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The α and β Subunits of *Cyanidium caldarium* Phycocyanin: Properties and Amino Acid Sequences at the Amino Terminus†

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ABSTRACT: Phycocyanin was isolated and purified from the unicellular alga, *Cyanidium caldarium.* Subunits were prepared on a Bio-Rex-70 column developed stepwise with urea solutions (pH 1.9). The α subunit eluted in 8 M urea and the β subunit eluted in 9 M urea. The α and β subunits displayed absorption maxima at 660, 354, and 277 nm in 8 M and 9 M urea. The α:β ratio of total absorbance under the 660-nm peak was 0.56 suggesting an α:β phycocyanobilin chromophore ratio of 1:2. On calibrated sodium dodecyl sulfate gels, the α subunit had an estimated molecular weight of 15,500 ± 1100 and the β subunit had an estimated molecular weight of 18,300 ± 300. Minimum molecular weights based on one histidine residue per subunit were 16,300 for the α subunit and 18,750 for the β subunit. Phycocyanin displayed a single visible absorption maximum at 625 nm and two positive circular dichroic bands at 632 and 610 nm. The α and β subunits displayed single visible absorption maxima at 618 and 600 nm and single positive circular dichroic peaks at 620 and 585 nm, respectively. Two-dimensional maps of tryptic digests of the α and β subunits revealed distinct patterns of peptides each of which was consistent with the lysine and arginine composition of these polypeptides. Maps of tryptic digests of phycocyanin contained 25 major peptides (a total of 27 lysine and arginine residues). Automated sequence analysis of separated subunits revealed a 70% homology within the first 27 residues at the amino terminus of the α and β subunits of *C. caldarium* phycocyanin.

Phycocyanin[1] is a bile pigment–protein complex which is found in the photosynthetic apparatus of the red (Rhodophyta), blue-green (Cyanophyta), and golden-brown (Cryptophyta) algae (OhEocha, 1965). This biliprotein is an accessory photosynthetic pigment which is a functional component of pigment system II (Myers, 1971). Phycocyanin occurs as a high molecular weight aggregate *in vivo,* called a "phycobilisome" (Gantt and Conti, 1966). In stained sections in the electron microscope phycobilisomes appear as granules, 350–450 Å in diameter, located on the stroma side of thylakoid membranes (Gantt and Conti, 1965). Phycocyanin from the golden-brown algae does not form high molecular weight aggregates (MacColl *et al.,* 1973) and is located within the intrathylakoid space rather than on the outer surface of thylakoid membranes (Gantt *et al.,* 1971).

The fundamental unit of phycocyanin aggregation is a monomer which has an estimated molecular weight of approximately 33,000 (Berns, 1971). The monomer can associate or dissociate into larger or smaller aggregates depending on the pH, ionic strength, and protein concentration (Hattori *et al.,* 1965; Berns, 1971). The monomer from

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[1] Abbreviations used are: phycocyanin, C-phycocyanin in which phycocyanobilin is the only chromophore; TPCK, L-tosylamido-2-phenylethyl chloromethyl ketone; dansyl, dimethylaminonaphthalenesulfonyl derivative.

some algal species has been resolved into α and β subunits in the 15,000 and 19,000 range, respectively, on urea or sodium dodecyl sulfate polyacrylamide gels (Binder *et al.*, 1971; Bennett and Bogorad, 1971; Torjesen and Sletten, 1972; Glazer and Fang, 1973a; Gantt and Lipschultz, 1974) and on Sephadex G-100 columns run in 0.1% sodium dodecyl sulfate–0.25% β-mercaptoethanol (Kobayashi *et al.*, 1972).

The immunochemical properties of phycocyanins have been studied by several laboratories (Bogorad, 1965; Berns, 1967; Glazer *et al.*, 1973). Interspecies sequence homology between phycocyanins was suggested by the observation that rabbit antiserum prepared against phycocyanin from a given algal species cross-reacted with heterologous phycocyanins from other blue-green and red algae. More recently, Glazer and Fang (1973b) have shown that the α and β subunits from unrelated blue-green algae could combine to form stable hybrid phycocyanins. This suggested that the polypeptide moieties of α and β subunits have homologous regions which are necessary to maintain the higher order of structure required for subunit association and monomer aggregation.

