Physical-Chemical Properties of C-Phycocyanin Isolated from an Acido-Thermophilic Eukaryote, *Cyanidium caldarium*

By ORANDA H. W. KAO, MERCEDES R. EDWARDS and DONALD S. BERNIS
*Division of Laboratories and Research, New York State Department of Health,
New Scotland Avenue, Albany, N.Y. 12201, U.S.A.*

(Received 30 September 1974)

C-Phycocyanin from an acido-thermophilic eukaryotic alga, *Cyanidium caldarium*, was characterized with respect to subunit structure, absorption spectrum and fluorescence properties and was found to be similar to C-phycocyanins from mesophilic sources. The pH-dependence of fluorescence polarization and the changes in sedimentation velocity as a function of pH, concentration and temperature indicate the presence of extremely large amounts of unusually stable 19S aggregates. It was not possible to disaggregate this phycocyanin completely to monomer under normal conditions. The amino acid composition is similar to that of phycocyanins from other thermophilic and halophilic sources. The isoelectric point of this C-phycocyanin was 5.11, an unusually high value. The properties of this C-phycocyanin suggest an increase in protein stability as its mode of adaptation to the environmental stress of high temperature.

The protein C-phycocyanin, an accessory photosynthetic pigment, has been isolated from extremely thermophilic organisms and studied by MacColl *et al.* (1974) and Bernis & Scott (1966). We have extended their observations to the study of *Cyanidium caldarium*, a unicellular eukaryotic organism of uncertain classification, which exists in acid, thermal habitats (Castenholtz, 1969; Brock & Brock, 1966). In the present paper we study the C-phycocyanin from *C. caldarium* (aggregation properties, visible absorption spectra, fluorescence spectra, circular dichroism, isoelectric point, amino acid composition and subunit structure) and compare our results with those obtained for the protein isolated from mesophilic and other thermophilic sources. This is the first extensive report of the physical properties of C-phycocyanin from a eukaryotic alga.

In a review of the properties of several proteins isolated from various thermophilic micro-organisms, Singleton & Amelunxen (1973) were unable to suggest a consistent molecular basis for thermophily. A study of the same protein from different thermophilic sources should help to answer this question, and C-phycocyanin offers unusual advantages in such an investigation. It is reported (Myers & Kratz, 1955) at times to constitute 25% of the total algal weight, and it plays an essential role in energy transfer (Teale & Dale, 1970) and electron movement (Ilani & Bernis, 1972). The phycobilisome structure (Edwards & Gantt, 1971), which is apparently essential for the physiological function of C-phycocyanin, requires specific protein-protein interactions. At least a portion of this interaction is retained *in vitro* in the smaller aggregates that have been isolated and purified. The characterization of these interactions and aggregates,

which are most probably intermediates in the complicated process leading to phycobilisome formation, has led to the proposal of a model to explain the phycobilisome structure (Edwards & Gantt, 1971).

Experimental

C. caldarium was isolated from an acid thermal area of Yellowstone National Park by Dr. W. N. Doemel of Wabash College, Crawfordsville, Ind., U.S.A. Unialgal cultures were grown in our laboratory in Ascione's medium (Ascione *et al.*, 1966) at pH 2–3 and 50–52°C. Florence flasks containing 10 litres of this culture medium were each inoculated with a dense suspension of cells to give a final concentration of 1×10^6 – 5×10^6 cells/ml. The culture was agitated with a magnetic stirrer, and $N_2 + CO_2$ (95:5) (Matheson Corp., Rutherford, N.J., U.S.A.) was bubbled into the flask at a steady rate of about 3 ml/min. The culture was illuminated with daylight fluorescent bulbs and tungsten lamps at 2200–5400 lx. Cells were generally harvested after 10–15 days in the late-exponential stage of growth. In some cases where harvesting was done after only 5–6 days, the results did not show any detectable difference in the characteristics or quantity of phycocyanin.

Algal cells were disrupted by a Braun mechanical cell homogenizer, model MSK (Bronwill Scientific, Rochester, N.Y., U.S.A.). About 6–8 g of cells were suspended in 30 ml of sodium phosphate buffer, pH 6.0, 1.0 mol/l, and transferred to a 60 ml Braun Melsungen glass flask containing 6–8 g of glass beads in a mixture of sizes (0.25, 0.45 and 1.0 mm diam.). The homogenizer was chilled with liquid CO_2 , and cell disintegration was continued in periods of 1–2 min for a total of 10–15 min. The supernatants were

