Ho, Y. K., Novak, L., & Bardos, T. J. (1978) in *Nucleic Acid Chemistry* (Townsend, S. B., & Tipson, R. S., Eds.) Part II, pp 813-816, Wiley, New York.
Hogan, M. E., & Jardetzkey, O. (1980) *Biochemistry 19*, 3460-3468.
Hogan, M. E., Wang, J., Austin, R. H., Monitto, C. L., & Hershkowitz, S. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3518-3522.
Hurley, I., Osei-Gyimah, P., Archer, S., Scholes, C. P., & Lerman, L. S. (1982) *Biochemistry 21*, 4999-5009.
Klevan, L., Armitage, I. M., & Crothers, D. M. (1979) *Nucleic Acids Res. 6*, 1607-1616.
Langemeier, P. W., & Bobst, A. M. (1981) *Arch. Biochem. Biophys. 208*, 205-211.
Luoma, G. A., Herring, F. G., & Marshall, A. G. (1982) *Biochemistry 21*, 6591-6598.
Mason, R. P., Polnaszek, C. F., & Freed, J. H. (1974) *J. Phys. Chem. 78*, 1324-1329.
McCain, D. C., Virudachalam, R., Santini, R. E., Abdel-Meguid, S. S., & Markley, J. L. (1982) *Biochemistry 21*, 5390-5397.
Meirovitch, E., Igner, D., Igner, E., Moro, G., & Freed, J. H. (1982) *J. Chem. Phys. 77*, 3915-3938.
Ozinskas, A. J., & Bobst, A. M. (1979) *Helv. Chim. Acta 62*, 1677-1681.
Ozinskas, A. J., & Bobst, A. M. (1980) *Helv. Chim. Acta 63*, 1407-1411.
Polnaszek, C. F. (1976) Ph.D. Thesis, Cornell University, Ithaca, NY.
Polnaszek, C. F., & Freed, J. H. (1975) *J. Phys. Chem. 79*, 2283-2306.
Polnaszek, C. F., Schreier, S., Butler, K. W., & Smith, I. C. P. (1978) *J. Am. Chem. Soc. 100*, 8223-8232.
Rauckman, E. J., Rosen, G. M., & Abou-Donia, M. B. (1976) *Org. Prep. Proced. Int. 8*, 159-161.
Robinson, B. H., & Dalton, L. R. (1980) *J. Chem. Phys. 72*, 1312-1324.
Robinson, B. H., Forgacs, G., Dalton, L. R., & Frisch, H. L. (1980a) *J. Chem. Phys. 73*, 4688-4692.
Robinson, B. H., Lerman, L. S., Beth, A. H., Frisch, H. L., Dalton, L. R., & Auer, C. (1980b) *J. Mol. Biol. 139*, 19-44.
Shindo, H., & McGhee, J. D. (1980) *Biopolymers 19*, 523-537.
Toppin, C. R. (1983) Ph.D. Thesis, University of Cincinnati, Cincinnati, OH.
Wallach, D. (1967) *J. Chem. Phys. 47*, 5258-5268.
Wang, J., Hogan, M. E., & Austin, R. H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 5896-5900.
Warwick, P. E., Hakam, A., Bobst, E. V., & Bobst, A. M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4574-4577.

