Formation of Hybrid Proteins from the α and β Subunits of Phycocyanins of Unicellular and Filamentous Blue-green Algae

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SUMMARY

Phycocyanins of two unicellular (Synechococcus sp., and Aphanocapsa sp.), and one filamentous (Anabaena sp.) blue-green algae, have been purified and characterized with respect to behavior on polyacrylamide gel electrophoresis in the absence of denaturing agents, isoelectric point, absorption spectra in the visible region, subunit molecular weights, and amino acid composition. The α and β subunits of each of the phycocyanins were separated by chromatography in urea solutions, and characterized in a similar manner. Each phycocyanin was reconstituted from its separated subunits.

Hybrid phycocyanins were formed from the α subunits of Synechococcus sp. phycocyanin and the β subunits of Aphanocapsa sp. and Anabaena sp. phycocyanins, and from the β subunit of Synechococcus sp. phycocyanin and the α subunits derived from the phycocyanins of the other two blue-green algae. The reconstituted and hybrid phycocyanins were purified by ion exchange chromatography, and recovered in an over-all yield of 40 to 60% based on the starting weight of subunit protein. The reconstituted and hybrid phycocyanins were characterized in the same manner as the native proteins.

The native, reconstituted, and hybrid phycocyanins, all consisted of α and β subunits in a ratio of 1:1, as established by quantitative amino acid analyses and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The chromatographic mobilities, absorption spectra, and behavior on isoelectric focusing, of both the reconstituted and hybrid phycocyanins, were consistent with the possession by these macromolecules of a three-dimensional structure very similar to that of the native proteins.

The divergence of unicellular and filamentous blue-green algae occurred over 2.5 billion years ago. The successful hybridization of subunits of phycocyanins from these two types of organisms indicates a conservation of structural features over a span of time considerably greater than had been involved in previous comparative studies of protein structure.

The phycobiliprotein phycocyanin is a component of the accessory photosynthetic pigment system of the prokaryotic Cyanobacteria (blue-green algae), and of the eukaryotic Rhodophyta (red algae) and Cryptophyta. All blue-green algae possess two phycobiliproteins—allophycocyanin (λmax ~ 630 nm), and phycocyanin (λmax ~ 625 nm), and many, a third pigment, phycoerythrin (λmax ~ 565 nm), as well (1). These proteins owe their intense absorption bands in the visible to covalently bound chromophores—phycocyanobilin in the case of phycocyanin and allophycocyanin, and phycoerythrobilin, in the case of phycoerythrin (9). These chromoproteins function as accessory pigments in photosynthesis with an energy transfer efficiency of close to 100% (3, 4). The phycobiliproteins exist in vivo as high molecular weight aggregates. These are assembled into discrete organelles, termed phycobilisomes, which are arranged in a regular array on the photosynthetic lamellae of the blue-green algal cell (5–7), or, on the thylakoids of the red algal chloroplast (8, 9). There is evidence suggesting that allophycocyanin is in more intimate contact with the thylakoid membrane than phycocyanin (10). This evidence, and the results of electron microcscope studies (5–9), indicate that the phycobiliproteins are assembled in a highly organized manner within the cell.

The phycobiliproteins are readily released upon breakage of algal cells and are highly water-soluble. The size of the aggregates found in vivo varies widely, depending both on the method of preparation and on protein concentration (1, 11–14). Phycocyanins from diverse unicellular and filamentous blue-green algae share a number of key characteristics in common. With respect to an antiserum directed against a specific cyanobacterial phycocyanin, all heterologous phycocyanins are immunologically identical, as shown by the fact that absorption with a given heterologous antigen simultaneously eliminates cross-reactions with other heterologous antigens (15). The monomeric unit of the phycocyanins is made up of two subunits, α and β, of unequal size, each carrying covalently bound chromophore(s) (16–20). Finally, the isoelectric points of phycocyanins from different organisms are very similar (16), as are their absorption spectra in the visible region (1).

A striking finding is that these similarities extend beyond the prokaryotic phycocyanins to those of the eukaryotic red algae (15). Parallel similarities have been found for allophycocyanins and phycoerythrins (15, 21).

