Characterization of Phycocyanin from *Chroomonas* Species*†

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SUMMARY

Phycocyanin, a chromoprotein from *Chroomonas* sp., is characterized in regard to its size, subunit structure, amino acid composition, and spectroscopic properties. It is a monodisperse protein of 50,000 daltons and is composed of two polypeptide chains of 10,000 and two chains of 16,000 daltons. The proposed structure of the “native” protein is $\alpha_2\beta_2$. The $s_{20\,w}$ is 4.4 S, and the partial specific volume is 0.73.

Unlike C-phycocyanin, a functionally related chromoprotein from blue-green and red algae, phycocyanin does not form a number of different aggregates. We suggest that the absence of these larger aggregates is related to its in vivo location. The amino acid composition of phycocyanin from *Chroomonas* sp. differs extensively from that of C-phycocyanin, with much greater amounts of serine, half-cystine, and lysine. Likewise, the circular dichroism and fluorescence spectra are very different, indicating major functional modifications.

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Phycocyanin, a chromoprotein found in Cryptophycean algae, is a protein about whose size and subunit structure little has hitherto been known (1, 2). This is in marked contrast to a functionally related protein, C-phycocyanin from blue-green and red algae, which has been studied extensively (3–6). Electron microscopic studies have revealed salient morphological differences between the state of C-phycocyanin (7, 8) and Cryptophycean phycocyanin (9) in the cell. The most important of these differences concerns location and aggregation. C-Phycocyanin and phycocyanin are located on the stromal side of the photosynthetic lamellae in the form of large, discrete aggregates called phycobilisomes. Cryptophycean phycocyanin from *Chroomonas* sp. is contained within the intrathylakoidal spaces, and there is no evidence of phycobilisomes. This paper presents that the physical properties and structure of phycocyanin from *Chroomonas* sp. are related to the intriguing morphological differences (7–9) between it and C-phycocyanin. This correlation is particularly evident when the differences in aggregation properties are considered.

In this paper, the term phycocyanin refers specifically to

*Chroomonas* sp. and C-phycocyanin to the chromoprotein from blue-green algae.

EXPERIMENTAL PROCEDURE

Purification of Phycocyanin—A culture of the eukaryotic alga, *Chroomonas* sp., was obtained from Dr. L. Provazali, Haskins Laboratory, New Haven, Conn. The alga was grown using white fluorescent lamps with an illumination of 80 foot-candles in 500 ml of DV media (10) in 2-liter Erlenmeyer flasks without agitation at 18° and was harvested by centrifugation. The cells were then resuspended in sodium phosphate buffer, pH 6.0, I 0.1.

Following a second centrifugation, the pelleted cells were stored frozen at $-20^\circ$. To extract the phycocyanin, the cells were suspended in small amounts of pH 6.0 buffer and subjected to three cycles of freezing and thawing. The protein which appeared in the supernatant was then purified either by ammonium sulfate gradient chromatography (11) or by differential ammonium sulfate fractionation. An $A_{450}:A_{650}$ ratio in the range of 5.5 to 6.2 was usually obtained by fractionation with ammonium sulfate. (Absorbance ratio > 5.0 was considered an index of purity.) These solutions were centrifuged at 90,000 × g to remove cell debris prior to each addition of ammonium sulfate. The purified protein was usually stored in 68% saturated ammonium sulfate at $4^\circ$.

In purification by ammonium sulfate gradient chromatography, the procedures were as follows. Twenty milliliters of crude *Chroomonas* sp. extract ($A_{450} = 480$) were adjusted to 80% saturated ammonium sulfate in sodium phosphate buffer, pH 6.0, I 0.1. This solution (100 ml) was mixed for 20 min with 6 g of Celite 545 and then poured into a column with a final bed size of $1 \times 12$ cm. Following an initial wash with 80% saturated ammonium sulfate, a linear ammonium sulfate gradient from 80 to 0% saturation in pH 6.0 buffer was passed through the column. The initial crude extract had an $A_{450}:A_{650}$ ratio of about 0.9. By following this procedure, about 90% of the phycocyanin was purified to a ratio of ≥5.0 (Fig. 1). The material which was initially washed from the column was soluble in 80% saturated ammonium sulfate and had an $A_{280}:A_{650}$ ratio of 1.93, indicative of a high proportion of nucleic acid. The biliprotein undoubtedly represents a large proportion of the soluble protein of the organism, since the elution profile revealed no other large protein peaks.