Phycocyanin 612: A Biochemical and Photophysical Study†

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ABSTRACT: Phycocyanin 612 was isolated from the cryptomonad *Hemiselmis virescens* and purified. Sedimentation equilibrium experiments indicated a molecular weight of 56 000, and sodium dodecyl sulfate gel electrophoresis yielded two bands of molecular weight 10 000 (α) and 18 000 (β) in a 1:1 molar ratio. A quaternary structure of $\alpha_2\beta_2$ was confirmed by sodium dodecyl sulfate gel electrophoresis after cross-linking the protein with dimethyl suberimidate. Analysis of the absorption spectrum of phycocyanin 612 in acidic urea showed that the protein has two types of tetrapyrrole chromophores: phycocyanobilin, A_{max} at 662 nm, and cryptoviolin, A_{max} at 590 nm. The $\alpha_2\beta_2$ structure has six phycocyanobilins and two cryptoviolin chromophores. The α and β subunits were completely separated by chromatography on a Sephacryl S-200 column in acidic urea. The α subunit has only phycocyanobilin; the β has both phycocyanobilin and cryptoviolin in a 2:1 ratio. Comparison of the total amounts of separated subunits produced a molar ratio of α to β phycocyanobilin of 1:2 and of α phycocyanobilin to β cryptoviolin of 1:1. The distribution of chromophores is then one phycocyanobilin per α subunit and two phycocyanobilins and one cryptoviolin per β. Fluorescence and fluorescence-polarization spectroscopy demonstrated very efficient excitation-energy transfer from the cryptoviolins to phycocyanobilins. Circular dichroism spectroscopy indicated that the phycocyanobilins were split, either by strong exciton coupling or by different tetrapyrrole conformations, into at least two types. Both the cryptoviolins and phycocyanobilins were in a more extended conformation in the native state, compared to their cyclic conformation in the denatured state.

Cryptomonads are flagellated protozoa that perform photosynthesis through a unique array of pigments: biliproteins, chlorophyll c_2, and chlorophyll *a*. Biliproteins are chromoproteins that have covalently bound linear tetrapyrrole chromophores that also occur in blue-green and red algae. The biliproteins from these sources have been studied much more extensively than those from the cryptomonads [for reviews, see Gantt (1979, 1981), Scheer (1981), and Troxler (1977)].

There are six spectroscopic classes of cryptomonad biliproteins—three phycocyanins and three phycoerythrins (O'hEocha et al., 1964)—with one class per organism. Although allophycocyanin has not yet been discovered in any cryptomonad, it occurs in several forms in all blue-green and red algae (Zilinskas et al., 1978; Troxler et al., 1980), where it has a vital function (Gantt et al., 1976; Gantt & Lipschultz, 1973). Three types of cryptomonad biliprotein—phycocyanin 645, phycoerythrin 545, and phycoerythrin 565—have been carefully investigated (Brooks & Gantt, 1973; Glazer & Cohen-Bazire, 1975; Glazer et al., 1971; Jung et al., 1980; MacColl et al., 1973, 1976; Mörschel & Wehrmeyer, 1975, 1977), while the remaining cryptomonad biliproteins including

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Table I: DV Medium (pH 7.6–8.0)

ingredient	amount per liter	ingredient	amount per liter
NaCl	18 g	biotin	0.001 mg
KCl	0.6 g	Tris[a]	1.0 g
$NaNO_3$	0.5 g	P II metals[b]	3 mL
$MgSO_4 \cdot 7H_2O$	5.0 g	H_3BO_3	11.4 g
$CaCl_2$	0.1 g	$FeCl_3$	0.29 g
K_2HPO_4	0.03 g	$MnSO_4 \cdot H_2O$	1.23 g
$NaSiO_3 \cdot 9H_2O$	0.2 g	$ZnSO_4 \cdot 7H_2O$	0.22 g
$FeCl_3 \cdot 6H_2O$	0.48 mg	$CoSO_4 \cdot 7H_2O$	0.048 g
B_{12}	0.003 mg	NaEDTA[a]	1.0 g
thiamin HCl	0.1 mg		

[a] Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid. [b] Dissolve P II metals to 1-L volume and then add 3 mL of this solution to make 1 L of DV medium.

phycocyanin 612 have been only very casually examined (Gantt, 1979). In this study, phycocyanin 612 was investigated to determine (a) its protein subunit structure, (b) its chromophore content, and (c) the protein–chromophore interactions that modulate its light-gathering and excitation energy transfer activities.

Experimental Procedures

Materials

A unialgal culture of *Hemiselmis virescens* (Plymouth No. 157) LB 984/5 was obtained from the Culture Center of Algae and Protozoa at Cambridge, England. The algae were grown in still cultures of 500 mL of DV medium (Provasoli & MacLaughlin, 1963), modified as shown in Table I, in 2000-mL cotton-plugged flasks. The cultures were incubated at between 13 and 18 °C on an alternating 12-h light, 12-h dark cycle for 3 weeks and then harvested by centrifugation for 10 min at 4 °C. The pellets were combined, resuspended in DV medium, centrifuged for 10 min, and stored at −20 °C until used.

