band of the complex in fraction II was considerably red-shifted and intensified relative to that of the allophycocyanin in fraction IV. More important, upon 580 nm excitation, the fraction II complex gave a fluorescence emission maximum at 680 nm with only a slight shoulder at 660 nm, similar to the emission spectrum of the intact 18 S particle. Comparison of the fraction II and IV complexes by sedimentation in sucrose density gradients indicated that the fraction II complex had a slightly higher molecular weight than the (αβ₃) allophycocyanin trimer. SDS-polyacrylamide gel electrophoresis (Fig. 1C) showed the presence of the α and β subunits of allophycocyanin, the 18.3K polypeptide and of the 40K and 11K polypeptides derived from the 75K polypeptide. The molar ratio of (AP-α + 18.3K):AP-β:40K:11K was 3:2:1:1. Isoelectric

![Fig. 2. Fluorescence emission and absorption spectra (inset) of complexes isolated from trypsin-treated 18 S particles. Spectra are shown for fraction I (Fig. 1), representing the complexes of phycocyanin with the 22 and 24K polypeptides derived from the 27K polypeptide (---), fraction II (Fig. 1), the AP-18.3K-40K-11K complex (——), and fraction IV (Fig. 1), the AP trimer (---). Fluorescence emission spectra were recorded with samples of equal absorbance at the excitation wavelength of 680 nm with excitation and emission slits of 6 and 4 nm, respectively.]

A small amount of phycocyanin (αβ)₂ 27K complex was isolated from intact 18 S particles as follows. The 18 S particles were transferred into 0.5 mM NaCl and the solution applied to hydroxylapatite equilibrated with 1 mM Na-phosphate-0.1 mM NaCl, pH 7.0. The (αβ)₂ 27K complex was eluted with 20 mM Na-phosphate-0.1 mM NaCl, pH 7.0. This undegraded complex is spectroscopically identical with the complexes containing >20K fragments of the 27K polypeptide obtained from trypsin-treated 18 S particles.
focusing in urea showed that AP-α:18.3K was 2:1. From the above results, the composition of the fraction II complex was deduced to be αββ (18.3K), (40K, 11K), with a calculated molecular weight of 140,000. The 40K fragment carried the bilin chromophore of the 75K polypeptide. It is not seen on the isoelectric focusing gel of Fig. 1B because it is too basic to enter the gel. The combination of the data on fractions II and IV accounts for the unexpected number of αβ allophycocyanin monomers and the single 18.3K polypeptide per 18 S particle.

The three complexes present in the 18 S particle will hereafter be designated: fraction I complex, the PC:27K trimer (no distinction will be made between this complex and the partially degraded form that is isolated after tryptic digestion when discussing spectroscopic data, since there appears to be no difference); fraction IV, the AP trimer; fraction II, the AP-18.3K:40K*:11K* trimer (or complex), the asterisk indicating that the 40K and 11K polypeptides are fragments resulting from the tryptic cleavage of the 75K polypeptide.

Further Characterization of the AP-18.3K:40K*:11K* Complex and the 18.3K Polypeptide—MacColl et al. (23) have recently shown that in the presence of moderate concentrations of chaotropic salts, allophycocyanin is converted partially degraded form that is isolated after tryptic digestion in urea at pH 6. The absorption spectrum of the dissociated state. This difference spectrum (Fig. 3) has two maxima, at 660 and 625 nm. We have previously shown that the 75K polypeptide has an absorption maximum at 665 nm and that the 40K polypeptide fragment carries the bilin chromophore (15). We assign, therefore, the long wavelength maximum to the 40K polypeptide. This point is discussed further below.

The 18.3K polypeptide has properties similar to those of other phycobiliprotein subunits with respect to molecular weight, isoelectric point, and the presence of one covalently-bound phycocyanobilin. The procedure for the separation of AP-α and AP-β subunits, gradient elution from CM-cellulose in urea at pH 5, could not be applied to the purification of the 18.3K polypeptide. Under these conditions, the 18.3K polypeptide coeluted with AP-α. The only successful isolation procedure developed thus far was preparative isoelectric focusing on polyacrylamide gels in urea followed by elution of the protein from the gel by diffusion into a buffer at neutral pH. The absorption spectrum of the 18.3K polypeptide isolated in this manner showed a maximum at 620 nm and closely resembled that of the AP-α subunit. Thus the shorter wavelength maximum in the difference spectrum of Fig. 3 is undoubtedly due to the 18.3K polypeptide. Since the spectrum of the 18.3K polypeptide is similar to that of the AP-α subunit (14), subtraction of the spectrum of the AP-α subunit from the upper spectrum in Fig. 3 should give the spectrum of the AP-18.3K:40K*:11K* complex.