The present investigation describes some properties and amino acid sequences of the amino terminal regions of the α and β subunits of phycocyanin from the unicellular alga, *Cyanidium caldarium*.

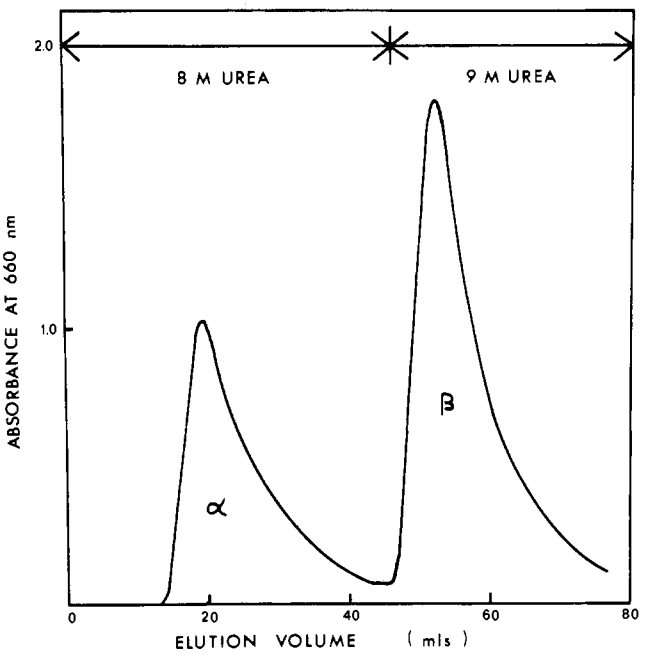

FIGURE 1: Separation of phycocyanin subunits by ion exchange chromatography on Bio-Rex-70 columns. Absorbance at 660 nm of 1.0-ml fractions of column eluate was monitored on a Beckman DB-GT spectrophotometer. Flow rate was 1.0 ml/min.

Materials and Methods

The Organism. C. caldarium is an eukaryotic alga of uncertain taxonomic position. Confusion surrounding the taxonomy of this organism arises because it contains phycocyanin and allophycocyanin, a characteristic of the Cyanophyta, but has a morphology and cytoplasmic organization similar to the unicellular chlorophyte, *Chlorella pyrenoidosa* (Doemel and Brock, 1971).

The *C. caldarium* strain used in the present work has been described (Allen, 1959) and the culture conditions employed for growth of this organism in the laboratory have been reported previously (Troxler and Lester, 1967).

Preparation of Phycocyanin. Algal cells were disrupted by sonic vibration in 0.01 M potassium phosphate buffer (pH 7.0) with a Branson sonic oscillator (Model W185). The sonically disrupted cells were centrifuged at 100,000*g* for 1 hr and the resulting supernatant was made 50% saturated with ammonium sulfate. The precipitate obtained was dissolved in distilled water, dialyzed against water at 4°, and chromatographed on a brushite column (2.5 × 20 cm) developed with potassium phosphate buffers (pH 7.0) of increasing ionic strength (Troxler, 1972). Phycocyanin eluted from the column in 0.01–0.025 M phosphate buffer and allophycocyanin eluted in 0.1–0.15 M phosphate buffer. Phycocyanin in the eluate was precipitated in 80% saturated ammonium sulfate, dialyzed against distilled water at 4°, and lyophilized.

Subunit Preparation. Purified phycocyanin (8 mg) was dissolved in 14.2 ml of distilled water, and an equal volume of 24% formic acid–0.002 M β-mercaptoethanol was added. This solution was applied to a Bio-Rex-70 column (Bio-Rad, Richmond, Calif.; minus 400 mesh; 1 × 10 cm) which had been equilibrated in 12% formic acid–0.001 M β-mercaptoethanol. The column was developed stepwise with 8 M and 9 M urea, pH 1.9 (Glazer and Fang, 1973a). Resolved subunits in the column eluate were dialyzed against distilled water at 4° and lyophilized.

Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed in 10% gels in which the ratio of *N,N'*-methylenebisacrylamide to acrylamide was 1:18.5 according to the procedure of Weber and Osborn (1969). Electrophoresis was performed at 8 mA/tube for 4–6 hr using 0.5 × 10 cm gels. The 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 in a solution of 10% methanol–7% acetic acid at room temperature.