Phycocyanin 612 was extracted from 250 mL of frozen cell pellets, which were thawed, suspended in an equal volume of pH 6.0, 0.1 ionic strength, sodium phosphate buffer (buffer A), and centrifuged for 20 min. The supernatant was decanted and reserved. The new pellet, resuspended in 500 mL of working buffer, was similarly frozen, thawed, and centrifuged. The two supernatants were pooled and treated with ammonium sulfate to 80% saturation. To remove cellular debris, the precipitate was resuspended in buffer A and centrifuged for 30 min. After each of four subsequent 80% fractionations and a final 55% fractionation, the precipitate was resuspended in buffer A and monitored for purity by absorbance measurements at 280 and 612 nm. The final resuspended pellet was centrifuged for 2 h to remove debris.

The phycocyanin 612 was eluted from a 2.5 × 43 cm column of Sepharose 6B (Pharmacia, Piscataway, NJ) with pH 6.0, 0.1 ionic strength, sodium phosphate buffer containing 0.5 M NaCl and 0.02% sodium azide (buffer B) at room temperature. Selected fractions were applied to a 2.5 × 20 cm column of Ultrogel AcA54 (LKB Instruments, Rockville, MD). The Sepharose 6B and Ultrogel AcA54 separations were repeated, and the purity of the protein was monitored in pH 6.5 solutions by sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis with 12% polyacrylamide gels (10 cm long) (Bio-Rad Laboratories, Richmond, CA). Electrophoresis was water cooled, and gels were stained with Coomassie brilliant blue and destained by diffusion. Purified protein was stored in 80% saturated ammonium sulfate at 4 °C or was dialyzed against double-distilled water and lyophilized.

Methods

Absorption spectra were recorded on a Perkin-Elmer 320 spectrophotometer. Fluorescence excitation and emission spectra were recorded on a Perkin-Elmer Model MPF44A fluorescence spectrophotometer. Fluorescence emission spectra were corrected for photomultiplier (Model R777HTV) response and emission monochromator effects by use of a calibrated tungsten lamp (secondary source, Perkin-Elmer Corp.). Fluorescence excitation spectra were corrected out to 610 nm by using a Rhodomine B quantum counter. The fluorescence excitation polarization spectrum was obtained with the same instrument, and degree of polarization was calculated as $p = (I_{vv} - GI_{vh})/(I_{vv} + GI_{vh})$, where $G = I_{hv}/I_{hh}$, a correction factor due to the optics, which varies with emission wavelength. The maximum absorbance of the solutions was <0.10 in a 1-cm light path, and reabsorption of emission was negligible. Circular dichroic spectra were recorded on a Cary 61. All spectral recordings were generated at room temperature.

Sedimentation studies utilized a Spinco Model E analytical ultracentrifuge. In the sedimentation velocity experiments, the schlieren photographic plates were measured with a Nikon Model 6 microcomparator. Protein solutions of 1.5–8.0 g/L in buffer A were centrifuged at 60 000 rpm and 20 °C. For molecular weight determinations by sedimentation equilibrium, the sample, $A_{280} = 0.3$, was centrifuged at 24 000 rpm and analyzed with the photoelectric scanner.

The α and β subunits were separated by gel filtration on a Sephacryl S-200 column (1.6 × 84 cm) at a 6 mL/min flow rate that was equilibrated and eluted with 8 M urea (Schwarz/Mann, ultrapure)–0.01 M 2-mercaptoethanol, pH 3.0, at room temperature. The cross-linking of phycocyanin 612 by dimethyl suberimidate and the scanning of stained $NaDodSO_4$ gels were performed as described previously (MacColl et al., 1976).

Results

Subunit Structure. The molecular weight from sedimentation equilibrium experiments with purified phycocyanin 612 in Buffer A is 55 800 ± 2600. The ln c vs. r^2 plots are linear. $NaDodSO_4$ gel electrophoresis after staining with Coomassie brilliant blue showed two bands of molecular weights 9600 ± 860 (α) and 18 400 ± 1900 (β). The stained gels were scanned, and the protein–bound dye ratio, measured from the areas under the two bands, was 1.6 ± 0.3 (β) to 1 (α). After reaction with the bifunctional cross-linking reagent dimethyl suberimidate, terminated by addition of $NaDodSO_4$, gel electrophoresis of the protein showed seven major bands. The sedimentation coefficient ($s^0_{20,w}$) was 4.1 S, and only a single boundary was observed.