This conservation of structure is particularly interesting since microfossil evidence indicates the presence of both unicellular and filamentous blue-green algae over 2.6 billion years ago (22, 23), and since contemporary blue-green algae differ enormously
from each other in the mole per cent guanine plus cytosine content of their DNAs (24).

The conformation of a phycobiliprotein, such as phycocyanin, must satisfy a number of requirements. The paramount need is to assure the correct orientation and distance of the chromophores within the phycobilisome relative to each other, as well as to the protein-chlorophyll complex within the thylakoid. For phycocyanin, this implies highly specified contacts between the  and  subunits, each of which carries covalently bound chromophore(s). Further restrictions are imposed by the necessity to form aggregates of defined structure, and the requirement to interact closely with allophycocyanin (inferred from the high efficiency of energy transfer to chlorophyll (4)).

The hypothesis may be put forward that these requirements place stringent constraints on the structure of phycocyanin and account for the remarkable evolutionary stability of this protein.

We have purified phycocyanins from three blue-green algae chosen to give a representative sample of this diverse group of organisms. Procedures were then developed for the separation of the  and  subunits of these phycocyanins, as well as for the reconstitution of each of the proteins from its separated subunits. In support of the hypothesis outlined above, we have demonstrated by hybridization of  and  subunits of phycocyanins from unicellular and from filamentous blue-green algae that the structural requirements for the formation of the  unit are satisfied by pairing subunits from totally unrelated organisms.

**EXPERIMENTAL PROCEDURE**

**Materials**

*Blue-Green Algae*—Two unicellular blue-green algae, *Synechococcus* sp. (strain 6301), and *Aphanocapsa* sp. (strain 6701), and a filamentous blue-green alga, *Anabaena* sp. (strain 6411), were obtained from the Berkeley collection (24). The original sources, culture conditions, and properties of the strains have been described previously (24, 25). The cells were grown to a density of 1 to 1.5 g per liter, harvested by centrifugation, and the pellets stored at 4°C.

*Chemicals*—Preswollen Whatman microgranular DEAE-cellulose DE52 was obtained from Reeve Angel, Clifton, New Jersey, and Bio-Rex 70 (minus 400 mesh) from Bio-Rad, Richmond, California. Ampholine carrier ampholytes (40% w/v, Batch 38) were obtained from Schwerz-Mann, Orangeburg, New York, and  mercaptoethanol from Nutritional Biochemicals Corp. Hydroxyapatite was prepared by the procedure of Fiegenman et al. (26) and stored at 4°C. All other chemicals were of reagent grade.

**Methods**

*Preparation of Phycocyanins*—These proteins were prepared by modification of procedures described earlier (16, 17). All of the buffers used contain 1 mM  mercaptoethanol.

For the preparation of the phycocyanin of *Synechococcus* sp. (strain 6301), the cells (40 g wet weight) were suspended in 200 ml of 0.05 M sodium acetate at pH 5.5, and broken by repetitive slow freezing and thawing. The resulting suspension was centrifuged at 23,500 x g for 30 min, the supernatant decanted, and the pellet washed once with acetate buffer. The pooled supernatants were brought to 65% of saturation with solid ammonium sulfate and allowed to stand at 4°C overnight. The precipitate was collected by centrifugation, dissolved in a minimum volume of 0.005 M phosphate buffer at pH 7.0, and dialyzed to equilibrium against the same buffer at 4°C. The dialyzed sample was applied to a column (1.5 x 27 cm) of DEAE-cellulose, equilibrated previously with the 0.005 M buffer. After washing the sample in with starting buffer, the column was developed, at 24°C, with a linear gradient of 200 ml of 0.005 M phosphate, pH 7.0, in the mixing chamber, and 200 ml of 0.2 M phosphate, pH 7.0, in the reservoir, at a flow rate of 30 to 40 ml per hour. The phycocyanin fraction was pooled and concentrated by ultrafiltration with a Diaflo PM 10 membrane (Amicon Corp., Lexington, Mass.). The concentrated solution was then dialyzed against 0.005 M phosphate, at pH 7.0, and 4°C, and rechromatographed under the same conditions. The elution profiles are shown in Fig. 1.

Phycocyanin of *Anabaena* sp. (strain 6411) was purified by a procedure similar to that described above. The sole significant difference was the use of a shallower gradient, 0.005 to 0.1 M phosphate, pH 7.0, for the development of the DEAE-cellulose column. The elution profile is shown in Fig. 2.