The freeze-thawing procedure is quite efficient in releasing phycocyanin from *Chroomonas* sp. For example, from a packed cell volume of 24.5 cm$^3$, 50 ml of soluble phycocyanin solution with an $A_{450}$ of 49.3 and an $A_{650}:A_{450}$ ratio of 0.92 was obtained.

† The abbreviation used is: I, ionic strength.
This corresponds to about 9 mg of phycocyanin per ml of packed cells (assuming \( A_{665}^{1%} = 114 \)). Recovery of biliprotein was even greater when the pellet was re-extracted several times.

**Ultracentrifuge Studies**—Sedimentation velocity experiments were performed in a Spinco model E ultracentrifuge with either schlieren or absorption optics. The photoelectric scanner was used for solutions of very low concentration, \( A_{665}^{1%} = 0.01 \) and 0.40. All experiments were performed at 25°C in pH 6.0 buffer, I 0.1. Sedimentation coefficients were calculated from schlieren plates analyzed with a Nikon microcomputer, model 6.

Sedimentation equilibrium experiments were performed by the high speed method introduced by Yphantis (12). The weight-average molecular weight \( (M_w) \) was calculated from the equation:

\[
M_w = \frac{2RT}{\omega^2 (1 - \frac{V}{V_o})} \cdot \frac{d\ln A}{d\gamma^2}
\]

where \( \omega \) is angular velocity; \( R \), the gas constant; \( T \), the temperature; \( V \), the partial specific volume; and \( \rho \), the solvent density. A least squares analysis of \( \ln A \) versus \( \gamma^2 \) was used to obtain \( d \) in \( \ln A/\gamma^2 \). A linear plot of \( \ln A \) against \( \gamma^2 \) is expected for a homogeneous and thermodynamically ideal solution. The photoelectric scanner, multiplexer, and monochromator attachment, set at either 580 or 620 nm, were used. All sedimentation equilibrium experiments were carried out in sodium phosphate buffer, pH 6.0, either in 100% H₂O, or in a mixture of 9% D₂O and 91% D₂O. The mixture was used to determine simultaneously the partial specific volume \( (\bar{V}) \) and molecular weight of the protein as demonstrated by Edelstein and Schachman (13). These experiments were performed at 20°C and 29,500 rpm in an AN-F rotor with 12-mm double sector Kel-F centerpieces and sapphire windows.

**Gel Electrophoresis in Sodium Dodecyl Sulfate**—Gel electrophoresis in the presence of sodium dodecyl sulfate, introduced by Shapiro et al. (14), was performed with the procedures of Weber and Osborn (15). Gels with both the normal and double the normal amounts of cross-linking reagent were used. Acrylamide (electrophoresis grade) was purchased from Eastman, sodium dodecyl sulfate (99%) was obtained from Sigma.

**Amino Acid Composition**—Phycocyanin solutions were dialyzed exhaustively into distilled water and lyophilized. The protein was treated by acid hydrolysis in 6 N HCl in evacuated tubes at 110°C for 24, 48, and 72 hours. The HCl was removed by rotary evaporation under reduced pressure at 40°C. Amino acid analyses were performed by the method of Spackman et al. (16). Separate analyses were done for cysteic acid (17).

The two subunits of phycocyanin were separated on the gels and eluted and prepared for amino acid analysis by the method of Weber and Osborn (15). The two bands were clearly visible on the gels; the faster migrating band was green and the slower, blue. Phycocyanin (0.1 mg) was placed on each gel, and the two bands were cut out of the gels and eluted overnight into a 0.1% sodium dodecyl sulfate solution at 37°C. The solutions were separately pooled and lyophilized. Distilled water was added to make a 1% sodium dodecyl sulfate solution, and 9 parts of acetone were added in the cold to dissolve the detergent. Two additional washings with acetone were performed at 0°C. The separated chains were then dissolved in 6 N HCl, and the amino acid analysis was accomplished as indicated above. A small amount of phenol (0.5 g per liter) was present in the 6 N HCl during acid hydrolysis of the α- and β-bands to prevent destruction of tyrosine.

