Allophycocyanin from the Filamentous Cyanophyte, *Phormidium luridum*†

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ABSTRACT: Allophycocyanin from the filamentous cyanophyte, *Phormidium luridum*, was purified by ammonium sulfate fractionation and ion exchange chromatography on brushite columns. The specific absorption coefficient ($E^{0.1\%}_{1\,cm}$) of purified allophycocyanin was 6.1 in distilled water and 7.3 in 0.05 *M* potassium phosphate buffer (pH 7). Absorption maxima of allophycocyanin occurred at 650, 618 (shoulder), 350, and 275 nm. Circular dichroic spectra displayed positive ellipticity bands at 655 and 625 nm, and a major negative ellipticity band at 340 nm. Computer analysis of the circular dichroic spectrum of allophycocyanin from 207 to 243 nm indicated that the secondary structure contained 60% α helix and 40% β form. The estimated molecular weight of allophycocyanin on Sephadex G-200 columns at pH 7.0 was 155,000. Electrophoretic examination of allophycocyanin on sodium dodecyl sulfate polyacrylamide gels revealed two subunits, α and β, with apparent molecular weights of 17,300 and 19,000, respectively. Densitometric analysis of unstained gels at 600 nm indicated that one phycocyanobilin chromophore was associated with each subunit. Treatment of allophycocyanin with 12% formic acid or 8 *M* urea and subsequent removal of the denaturant yielded a derivative with spectroscopic characteristics similar to phycocyanin. Subsequent incubation in phosphate buffer (pH 7), but not in acetate buffer (pH 5) or in water, was accompanied by a progressive reappearance of absorption maxima at 650 and 618 nm (shoulder), and positive ellipticity bands at 655 and 617 nm. Automated sequence analysis of allophycocyanin (a) showed that the sequence of amino acids at the amino terminus of the α and β subunits is different, (b) showed that the subunits occur in a ratio of 1:1, and (c) demonstrated sequence homology at the amino terminus of allophycocyanin, phycocyanin, and phycoerythrin.

Allophycocyanin, phycocyanin, and phycoerythrin are bile pigment–protein complexes found in the photosynthetic apparatus of red (Rhodophyta), blue-green (Cyanophyta), and cryptomomad (Cryptophyta) algae (O hEocha, 1965). Algal biliproteins are accessory photosynthetic pigments (Myers, 1971). Biliproteins occur in vivo as large aggregates, called phycobilisomes, which are located on the outer surfaces of thylakoid membranes in red and blue-green algae (Gantt and Conti, 1966). In vitro, biliproteins form aggregates of different size depending on the pH, ionic strength, and protein concentration (Berns, 1971).

Phycocyanin is the most thoroughly investigated biliprotein. The phycocyanin monomer[1] is comprised of two dissimilar polypeptide subunits, designated α and β, which can be separated electrophoretically on urea or sodium dodecyl sulfate polyacrylamide gels or by ion exchange chromatography (Bennett and Bogorad, 1971; Binder et al., 1972; Kobayashi et al., 1972; Torjesen and Sletten, 1972; Glazer and Fang, 1973a; Gantt and Lipschultz, 1974; Troxler et al., 1975). The chromophore of phycocyanin is phycocyanobilin (Cole et al., 1968; Crespi et al., 1968). This linear tetrapyrrole is covalently attached to phycocyanin apoprotein (Chapman, 1973). The α subunit of phycocyanin contains one, and the β subunit of phycocyanin contains two, residues of phycocyanobilin (Bennett and Bogorad, 1971; Glazer and Fang, 1973a; Troxler et al., 1975). Phycocyanobilin is structurally related to bilirubin, the principal bile pigment derived from hemoglobin in senescent erythrocytes (Lester and Troxler, 1969).

Relatively little is known about the composition and properties of allophycocyanin. This biliprotein was originally thought to be a breakdown product of phycocyanin (Lemberg and Bader, 1933) but reports of its wide distribution and successful extraction and purification (Haxo et al., 1955) support its position as a unique biliprotein. Phycocyanin can constitute up to 40% of the total soluble protein in certain algae (Myers and Kratz, 1955). Allophycocyanin has been reported to constitute 9.5–16% of the soluble protein in the filamentous cyanophyte, *Fremyella diplosiphon* (Bennett and Bogorad, 1973) and 5% of the soluble protein in the unicellular rhodophyte, *Porphyridium cruentum* (Gantt and Lipschultz, 1974).

Although allophycocyanin and phycocyanin are normally distinguished from one another by their characteristic absorption bands at 650 and 620 nm, respectively, phycocyanobilin is the only chromophore in both biliproteins (Chapman et al., 1968). The number of phycocyanobilin residues attached to the subunits and the actual number and distribution of subunits in allophycocyanin is uncertain (Bennett and Bogorad, 1971; Glazer and Cohen-Bazire, 1971; Glazer et al., 1973; Gantt and Lipschultz, 1974; Gysi and Zuber, 1974).

Recent studies have demonstrated sequence homology at the amino terminus of the α and β subunits of phycocyanin and phycoerythrin (Williams et al., 1974; Harris and Berns, 1975; Troxler et al., 1975). The sequence of amino acids at

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[1] The term, monomer, refers to the fundamental unit of biliprotein aggregation in vitro and is comprised of one α subunit and one β subunit. Free α and β subunits have not been observed in extracts of disrupted algae cells or in dilute solutions of purified biliproteins.