### Fig. 3. Calculated absorption spectra for the 40K and 18.3K polypeptides in 0.5 NH4SCN at pH 7.0. The solid line represents the sum of the absorption spectra of the 40K and 18.3K polypeptides obtained by subtracting ½ times the absorbance of the AP trimer in 0.5 M NH4SCN-0.05 M Na-phosphate, pH 7.0, from the spectrum of an equimolar amount of the AP-18.3K:40K*:11K* complex in the same solvent (for the absorption spectra, see Fig. 2, inset); the rationale for the calculation is presented in the text. The dotted line shows the calculated spectrum for the 40 K polypeptide, obtained by subtracting the spectrum of the AP-α subunit (see Ref. 14) from the spectrum calculated for the sum of the molar absorbances of the 40K and 18.3K polypeptides (see text).

40K polypeptide alone. The latter spectrum is indicated by the dotted line in Fig. 3.

In spite of general similarities to other biliprotein subunits, the 18.3K polypeptide is a unique biliprotein subunit. Peptide maps of the 18.3K polypeptide, AP-α, and AP-β, obtained by the method of Cleveland et al. (21) after S. aureus V8 protease digestion, or after cyanogen bromide cleavage, were all different. The NH2-terminal sequence of the 18.3K polypeptide shows homology to, but is clearly different from those of the other Synechococcus 6301 biliprotein β subunits (see Fig. 4).

Reconstitution experiments with the 18.3K polypeptide and AP-α and AP-β subunits, all isolated from the AP-18.3K:40K*:11K* complex, were carried out. Slices from isoelectric focusing gels were chosen to give equal molar amounts of the appropriate subunits and were extracted together. When the AP-α and AP-β subunits were recombined, a complex was formed with an absorption maximum at 650 nm. This indicated that even though there is only an (αβ); dimer of allophycocyanin in the AP-18.3K:40K*:11K* complex, these allophycocyanin subunits are still capable of forming a complex with spectroscopic properties characteristic of an (αβ); allophycocyanin trimer (8). Thus, there appears to be no difference between the allophycocyanin subunits in the two domains of the 18 S particle.

When the 18.3K polypeptide was mixed with either the AP-α or AP-β subunit, the absorption and fluorescence emission spectra of the mixtures were just the sum of those of the individual polypeptides. However, when the 18.3K polypeptide was mixed together with both AP-α and AP-β subunits, a decreased yield of 650 nm absorbing complex (see above) was obtained, suggesting that it interacted with one or both of the allophycocyanin subunits and inhibited formation of allophycocyanin trimers. Further studies will be required to examine the nature of the interaction of the 18.3K polypeptide with allophycocyanin subunits. Because of the low yield in which the 40K polypeptide was isolated, and its poor...
have found for the detection of the isoelectric focusing in polyacrylamide gels in the presence of complexes were fractionated. The only reliable procedure we
peptide co-migrates with the cellulose showed a broad shoulder eluting after the separation of the subunits of units in AP-I was reported to be fragment in
inter-relationship in the somes in which only the basal phycocyanin disc of the rod plex appears to be very similar to bilin-bearing polypeptide of et al. that would correspond to the polypeptide we
applied for the AP-I polypeptide had a NH-terminal sequence of the 18.3K polypeptide with those of the other Synecoccus 6301 biliproteins. AP-B, allophycocyanin B, AP, allophycocyanin; PC, phycocyanin; AP-B a, a subunit of allophycocyanin B, etc.

DISCUSSION

Synecoccus 6301 mutant AN112 produces phycobilisomes in which only the basal phycocyanin disc of the rod substructures is present (7, 12). In an earlier report, we described the products of partial dissociation of this "simple" phycobilisome (12). Among these, a particularly important subassembly particle was isolated, the 18 S complex, which contained portions of both rod and core substructures (12). The present study describes the polypeptides of the 18 S complex, and their disposition within the particle.