**Spectroscopic Measurements**—Fluorescence measurements were performed on a Baird Atomic fluorescence spectrometer, model SF-1, as described previously (18). Fluorescence measurements were made in buffer, pH 6.0, I 0.1. Visible and ultraviolet absorption and CD spectra were obtained on a Cary 14 spectrophotometer and a Cary 61 circular dichrometer, respectively.

The extinction coefficient of phycocyanin was determined at 645 nm. After the absorption of a purified sample in pH 6.0 buffer was measured, the solution was exhaustively dialyzed into distilled water. Aliquots were pipetted into weighed vessels, dried at 110°C, and weighed. A 1 mg per ml solution was calculated to possess an absorption of 11.4 for a 1-cm path length.

**Gel Filtration in Guanidine Hydrochloride**—The subunit molecular weights were estimated by gel filtration in guanidine hydrochloride (Heico, Delaware Water Gap, Pa.) by the method of Fish et al. (19). A column, 2.1 x 41 cm, of Sepharose 6B, equilibrated with 6 M guanidine hydrochloride and 0.1 M 2-mercaptoethanol, was used in these experiments. The column was operated in an upward flow configuration at room temperature, and fractions of 60 drops (~1.6 ml) were collected at a flow rate of 6 ml per hour. Elution positions were expressed as the distribution coefficient, \( K_d \), where \( K_d = (V_e - V_i)/(V_e - V_o) \). Blue dextran and 2,4-dinitrophenyl alanine were used to determine the void \( (V_o) \) and internal \( (V_i) \) volumes, respectively. \( V_e \) is the elution position of the polypeptides. Samples containing 5 to 10 mg of lyophilized protein were denatured with 1.0 to 1.5 ml of the elution solvent for a minimum of 4 hours prior to application on the column.

**RESULTS**

**Size of Native Protein**—The molecular weight of phycocyanin in the absence of denaturation agents was determined by sedimentation equilibrium and Sephadex G-200 gel filtration. The average molecular weight obtained from sedimentation data from different samples in buffer, pH 6.0, I 0.1, was \( 4.9 \times 10^4 \pm 980 \) (Fig. 2). From gel filtration in phosphate buffer, pH 7.0, a molecular weight of \( 5.3 \times 10^4 \) was obtained (Fig. 3). Phycocyanin was eluted from the Sephadex G-200 column as a single symmetrical peak, and there was no indication of the size heterogeneity commonly seen with C-phycocyanin (3). Identical molecular weights were obtained with sedimentation equilibrium in pH 6.0 buffer composed of either 100% H₂O or a mixture of 9% H₂O and 91% D₂O. The partial specific volume determined from these studies was 0.724. In addition, values in the vicinity of \( 4.9 \times 10^4 \) daltons were observed, whether the monochromator...
was set at 580 or 620 nm. Spectrophotometric measurements at 580 and 620 nm showed a linear relationship between absorption and concentration over the range of protein concentrations at which equilibrium experiments were performed.

Sedimentation velocity experiments with both schlieren and absorption optics (Fig. 4) showed a single symmetrical boundary. Several experiments over a 100-fold concentration range between 0.04 and 5 g per liter yielded the same sedimentation coefficient. The $s_{20,w}$ is 4.4 S. This independence of the concentration of the sedimentation coefficient suggests that a single species is present. The sedimentation equilibrium data produced a linear $\ln A$ versus $r^2$ plot (Fig. 2), which also indicates the presence of a single phycocyanin species. To test this conclusion further, sedimentation equilibrium data were analyzed to obtain number and weight point-average slopes at various distances from the center of rotation, as indicated in the following equations (12, 20).

$$M_n(r) = \frac{RT}{\omega^2(1 - \bar{V}_p)} \cdot \frac{0.1}{\bar{v}_n} \left( 2 \ln A_2 + \ln A_1 - \ln A_{1.4} - 2 \ln A_{1.2} \right)$$

$$M_w(r) = \frac{RT}{\omega^2(1 - \bar{V}_p)} \cdot \frac{A(r_2)}{\bar{v}_w} + \frac{\Delta \Sigma}{2} \sum_{i=2}^{4} \left[ r_i A_i + r_0 A_0 \right]$$

Jeffrey and Pont (20) have demonstrated that this method provides a better criterion of homogeneity, since a linear $\ln A$ versus $r^2$ plot may be misleading in certain situations. The results of these analyses showed no variation in either number- or weight-average molecular weights at any distance from the center of rotation. In addition, the number average molecular weight is essentially identical with the weight-average molecular weight. Thus all these studies suggest that phycocyanin has a single quaternary structure under the conditions of these experiments.