separated from the glass beads and cell membranes by repeated centrifugation at 30 min intervals for a total of 4–6 h in the SS34 rotor of an RC-2B Sorvall centrifuge at 18000g at about 4°C. The clear blue supernatant was treated with 2% (w/v) protamine sulphate solution for 30 min and centrifuged at 18500g for 15 min. The supernatant solution was then adjusted to 50% (w/v) saturation with crystalline $(\text{NH}_4)_2\text{SO}_4$ at pH 6.0, and the phycocyanin was precipitated. The phycocyanin fraction was then dialysed repeatedly into 30%-satd. $(\text{NH}_4)_2\text{SO}_4$ in sodium phosphate buffer, pH 6.0, 10.1 mol/l, followed by extensive centrifugation of the purer phycocyanin fraction at 20000g at 30 min intervals for a total of 3 h or more. This procedure was continued until the E_{620}/E_{280} ratio of phycocyanin in phosphate buffer was 4.0 or greater. Purification by the same procedure but excluding the protamine sulphate treatment yielded an identical phycocyanin, but this treatment accelerated the process significantly. C-Phycocyanin from *Plectonema calothricoides* was purified as reported by Bernis & Morgenstern (1966).

Isoelectric focusing of crude C-phycocyanin extracts and purified C-phycocyanin was performed on a 110-ml LKB Ampholine column (8101) with a sucrose gradient as a stabilizing system (Haglund, 1971). A narrow-range (pH 4.0–6.0) synthetic ampholyte (LKB Instruments, Bromme, Sweden) was used for this study; the pH was measured on a Radiometer PHM 63 digital pH-meter. About 6 mg of protein was used on the column. Concentrations of phycocyanin were estimated from the extinction coefficient at 620 nm, $E_{1\text{cm}}^{1\%} = 60$, in sodium phosphate buffer, pH 6.0, 10.1 mol/l. Absorption for each fraction was determined on a Gilford model 2400 spectrophotometer.

In general, analytical ultracentrifugation, amino acid analysis and fluorescence measurements were performed as described (Kao *et al.*, 1973). A Hamamatsu TV photomultiplier tube, R446 selected, was used in the spectrofluorimeter. Absorption spectra were measured on a Cary 14 spectrophotometer and c.d. (circular-dichroism) spectra on a Cary 61 CD spectropolarimeter, both at room temperature (approx. 23°C).

Sedimentation-velocity studies were designed to provide information on quaternary structure at high protein concentrations (≥ 10 mg/ml), at a low protein concentration (0.02 mg/ml), at room temperature (23°C), at the normal growth temperature of the alga (50°C) and over a range of pH values (3.9–8.0).

Sodium dodecyl sulphate–polyacrylamide-gel electrophoresis was performed by the method of Weber & Osborn (1969).

Results

The amino acid composition of C-phycocyanin from *C. caldarium* as listed in Table 1 is based on a

molecular weight of 30000, the weight of the 3S monomer for other C-phycocyanins as determined by sedimentation equilibrium at pH 3.9 (Kao *et al.*, 1971). The partial specific volume, 0.731, was calculated from the amino acid composition by the method of Cohn & Edsall (1943). Table 2 collates our data on amino acid composition with those first reported for C-phycocyanin from this organism by Bernis *et al.* (1964) and with results for another thermophile, *S. lividus*, a halophile, *C. elabens*, and a mesophile, *P. calothricoides*. The differences between the two *C. caldarium* C-phycocyanins may be indicative of differences between strains of the same organism.

Like other phycocyanins (Glazer & Cohen-Bazire, 1971; Bennett & Bogorad, 1971; O'Carra & Killilea, 1971), C-phycocyanin from *C. caldarium* forms two blue bands on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. The molecular weights of these two polypeptide chains were determined by the relative mobilities of these chains compared with those of several proteins of known molecular weight. Proteins used as standards were bovine serum albumin (mol.wt. 65000), ovalbumin (43000), pepsin (35000), trypsin (23500), chymotrypsinogen (25000), ribonuclease (13700) and cytochrome *c* (11700). The relative mobilities of the calibrating proteins were fitted by a regression analysis of a least-squares plot with the resulting equation:

$$\log(\text{mol.wt.}) = (5.071 \pm 0.029) - (1.223 \pm 0.052) (\text{relative mobility})$$

Table 1. Amino acid composition of phycocyanin from *C. caldarium*

Results are averages of determinations (one 24 h, one 48 h and one 72 h hydrolysate), except that serine and threonine values are extrapolated to zero time. Half-cystine was measured as cysteic acid (Spencer & Wold, 1969).

Amino acid	Content (% w/w)
Asp	10.80 ± 0.10
Thr	5.15
Ser	5.91
Glu	11.17 ± 0.05
Pro	3.16 ± 0.03
Gly	4.08 ± 0.01
Ala	8.28 ± 0.07
½ Cys	1.88 ± 0.10
Val	6.23 ± 0.20
Met	3.36 ± 0.08
Ile	5.50 ± 0.18
Leu	9.20 ± 0.06
Tyr	7.14 ± 0.04
Phe	4.53 ± 0.05
Lys	4.75 ± 0.10
His	0.87 ± 0.03
Arg	9.53 ± 0.22
Total	101.54

Table 2. Amino acid composition of C-phycocyanins

Each analysis is based on mol.wt. 30000. Half-cystine was determined as cysteic acid (Spencer & Wold, 1969).