For the preparation of the phycocyanin of *Aphanocapsa* sp. (strain 6701), the cells (10 g wet weight) were suspended in 75 ml of 0.05 M sodium acetate at pH 5.5, and broken by repeated slow freezing and thawing. The resulting suspension was centrifuged at 25,500 x g for 30 min, the supernatant decanted, and the pellet washed once with the pH 5.5 buffer. The pooled supernatant was brought to 65% of saturation with solid ammonium sulfate and allowed to stand at 4°C overnight. The precipitate was collected by centrifugation, dissolved in a minimum amount of 0.05 M sodium acetate at pH 5.5, and dialyzed exhaustively against 0.001 M phosphate-0.1 M NaCl at pH 7.0, at 4°C. The dialyzed sample was brought to room temperature, and applied to a column of hydroxyapatite (2.7 x 6 cm), pre-equilibrated with 0.001 M phosphate 0.1 M NaCl at pH 7.0. After washing with the starting buffer, the column was developed, at 24°C, by increasing the phosphate concentration stepwise. A flow rate of 50 to 60 ml per hour was maintained. The major phycocyanin fraction was eluted with 0.025 M phosphate-0.1 M NaCl, pH 7.0, as shown in Fig. 3. This fraction was concentrated by ultrafiltration, dialyzed against 0.001 M phosphate-0.1 M NaCl, at pH 7.0, and rechromatographed on a column (2.0 x 6 cm) of hy-
d rosophyllite under similar conditions. Prolonged elution with 0.015 M phosphate was utilized to recover the phycocyanin, free of residual impurities. The elution profile is shown in Fig. 4.

Separation of Subunits—Phycocyanin subunits were separated by a modification of the procedure described by Chernoff (27) for the separation of the $\alpha$ and $\beta$ chains of hemoglobin. In a typical experiment, a solution containing 32 mg of purified phycocyanin of Anabaena sp. in 0.1 M phosphate buffer at pH 7.0, was adjusted to pH 3.0 with glacial acetic acid and $\beta$-mercaptoethanol was added to a final concentration of 10 mM. This solution was applied to a column (2.2 x 13 cm) of Bio-Rex-70, pre-equilibrated with 0.04 M acetic acid at pH 3.0. The column was washed extensively with 2 M urea-10 mM $\beta$-mercaptoethanol at pH 3.0, and developed by increasing the urea concentration stepwise. The $\alpha$ subunit of phycocyanin was eluted with 8 M urea-10 mM $\beta$-mercaptoethanol, pH 3.0, and the $\beta$ subunit with 9 M urea-10 mM $\beta$-mercaptoethanol, pH 3.0, as shown in Fig. 5. The fractions containing each of the subunits were pooled, dialyzed exhaustively against water, and lyophilized. The subunits of all three phycocyanins examined in this study could be separated in this manner.

Acrylamide Gel Electrophoresis—Electrophoresis at pH 8.1 on 7.5, 11.25, and 12.5% polyacrylamide gels (8 cm long) was performed by the method of Davis (28). Gels were run at 2 ma per gel for 4 hours at room temperature. Sodium dodecyl sulfatopolyacrylamide gel electrophoresis was performed in 10.5 cm long gels, prepared with double the normal amount of cross-linker, as described by Weber and Osborn (29). Electrophoresis was performed at 4 ma per gel for 10 hours.

Isoelectric Focusing—A modified version (21) of the procedure described by Wrigley (30) was used. Gels were photopolymerized, and then electrophoresed at 2 ma per gel for 4 hours at room temperature. Sodium dodecyl sulfate—polyacrylamide gel electrophoresis was performed in 10.5 cm long gels, prepared with double the normal amount of cross-linker, as described by Weber and Osborn (29). Electrophoresis was performed at 4 ma per gel for 10 hours.
ized in the presence of riboflavin. More reproducible polymerization rates were obtained with riboflavin solutions which had been stored at 4°C for several weeks. Seven per cent polyacrylamide gels, 11 cm long, containing Ampholine carrier ampholytes (pH range 3 to 10) were maintained at a constant voltage of 180 volts for 16 hours at room temperature. Isoelectric points were determined by cutting out the protein bands (1 to 2 mm in width) from the gels and eluting with 2 ml of 0.05 M NaCl for 16 hours at 4°C.