Table I: Mean Residue Ellipticity, $[\theta]$, of *P. luridum* Allophycocyanin (APC), and Phycocyanin (PC-1, PC-2).[a]

Wavelength (nm)	Biliproteins $[\theta]$			Basis Spectra[b] $[\theta]$		
	APC	PC-1	PC-2	X_H	X_β	X_R
207	−20090	−20400	−20140	−24300	−8220	−5590
210	−20470	−21300	−21200	−28300	−10400	−4210
213	−20410	−21250	−21730	−26400	−9680	−3500
216	−21220	−21730	−22260	−28100	−9320	−2480
219	−21980	−22360	−23210	−30500	−6850	−2350
222	−21350	−22260	−22790	−31500	−3670	−2780
225	−18400	−19080	−20140	−30000	+2000	−3380
228	−13190	−14310	−14840	−25000	+4130	−3120
231	−10050	−10600	−11600	−17900	+3920	−2470
234	−5650	−6250	−6730	−11300	+3740	−1510
237	−3140	−3870	−3970	−6100	+2830	−1160
240	−1880	−2230	−2230	−2780	+3450	−1260
243	−1010	−1060	−1210	−1110	+2000	−900

[a] The basis spectra, expressed in terms of $[\theta]$, represent the values expected if a protein was comprised of 100% α helix, β form, or unordered form. [b] Data taken from Chen et al. (1972).

the amino terminus of allophycocyanin has not been described. Gysi and Zuber (1975) reported that the amino terminal residues of allophycocyanin from *Mastigocladus laminosus* allophycocyanin were blocked.

The present paper describes some physical-chemical properties and the amino terminal sequence of allophycocyanin from the filamentous cyanophyte, *Phormidium luridum*.

Materials and Methods

The Organism. Phormidium luridum is a filamentous blue-green alga which was obtained from the Indiana University Culture Collection (426). Chlorophyll a, phycocyanin, and allophycocyanin are the principal photosynthetic pigments in this organism.

Culture Conditions. P. luridum was grown at 37° in 50 l. of "C" medium (Kratz and Myers, 1955) contained in 55-l. polyethylene carboys. The algal cells were agitated by vigorous aeration and were illuminated with a bank of fluorescent lights (General Electric, power groove, F17PG, CW). After 7 days, the cells were harvested by centrifugation in a Szent-Gyorgi and Blum continuous flow system.

Preparation of Biliproteins. Algal cells were washed twice with 0.1 *M* potassium phosphate buffer (pH 7) and disrupted by sonic vibration with a Branson sonic oscillator (Model W185). Cells which had been disrupted by sonic vibration were centrifuged for 20 min 30,000*g* and the biliproteins in the resulting supernatant were precipitated by the addition of solid ammonium sulfate to 50% saturation. The precipitate was dissolved in water, dialyzed against water at 4°, and centrifuged at 100,000*g* for 1 hr. The dialyzed sample was chromatographed on a 2.5 × 20 cm brushite column (Siegelman and Firer, 1964), which was developed with potassium phosphate buffers (pH 7) of increasing ionic strength as described previously (Troxler and Lester, 1967). Allophycocyanin in the brushite column eluate in which the A_{650}/A_{620} and A_{650}/A_{280} ratios were greater than 1.5 and 3.8, respectively, was precipitated in 80% saturated ammonium sulfate, dialyzed against water at 4°, and stored at −20°. Fractions not meeting these criteria were rechromatographed under the same conditions on a second brushite column, precipitated, dialyzed, and stored as above.

The specific absorption coefficient ($E^{0.1\%}_{1\,cm}$) of *P. luridum* allophycocyanin was determined by weighing aliquots (which were dried on a hot plate) of a solution of known absorbance at 650 nm in tared aluminum pans on a Cahn microbalance. The specific absorption coefficient of allophycocyanin at 650 nm was 6.1 in distilled water and 7.3 in 0.05 *M* potassium phosphate buffer (pH 7).

Spectral Measurements. Absorption spectra of biliproteins were performed on a Beckman DB-GT recording spectrophotometer. Circular dichroic (CD) spectra were determined on a Cary 61 spectropolarimeter calibrated with a *d*-10-camphorsulfonic acid standard (1 mg/ml; Eastman Kodak Co.) in water. All CD measurements were performed at 27°. Mean residue ellipticity, $[\theta]$, in deg cm^2 dmol^{-1}, was determined using mean residue weights of 108 and 106 for allophycocyanin and phycocyanin, respectively. The CD of these biliproteins from 207 to 243 nm was analyzed by the method of Chen et al. (1972). Briefly, CD spectra at selected wavelengths (see Table I) were fitted with basis spectra by a standard multiple regression analysis–linear least-squares method. The relationship between the CD of allophycocyanin at selected wavelengths, basis spectra at these wavelengths, and secondary structure is shown in

$$X = f_H X_H + f_\beta X_\beta + f_R X_R \quad (1)$$

$X = [\theta]$ in deg cm^2 dmol^{-1} of allophycocyanin, X_H, X_β, and X_R are values of $[\theta]$ (basis spectra) expected if the secondary structure were 100% α helix, β form, or unordered form (based on five protein standards whose secondary structure has been determined by X-ray diffraction), and the f's are the fraction of α helix, β form, or unordered form. The conditions set in the analysis are $\sum f\text{'s} \geq 0$ and $f_H + f_\beta + f_R = 1$. Therefore

$$X = f_H X_H + f_\beta X_\beta + (1 - f_H - f_\beta) X_R \quad (2)$$

and

$$X = f_H X_H + f_\beta X_\beta + (X_R - X_R f_H - X_R f_\beta) \quad (3)$$

or

$$X - X_R = f_H(X_H - X_R) + f_\beta(X_\beta - X_R) \quad (4)$$

The computer program is designed to solve for f_H and f_β, and f_R is obtained from the expression $f_H + f_\beta + f_R = 1$. Analyses were performed on an IBM 370/145 computer.