**Subunit Composition—Gel filtration in 6 M guanidine hydrochloride and sodium dodecyl sulfate gel electrophoresis experiments both showed that phycocyanin is composed of two polypeptide chains. In the experiments with detergent (Fig. 5), two bands were visible, one blue and the other green. No additional protein bands were detected upon staining with Coomassie blue. When the mobilities of the two phycocyanin bands were compared with those of several proteins with known molecular weights (Fig. 6), the green band (α chain) was calculated to have a molecular weight of $9.9 \times 10^3 \pm 510$ and the blue band (β chain) one of $1.59 \times 10^4 \pm 570$. The standard deviations were calculated from the results of six separate experiments. Identical results were obtained with a ratio of 22.2 g of acrylamide to either 0.6 or 1.2 g of methylenebisacrylamide. When 2-mercaptoethanol was omitted from the solutions, the two bands were still observed, indicating that they are not joined by disulfide bonds.

Gel filtration in 6 M guanidine hydrochloride resolved the phycocyanin clearly into two peaks (Fig. 7) corresponding to 19,900 and 9,900 daltons (Fig. 8). The larger of these appeared blue-green and, despite a high absorbance at 400 nm, possessed a clearly defined absorption maximum at 600 nm. The slower, yellow component had a single visible absorption band at 400 nm. Examination of this same protein by gel filtration in 0.1 M phosphate buffer, pH 7.0, containing 0.1% sodium dodecyl sulfate, yielded two partially resolved polypeptides with absorp-
Amino acid compositions of phycocyanin from Chroomonas sp. and its subunits

VINE SERUM ALBUMIN

\[
\begin{array}{c|c|c|c}
\text{Amino acid} & \text{Phycocyanin} & \alpha \text{ chain} & \beta \text{ chain} \\
& (24,000) & (9,000) & (15,000) \\
\hline
\text{Lysine} & 16.6 \pm 17 & 7.0 \pm 8 & 7.7 \pm 8 \\
\text{Histidine} & 0.90 \pm 1 & 0.61 \pm 1 & \text{Trace} \text{ Trace} \\
\text{Arginine} & 8.9 \pm 9 & 3.7 \pm 4 & 7.4 \pm 7 \\
\text{Aspartic acid} & 23.3 \pm 28 & 10.5 \pm 11 & 16.5 \pm 17 \\
\text{Serine} & 10.1 \pm 10 & 4.0 \pm 4 & 5.8 \pm 6 \\
\text{Glutamic acid} & 16.9 \pm 17 & 8.0 \pm 9 & 8.4 \pm 8 \\
\text{Proline} & 6.4 \pm 6 & 3.3 \pm 3 & 4.2 \pm 4 \\
\text{Alanine} & 19.8 \pm 19 & 6.7 \pm 7 & 13.4 \pm 13 \\
\text{Valine} & 33.4 \pm 33 & 10.0 \pm 10 & 20.2 \pm 20 \\
\text{Methionine} & 16.4 \pm 16 & 5.3 \pm 5 & 9.5 \pm 10 \\
\text{Isoleucine} & 4.1 \pm 4 & 1.6 \pm 2 & 2.0 \pm 2 \\
\text{Leucine} & 9.8 \pm 10 & 4.2 \pm 4 & 6.0 \pm 6 \\
\text{Tyrosine} & 18.6 \pm 19 & 4.8 \pm 5 & 13.3 \pm 13 \\
\text{Phenylalanine} & 6.7 \pm 7 & 2.4 \pm 2 & 4.9 \pm 5 \\
\text{Half cystine} & 5.3 \pm 5 & 1.7 \pm 2 & 3.0 \pm 3 \\
\text{Cysteic acid} & 11.1 \pm 11 & 3.2 \pm 3 & 5.0 \pm 6 \\
\end{array}
\]

* Residues calculated from analyses of 24-, 48-, and 72-hour hydrolysates for phycocyanin and 24- and 72-hour for \(\alpha\) and \(\beta\) chains.

* Extrapolated to zero hydrolysis time.

* Measured as cysteic acid (17).