Chromophore Assay. The chromophores were studied in 8.0 M urea (pH 3.0)–10^{-2} M 2-mercaptoethanol. When the spectrum of the six phycocyanobilins (i.e., C phycocyanin) was subtracted from that of phycocyanin 612, the residue had a near-ultraviolet maximum near 333 nm (Figure 1).

Sephacryl S-200 chromatography gave two bands for phycocyanin 612 (Figure 2). $NaDodSO_4$ gel electrophoresis shows that the faster eluting band is the pure β subunit and the slower is pure α (Figure 2). When either α or β fractions are separately pooled and individually rerun on this column, single bands are observed. The absorption spectra of α and β from this column are quite different (Figure 3).

From the extinction coefficients of phycocyanobilin and cryptoviolin (Bryant et al., 1976), a plot of A_{662}/A_{590} vs. the possible molar ratios of phycocyanobilin to cryptoviolin was constructed (Figure 4). The spectra of phycocyanin 612, β, and α had A_{662}/A_{590} of 1.21 ± 0.04, 1.03 ± 0.03, and 2.09

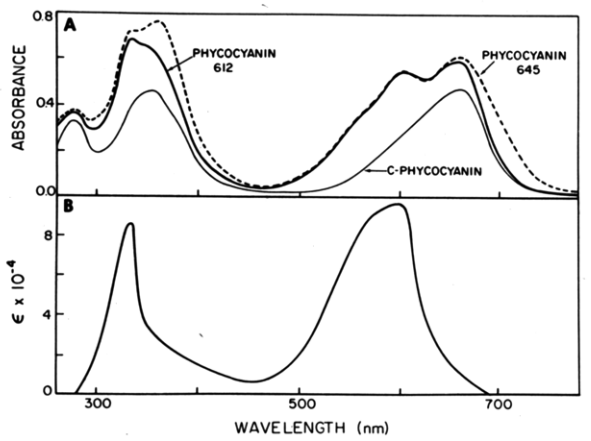

FIGURE 1: Biliprotein absorption spectra in acidic urea. (A) Spectra of C phycocyanin, phycocyanin 645, and phycocyanin 612. (B) Spectrum of phycocyanin 612 minus that of six phycocyanobilins.

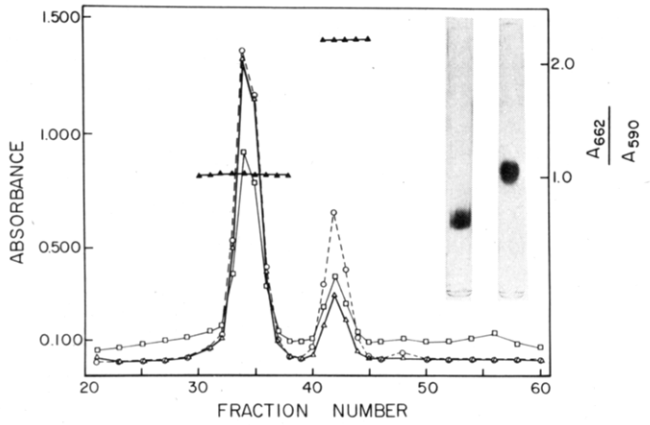

FIGURE 2: Effect of chromatography through a Sephacryl S-200 column on phycocyanin 612. Solvent was 8.0 M urea (pH 3.0), 10^{-2} M 2-mercaptoethanol, and 0.02% NaN_3. $NaDodSO_4$ gels demonstrate that chromatography has completely separated the α and β subunits. Absorbance was measured at 662 (○), 590 (△), and 280 (□) nm. A_{662}/A_{590} (▲).