Gel Filtration Chromatography. Sephadex G-200 was equilibrated in 0.05 *M* potassium phosphate buffer (pH 7) containing 0.02% sodium azide and poured into a 1.25 × 50 cm column. The column was calibrated with cytochrome *c*, equine hemoglobin and myoglobin, ovalbumin, and catalase (Sigma Chemical Corp.) according to the procedure of Andrews (1965). Flow rate was maintained at 20 ml/hr by pumping from the bottom of the column.

Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed on 10% gels (0.5 × 9 cm) according to the method of Weber and Osborn (1969) using a ratio of *N,N'*-methylenebisacrylamide to acrylamide of 1:18.5. The gels were run for 4–6 hr at 8 mA/tube. All reagents were purchased from Bio-Rad Laboratories. Densitometric analysis of unstained gels was performed at 600 nm in a Gilford spectrophotometer (2400-S) equipped with a linear transport attachment (2410-S). Gels were stained with 0.25% Coomassie Brilliant Blue in 10% methanol–7% acetic acid for 1 hr at 37°. Diffusion destaining was carried out overnight at 37° in the same solvent.

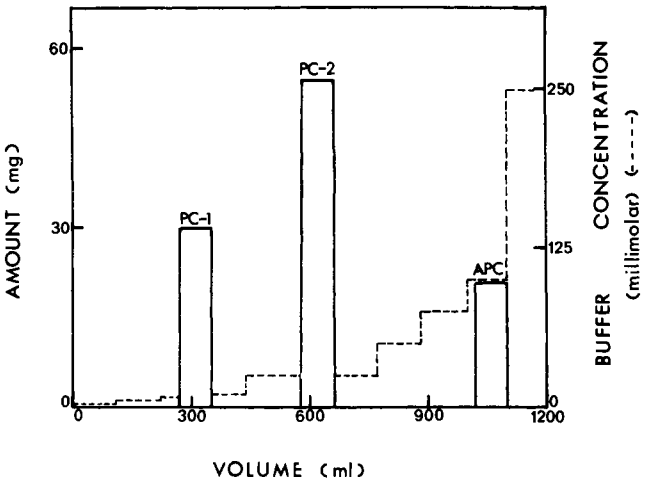

FIGURE 1: Stepwise elution of allophycocyanin (APC) and phycocyanin (PC) from a 2.5 × 20 cm brushite column developed with potassium phosphate buffers (pH 7) of increasing ionic strength. Flow rate was 1.0 ml/min. The amount (mg) of each biliprotein in the respective fractions was determined spectrophotometrically: allophycocyanin, $E^{0.1\%}_{1cm} = 7.3$; phycocyanin, $E^{0.1\%}_{1cm} = 7.7$. It should be mentioned that the elution pattern of PC-1 and PC-2 was variable depending on the batch of brushite (Siegelman and Firer, 1964) employed.

Amino Acid Analysis. Allophycocyanin and phycocyanin were hydrolyzed in vacuo in 3 *N* *p*-toluenesulfonic acid for 22 hr at 110° (Liu and Chang, 1971). Hydrolysates were chromatographed on a Jeolco 6AH automated analyzer employing a two column system.

Automated Sequence Analysis. Sequencing was carried out with automatic stepwise degradation (Beckman Model 890C sequencer) according to the procedure of Edman and Begg (1967). An 8-mg sample of allophycocyanin was subjected to automated sequencing utilizing a Quadrol buffer system (Beckman Sequencer Manual, 1969). PTH-norleucine served as an internal standard in all samples. All fractions were converted to the PTH-amino acid derivative and extracted into ethyl acetate and an aliquot (10% of the ethyl acetate) was identified on a Beckman Model 65 gas chromatograph according to a modification of the method of Pisano and Bronzert (1969). The remainder of the ethyl acetate layer and the aqueous layer were combined, hydrolyzed with hydriodic acid (Smithies et al., 1971), and subjected to amino acid analysis on a Beckman 119 analyzer employing a one-column system.

Results

The elution pattern of *P. luridum* biliproteins from a 2.5 × 20 cm brushite column is shown in Figure 1. Phycocyanin eluted in two fractions designated PC-1 and PC-2. Allophycocyanin eluted as a single component in 0.1 *M* potassium phosphate buffer (pH 7). Each biliprotein fraction was dialyzed against 0.05 *M* potassium phosphate buffer (pH 7) at 4° and the absorption and CD spectra were determined. Allophycocyanin displayed absorption maxima at 650, 618 (shoulder), 350, and 275 nm; positive ellipticity bands occurred at 655 and 625 nm and a major negative ellipticity band was observed at 340 nm (Figure 2). The main absorption band of PC-1 and PC-2 occurred at 632 and 618 nm, respectively, with otherwise identical absorption maxima at 350 and 275 nm. Both phycocyanin fractions displayed positive ellipticity bands at 633 and 595 nm (shoulder) and a major negative ellipticity band at 340 nm. The ellipticity bands due to the chromophore of both allophycocyanin and phycocyanin were not observed when these biliproteins were