### Table I: Amino acid compositions of phycocyanin from Chroomonas sp. and its subunits

| Amino acid | Phycocyanin (24,000) | \(\alpha\) chain (9,000) | \(\beta\) chain (15,000) |
|------------|---------------------|------------------------|------------------------|
| Lysine     | 16.6 \pm 17         | 7.0 \pm 8              | 7.7 \pm 8              |
| Histidine  | 0.90 \pm 1          | 0.61 \pm 1             | Trace                  |
| Arginine   | 8.9 \pm 9           | 3.7 \pm 4              | 7.4 \pm 7              |
| Aspartic acid | 23.3 \pm 28     | 10.5 \pm 11            | 16.5 \pm 17            |
| Serine     | 10.1 \pm 10         | 4.0 \pm 4              | 5.8 \pm 6              |
| Glutamic acid | 16.9 \pm 17    | 8.0 \pm 9              | 8.4 \pm 8              |
| Proline    | 6.4 \pm 6           | 3.3 \pm 3              | 4.2 \pm 4              |
| Alanine    | 19.8 \pm 19         | 6.7 \pm 7              | 13.4 \pm 13            |
| Valine     | 33.4 \pm 33         | 10.0 \pm 10            | 20.2 \pm 20            |
| Methionine | 16.4 \pm 16         | 5.3 \pm 5              | 9.5 \pm 10             |
| Isoleucine | 4.1 \pm 4           | 1.6 \pm 2              | 2.0 \pm 2              |
| Leucine    | 9.8 \pm 10          | 4.2 \pm 4              | 6.0 \pm 6              |
| Tyrosine   | 18.6 \pm 19         | 4.8 \pm 5              | 13.3 \pm 13            |
| Phenylalanine | 6.7 \pm 7     | 2.4 \pm 2              | 4.9 \pm 5              |
| Half cystine | 5.3 \pm 5         | 1.7 \pm 2              | 3.0 \pm 3              |
| Cysteic acid | 11.1 \pm 11       | 3.2 \pm 3              | 5.0 \pm 6              |

* Numbers in headings refer to an algal source of C-phycocyanin designated in first column.

### Table II: Difference index for phycocyanin from Chroomonas sp. and various C-phycocyanins

The difference index (DI) was calculated as follows: 

\[ DI = \frac{\sum_{i=1}^{n} (X_{i,A} - X_{i,B})}{\sum_{i=1}^{n} X_{i,B}} \]

where \(X_{i,A}\) is the mole fraction of the \(i\)th amino acid in Protein \(A\) and \(X_{i,B}\) is the mole fraction of the \(i\)th amino acid in Protein \(B\). References for the amino acid compositions are 3, 5, 18, 22, and 25.

### Spectroscopic Characteristics

Functionally, phycocyanin is an accessory pigment affiliated with Photosystem 2 in photosynthesis. Ilani and Berns (26) have also indicated a possible role for C-phycocyanin in electron transfer. The spectroscopic properties of this protein are therefore related to its functions. Fluorescence spectra (excitation, emission, and polarization) were obtained for a sample at pH 6.0 and \(A_{600} = 0.20\) (Fig. 9). Excitation bands were found with maxima at 664, 585, and 543 (shoulder) nm. The maximum of the apparently symmetrical

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**Fig. 6.** Calibration curve from sodium dodecyl sulfate gel electrophoresis experiment.

**Fig. 7 (left).** Elution pattern from a guanidine hydrochloride column showing two bands for phycocyanin monitored at 400 nm. **Fig. 8 (right).** Calibration curve for guanidine hydrochloride column.

**Amino Acid Composition**—The amino acid compositions of phycocyanin and its two component polypeptide chains are given in Table I. The partial specific volume of phycocyanin, calculated by the method of Cohn and Edsall (31), was 0.724; this compared favorably with the 0.724 determined from sedimentation equilibrium. A value of 0.73 was subsequently used in all calculations. The residues in the \(\alpha\) and \(\beta\) chains were evaluated, based on 9,000 and 15,000, respectively. These molecular weights were based on the sodium dodecyl sulfate gel electrophoresis and gel filtration results. If equal numbers of each chain are present in the native protein, the sum of the \(\alpha\) and \(\beta\) residues, or an integral multiple thereof, should equal that of the whole protein, provided the choice of molecular weights was judicious.