The three different types of complexes present in the 18 S particle are shown schematically in Fig. 5C. These complexes constitute the entire 18 S particle as corroborated by the stoichiometry determined from 14C-labeling; their possible inter-relation ship in the 18 S complex is indicated by the model in Fig. 5B and in the intact AN112 phycobilisome in Fig. 5A.

The elucidation of the substructure of the 18 S particle sheds light on a number of previously unresolved points important to the understanding of phycobilisome structure: (a) the relationship between the AP-18.3K-40K*-11K* complex and AP-I, a previously isolated core component in Nostoc sp. phycobilisomes (28); (b) the nature and function of the 18.3K polypeptide; (c) the domain structure of the 75K polypeptide; (d) the presence of a strongly red-shifted phycocyanin species in the 18 S particles (12); (e) the nature of the rod-core junction; (f) the nature of the absorption spectrum of the allophycocyanin trimer when bound within the core substructure. Each of these points is discussed below.

Fig. 5C3 shows a proposed structure for the 680 nm emitting allophycocyanin complex, AP-18.3K-40K*-11K*. This complex appears to be very similar to AP-I isolated by Zilinskas et al. (29) from Nostoc sp. However, a number of points remain to be clarified. Troxler et al. (29) claimed that a 37K bilin-bearing polypeptide of AP-I was a constituent of the intact Nostoc sp. phycobilisomes, while we found that the corresponding 40K polypeptide could only be generated by proteolysis. No evidence was seen for the presence of the 11K fragment in AP-I (28), nor was there a report of a polypeptide in AP-I that would correspond to the 18.3K polypeptide we observed. However, the ratio of α to β allophycocyanin subunits in AP-I was reported to be 3:2 (28), exactly as we would assume, if we were unaware of the fact that the 18.3K polypeptide co-migrates with the α subunit of allophycocyanin. Furthermore, the profile obtained by Troxler et al. (29) for the separation of the subunits of AP-I in urea on DEAE-cellulose showed a broad shoulder eluting after the AP-α subunit peak that was absent when other allophycocyanin complexes were fractionated. The only reliable procedure we have found for the detection of the 18.3K polypeptide is isoelectric focusing in polyacrylamide gels in the presence of urea. It is highly likely that AP-I from Nostoc sp. and the AP-

18.3K-40K*-11K* complex described here are structurally analogous.

What is the function of the 18.3K polypeptide? It is clearly similar in physical properties to allophycocyanin subunits and its NH-terminal sequence is homologous to that of the β subunit of allophycocyanin. A role for the 18.3K polypeptide is suggested in the schematic drawing of Fig. 5C3. It is inferred that the 18.3K polypeptide mediates the interaction of the 75K polypeptide with allophycocyanin. A nearest-neighbor relationship between the 18.3K polypeptide and the 40K*11K* polypeptides is consistent with the fact that the 18.3K polypeptide can inhibit the reassociation of AP-α and AP-β, most likely because it can interact with an allophycocyanin subunit. Further, the 18.3K polypeptide failed to replace either an AP-α or AP-β subunit in the formation of an allophycocyanin-like trimeric complex, e.g. (18.3K-β)*.

Approximately 51 kilodaltons (40K + 11K) of the 75K...
polypeptide remain tightly bound within the AP-18.3K-40K*•11K* complex after trypsin digestion. The function of the 11K fragment is not known. The 40K fragment contains the phycocyanobilin chromophore of the 75K polypeptide and, based on Fig. 3, has an absorption maximum near 660 nm in 0.5 M NH₄SCN; purified 75K polypeptide has an absorption maximum at 665 nm (15). The extinction coefficient of the 40K polypeptide, ~105 mM⁻¹cm⁻¹ at 660 nm (Fig. 3), is quite similar to those determined for other isolated biliprotein subunits (14). The red-shifted spectrum of the 40K fragment of the 75K polypeptide is reminiscent of that of the allophycocyanin B α subunit which has an absorption maximum near 650 nm (14). In contrast, α and β subunits of Synechococcus 6301 allophycocyanin and phycocyanin have absorption maxima between 610 and 620 nm. All complexes containing the allophycocyanin B α subunit, whether isolated from phycobilisomes or reconstituted in vitro, show a 680 nm emission maximum. It is reasonable to assume that the allophycocyanin B α subunit, and the 75K polypeptide, are the terminal fluorescing entities in their respective complexes and in the phycobilisome. This assumption has not been rigorously proved for either case and indeed represents an oversimplification. Spectroscopic studies show that the properties of the allophycocyanin B α subunit and of the 75K polypeptide are influenced by their environment within the native “trimeric” complexes. In such complexes, there is an increase in extinction per bilin chromophore, further red shift, and a large increase in fluorescence quantum yield.