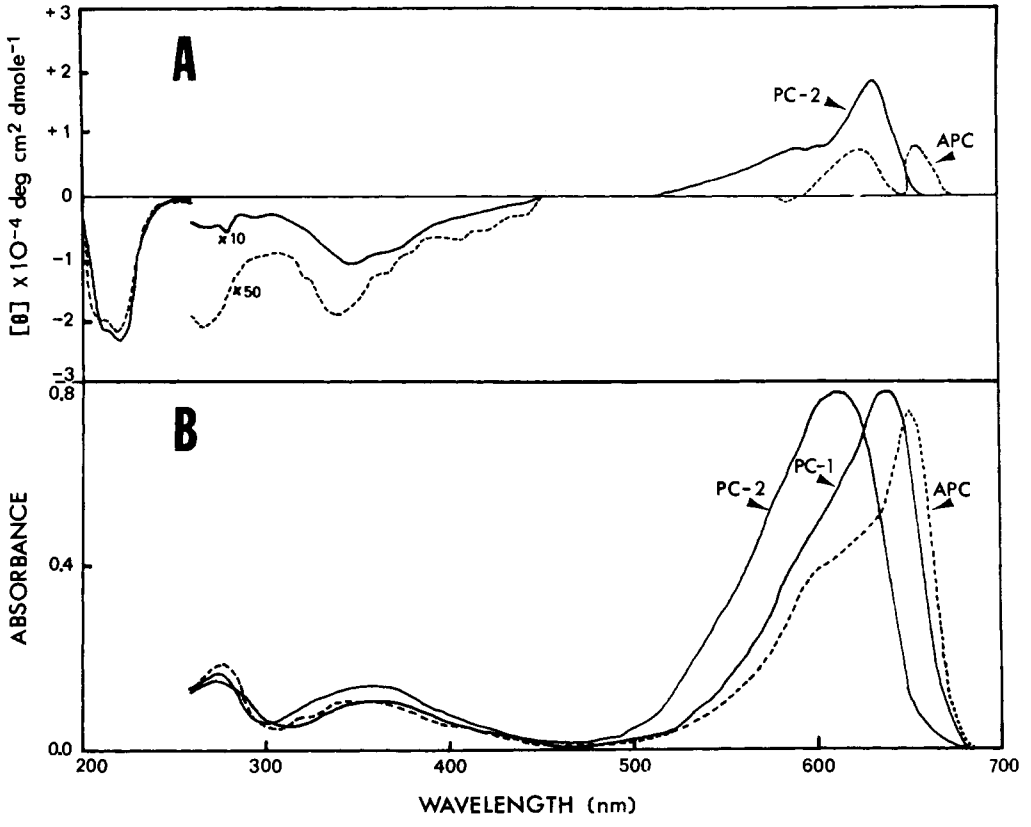

FIGURE 2: Absorption and circular dichroic spectra of allophycocyanin (APC) and phycocyanin (PC-2). Both biliproteins were dialyzed against 0.05 *M* potassium phosphate buffer (pH 7) and the concentration of protein was adjusted to 0.1 mg/ml.

Table II: Evaluation of Secondary Structure of *P. luridum* Allophycocyanin and Phycocyanin Obtained from Analysis of CD Curves in the Ultraviolet at Selected Wavelengths from 207 to 243 nm.

Protein	Form	Percent
Allophycocyanin	α helix	59
	β form	40
Phycocyanin–PC-1	α helix	61
	β form	38
Phycocyanin–PC-2	α helix	64
	β form	33

Protein	Form	X-Ray Diffraction[a]	Percent[b]
Myoglobin	α helix	77	77 (77)
	β form	0	2 (2)
Ribonuclease	α helix	19	18 (18)
	β form	38	44 (44)
Lactic dehydrogenase	α helix	29	33 (31)
	β form	20	4 (6)
Lysozyme	α helix	29	26 (29)
	β form	16	17 (16)
Papain	α helix	21	21 (21)
	β form	5	11 (10)

[a] Quoted by Chen et al. (1972). [b] The numbers in parentheses are the data of Chen et al. (1972) who employed the BMDX 85T, UCLA (gauss-newton stepwise integration) computer program. Our results were obtained using a conventional multiple regression analysis–linear least-squares method.

Table III: A Comparison of Molecular Weights of *P. luridum* Biliproteins, as Determined by Gel Filtration on Calibrated Sephadex G-200 Columns, at pH 7.0.

Biliprotein	Molecular Weight	Percent of Total Biliprotein	Deduced Aggregate
Allophycocyanin	155,000	100	Tetramer
Phycocyanin (PC-1)	230,000	79	Hexamer
	107,000	12	Trimer
	23,000	9	Monomer
Phycocyanin (PC-2)	115,000	100	Trimer

placed in 8 *M* urea, 0.1% sodium dodecyl sulfate, or 0.01 *M* HCl.

The mean residue ellipticity, $[\theta]$, at selected wavelengths from 207 to 243 nm of the *P. luridum* biliproteins and the values of $[\theta]$ at these wavelengths of basis spectra (Chen et al., 1972) are shown in Table I. The results of linear least-squares fitting of biliprotein CD curves is indicated in Table II. The data suggest that allophycocyanin and phycocyanin apoproteins are comprised of approximately 60% α helix and 40% β-pleated sheet. The results obtained with our computer program with five protein standards using data obtained or quoted by Chen et al. (1972) is also given in Table II.