Absorption Spectra—Absorption spectra were obtained with a Cary model 15 recording spectrophotometer, and λ_max and A_max values verified with a Zeiss PMQ-II spectrophotometer.

Amino Acid Analyses—These were performed by conventional procedures (31). Amino acid compositions were calculated to give the best fit to integral numbers of residues and to the monomer molecular weights, as determined by SDS-polyacrylamide gel electrophoresis.

Reconstitution and Hybridization Procedures—Murphy and O’Carra (32) reported that the denaturation of C-phycocyanin by 8 M urea at pH 8 resulted in a loss of fluorescence of the protein, marked alteration in the chromophore absorption spectrum, and an increase in intrinsic viscosity. Renatured phycocyanin could be recovered in ~50% yield by dialysis of the phycocyanin-8 M urea solution to 3 M urea, followed by dialysis against 0.01 M phosphate at pH 0.5, and chromatography on tricalcium phosphate gel (32). The extent of renaturation was reported to be pH-dependent with a broad maximum near pH 7. Although this work was performed prior to the realization that C-phycocyanin consists of two distinct subunits (16), it was used as a guide in choosing the conditions for the reconstitution and hybridization experiments described below.

The amounts of the α and β subunits used for reconstitution experiments were based on the subunit molecular weights as determined by SDS polyacrylamide gel electrophoresis. These were as follows: α_max 15,900, β_max 19,100 (16); α_max 16,600, β_max 20,200 (16); α_max 17,100, β_max 19,000. The abbreviations employed in this paper for the subunits of the various phycocyanins, the phycocyanins themselves, reconstituted phycocyanins, and hybrid phycocyanins are defined in Table I.

In a typical experiment, 2.37 ml of lyophilized α_max and 2.63 mg of β_max were weighed out on a Cahn electrobalance, model G (Ventron Corp., Paramount, Calif.) and dissolved together in 12.5 ml of 8 M urea-0.005 M β-mercaptoethanol at pH 8.0. The resulting solution was dialyzed against 400 ml of 3 M urea-0.005 M β-mercaptoethanol-0.005 M phosphate at pH 6.7, and 4°C, for 4 hours. It was then dialyzed against 400 ml of 0.01 M phosphate-0.005 M β-mercaptoethanol at pH 6.5 for 20 hours at 4°C, with one change of dialysis buffer. Progressive gain of color and fluorescence was noted throughout the dialysis procedure. After a final dialysis against 0.005 M phosphate-0.001 M β-mercaptoethanol, at pH 7.0, the protein was applied to a column (0.9 × 6 cm) of DEAE-cellulose, pre-equilibrated with the same buffer. After washing with the starting buffer, the column was developed with a linear gradient of 25 ml of 0.005 M phosphate-0.001 M β-mercaptoethanol, pH 7.0, in the mixer, and 25 ml 0.2 M phosphate-0.001 M β-mercaptoethanol, pH 7.0, in the reservoir. Reconstituted phycocyanin emerged as a single peak near the middle of the gradient. Some of the material remained irreversibly adsorbed to the top of the column.

In all recombination experiments, protein concentration was maintained at 0.4 mg per ml or lower. At higher concentrations,

\[ 1 \text{ The abbreviation used is: SDS, sodium dodecyl sulfate.} \]
Fig. 6. SDS-polyacrylamide gel electrophoresis of purified phycoerythrin and their separated subunits, as well as of reconstituted and hybrid phycoerythrin. A t, PC-6361; 2, p6361; 3, 6p631; 4, PC-6701: 5, 6p631; 6, p670r. Bt, PC-6411; 2, PC-6701; 3, PC-6411; 4, PC-6411; 5, PC-6411; 6, PC-6411. For explanation of the abbreviations used, see Table I.

Separation of Subunits—The subunits of the phycoerythrin were separated by stepwise elution from Bio-Rex 70 with increasing concentrations of acid urea. The purity of the subunits, and the freedom from cross-contamination were established by SDS-polyacrylamide gel electrophoresis (Fig. 6, A and B), and by quantitative amino acid analysis (Table III). The amino acid analyses also confirm that the phycoerythrin consist of the α and β subunits in a ratio of 1:1 (Table III).