The amino acid composition of phycocyanin differs from the reported analyses of C-phycocyanin (3-6, 18, 22, 23). To compare the two proteins quantitatively, the method introduced by Metzger et al. (24) was used to calculate a difference index based on their amino acid compositions (Table II). Its implications are discussed below.

**Spectroscopic Characteristics**—Functionally, phycocyanin is an accessory pigment affiliated with Photosystem 2 in photosynthesis. Ilani and Berns (26) have also indicated a possible role for C-phycocyanin in electron transfer. The spectroscopic properties of this protein are therefore related to its functions. Fluorescence spectra (excitation, emission, and polarization) were obtained for a sample at pH 6.0 and \(A_{600} = 0.20\) (Fig. 9). Excitation bands were found with maxima at 664, 585, and 543 (shoulder) nm. The maximum of the apparently symmetrical
emission band was at 655 nm. A much lower polarization was found within the wave length range of the band with an A_{max} of 585 nm, compared with that of an A_{max} of 635 nm; and the emission maximum and the shape of the band were unaffected by the selection of an excitation wave length (520 to 630 nm). Both of these facts are consistent with the suggestion that the band at 585 nm transfers its energy to the 635-nm band prior to emission.

The visible ultraviolet and CD spectra are illustrated in Fig. 10. The CD spectrum correlates well with the visible absorption maxima.

**DISCUSSION**

Phycocyanin from *Chroomonas sp.* is a single species with a particle weight of 5.0 x 10^{4} and is composed of two different polypeptide chains of about 1.0 x 10^{4} and 1.6 x 10^{4} daltons. These data have led us to conclude that the native protein has a quaternary structure of \( \alpha_2\beta_2\). The 1:1 ratio of \( \alpha \) and \( \beta \) chains is an assumption supported by the amino acid data. From the Svedberg equation, using the particle weight (50,000), \( s_{20, w} \) (4.4 x 10^{-13} S), and partial specific volume (0.73), a \( D \) of 7.9 x 10^{-7} cm^{2} s^{-1} was calculated. Subsequent calculation of a frictional ratio (\( f/f_{\text{max}} \)) yielded 1.11, a value typical for globular proteins (27). The physical properties of phycocyanin are summarized in Table III.

| Property                          | Method                          | Result       |
|----------------------------------|---------------------------------|--------------|
| Size of protein                  | Sedimentation equilibrium       | 4.9 x 10^{4} daltons |
| Subunit molecular weight         | Sodium dodecyl sulfate gel electrophoresis | 5.3 x 10^{5} daltons |
|                                  | Gel filtration with 6 M guanidine hydrochloride | 9.9 x 10^{4} and 1.6 x 10^{5} |
|                                  | Sedimentation velocity          | 9.9 x 10^{4} and 2.0 x 10^{4} |
|                                  | Amino acid composition          | 4.4 x 10^{-15} S |
| Partial specific volume (\( V \)) | Sedimentation equilibrium       | 0.729 ml/g   |
|                                  | Svedberg equation               | 0.724 ml/g   |
|                                  |                                   | 7.9 x 10^{-7} cm^{2} s^{-1} |
| D Fluorescence polarization (620 nm) |                                   | +0.10        |

The loss of this aggregation trait is produced by a significantly distinct amino acid composition, as shown by the difference index. The lower the index, the more closely related are the two proteins. Fondy and Holohan (29) have used this index on other functionally related families of proteins, the pyridine-nucleotide-
linked dehydrogenases. For glyceraldehyde 3-phosphate dehydrogenase, for example, taking the enzyme from pig muscle as a base, they found the following values: rabbit muscle 2.7, chicken muscle 3.5, halibut muscle 4.6, yeast 5.8, and Escherichia coli 8.3. The indices for cytochrome c were greater; with rabbit's heart as a base, they found pig and chicken 3.8, tuna 9.2, and bakers' yeast 10.2.

Compared with these selected values, phycocyanin is seen to be very different from the C-phycocyanin, since the average difference index is 12.0 (Table II). This result also indicates some residual homology, however, since Metzger et al. (24) found that 75% of all the randomly selected proteins they tested had difference indices above 20. The principal contributors to this difference index are the much larger quantities of serine, half-cystine, and lysine in phycocyanin. The α and β chains of phycocyanin differ from each other. The α chain is richer in glutamic acid and lysine; the β chain is richer in serine and leucine. It was noted that the α chain possesses a higher proportion of polar amino acids (glutamic acid, aspartic acid, serine, threonine, lysine, histidine, and arginine).