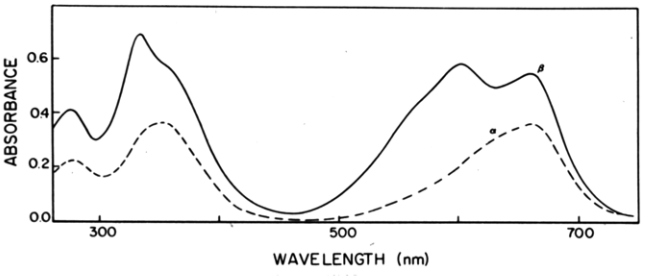

FIGURE 3: Absorption spectra of α and β subunits of phycocyanin 612. Subunits were separated by chromatography through the Sephacryl S-200 column. The solvent was acidic urea. Spectra are for total pools of α (9.90 mL) and total β (17.20 mL).

± 0.10, respectively, and could be analyzed from Figure 4. Beer's law equations for a binary mixture of phycocyanobilins and cryptoviolin could be derived

$$C_{PCB} = \frac{(4.30 \times 10^4)A_{662} - (3.33 \times 10^3)A_{590}}{1.47 \times 10^9} \quad (1)$$

$$C_{CV} = \frac{A_{662} - (3.55 \times 10^4)C_{PCB}}{3.33 \times 10^4} \quad (2)$$

where C is molar concentration, PCB is phycocyanobilin, and CV is cryptoviolin.

All the fractions containing α or β subunits from a Sephacryl column were separately and quantitatively pooled, and the

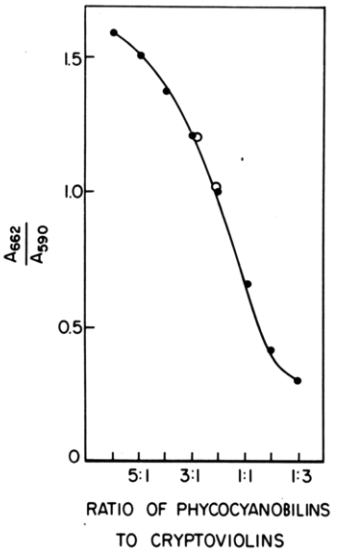

FIGURE 4: Relationship between A_{662}/A_{590} and ratio of phycocyanobilin to cryptoviolin. The solvent was acidic urea. Points are calculated for a given chromophore ratio (●); experimental results (○) are for phycocyanin 612 and its β subunit.

molarities of their chromophores were calculated from eq 1 and 2. From these molarities, the number of moles of each type of bilin was obtained, and the molar ratios of the number of chromophores between subunits were calculated: phycocyanobilins on β/α = 2.3 ± 0.2; phycocyanobilins on β/cryptoviolins on β = 2.2 ± 0.2; phycocyanobilins on α/cryptoviolins on β = 0.98 ± 0.10.

Solutions of phycocyanin 612 could also be analyzed from eq 1 and 2 when the weight of protein and the volume of acidic urea solvent were known. From division of the molarity of $\alpha_2\beta_2$ into the molarities of the chromophores found from eq 1 and 2, the molar ratios were phycocyanobilin/$\alpha_2\beta_2$ = 5.73 ± 0.55 and cryptoviolin/$\alpha_2\beta_2$ = 2.07 ± 0.15.

Protein–Chromophore Interactions. The fluorescence excitation and emission spectra of phycocyanin 612 were obtained in buffer A (Figure 5A). Excitations at a series of wavelengths between 480 and 600 nm all yielded the same emission spectrum. The corrected emission maximum was at 634 nm. Fluorescence polarization and circular dichroism spectra are shown in Figure 5B. The circular dichroism spectrum shows positive bands at 579 and 598 nm, a negative band at 634 nm, and the zero crossover at 620 nm.

The ratio of the oscillator strengths (f = constant $\int \epsilon \bar{\nu}$) of the visible to near-ultraviolet absorption bands was 1.9 for native and 0.5 for urea-denatured protein (Figure 6A). For these measurements, spectra similar to those in Figure 6A were recorded on a wavenumber (cm^{-1}) scale. Similarly, if $NaClO_4$ (0.2–1.0 M) was present in solutions of phycocyanin 612 at pH 6.0, both of the visible bands declined steadily, and there was a corresponding hyperchromicity in the near-ultraviolet absorbance (Figure 6B).