Fig. 5C shows the proposed structure for the phycocyanin-27K polypeptide trimer. This complex was actually isolated from the trypsin-treated 18 S particle with a 22- or 24-kilodalton fragment of the 27K polypeptide. The partial degradation of the 27K polypeptide did not lead to detectable changes in spectroscopic properties of this complex. The absorption maximum of the phycocyanin-27K trimer, 640 nm, explains why the spectrum of the 18 S particle has a maximum at 649 nm, and lacks a maximum or pronounced shoulder near 620 nm expected for phycocyanin and certain of its complexes (12). The red-shifted absorption of the phycocyanin-27K trimer is reflected in the position of its fluorescence emission peak, 654 nm as opposed to 645 nm for uncomplexed phycocyanin. This red-shift in the emission increases the overlap integral between phycocyanin and allophycocyanin with consequent increase in the rate of energy transfer from phycocyanin to allophycocyanin.

The 27K polypeptide has been proposed to join the rod and core elements in phycobilisomes (7, 12). The results of Fig. 1 support this view. As mentioned above, short trypsin digestion cleaves only the 75K polypeptide without dissociation of the 18 S particle. In contrast, trypptic digestion of longer duration, leading to cleavage of the 27K polypeptide (see Fig. 7 of Ref. 12), causes the dissociation of the 18 S particle to complexes of ~100,000 daltons or less. A complex sedimenting slightly slower than 18 S can be isolated after intermediate times of digestion in which the phycocyanin content is reduced from 6 to 3 ąb monomers, the 27K is partially digested to 25K, and the number of 27 + 25K polypeptides is reduced from 2 to 1. However, fragments of the 27K polypeptide smaller than 25K were not detected in trypsin-treated re-isolated “18 S” particles; fragments of the 27K polypeptide between 20 and 25 kilodaltons are found on such gradients migrating with phycocyanin, allophycocyanin, and the 18.3K polypeptide in the M₉ = 100,000 range. These observations suggest that a small portion of one of the 27K polypeptide may be responsible for holding together the two allophycocyanin-containing complexes of the 18 S particle.

The third complex obtained from trypsin-treated 18 S particles is an (ąb)₃ allophycocyanin trimer. The spectroscopic properties of the isolated complex are similar to those reported previously for pure Synechococcus 6301 allophycocyanin (8). However, there is evidence indicating that the spectrum of this (ąb)₃ allophycocyanin trimer is perturbed when it is a part of the 18 S particle. Fig. 6 shows a comparison, on a molar basis, of the spectrum of the 18 S particle (assuming 30 bilins per particle) and the spectrum of the sum of two parts of PC-27K complex, one part of AP-18.3K-40K*•11K* complex and one part of allophycocyanin trimer (data from Fig. 2). The latter spectrum is clearly blue-shifted relative to that of the intact 18 S particle. The difference between the two spectra, shown in the inset to Fig. 6, shows a maximum at 660 nm. Hence, this difference is more likely to be due to a perturbation in the spectrum of allophycocyanin rather than phycocyanin.

Characterization of the complexes present in the 18 S par-
article leads to the following pathway (solid arrows) of energy transfer within this complex:

\[
\text{[AP]} \quad \text{[PC.27K]} \quad \text{[AP.18.3K]} \quad \text{75K} \quad \text{chlorophyll a}
\]

The major fluorescence emission peak of 18 S particles is at 680 nm, but there is a significant shoulder at 660 nm (12). On the basis of the fluorescence spectra of Fig. 2, and assuming no significant phycocyanin fluorescence, it can be calculated that 20–30% of the emission originates from allophycocyanin. The single bilin chromophore of the 75K polypeptide is postulated to be the source of the 680 nm fluorescence emission. Since ten allophycocyanin bilins (excluding the chromophore on the 18.3K polypeptide) are present for each bilin on the 75K polypeptide, the 660 nm emission may simply be due to a small but significant rate of back transfer to allophycocyanin (see scheme above). In vivo this back transfer would be greatly decreased if the rate of energy transfer from the 75K polypeptide to chlorophyll a is very fast. Whole cell studies on Synechococcus 6301 show a small amount of uphill energy transfer to allophycocyanin (30, 31).