Amino Acid Analysis. Protein samples were hydrolyzed in 3 N *p*-toluenesulfonic acid for 22 hr at 108° *in vacuo* according to the procedure of Liu and Chang (1971). The hydrolysates were chromatographed on the column (Type A resin, 130 × 0.6 cm) of a Technicon automated analyzer (Hamilton, 1963).

Spectral Measurements. Absorption spectra were determined on a Zeiss PMQII spectrophotometer or a Beckman DB-GT recording spectrophotometer. Circular dichroic spectra (CD) were determined at 27° on a Cary (Model 61) recording spectropolarimeter which had been calibrated with a *d*-10-camphorsulfonic acid standard (Eastman, Rochester, N.Y.) in water. Mean residue ellipticity $[\theta]$ given in deg cm^2 dmol^{-1} for phycocyanin, the α subunit, and the β subunit was calculated using mean residue weights of 105, 103, and 106, respectively.

Peptide Mapping. Lyophilized samples (1–2 mg) were suspended in 0.4 ml of 0.1 M NH_4HCO_3 (pH 8.0); 40 μl of a freshly prepared TPCK-trypsin (Worthington Biochemical Corp., Freehold, N.J.; 1 mg/ml) in 0.01 M Tris buffer (pH 8.0) was added and digestion allowed to proceed overnight with gentle mixing at 37°. Following the addition of a second and equal quantity of freshly prepared trypsin solution to give a final enzyme to protein ratio of 1:25, the digestion was continued for an additional 2 hr and centrifuged prior to lyophilization.

Lyophilized tryptic digests were suspended in 150 μl of water and aliquots containing *ca.* 75 μg of digest were spotted on the lower left-hand corner of silica gel G plates (An-

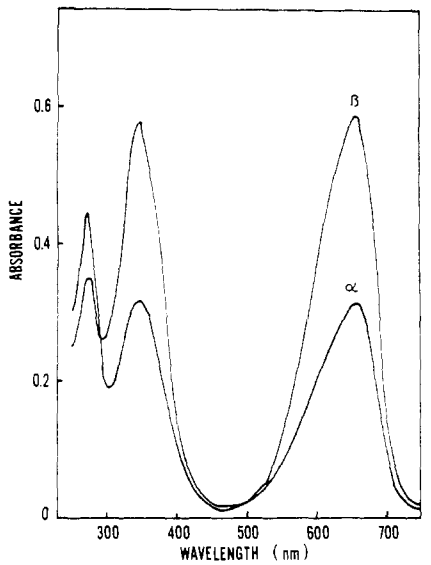

FIGURE 2: Absorption spectrum of phycocyanin subunits in 8 M (α subunit) and 9 M (β subunit) urea. Total area under the 660-nm peak was determined by cutting and weighing the chart paper (700 nm to 500 nm). The $\alpha:\beta$ ratio was 0.56.

altech, Newark, Del., 20 × 20 cm). Thin-layer peptide mapping was performed according to the method of Ritschard (1964). Chromatography in the first dimension was carried out in an ascending system of chloroform-methanol-30% ammonium hydroxide (2:2:1). The plates were air dried and sprayed with pyridine-glacial acetic acid-water (1:10:489), pH 3.5. Electrophoresis was carried out in the same buffer at 1000 V for 80 min using a Shandon thin-layer electrophoresis unit (SAE-3230-Mk 11). The maps were sprayed with fluorescamine (Fluram, Fisher Scientific, Boston, Mass.; 3 mg/20 ml of acetone) and examined under ultraviolet light (3600 Å).

Sequencing Procedure. Sequencing was carried out with automatic stepwise degradation (Beckman Model 890C sequencer) according to the procedure of Edman and Begg (1967); 5–10-mg samples were subjected to automated sequencing utilizing a Quadrol buffer system (Beckman Sequencer Manual, 1969). All fractions were converted to PTH-amino acids in the usual manner (Edman and Begg, 1967) and extracted into ethyl acetate. An aliquot (10%) of each cycle was run on a Beckman (Model 65) gas chromatograph according to the method of Pisano and Bronzert (1969). Silylation of residues was performed in *N,O*-bis(trimethylsilyl)acetamide (Pisano, 1972). The remainder of the ethyl acetate and the aqueous layers were hydrolyzed with hydriodic acid (Smithies *et al.*, 1971) and analyzed on a Jeolco 6AH amino acid analyzer.