The fluorescence emission spectrum of phycocyanin 612 in acidic urea varied with excitation at different wavelengths. Excitation at 550 nm produced emission with a 608-nm maximum, whereas excitation at 620 nm clearly showed emission in the red region (692-nm maximum). Excitation of C phycocyanin in acidic urea produced the identical emission spectrum from its phycocyanobilins as was produced by 620-nm excitation of phycocyanin 612.

Discussion

Evaluation of the molecular weight (56000) and $NaDodSO_4$ gel electrophoresis data (molecular weights 10000 and 18000)

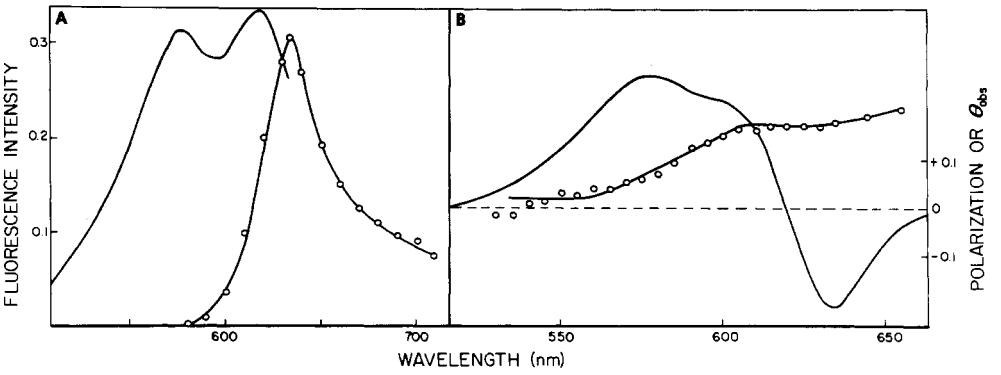

FIGURE 5: Spectroscopic properties of phycocyanin 612. (A) Fluorescence excitation and emission spectra (corrected) of phycocyanin 612 in buffer A (see Materials). The sample was excited at 500 nm for emission and monitored at 640 nm for the excitation spectrum. (B) Circular dichroism and fluorescence polarization spectra of phycocyanin 612 in buffer A (see Materials). The scale on the right ordinate is that for polarization, and the zero line is for both circular dichroism and polarization.

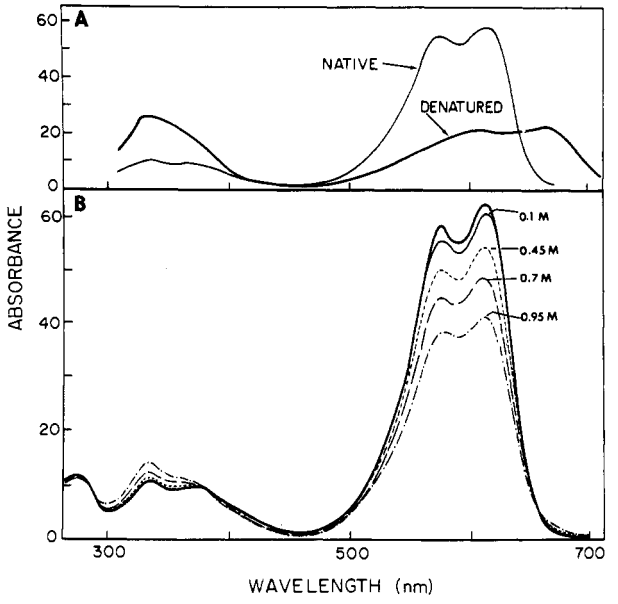

FIGURE 6: Effects of urea and sodium perchlorate on absorption spectrum of phycocyanin 612. (A) Native protein in buffer A (see Materials) vs. denatured protein in acidic 8.0 M urea. (B) Native protein vs. sodium perchlorate at pH 6.0.

from phycocyanin 612 indicates that the subunit structure is $\alpha_2\beta_2$. The 1:1 molar ratio is also suggested by scanning of stained gels, where the β to α ratio of 1.6:1 is close to the molecular weight ratio of 1.8:1. Dimethyl suberimidate cross-linking of an $\alpha_2\beta_2$ structure can maximally give eight bands on $NaDodSO_4$ gels, and the observation of seven tends to support this structure ($\beta_2\alpha$, e.g., could produce a maximum of five). A sedimentation coefficient of 4.1 S is consistent with a globular protein of this size.