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REFERENCES

1. Gantt, E. (1980) Int. Rev. Cytol. 66, 45-80
2. Glazer, A. N. (1982) Annu. Rev. Microbiol. 36, 171-196
3. Glazer, A. N. (1981) in The Biochemistry of Plants (Hatch, M. D., and Boardman, N. K., eds) Vol. 8, pp. 51-96, Academic Press, New York
4. Bryant, D. A., Glazer, A. N., and Eiserling, F. A. (1976) Arch. Microbiol. 110, 61-75
5. Lundell, D. J., Williams, R. C., and Glazer, A. N. (1981) J. Biol. Chem. 256, 3580-3592
6. Yamanaka, G., and Glazer, A. N. (1981) Arch. Microbiol. 130, 23-30
7. Yamanaka, G., Glazer, A. N., and Williams, R. C. (1980) J. Biol. Chem. 255, 11004-11010
8. Cohen-Bazire, G., Beguin, S., Rimony, S., Glazer, A. N., and Brown, D. M. (1977) Arch. Microbiol. 111, 225-238
9. Lemasson, C., Tandieu de Marsac, N., and Cohen-Bazire, G. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 3130-3133
10. Glazer, A. N., and Bryant, D. A. (1975) Arch. Microbiol. 104, 15-22
11. Ley, A. C., Butler, W. L., Bryant, D. A., and Glazer, A. N. (1977) Plant Physiol. 59, 974-980
12. Yamanaka, G., Lundell, D. J., and Glazer, A. N. (1982) J. Biol. Chem. 257, 4077-4086
13. Gantt, E. (1981) Annu. Rev. Plant Physiol. 32, 327-347
14. Lundell, D. J., and Glazer, A. N. (1981) J. Biol. Chem. 256, 12600-12606
15. Lundell, D. J., Yamanaka, G., and Glazer, A. N. (1981) J. Cell Biol. 91, 315-319
16. Yamanaka, G., Glazer, A. N., and Williams, R. C. (1978) J. Biol. Chem. 253, 8303-8310
17. Morschel, E., Koller, K.-P., and Wehrmeyer, W. (1980) Arch. Microbiol. 123, 43-51
18. Bryant, D. A., Glazer, A. N., and Eiserling, F. A. (1976) Arch. Microbiol. 110, 61-75
19. Stanier, R. Y., Kunisawa, R., Mandel, M., and Cohen-Bazire, G. (1971) Bacteriol. Rev. 35, 171-205
20. Glazer, A. N., and Fang, S. (1973) J. Biol. Chem. 248, 659-662
21. Cleveland, D. W., Fischer, S. G., Kirchner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102-1106
22. Yu, M.-H., Glazer, A. N., and Williams, R. C. (1981) J. Biol. Chem. 256, 13130-13136
23. MacColl, R., Csartorday, K., Berns, D. S., and Traeger, E. (1981) Arch. Biochem. Biophys. 206, 42-46
24. Bryant, D. A. (1977) Ph.D. Thesis, University of California at Los Angeles
25. Glazer, A. N., Apell, G. S., Hixson, C. S., Bryant, D. A., Rimony, S., and Brown, D. M. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 428-431
26. Walsh, R. G., Wingfield, P., Glazer, A. N., and DeLange, R. J. (1980) Fed. Proc. 39, 1998
27. Freidenreich, P., Apell, G. S., and Glazer, A. N. (1978) J. Biol. Chem. 253, 212-219
28. Zilinskas, B. A., Zimmerman, B. K., and Gantt, E. (1978) Photosynthetica 34, 427-437
29. Troxler, R. F., Greenwald, L. S., and Zilinskas, B. A. (1980) J. Biol. Chem. 255, 9380-9387
30. Murata, N. (1977) in Photosynthetic Organelles (Miyachi, S., Katoh, S., Fujita, Y., and Shibata, K., eds), pp. 9-13, Japanese Society of Plant Physiologists, Tokyo
31. Wang, R. T., and Myers, J. (1977) in Photosynthetic Organelles (Miyachi, S., Katoh, S., Fujita, Y., and Shibata, K., eds), pp. 3-7, Jpn. Soc. Plant Physiol., Tokyo