The results of gel filtration chromatography of *P. luridum* biliproteins on calibrated Sephadex G-200 columns at pH 7 are shown in Table III. The estimated molecular weight of allophycocyanin was 155,000. PC-1 eluted from the column in three fractions with apparent molecular weights of 230,000, 107,000, and 23,000. PC-2 eluted as a single component with an estimated molecular weight of 115,000.

The electrophoretic behavior of the *P. luridum* biliproteins on sodium dodecyl sulfate polyacrylamide gels is shown in Figure 3. Allophycocyanin and phycocyanin have two polypeptide subunits. Densitometric analysis at 600 nm of unstained gels suggests that each of the two allophycocyanin subunits contains one phycocyanobilin chromophore per polypeptide chain. On calibrated gels, the more rapidly migrating α subunit and the more slowly migrating β subunit of allophycocyanin had apparent molecular weights of ca. 17,300 and 19,000, respectively (Table IV). Two rather broad bands were observed when allophycocyanin and phycocyanin were examined electrophoretically on the same gel.

The amino acid composition of allophycocyanin and phycocyanin is shown in Table V. The results are expressed in residues/1000 residues to more clearly illustrate differences in the amino acid composition of these biliproteins since the monomer ($\alpha_1\beta_1$) molecular weights are slightly different. Allophycocyanin contains fewer residues of aspartic acid, serine, alanine, methionine, and phenylalanine and more residues of threonine, glutamic acid, proline, tyrosine, and valine than does phycocyanin.

The α and β subunits of phycocyanin from several algae have been separated by ion exchange chromatography on Bio-Rex 70 columns (Glazer and Fang, 1973a; Troxler et al., 1975). When *P. luridum* phycocyanin was chromatographed on a Bio-Rex 70 column (Bio-Rad Laboratories), the α subunit eluted in 8 *M* urea and the β subunit eluted in 9 *M* urea. This procedure failed to separate the subunits of

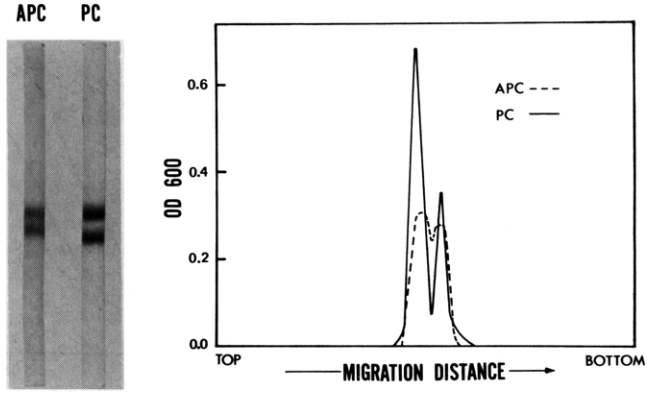

FIGURE 3: Electrophoresis of allophycocyanin (APC) and phycocyanin (PC) on sodium dodecyl sulfate polyacrylamide gels. Densitometric scans were performed at 600 nm prior to staining. Scans of stained gels (not shown) at 635 nm indicated a 1:1 ratio of α and β subunits in both allophycocyanin and phycocyanin.

Table IV: Subunit Molecular Weights of *P. luridum* Biliproteins as Determined Electrophoretically on Sodium Dodecyl Sulfate Polyacrylamide Gels.

Biliprotein		Molecular Weight[a]	Monomer Molecular Weight[b]
Allophycocyanin	α subunit	17,300 ± 120	36,300
	β subunit	19,000 ± 150	
Phycocyanin (PC-1)	α subunit	16,900 ± 500	36,900
	β subunit	20,000 ± 500	
Phycocyanin (PC-2)	α subunit	16,300 ± 300	35,450
	β subunit	19,150 ± 350	

[a] Average of five determinations. [b] Assuming the monomer is comprised of one α and one β subunit. It should be noted that the accuracy of the electrophoretic method employed is stated to be approximately ±5% (Weber and Osborn, 1969). Therefore, the data suggest that the molecular weights of α and β subunits of allophycocyanin and phycocyanin are similar.

Table V: Amino Acid Composition of *P. luridum* Biliproteins.

Amino Acid	Residues/1000 Residues		Residues/Monomer[a]	
	Allophycocyanin	Phycocyanin	Allophycocyanin	Phycocyanin
Aspartic acid	92	110	29.5	34.4
Threonine	89	55	28.6	17.3
Serine	66	76	21.2	23.8
Glutamic acid	89	66	28.6	20.5
Proline	42	32	13.5	10.0
Glycine	86	88	27.6	27.6
Alanine	112	161	35.9	50.4
Valine	70	57	22.5	17.7
Half-cystine	8	9	2.6	2.8
Methionine	26	35	8.3	10.8
Isoleucine	43	47	13.8	14.6
Leucine	85	75	27.3	23.4
Tyrosine	63	50	20.2	15.6
Phenylalanine	21	31	6.7	9.7
Lysine	47	46	15.1	14.4
Histidine	5	8	1.6	2.4
Arginine	56	56	18.0	17.4

[a] Based on monomer molecular weights of allophycocyanin and phycocyanin (PC-2) indicated in Table IV, and assuming that the former contains two residues of phycocyanobilin (mol wt 584) and that the latter contains three residues of phycocyanobilin, i.e., one chromophore per α subunit and two chromophores per β subunit (Troxler et al., 1975). The values given are the average of three determinations.