Phycocyanin 612 is composed of both phycocyanobilin and cryptoviolin (Figure 1). C phycocyanin contains only phycocyanobilin, and phycocyanin 645 contains three bilins—phycocyanobilin, cryptoviolin, and a novel low-energy chromophore with a 697-nm maximum. The 697-nm chromophore is not found on phycocyanin 612 (Figure 1). The spectrum in Figure 1B is nearly identical with that of authentic cryptoviolin (near-ultraviolet maximum at 333 nm and A_{555}/A_{590} = 0.86) and is the residue after the spectra of six phycocyanobilins are subtracted from phycocyanin 612. From Figure 4, the ratio of phycocyanobilin to cryptoviolin is 3:1 for phycocyanin 612 and 2:1 for the β subunit. The α subunit has the spectrum of pure phycocyanobilin.

Analysis by eq 1 and 2 of the total amounts of α and β eluted from the Sephacryl S-200 column shows that the ratio of phycocyanobilin on α to phycocyanobilin on β is close to 1:2 and the ratio of phycocyanobilin on α to cryptoviolin on β is close to 1:1. Several distributions of chromophores could yield these ratios, depending on the total numbers of the two bilins. Two-component Beer's law analyses of phycocyanin 612 solutions by eq 1 and 2 prove that each $\alpha_2\beta_2$ structure contains six phycocyanobilins and two cryptoviolins. If one assumes symmetrical distribution of chromophores, each α must consist of one phycocyanobilin and each β of two phycocyanobilins and one cryptoviolin.

Cryptoviolin is a little-studied and infrequently encountered biliprotein chromophore. It is named for its spectral resemblance to the violin class of tetrapyrroles (O'Carra et al., 1980) but its true chemical structure is unknown. A 333-nm absorption maximum in acidic urea is characteristic of its presence. It is the only chromophore found on the α subunit of phycoerythrocyanin, which is present in only a few blue-green algae (Bryant et al., 1976).

The absorption and fluorescence excitation spectra of phycocyanin 612 show two visible maxima, but the fluorescence emission spectrum has only a single maximum, which is independent of excitation wavelength (Figure 5). This suggests that the 585-nm band, which contains the cryptoviolin transition, probably transfers its excitation energy to phycocyanobilin with very high efficiency. In the presence of urea, this energy transfer is abolished, and the individual chromophores emit independently. The fluorescence polarization spectrum of phycocyanin 612 in buffer A has a transition between plateaus in the exact wavelength interval between the cryptoviolin and phycocyanobilin maxima, which confirms the energy-transfer assignment. The circular dichroism spectrum of phycocyanin 612 in the visible region is composed of at least three bands. Comparison to known spectra (Teale & Dale, 1970; Vernotte, 1971; Bryant et al., 1976) demonstrates that the highest energy visible circular dichroism band is from cryptoviolin and the two lower energy bands are phycocyanobilins. C phycocyanin has only phycocyanobilins for its chromophores but has a two-plateau fluorescence polarization spectrum (Teale & Dale, 1970). Teale & Dale (1970) interpreted this datum as resulting from bilins in two conformational states. This explanation is viable for the two phycocyanobilin bands in phycocyanin 612. A second possible explanation is that very closely spaced phycocyanobilins result in a strongly coupled exciton interaction, which would mandate the negative–positive Cotton effects in the circular dichroism spectrum. The 634-nm emission maximum is at a higher energy than for most other phycocyanobilin-containing proteins, and this might argue against strong exciton coupling, since such coupling would produce a bathochromic shift in

absorbance and, hence, a longer wavelength emission.

Quantum chemical calculations and model-compound data on tetrapyrroles suggest that the ratio of the oscillator strengths of the visible to near-ultraviolet absorption bands is indicative of their degree of extension (Blauer & Wagnière, 1975; Burke et al., 1972; Chae & Song, 1975; Scheer, 1981; Suzuki et al., 1975). The higher this ratio, the more extended are the chromophores. For phycocyanin 612, the visible band is decreased, and the near-ultraviolet becomes hyperchromic as the protein is denatured (Figure 6). The chaotropic salt $NaClO_4$ also produces this effect with phycocyanin 612 and has been shown to effectively dissociate other biliproteins (MacColl, 1983). The data saliently show that both the phycocyanobilins and cryptoviolins become more cyclic as the protein is denatured and as the bilins can presumably assume a more stable conformation. Clearly, a function of the polypeptides in the native protein is to hold these chromophores in an extended conformation in which their light-harvesting (Figure 6) and excitation energy transfer faculties (Figure 5A) are maximized.