The colorless contaminant in PC-6701 (see above) eluted from Bio-Rex 70 with the β subunit (see Fig. 6A, Gel B). However, in one experiment, it eluted ahead of the β subunit, permitting the determination of the amino acid composition of a pure preparation of the latter.

Reconstitution of Phycoerythrin—Successful reconstitution of all three phycoerythrin was achieved by dissolution of the α and β subunits, in equimolar amounts, in 8 M urea, followed by stepwise dialysis and column chromatography (see “Methods”). The reconstituted proteins exhibited the strong red fluorescence characteristic of native phycoerythrin. The absorption spectra in the visible region exhibited a small blue shift relative to those of the native proteins (Fig. 8). Such a shift has been observed on disaggregation of the phycoerythrin (12, 14, 17). Electrophoresis of the reconstituted phycoerythrin at pH 8.1 on polyacrylamide gels of varying concentration (7.5 to 12.5%) suggested that the reconstituted material indeed consisted of smaller aggregates than those present in the native preparation (Fig. 7A, 7 and 5).

With respect to amino acid analyses (Table III), isoelectric points (Table II), and SDS-polyacrylamide gel electrophoresis (Fig. 6, C to E), the properties of the reconstituted proteins were in excellent agreement with those of their native counterparts.
FIG. 7. Electrophoresis at pH 8.1, and isoelectric focusing, in polyacrylamide gels, of purified phycoerythrin, reconstituted phycoerythrin, and hybrid phycoerythrin. A, electrophoresis at pH 8.1, in 7.5% (Gels 1 to 4), and 11.25% (Gels 5 to 8) polyacrylamide gels. Gels 1 and 5, PC-6701; Gels 2 and 6, PC-6301; Gels 3 and 7, a mixture of PC-6701 and PC-6301; Gels 4 and 8, PC-αβ. A1, gels photographed before staining; A2, gels photographed after staining with Amido black, and destaining with 7% aqueous acetic acid. Arrows in A1; B, and C1 indicate the bromphenol blue tracking dye front. B, electrophoresis at pH 8.1, in 7.5% gels, of 1, PC-6301; 2, PC-αβ; 3, PC-αβ; 4, PC-6301 plus PC-6411; 5, PC-6411. C, electrophoresis at pH 8.1, in 12.5% gels, of 1, PC-6301; 2, PC-αβ; 3, PC-αβ; 4, PC-6301 plus PC-6411; 5, PC-6411. D to G isoelectric focusing. D1, PC-6701: 2, PC-αβ; 3, PC-αβ; 4, PC-αβ; 5, PC-6301; 6, PC-6301 plus PC-6701. E1, PC-6701; 2, PC-αβ; 3, PC-αβ; 4, PC-6301 plus PC-6701; 5, PC-6301; 6, PC-6301 plus PC-6701; 7, PC-6701; 8, PC-αβ. F1, PC-6411: 2, PC-αβ; 3, PC-αβ; 4, PC-6301 plus PC-6411; 5, PC-6411 plus PC-6301; 6, PC-6411; 7, PC-6301 plus PC-6411; 8, PC-αβ. G1, PC-6301 plus PC-αβ; 2, PC-αβ; 3, PC-6411; 4, PC-6411. All abbreviations used are defined in Table I.

It is noteworthy that the colorless component, present in the purified preparations of PC-6701, was totally eliminated from purified PC-αβ (Fig. 6D, compare Gels 1 and 2).