Results

Phycocyanin samples used in this work had A_{620}/A_{280} ratios greater than 4.2. The α and β subunits were separated by ion exchange chromatography on Bio-Rex-70 columns according to the method of Glazer and Fang (1973a). The α subunit eluted in 8 M urea and the β subunit eluted in 9 M urea (Figure 1). Fractions 15–44 (α subunit) and 45–77 (β subunit) in the experiment shown in Figure 1 were separately pooled and the absorption spectrum was determined (Figure 2). The subunits displayed absorption maxima at 660, 354, and 277 nm in 8 M and 9 M urea, respectively. The ratio of extinction of phycocyanobilin at 660 and 354 nm was approximately 1.0. This indicates that chromophore–protein interaction had been completely disrupted in the separated subunits because the A_{662}/A_{375} ratio of the chromophore free acid in acid chloroform is about 1.0 (Cole *et al.*, 1968). The total area under the 600-nm peak of the α subunit was nearly one-half the total area under the corresponding peak of the β subunit. This is in agreement with densitometric analyses of algal biliproteins in stained and unstained gels (Bennett and Bogorad, 1971; Gantt and Lipschultz, 1974) and with the spectral properties of isolated subunits (Glazer and Fang, 1973) which indicates an $\alpha:\beta$ chromophore ratio of 1:2.

The α subunit, the β subunit, and phycocyanin were subjected to electrophoresis on sodium dodecyl sulfate polyacrylamide gels. Gel electrophoresis showed that phycocyanin is comprised of two subunits, which when isolated on Bio-Rex-70 columns migrate on sodium dodecyl sulfate gels as single bands. In addition, it was shown that the faster and slower migrating bands of phycocyanin observed on sodium dodecyl sulfate gels correspond to the subunits designated α and β obtained from the Bio-Rex-70 column. On calibrated gels, the estimated molecular weight of the α subunit was 15,500 ± 1100 and that for the β subunit was 18,300 ± 300 (average of 10 determinations).

The absorption and CD spectra from 700 to 500 nm of phycocyanin, the α subunit, and the β subunit in 0.01 M sodium phosphate buffer (pH 7.0) are shown in Figure 3. Phycocyanin displayed a single absorption maximum at 625 nm and two positive CD bands at 632 and 610 nm (Table I). The α and β subunits had single absorption maxima at

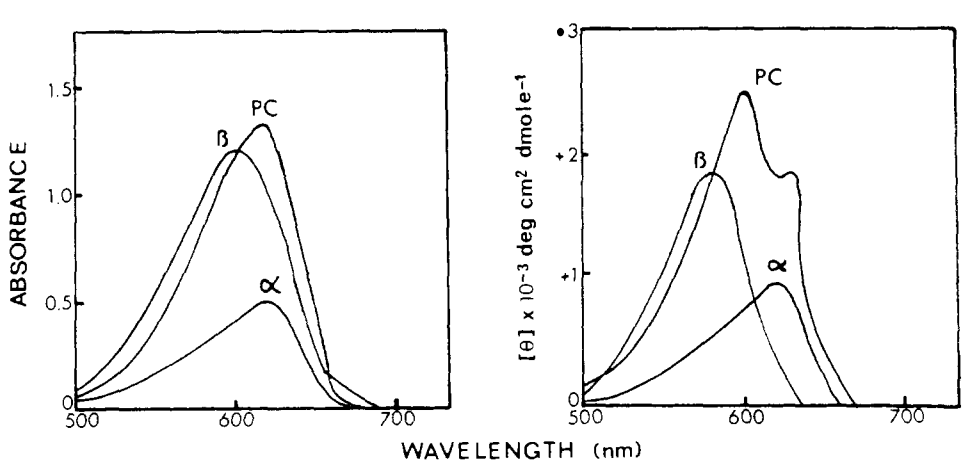

FIGURE 3: Absorption and circular dichroic spectra of phycocyanin and subunits.

Table I: Spectral Properties of the α and β Subunits of *C. caldarium* Phycocyanin.[a]

Sample	Absorption[b] Max (nm)	$\epsilon \times 10^{-5}$	CD Maximum (nm)	Mean Residue Ellipticity $[\theta] \times 10^{-3}$
Phycocyanin	625	2.63	632	2.41
			610	2.47
α subunit	618	0.46	620	0.91
β subunit	600	1.28	585	1.79

[a] Absorption and CD measurements were made as described in Methods. [b] The concentration of each sample employed for absorption and CD measurements was 0.17 mg/ml. Molecular weights were determined on calibrated sodium dodecyl sulfate gels (see text). [c] Mean residue ellipticity given in deg cm^2 dmole^{-1}.