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Registry No. Cryptoviolin, 75026-32-3.

References

Blauer, G., & Wagnière, G. (1975) *J. Am. Chem. Soc. 97*, 1949–1954.

Brooks, C., & Gantt, E. (1973) *Arch. Mikrobiol. 88*, 193–204.

Bryant, D. A., Glazer, A. N., & Eiserling, F. A. (1976) *Arch. Microbiol. 110*, 61–75.

Burke, M. J., Pratt, D. C., & Moscowitz, A. (1972) *Biochemistry 11*, 4025–4031.

Chae, Q., & Song, P.-S. (1975) *J. Am. Chem. Soc. 97*, 4176–4179.

Gantt, E. (1979) in *Biochemistry and Physiology of Protozoa* (Levandowsky, M., & Hutner, S. A., Eds.) 2nd ed., Vol. 1, pp 121–137, Academic Press, New York.

Gantt, E. (1981) *Annu. Rev. Plant Physiol. 32*, 327–347.

Gantt, E., & Lipschultz, C. A. (1973) *Biochim. Biophys. Acta 292*, 858–861.

Gantt, E., Lipschultz, C. A., & Zilinskas, B. (1976) *Biochim. Biophys. Acta 430*, 375–388.

Glazer, A. N., & Cohen-Bazire, G. (1975) *Arch. Microbiol. 104*, 29–32.

Glazer, A. N., Cohen-Bazire, G., & Stanier, R. Y. (1971) *Arch. Mikrobiol. 80*, 1–18.

Jung, J., Song, P.-S., Paxton, R. J., Edelstein, M. S., Swanson, R., & Hazen, E. E., Jr. (1980) *Biochemistry 19*, 24–32.

MacColl, R. (1983) *Arch. Biochem. Biophys. 223*, 24–32.

MacColl, R., Habig, W., & Berns, D. S. (1973) *J. Biol. Chem. 248*, 7080–7086.

MacColl, R., Berns, D. S., & Gibbons, O. (1976) *Arch. Biochem. Biophys. 177*, 265–275.

Mörschel, E., & Wehrmeyer, W. (1975) *Arch. Microbiol. 105*, 153–158.

Mörschel, E., & Wehrmeyer, W. (1977) *Arch. Microbiol. 113*, 83–89.

O'Carra, P., Murphy, R. F., & Killilea, S. D. (1980) *Biochem. J. 187*, 303–309.

O'hEocha, C., O'Carra, P., & Mitchell, D. (1964) *Proc. R. Ir. Acad., Sect. B 63B*, 191–200.

Provasoli, L., & MacLaughlin, J. J. (1963) in *Symposium in Marine Microbiology* (Oppenheimer, C. H., Ed.) pp 105–112, Charles C Thomas, Springfield, IL.

Scheer, H. (1981) *Angew. Chem., Int. Ed. Engl. 20*, 241–261.

Suzuki, H., Sugiomoto, T., & Ishikawa, K. (1975) *J. Phys. Soc. Jpn. 38*, 1110–1118.

Teale, F. W. J., & Dale, R. E. (1970) *Biochem. J. 116*, 161–169.

Troxler, R. F. (1977) in *Chemistry and Physiology of Bile Pigments* (Beck, P. D., & Berlin, H. I., Eds.) pp 431–454, DHEW Publication No. 77-1100.

Troxler, R. F., Greenwald, L. S., & Zilinskas, B. A. (1980) *J. Biol. Chem. 255*, 9380–9387.

Vernotte, C. (1971) *Photochem. Photobiol. 14*, 163–173.

Zilinskas, B. A., Zimmerman, D. K., & Gantt, E. (1978) *Photochem. Photobiol. 27*, 587–595.