**Properties of α and β Subunits**—The isolated α and β subunits were subjected, separately, to the procedure employed for the reconstitution of phycoerythrin, but with omission of the final chromatography step (see "Methods"). The subunits tended to precipitate on dialysis and storage. As might be expected, precipitation was more rapid at high protein concentrations, but, even at very low concentrations (<0.5 mg per ml), precipitation occurred on storage at 4°C. Whereas, in 8 M urea at pH 3.0, the absorption spectra of the α and β subunits in the visible region were qualitatively identical, in 0.01 M phosphate-0.005 M β-mercaptoethanol at pH 6.5, they differed grossly from each other and from that of native phycoerythrin. The limited solubility of these materials at near neutral pH, and tendency to precipitate from solution, hampered studies of their state of aggregation and the determination of quantitative absorption spectra. The re-
TABLE II
Isoelectric points of phycocyanins, hybrid phycocyanins, and their subunits as determined by isoelectric focusing in polyacrylamide gels

<table>
<thead>
<tr>
<th>Protein</th>
<th>pI</th>
<th>Subunit a</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-6301</td>
<td>4.76</td>
<td>4.90</td>
<td>5.20</td>
</tr>
<tr>
<td>PC-(\alpha_{\alpha}6301)</td>
<td>4.62</td>
<td>4.80</td>
<td>5.17</td>
</tr>
<tr>
<td>PC-(\beta_{\beta}6301)</td>
<td>4.66</td>
<td>Precipitate</td>
<td>5.17, 5.51, 5.89</td>
</tr>
<tr>
<td>PC-(\alpha_{\alpha}6411)</td>
<td>4.56</td>
<td>4.35, 4.60, 5.09</td>
<td>5.06</td>
</tr>
<tr>
<td>PC-(\beta_{\beta}6411)</td>
<td>4.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The isolated subunits were subjected to the procedure used for reconstitution and hybridization experiments (see "Methods"), but the final chromatography on DEAE-cellulose was omitted.

b In contrast to the strongly red fluorescent bands obtained from the other phycocyanins, this hybrid gave very weak fluorescent bands (see Fig. 2G, Gel 2).

c Values calculated from 24-, 48-, and 72-hour hydrolysates.

d Uncorrected values obtained for cysteic acid after performic acid oxidation by the method of Hirs (34).

e N.D., not determined.

f Values calculated from 24-, and 48-hour hydrolysates.

g Values obtained by linear extrapolation to zero time.

h Uncorrected values obtained for cysteic acid after performic acid oxidation by the method of Hirs (34).

i N.D., not determined.

j Tryptophan was determined by the method of Liu and Chang (35).

suits of isoelectric focusing of the individual subunits are given in Table II. The isoelectric points of the subunits were found to be generally higher than those of the intact phycocyanins.

Characterization of Hybrid Phycocyanin—A total of four hybrid phycocyanins was prepared by combining the \(\alpha\) subunit of Synechococcus sp. phycocyanin with the \(\beta\) subunit of the phycocyanins of \(\alpha\) and \(\beta\) subunits derived from the other two phycocyanins. The hybrids were obtained in the same overall yield as phycocyanins formed by recombination of their constituent subunits. With respect to visible absorption spectra (Fig. 8), the hybrid phycocyanins were indistinguishable from those formed by recombination of \(\alpha\) and \(\beta\) subunits derived from a single phycocyanin. Likewise, SDS-polyacrylamide gel electrophoresis (Fig. 6, D and E), and amino acid analyses (Table IV) demonstrated unambiguously that each of the hybrids consisted of the appropriate \(\alpha\) and \(\beta\) chains in a molar ratio of 1:1. With one exception (see below), the hybrides gave sharp bands on isoelectric focusing (Fig. 7, D to F). The isoelectric points of the hybrids were distinct from those of the parent phycocyanins (Table II). The hybrid, PC-\(\alpha_{\alpha}6411\), exhibited anomalous behavior on isoelectric focusing (Table II, Fig. 7G, Gel 2), suggestive of dissociation. Presumably the interaction of the subunits in this hybrid is weaker than in the other hybrids examined.