618 and 600 nm and single positive CD maxima at 620 and 585 nm, respectively.

The amino acid composition of the α subunit, the β subunit, and phycocyanin is indicated in Table II. The numerical sum of the residues in the subunits is in good agreement with the total residues in phycocyanin. Calculated minimum molecular weights based on 1 histidine residue per polypeptide chain were 16,300 and 17,750 for the α and β subunit, respectively, assuming an α:β chromophore ratio of 1:2.

Composite drawings of two-dimensional maps of tryptic digests of the α subunit, the β subunit, and phycocyanin are shown in Figure 4. On maps of each subunit, 14 major tryptic peptides were observed and on maps of phycocyanin 25 major peptides were evident. Thus, the number of tryptic peptides observed on maps of the subunits and of phycocyanin were what would be expected from the lysine and arginine composition of these proteins (see Table II). One blue peptide was observed on maps of the α subunit and two partially separated blue peptides were seen on maps of the β subunit. The blue peptides on maps of phycocyanin were consistent with those on maps of the subunits. Trypsin alone displayed no fluorescamine reactive material over the entire map. One major peptide (hatched lines) was observed on all maps of phycocyanin digests but was never observed on maps of the subunits.

The α and β subunits were subjected to automated sequence analyses. The first 27 residues at the amino terminus of the subunits are indicated in Table III and the basis of residue identification and the recovery at each sequencer cycle is shown in Table IV. Methionine is the amino terminal residue in each subunit. In both subunits residues 10–27 are identical. Differences are observed at residues 2–9. A 70% homology is observed within the first 27 residues from the amino terminus of the α and β subunits of phycocyanin from this alga. The amino acid differences at residues 3–9 can be accounted for by single base changes in the codon and are conservative substitutions with respect to the side groups on the amino acids.

Discussion

The present investigation has shown that phycocyanin from the unicellular alga, *Cyanidium caldarium*, is comprised of two dissimilar polypeptide subunits in which the chromophore composition, amino acid composition, absorption spectrum, CD spectrum, and electrophoretic mobility on sodium dodecyl sulfate polyacrylamide gels are consistent with that described for cyanophytan and rhodophytan phycocyanins (Binder *et al.*, 1971; Bennett and Bogorad,

Table II: Amino Acid Composition of the α and β Subunits of *C. caldarium* Phycocyanin.

Amino Acid	Average or Extrapolated Value[a] α Subunit[c]	β Subunit[c]	α:β	Phycocyanin[c]
Aspartic acid	14.8	20.2	35.0	33.9
Threonine[b]	8.9	9.3	18.2	18.8
Serine[b]	11.6	13.8	25.4	24.5
Glutamic acid	15.1	14.4	29.5	29.2
Proline	5.9	5.1	11.0	12.2
Glycine	11.6	12.8	24.4	22.6
Alanine	22.4	25.6	48.0	44.2
Valine	6.2	9.9	16.1	16.0
Half-cystine[d]	1.1	1.6	2.7	2.8
Methionine	3.5	5.4	8.9	8.5
Isoleucine	7.0	6.4	13.4	13.2
Leucine	11.9	13.5	25.4	24.5
Tyrosine	8.6	5.1	13.7	14.1
Phenylalanine	3.0	4.2	7.2	8.5
Lysine	5.1	5.5	10.6	10.4
Histidine	1.0	0.98	1.98	1.9
Arginine	7.5	10.3	17.8	16.9
Sum	145.2	164.1	309.5	302.5

[a] Residues calculated assuming mol wt of 15,500 and 18,300 for α and β subunits, respectively. [b] Values obtained by linear extrapolation to zero time. [c] Values based on 24-, 48-, and 72-hr hydrolysates. [d] Determined by the procedure of Spencer and Wold (1969).