P. luridum allophycocyanin. The entire allophycocyanin sample eluted from the column in 8 *M* urea.

Surprisingly, allophycocyanin after chromatography on the Bio-Rex 70 column and dialysis against water had spectral properties which were more like those of phycocyanin than allophycocyanin (Figure 4A). The absorption maximum at 650 nm disappeared and a new absorption band was observed at 622 nm. The positive ellipticity bands at 655 and 625 nm had been replaced by a single positive ellipticity band at 615 nm. These spectral changes were partially reversible. When allophycocyanin which had been treated with 12% formic acid and 8 *M* urea, and dialyzed, was incubated in 0.05 *M* phosphate buffer (pH 7), the original absorption maxima at 650 and 618 nm reappeared, as did the positive ellipticity band at 655 nm. A new positive ellipticity band was observed at 617 nm (Figure 4B; Table VI). The absorption and CD spectra of allophycocyanin which had been treated with 12% formic acid and 8 *M* urea and dialyzed against water remained essentially unchanged when placed in 0.2 *M* acetate buffer (pH 5) or when left standing in water for 24 hr at 4°. An identical set of spectral shifts to those described above was observed when allophycocyanin (lyophilized) was put into 8 *M* urea–0.001 *M* β-mercaptoethanol, incubated at room temperature for 1 hr, dialyzed against water, and incubated in 0.05 *M* phosphate buffer (pH 7).

Allophycocyanin was subjected to automated sequence analysis. The first 18 residues at the amino terminus and the criteria for residue identification are indicated in Table VII. These results confirm that there are two distinct subunits in *P. luridum* allophycocyanin and that the ratio of subunit polypeptides is 1:1. Within the first 18 residues at the amino terminus of the α and β subunits, 30% of the amino acids are identical and the differences observed in an additional 40% of the amino acids could be explained by a single base change in the genetic code.

Discussion

The present investigation has shown that the monomer molecular weight, subunit molecular weight, and circular dichroic spectrum from 207 to 243 nm of allophycocyanin and phycocyanin from the filamentous cyanophyte, *P. luridum*, are remarkably similar. The chromophore and amino acid composition of these biliproteins is comparable to that described for the corresponding biliproteins in other cyanophytan and rhodophytan algae (Berns et al., 1964; Craig and Carr, 1969; Glazer and Cohen-Bazire, 1971; Binder et al., 1972; Torjesen and Sletten, 1972; Chapman, 1973; Gysi and Zuber, 1974; Troxler et al., 1975).

Gel filtration chromatography on calibrated Sephadex G-200 columns at pH 7.0 indicated that the molecular weight of *P. luridum* allophycocyanin was 155,000. This value is in general agreement with previously reported values of 134,000 (Hattori and Fujita, 1959), 138,000 (Nolan and O hEocha, 1967), and 120,000 (Gantt and Lipschultz, 1974). However, the molecular weights of *Synechococcus* sp. and two strains of *Synechococcus lividus* allophycocyanin were reported to be 96,000 and 103,000, respectively (Glazer and Cohen-Bazire, 1971; MacColl et al., 1974). These discrepancies may result in part from species differences, the conditions employed during purification, the method of molecular weight determination (gel filtration chromatography vs. sedimentation analyses), and the conditions under which analyses were performed (pH, ionic strength, allophycocyanin concentration). We estimated the molecular weights of the *P. luridum* biliproteins to compare the aggregation behavior of allophycocyanin and phycocyanin. Although our results show that the aggregation properties of allophycocyanin and phycocyanin are clearly different, the functional implications of biliprotein aggregation in vitro are still difficult to assess because (a) in vivo, allophycocyanin, phycocyanin, and phycoerythyrin (when present) occur together in a large heterogeneous aggregate called a phycobilisome (Gantt and Lipschultz, 1972), and (b) biliprotein subunits self-aggregate in vitro (Glazer and Fang, 1973b).

The α and β subunits of both allophycocyanin and phycocyanin from *P. luridum* had molecular weights of approximately 17,000 and 19,000, respectively. Densitometric analyses of unstained polyacrylamide gels indicated that the α and β subunits of allophycocyanin each contained one res-