The larger aggregates of C-phycocyanin have an increased ability for energy transfer, as indicated by their greater relative fluorescence efficiency and lower fluorescence polarization (3, 30). Since phycocyanin from Chromonas sp. is smaller, it might be expected to be comparatively inefficient in energy transfer and this would be reflected in a high fluorescence polarization with respect to C-phycocyanin of similar size. However, its fluorescence polarization at 620 nm is +0.10, compared to a p value of +0.29 for the 6 S subunit of C-phycocyanin (30). Since lower polarization means more extensive internal energy transfer (31) and since this is combined with a smaller size, phycocyanin has apparently been equipped with a system whereby energy transfer is more efficient.

It is significant that the $A_{645}$:$A_{385}$ ratio is constant across the entire phycocyanin peak obtained by ammonium sulfate chromatography. This indicates either that the chromophores responsible for these maxima are present in a fixed ratio in the native protein or that they are inseparable by this technique. Other experiments provide support for the first hypothesis: no spectral changes were observed when Chromonas extracts were examined under non-denaturing conditions by centrifugation, gel filtration, or isoelectric focusing. This may indicate a very high association constant for the subunits.

Ohto et al. (32) have reported purification of phycocyanin from the Cryptomonad Hemialesis vireocens, which is spectrally similar to the billprotein in Chromonas. Phycocyanin from H. vireocens had an $A_{645}$:$A_{385}$ ratio of about 1.1 (compared to 1.05 for ours) but an $A_{455}$:$A_{380}$ ratio of only 3.5 (calculated from Fig. 1, Ref. 32). The ammonium sulfate gradient purification procedure yielded peak fractions with $A_{455}$:$A_{380}$ ratios greater than 5.5, while more than half of the phycocyanin applied was recovered with a ratio greater than 5.0.

Phycocyanin possesses two positive CD bands, at 630 and 595 nm, while phycocyanin has a negative band at 507 nm and a positive band at 548 nm (33, 34). The CD spectrum of phycocyanin exhibits a strong negative band at 560 nm, a strong positive at 588 nm, and a positive shoulder at 613 nm. Both the CD and the absorption spectra indicate at least two different types of chromophores, or distinctly different environments for similar tetrapyrrole chromophores, as was found for C-phycocyanin and allophycocyanin (35). The molar ellipticity $[\theta]_{630 \text{ nm}}$ was calculated to be $-20,200$ deg cm$^2$/dml. This corresponds to about 55% α helix using poly(L-lysine) as a standard (36) and assuming that the chromophore makes no contribution to the dichroism at 208 nm.

The behavior of phycocyanin in denaturing solvents differs from that of C-phycocyanin. C-Phycocyanin in sodium dodecyl sulfate gel electrophoresis yields two bands (22, 35-39); but the α-band of phycocyanin seems from our experiments to have a significantly smaller molecular weight than the corresponding band in C-phycocyanin, whereas the β-bands may be similar in molecular weight.

The monodisperse nature of phycocyanin is not a result of the purification procedures, since crude solutions examined by ultracentrifugation with schlieren optics also showed the 4.4 S boundary as the principal component. In contrast, fresh extracts of C-phycocyanin possess an even greater proportion of larger aggregates than does purified C-phycocyanin (40).

Isoelectric focusing in sucrose density gradients revealed a complex pattern of bands which are isoelectric in the range of pH 4.2 to 6.9. This complex pattern is apparently not artificial, for several of the phycocyanin zones have been re-electrofocused and found to return to their original isoelectric points. Nor is this complex pattern due to photodegradation, as has been reported for phycocyanin from another Cryptophycean alga (41). Other studies in this laboratory have indicated that the degree of charge heterogeneity seen with phycocyanin is not observed with the C-phycocyanins, even though the latter have multiple agglomerate forms.

Chromonas sp. is a eukaryotic and flagellated organism representative of the Cryptophyta. The experiments presented here show that its chromoprotein is quite different, in terms of its properties and amino acid composition from the chromoprotein from the prokaryotic, blue-green algae. This supports the earlier observation (42) that the Cryptophyta have evolved to a significantly different phylogenetic position than the blue-green algae, a position more distant from the prokaryotic than another eukaryote, the red algae.

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