TABLE III
Amino acid composition of phycocyanins, their \(\alpha\) and \(\beta\) subunits, and of "reconstituted" phycocyanins, of Synechococcus sp. (strain 6301), \(\alpha\) and \(\beta\) subunits derived from the other two phycocyanins (strain 6411), and Anabaena sp. (strain 6411)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Synechococcus sp. phycocyanin (PC-6301)</th>
<th>(\alpha) Subunit ((\alpha_{\alpha}))</th>
<th>(\beta) Subunit ((\beta_{\beta}))</th>
<th>Reconstituted phycocyanin (PC-(\alpha_{\alpha}6301))</th>
<th>(\alpha) Subunit ((\alpha_{\alpha}))</th>
<th>(\beta) Subunit ((\beta_{\beta}))</th>
<th>Reconstituted phycocyanin (PC-(\alpha_{\alpha}6301))</th>
<th>(\alpha) Subunit ((\alpha_{\alpha}))</th>
<th>(\beta) Subunit ((\beta_{\beta}))</th>
<th>Reconstituted phycocyanin (PC-(\alpha_{\alpha}6301))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>13.4</td>
<td>7.3</td>
<td>5.2</td>
<td>12.6</td>
<td>12.0</td>
<td>6.8</td>
<td>11.3</td>
<td>11.3</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Arginine</td>
<td>20.1</td>
<td>7.0</td>
<td>13.0</td>
<td>19.5</td>
<td>17.8</td>
<td>7.0</td>
<td>11.0</td>
<td>10.3</td>
<td>7.0</td>
<td>10.3</td>
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<tr>
<td>Aspartic acid</td>
<td>41.8</td>
<td>18.1</td>
<td>21.2</td>
<td>28.8</td>
<td>21.3</td>
<td>19.9</td>
<td>21.9</td>
<td>21.9</td>
<td>12.0</td>
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</tr>
<tr>
<td>Threonine</td>
<td>18.8</td>
<td>9.8</td>
<td>9.0</td>
<td>18.4</td>
<td>21.1</td>
<td>19.5</td>
<td>20.5</td>
<td>21.3</td>
<td>19.0</td>
<td>20.0</td>
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<tr>
<td>Serine</td>
<td>24.0</td>
<td>12.3</td>
<td>11.2</td>
<td>23.0</td>
<td>14.8</td>
<td>14.8</td>
<td>14.8</td>
<td>20.8</td>
<td>8.7</td>
<td>20.6</td>
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<tr>
<td>Glutamic acid</td>
<td>22.0</td>
<td>11.1</td>
<td>11.1</td>
<td>21.9</td>
<td>17.7</td>
<td>17.7</td>
<td>17.7</td>
<td>21.7</td>
<td>15.0</td>
<td>21.7</td>
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<tr>
<td>Proline</td>
<td>20.9</td>
<td>6.3</td>
<td>4.0</td>
<td>10.0</td>
<td>9.6</td>
<td>5.3</td>
<td>3.6</td>
<td>9.3</td>
<td>5.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>29.9</td>
<td>12.7</td>
<td>13.1</td>
<td>23.9</td>
<td>12.5</td>
<td>10.5</td>
<td>24.9</td>
<td>24.9</td>
<td>11.5</td>
<td>24.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>30.4</td>
<td>25.1</td>
<td>31.9</td>
<td>58.0</td>
<td>50.7</td>
<td>21.9</td>
<td>51.5</td>
<td>44.4</td>
<td>20.5</td>
<td>25.3</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>1.3</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.5</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
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</table>

a Values calculated from 24-, 48-, and 72-hour hydrolysates.

b Values calculated from 24-, and 48-hour hydrolysates.

c Values obtained by linear extrapolation to zero time.

d Uncorrected values obtained for cysteic acid after performic acid oxidation by the method of Hirs (34).

e N.D., not determined.

f Tryptophan was determined by the method of Liu and Chang (35).
FIG. 8. Absorption spectra in the visible region, of (A) purified phycocyanins; (B) and (C), of various reconstituted and hybrid phycocyanins. All spectra were obtained in 0.20 M phosphate buffer, 0.001 M β-mercaptoethanol at pH 7.0. The abbreviations used are defined in Table I.

TABLE IV

Amino acid analyses of hybrids of the α and β subunits of Synechococcus sp. (strain 6301) phycocyanin with complementary subunits of Aphanocapsa sp. (strain 6701) and Anabaena sp. (strain 6411) phycocyanins

Amino acid analyses were performed as described in Table III. The values given for the hybrid phycocyanins were calculated from analyses of 24- and 48-hour hydrolysates. The "expected" analytical values for each hybrid represent the sum of the amino acid compositions of the appropriate pair of subunits, and are derived from the data in Table III. Determinations of half-cystine and tryptophan content were not performed.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Hybrids of Synechococcus sp. and Aphanocapsa sp. phycocyanins</th>
<th>Hybrids of Synechococcus sp. and Anabaena sp. phycocyanins</th>
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<tr>
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<td>φ ω1 + β ω2</td>
<td>ω1 + β ω2</td>
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<tr>
<td>Phenylalanine</td>
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</table>