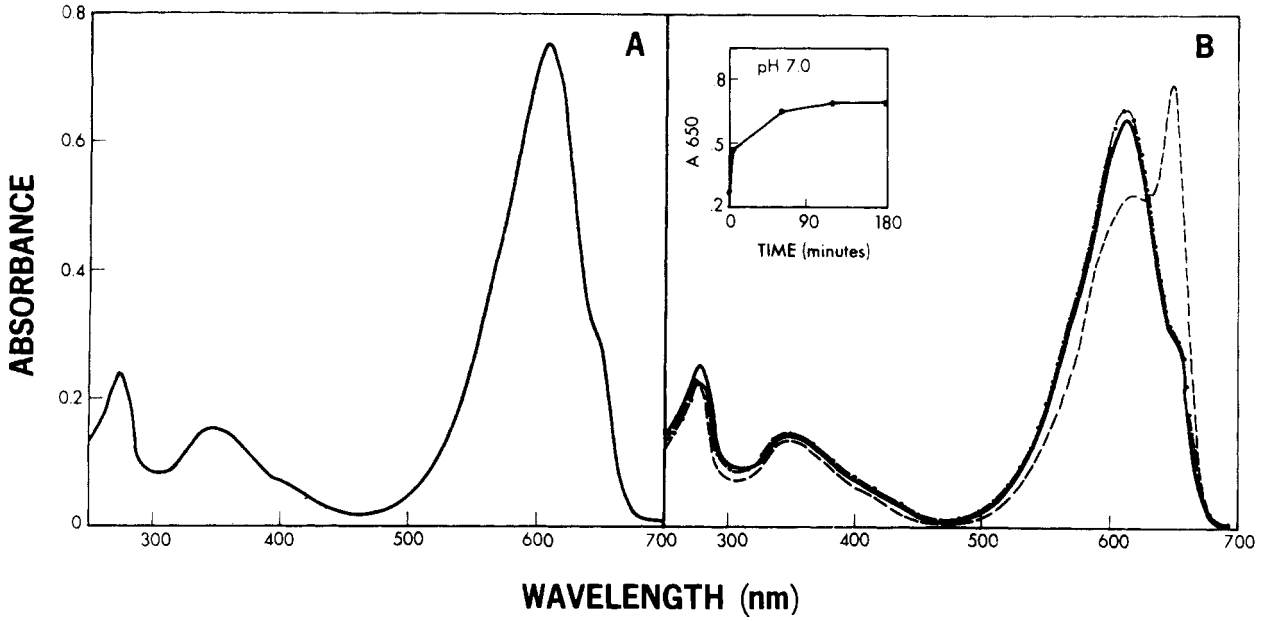

FIGURE 4: (A) Absorption spectrum of allophycocyanin in water after ion exchange chromatography on a Bio-Rex 70 column as described previously (Troxler et al., 1975). (B) Absorption spectrum of allophycocyanin in (A) after incubation for 3 hr at 25° in water (—), 0.2 *M* sodium acetate buffer, pH 5 (- · · ·), or 0.05 *M* potassium phosphate buffer (pH 7) (- - - -).

Table VI: Spectral Properties of Allophycocyanin before and after Denaturation.[a]

Sample	Conditions	Absorption Band (nm)	Ellipticity Band (nm)	$[\theta]^b$
1. Allophycocyanin	0.05 *M* phosphate buffer, pH 7	650 618	655 625	165 151
2. Phycocyanin (PC-2)	0.05 *M* phosphate buffer, pH 7	618	633	1860
3. Allophycocyanin	Water, after 12% formic acid and 8 *M* urea	622	615	N.D.
4. Allophycocyanin	Same as (3), 3 hr at 25° in 0.05 *M* phosphate buffer, pH 7	650 618	655 617	610 396
5. Allophycocyanin	Same as (3), 3 hr at 25° in water or 0.2 *M* sodium acetate buffer, pH 5	622	615	1430

[a] The protocol employed is described in the text. [b] $[\theta]$ = deg cm^2 dmol^{-1}.

Table VII: Sequential Degradation of the Amino Terminus of *P. luridum* Allophycocyanin.

Step	Deduced Residues[a]	SP 400[b] Residue	SP 400[b] nmoles	Amino Acid Analysis[c] Residue	Amino Acid Analysis[c] nmoles
1	Met + Met	Met	140	N.D.	
2	Ile + Glx	Ile	68	Glx	50
3	Asx + Val	Val	70	Asx	42
4	Ala + Thr	Ala	62	Thr	30
5	Ile + Lys	Ile	42	Lys	31
6	Thr + (Thr)	Gly/Pro[d]	34	Thr	28
7	Ile + Ile	Ile	92	N.D.	
8	Val + Val	Val	99	N.D.	
9	Asx + Ile	Ile	38	Asx	27
10	Asx + Ala	Ala	35	Asx	25
11	Thr + (Asx)	X		Asx + Thr	24 + 23
12	Ala + (Ala)	Ala	52	N.D.	
13	Asx + Glx	X		Asx + Glx	28 + 24
14	Ala + (Thr)	Ala	30	Thr	10
15	Gly + Glx	Gly	10	Glx	14
16	Tyr + Gly	Gly	11	Tyr	6
17	Leu + X	Leu	8	X	
18	Tyr + X	X		Tyr	4

[a] X signifies an unknown amino acid. [b] Packing material present in gas chromatography columns. [c] HI hydrolysis. [d] Degradation products of threonine on GC are Gly/Pro.

idue of phycocyanobilin. Phycocyanin, on the other hand, has one chromophore associated with the α subunit and two chromophores attached to the β subunit (Bennett and Bogorad, 1971; Glazer and Fang, 1973a; Troxler et al., 1975). The relation of biliprotein chromophore composition to the structural requirements for energy transduction in algal photosynthesis is not presently understood.

We observed that *P. luridum* allophycocyanin was comprised of a more rapidly migrating α subunit and a more slowly migrating β subunit on sodium dodecyl sulfate polyacrylamide gels. The subunits were more readily separated in 10% gels containing twice the normal amount of *N,N'*-methylenebisacrylamide (Weber and Osborn, 1969). Separation was largely obscured on gels containing more than 50 μg of lyophilized sample. Previous reports have indicated that allophycocyanin contains one (Bennett and Bogorad, 1971; Gantt and Lipschultz, 1974; Rice and Briggs, 1973), two (Glazer et al., 1971; Glazer and Fang, 1973b; Gysi and Zuber, 1974), or three (Glazer and Cohen-Bazire, 1971) subunits. We suggest that these discrepancies may be related in part to the choice of methodology but do not exclude the possibility that allophycocyanin in certain algal species may contain a single polypeptide subunit.