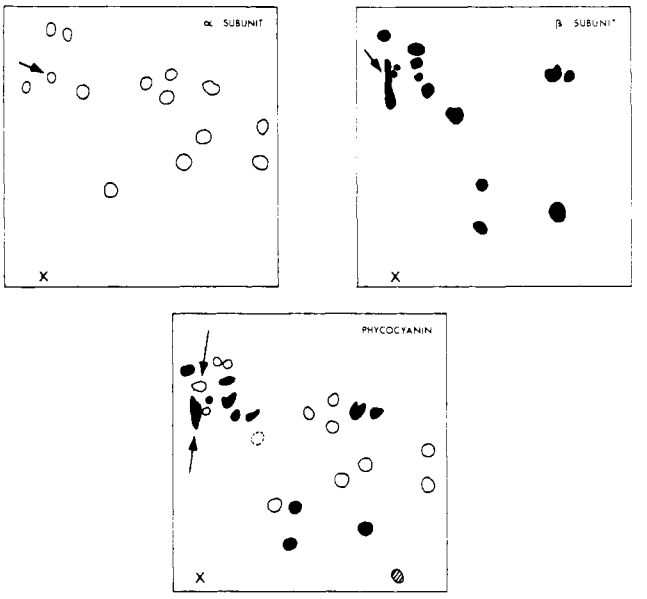

FIGURE 4: Two-dimensional peptide maps of tryptic digests of phycocyanin and subunits. Trypsin digestion and thin-layer electrophoresis were performed as described. The arrows indicate blue peptides. Chromatography was performed in the vertical direction and electrophoresis was performed in the horizontal direction with the cathode at the right.

Table III: Amino Acid Sequences of the Amino Terminus of the α and β Subunits of *C. caldarium* Phycocyanin.[a]

α Subunit

1 5 10 15 20

Met-Lys-*Thr-Pro-Ile-Thr-Glu-Ala-Ile*-Ala-Ala-Ala-Asn-Ala-Arg-Gly-Glu-Phe-Leu-(Ser)-Asx-Thr-Glx-(Tyr)-

25

Leu-Ala-Val-

β Subunit

1 5 10 15 20

Met-Leu-*Asn-Ala-Phe-Ala-Lys-Val-Val*-Ala-Ala-Ala-Asn-Ala-Arg-Gly-Glu-Phe-Leu-(Ser)-Asx-Thr-Glx-(Tyr)-

25

Leu-Ala-Val-

[a] Parentheses indicate that the residue is suspected but not definitively proven. Amino acid substitutions which can be accounted for by a single base change in the codon are italicized.

Table IV: Sequential Degradation of Amino Terminal Regions of the α and β Subunits of *C. caldarium* Phycocyanin.[a]

	α Subunit						β Subunit				
		SP400[b]		Amino Acid Analysis[c]				SP400[b]		Amino Acid Analysis[c]	
Step	Deduced Residue	Residue	nmoles	Residue	nmoles	Step	Deduced Residue	Residue	nmoles	Residue	nmoles
1	Met	Met	242	N.D.		1	Met	Met	535	N.D.	
2	Lys	X		Lys	160	2	Leu	Leu	498	Leu	357
3	Thr	Gly/Pro	184/90	AAB[d]	497	3	Asn	X		Asp	299
4	Pro	Pro	389	Pro	128	4	Ala	Ala	404	N.D.	
5	Ile	Ile	450	Ile	185	5	Phe	Phe	410	N.D.	
6	Thr	Gly/Pro	44/21	AAB[d]	164	6	Ala	Ala	404	N.D.	
7	Glu	Glu[e]	135	Glu	147	7	Lys	X		Lys	110
8	Ala	Ala	160	Ala	137	8	Val	Val	331	N.D.	
9	Ile	Ile	340	Ile	85	9	Val	Val	447	Val	223
10	Ala	Ala	230	Ala	107	10	Ala	Ala	233	N.D.	
11	Ala	Ala	160	Ala	160	11	Ala	Ala	299	N.D.	
12	Ala	Ala	401	Ala	199	12	Ala	Ala	578	N.D.	
13	Asn	X		Asp	83	13	Asn	X		Asp	217
14	Ala	Ala	200	Ala	50	14	Ala	Ala	318	Ala	162
15	Arg	X		Arg	86	15	Arg	X		Arg	86
16	Gly	Gly	190	Gly	99	16	Gly	Gly	178	Gly	168
17	Glu	Glu[e]	139	Glu	68	17	Glu	X		Glu	160
18	Phe	Phe	174	Phe	66	18	Phe	Phe	87	Phe	69
19	Leu	Leu	195	Leu	90	19	Leu	Leu	123	Leu	69
20	(Ser)	Ser[e]	101	Ala	61	20	(Ser)	Ser[e]	51	Ala	83
21	Asx	X		Asp	51	21	Asx	X		Asp	51
22	Thr	Gly/Pro	15/9	AAB[d]	66	22	Thr	Gly/Pro	24/14	AAB[d]	45
23	Glx	X		Glu	66	23	Glx	X		Glu	58
24	(Try)	X		Tyr	25	24	(Tyr)	X		Tyr	15
25	Leu	Leu	57	Leu	53	25	Leu	Leu	60	Leu	41
26	Ala	Ala	43	Ala	45	26	Ala	Ala	73	Ala	51
27	Val	Val	61	Val	48	27	Val	Val	50	Val	20