* Value obtained by linear extrapolation to zero time.

b Value from 48-hour hydrolysate.

discussion

The isoelectric points, native molecular weights, and subunit molecular weights of the three phycocyanins examined in the present study, are remarkably similar. These findings are in accord with the high degree of conservation of molecular properties in this class of proteins indicated by our earlier physicochemical (10) and immunological studies (15), and are extended further by the demonstration of the remarkable resemblance of the amino acid compositions of these phycocyanins (Table III). Monomer molecular weights reported for phycocyanin from various blue-green algal sources range from 26,000 to 39,000 (16–20). Those of the three phycocyanins included in this study lie at the upper end of this range. The considerable similarity between the amino acid compositions of the α and β subunits, derived from a given phycocyanin, is suggestive of a common evolutionary origin for these chains; a situation parallel to that observed...
for the α and β chains of hemoglobin. An interesting feature of the amino acid analyses (Table III) is the absence of histidine from the β subunits of phycocyanins, and its invariable presence in the α subunits.

The reconstitution of phycocyanin from its separated subunits represents but a step on the way to the elucidation of the mode of assembly of a phycobilisome. A more detailed comparison will be performed of the properties of reconstituted and native phycocyanins, in particular, of their circular dichroism, as well as a study of the conditions required for the formation of the high molecular weight aggregates characteristic of the state of this protein in vivo.

The blue-green algae, used as sources of phycocyanin, were selected on the basis of their wide diversity. Blue-green algae fall into two distinctive morphological lines, the unicellular and filamentous types. The two members of the Chroococcaceae, Synechococcus sp., and Aphanocapsa sp., represent the first type, while Anabaena sp. represents the second. Synechococcus sp. and Aphanocapsa sp. belong to two very different typological groups (24).

Their DNAs differ from each other by about 20% in guanine plus cytosine content (24). Aphanocapsa sp. (strain 6701) produces allophycocyanin, phycocyanin, and phycoerythrin, while Synechococcus sp. (strain 6301) does not produce phycoerythrin (13–15). The filamentous alga, Anabaena sp. (strain 6411), produces all three phycobiliproteins. However, whereas the Aphanocapsa sp. alga produces comparable amounts of phycocyanin and phycoerythrin, the latter chromoprotein is a minor component in this filamentous alga. Anabaena sp. (strain 6411) is a heterocyst former, capable of aerobic nitrogen fixation, a character shared by neither of the unicellular organisms (25, 37).

Microfossils of unicellular and filamentous organisms, over 2.6 billion years old, closely resemble many extant blue-green algae (22, 23). The separation of the evolutionary lines represented by the unicellular and filamentous blue-green algae is therefore a very ancient event, antedating the appearance of eukaryotic life (22).

The ability to form hybrid phycocyanins by utilizing subunits from proteins of filamentous and unicellular organisms is remarkable. It is evident that the evolutionary constraints, imposed upon the protein precursor of contemporary phycocyanins, permitted only those amino acid substitutions which maintained the conformation and contacts necessary for intersubunit association.

No other hybridization studies have been performed on proteins derived from organisms separated by a comparable span of time. The closest available comparison is provided by studies with lactic dehydrogenase (38). Catalytically functional hybrids were formed between the A and B subunits originating from enzymes obtained from different classes of vertebrates. For example, hybrid isozymes were formed between horse and lamprey lactic dehydrogenases (38); a noteworthy finding, since lampreys belong to the Cyclostomi, the oldest vertebrates which developed approximately 450 million years ago. Other striking instances are represented by the ability to form active hybrids of the alkaline phosphatases of Escherichia coli and Serratia marcescens, organisms belonging to two separate genera (39), and the well known hybridization studies on hemoglobin of human and canine origin (40). However, none of these proteins is of comparable antiquity to the blue-green algal phycocyanins.

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Formation of Hybrid Proteins from the $\alpha$ and $\beta$ Subunits of Phycocyanins of Unicellular and Filamentous Blue-green Algae
Alexander N. Glazer and Suen Fang


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