Allophycocyanin and phycocyanin are normally distinguished from one another by their characteristic absorption bands at 650 (shoulder at 618 nm) and 620 nm, respectively (Haxo et al., 1955; O hEocha, 1965). Phycocyanobilin is the chromophore of both biliproteins (Chapman et al.,

Table VIII: Amino Terminus of Algal Biliproteins.[a]

Organism	Phylum	Bili-protein[b]	Sequence 1	2	3	4	5	6	7	8	9	10	11	12	Homology (%) Obsd	One Base Change in Co-don
α-Subunit																
P. luridum	Cyanophyta	APC	Met	Glx	Val	Thr	Leu	Thr	Ile	Val	Ile	(Ala) or (Asx)	(Thr) or (Asx)	Ala-		
P. luridum[c]	Cyanophyta	PC	Met	Lys	Thr	Pro	Leu	Thr	Glu	Ala	Val	Ala-			30	60
A. nidulans[d]	Cyanophyta	PC	Ser	Lys	Thr	Pro	Leu	( )	Glu	Ala	Val	Ala	Ala	Ala-	33	66
P. cruentum[c]	Rhodophyta	PE	Met	Lys	Ser	( )	Ile	Thr-							40	80
C. caldarium[e]	Uncertain	PC	Met	Lys	Thr	Pro	Ile	Thr	Glu	Ala	Ile	Ala	Ala	Ala-	42	83
β Subunit																
P. luridum	Cyanophyta	APC	Met	Ile	Asx	Ala	Lys	Thr	Ile	Val	Asx	(Ala) or (Asx)	(Thr) or (Asx)	Ala-		
P. luridum[c]	Cyanophyta	PC	Met	Leu	Asp	Ala	Phe	Thr	Lys	Val	Val-				44	78
A. nidulans[d]	Cyanophyta	PC	Thr	Phe	Asp	Ala	Phe	Thr	Lys	Val	Val	Ala	Gln	Ala-	33	75
P. cruentum[c]	Rhodophyta	PE	Met	Leu	( )	Ala	Phe	Ser-							40	80
C. caldarium[e]	Uncertain	PC	Met	Leu	Asn	Ala	Phe	Ala	Lys	Val	Val	Ala	Ala	Ala-	33	75

[a] The sequence given for *P. luridum* allophycocyanin subunits is *proposed* on the basis of homology with phycocyanin and phycoerythrin. [b] Allophycocyanin, APC; phycocyanin, PC, phycoerythrin, PE. [c] Data from Harris and Berns (1974). [d] Data from Williams et al. (1974). [e] Data from Troxler et al. (1975). It should be noted that *C. caldarium* is a unicellular eukaryote which has been classified as an anomalously pigmented chlorophyte based on cell morphology and a rhodophyte based on sterol composition (Seckback and Ikan, 1972).

1968). Since there is ample evidence that the physical-chemical properties of these biliproteins are different (O hEocha, 1965; Berns, 1971; Chapman, 1973), differences in their chromophore absorption bands have been ascribed to the nature of the hydrophobic environment of phycocyanobilin peculiar to the respective subunit polypeptides. The existence of the positive ellipticity bands associated with the characteristic absorption bands is compatible with this hypothesis. It should be noted that the magnitude of the ellipticity bands of allophycocyanin and phycocyanin differs considerably. The factors underlying these differences are currently being investigated.

The spectral behavior of denatured allophycocyanin is unique. After removal of denaturants by dialysis against water, the absorption and ellipticity bands (due to chromophore) of allophycocyanin were more like those of phycocyanin. It has been noted that allophycocyanin from *Anacystis nidulans* after denaturation undergoes a similar spectral shift (Erokchina and Krasnovskii, 1974). The spectral shifts observed in the present study were partially reversible at pH 7 but not at pH 5. It is felt that the pH dependency of these spectral shifts observed with allophycocyanin may serve as a model system for determining the factors involved in subunit aggregation into the biliprotein monomer. In contrast, phycocyanin which has been exposed to denaturants and dialyzed does not undergo the spectral perturbations observed with allophycocyanin (Murphy and O'Carra, 1970). In fact, hybrid phycocyanins prepared from separated α and β subunits from different algae are nearly identical as spectral entities to the parent phycocyanins (Glazer and Fang, 1973b). This illustrates another physical-chemical parameter by which chromophore-apoprotein interaction in allophycocyanin and phycocyanin can be distinguished.

Automated sequence analysis of *P. luridum* allophycocyanin showed that the subunit polypeptides are different. The recovery of PTH-amino acids from each sequencer cycle of the allophycocyanin sample demonstrates that the subunits occur in a 1:1 ratio. A *proposed* sequence of allophycocyanin subunits at the amino terminus based on homology with phycocyanin and phycoerythrin is given in Table VIII. The amino acid sequence of the amino terminus of allophycocyanin and phycocyanin from the same alga shows that the subunit polypeptides of each are different as well. Since allophycocyanin is thought to mediate energy transfer between other biliproteins and chlorophyll a (Gantt and Lipschultz, 1973), it may be the evolutionary antecedent of phycocyanin and phycoerythrin.

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