[a] X signifies an unknown amino acid; parentheses indicate a suspected residue; N.D. means not done. [b] Packing material present in gas chromatography columns. [c] HI hydrolysis. [d] α-Aminobutyric acid derivative produced during HI hydrolysis of PTH-threonine. [e] Identified as silylated derivative.

1971; Torjesen and Sletten, 1972; Glazer and Fang, 1973a; Glazer *et al.*, 1973; Gantt and Lipschultz, 1974).

The spectral properties of *C. caldarium* phycocyanin deserve further comment. The single long wavelength absorption band at 625 nm and the two positive CD bands at 632 and 610 nm are similar to those described for cyanophytan phycocyanins (Pecci and Fujimori, 1969; Glazer *et al.*, 1973). The two positive CD bands are consistent with the fluorescence polarization spectrum of phycocyanin which suggests two chromophores in different environments (Teale and Dale, 1970). Since phycocyanobilin is the only chromophore on phycocyanin (Chapman *et al.*, 1968), it is tempting to suggest that the two positive bands can be attributed to phycocyanobilin in different hydrophobic environments in the subunit polypeptides. Nevertheless, Glazer *et al.* (1973) have convincingly demonstrated that the mag-

Table V: Amino Terminal Regions of Algal Biliprotein Subunits.[a]

Organism	Phylum	Biliprotein[e]	Sequence 1 5 10 15	Homology %
α Subunit				
C. caldarium	Uncertain	PC	Met-Lys-Thr-Pro-Ile-Thr-Glu-Ala-Ile-Ala-Ala-Ala-Asn-Ala-Arg-Gly-	
C. caldarium	Uncertain	PC[b]	Met-Lys-Thr-Pro-Ile-Thr-	100
P. cruentum	Rhodophyta	PE[b]	Met-Lys-Ser-( )-Ile-Thr-	80
O. agardhii	Cyanophyta	PC[d]	Met-Lys-Thr-Pro-Leu-Ser-Glu-Ala-Val-Ser-Ser-Ala-Asp-	54
A. nidulans	Cyanophyta	PC[c]	Ser-Lys-Thr-Pro-Leu-( )-Glu-Ala-Val-Ala-Ala-Ala-Asx-( )-( )-Gly-	69
β Subunit				
C. caldarium	Uncertain	PC	Met-Leu-Asn-Ala-Phe-Ala-Lys-Val-Val-Ala-Ala-Ala-Asn-Ala-Arg-Gly-Glu-Phe-Leu	
C. caldarium	Uncertain	PC[b]	Met-Ile-Asx-Ala-Phe-Ser-Lys-Val-	63
P. cruentum	Rhodophyta	PE[b]	Met-Leu-( )-Ala-Phe-Ser-	80
O. agardhii	Cyanophyta	PC[d]	Met-Phe-Asp-Ala-Phe-Ser-Lys-Val-Val-Ala-Gln-Ala-Asp-	62
A. nidulans	Cyanophyta	PC[c]	Thr-Phe-Asp-Ala-Phe-Thr-Lys-Val-Val-Ala-Gln-Ala-Asp-Ala-Arg-Gly-Glu-Phe-Leu-	68

[a] Homology with the subunits of *C. caldarium* phycocyanin employed in the present work is given in the column at the right. [b] From Harris and Berns (1974). [c] From Williams *et al.* (1974). [d] From Torjesen and Sletten (1972) (although phycocyanin from *O. agardhii* was sequenced directly, the proposed sequences are based on the observed homology with *C. caldarium* and *A. nidulans* phycocyanin. [e] PC, phycocyanin; PE, phycoerythrin. It should be mentioned that Harris and Berns (1974) employed a different isolate of *C. caldarium* to that used in the present work (Allen, 1959).

nitude of the two positive CD bands in *Anacystis nidulans* phycocyanin can be correlated with the aggregation state of this biliprotein, *i.e.*, the 632-nm CD band is enhanced under conditions which favor the 11S hexamer and the 610-nm band is enhanced in dilute solutions where the 3S monomer predominates (Hattori *et al.*, 1966; Neufeld and Riggs, 1969; MacColl *et al.*, 1971). Our sedimentation velocity studies indicate that at pH 7.0 at concentrations of 1–3 mg/ml, *C. caldarium* phycocyanin exists entirely as a 5S aggregate (dimer). Our CD measurements were made at phycocyanin concentrations of 0.1–0.4 mg/ml and a concentration dependency of the mean residue ellipticity $[\theta]$ at 632 and 610 nm was never observed. Therefore, we are uncertain as to whether the two positive CD bands of *C. caldarium* phycocyanin are related to chromophore–protein interaction within the subunits per se, or to monomer aggregation.

Cope *et al.* (1967) reported that the pattern of tryptic peptides on two-dimensional maps of *Phormidium luridum* and *Plectonema calothricoides* phycocyanin was nearly identical. Torjesen and Sletten (1972) noted a similarity between the pattern of tryptic peptides on maps of the α and β subunits of *Oscillatoria agardhii* phycocyanin. Our peptide mapping experiments in which the subunits from *C. caldarium* phycocyanin were employed show that each subunit can be clearly distinguished by this technique. In fact, the number of major peptides on maps of phycocyanin was nearly the same as the total lysine and arginine residues in this biliprotein. This suggests that few, if any, tryptic peptides derived from the α and β subunits comigrated under the conditions of chromatography and thin-layer electrophoresis employed.

Harris and Berns (1974) have performed dansyl-Edman degradations on the subunits of biliproteins from eight species of the Cyanophyta and from two species of the Rhodophyta. Williams *et al.* (1974) have determined the first 16 and 19 residues of the α and β subunits of *A. nidulans* phycocyanin by automated sequence analysis. Torjesen and Sletten (1972) have described the results of dansyl-Edman degradations of *O. agardhii* phycocyanin (subunits not separated). These results are compared to our sequence data in Table V, and may be summarized as follows: (a) our results on *C. caldarium* phycocyanin are in good agreement with those of Harris and Berns (1974), (b) interspecies and intraspecies homology at the amino terminus is evident between phycocyanin subunits, (c) the amino terminal region of phycocyanin is nearly identical with that of *Porphyridium cruentum* phycoerythrin, and (d) sequence homology is observed between biliproteins of cyanophytan prokaryotes (*O. agardhii, A. nidulans*), the rhodophytan eukaryote, *P. cruentrum*, and *C. caldarium* (eukaryote of uncertain taxonomic position).

The observed homology between biliproteins of modern-day cyanophytes and rhodophytes indicates that the gene for phycocyanin was present in the ancestral progenitor of prokaryotic and eukaryotic organisms (Williams *et al.*, 1974). The fossil record shows that organisms which resemble modern blue-green algae lived over 2.6 billion years ago prior to the appearance of eukaryotic life (Schopf, 1970). One could argue, therefore, that the sequence homology between biliproteins is consistent with the symbiotic theory for the origin of eukaryotic cells (Margulis, 1970). Though limited, the information available on the sequences of phycocyanin and phycoerythrin suggests the possibility that the

latter may have arisen directly from the former in view of the functional relationship between these accessory pigments in algal photosynthesis (Myers, 1971).

The homology between α and β subunits of phycocyanins provides circumstantial evidence that one subunit may have arisen from the other by gene duplication. This mode of protein evolution has been recently reviewed with respect to vertebrate hemoglobins, cytochromes, and enzymes (Smith, 1970; Wu *et al.*, 1974). Finally, the different chain length of the polypeptide moiety of algal biliprotein subunits and the observed homology at the amino terminus raise interesting questions as to which subunit occurred first in evolution, and to the processes involved in the formation of one from the other, *e.g.*, amino acid addition, amino acid deletion.

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