	Number of residues				
	<i>Cyanidium caldarium</i> (This study)	<i>Cyanidium caldarium</i> (Berns <i>et al.</i> , 1964)	<i>Coccochloris elabens</i> (Kao <i>et al.</i> , 1973)	<i>Synechococcus lividus</i> (Sy I) (MacColl <i>et al.</i> , 1974)	<i>Plectonema calothricoides</i> (Berns <i>et al.</i> , 1964)
Polar residues					
Acidic					
Asp	28	33	30	27	30
Glu	25	28	29	30	19
Basic					
Lys	11	10	9	10	11
His	2	1	2	3	2
Arg	18	17	17	20	15
Hydroxy					
Thr	15	14	15	14	15
Ser	19	13	14	17	17
Non-polar residues					
Aliphatic					
Gly	21	22	21	21	21
Ala	34	47	32	41	43
Val	18	18	15	16	17
Ile	14	17	10	15	15
Leu	24	25	22	26	24
Pro	10	11	8	11	9
Met	8	8	8	6	9
½Cys	6	2	2	6	2
Tyr	13	11	11	12	11
Phe	9	7	7	9	9

Fig. 1. Absorption spectrum of C-phycocyanin from *C. caldarium* in phosphate buffer, pH6.0, 10.1 mol/l, concn. 0.14 mg/ml

For details see the text.

Fig. 2. Fluorescence excitation, emission and polarization spectra of C-phycocyanin from *C. caldarium*

The excitation spectrum (—) was obtained by monitoring emission at 670 nm and the emission spectrum (----) by exciting at 620 nm. Polarization values (○) were calculated as described by Chen & Bowman (1965).

In one experiment, where the relative mobilities of two chains were 0.702 and 0.796, the calculated molecular weights were 16290 ± 800 and 12500 ± 630 respectively. The average of five experiments suggested molecular weights of 16500 ± 800 and 12500 ± 600 .

The absorption spectrum of C-phycoerythrin from *C. caldarium* (Fig. 1) at pH 6.0, $I_{0.1}$, is similar to that of C-phycoerythrin from *P. calothricoides*; the absorption maximum in the visible range is at 624 nm. The band is narrower at half-height for *C. caldarium* phycoerythrin than for *P. calothricoides* phycoerythrin (56 nm versus 80 nm at $E_{620} = 0.82$). The fluorescence spectra for C-phycoerythrin from *C. caldarium* (Fig. 2) are also very similar to those for C-phycoerythrins from other mesophiles. The single symmetrical emission peak at about 650 nm and the excitation maximum at 625 nm, with shoulders at 580 and 540 nm, are characteristic. The c.d. spectra at pH 6.0 and 8.0

Fig. 3. C.d. spectra of C-phycoerythrin from *C. caldarium*. Spectra were obtained with a 10 mm-light-path cuvette from 700 nm to 520 nm and with a 0.5 mm-light-path cuvette from 250 nm to 195 nm. —, In phosphate buffer, pH 6.0, $I_{0.1}$ mol/l, concn. 0.13 mg/ml. Sample consists of 33% 19S species, 28% 11S species and 39% 6S species. ----, In phosphate buffer, pH 8.0, $I_{0.1}$ mol/l, concn. 0.12 mg/ml. Sample consists of 75% 6S species.

are shown in Fig. 3. As the pH increased, the maximum in the visible portion shifted from 626 to 618 nm, with a shoulder at 570 nm.

Isoelectric focusing of crude extracts of C-phycoerythrin from *C. caldarium* produced separate bands with pI values of 4.75, 5.10, 5.27 and 5.50 (Fig. 4a). The purified protein, however, retained only the major band at pI 5.11 (Fig. 4b). However, if the C-phycoerythrin was dialysed first in sodium carbonate buffer, pH 9.0, $I_{0.1}$ mol/l, for 2 h and then in sodium phosphate buffer, pH 6.0, $I_{0.1}$ mol/l, for 2.5 h, a major band at pI 4.69 was formed (Fig. 5).

Quaternary structure was extensively examined by using fluorescence-polarization and sedimentation-velocity measurements. Owing to internal energy transfer among tetrapyrrole chromophores, an inverse relationship exists between aggregate size and fluorescence polarization (Vernotte, 1971; Goedheer & Birnie, 1965). Fluorescence-polarization measurements (Chen & Bowman, 1965) over a range of pH values are given in Fig. 6 for C-phycoerythrin from *C. caldarium* and from the mesophilic *P. calothricoides*.

The concentration-dependence of the sedimentation coefficients was investigated with sodium phosphate buffer, pH 6.0, $I_{0.1}$ mol/l. The $s_{20,w}^0$ values were 5.85, 10.75 and 17.30S. However, the usual descriptions are retained in this study: 3S (monomer), 6S (trimer), 11S (hexamer) and 19S (tentatively dodecamer). The 19S peak in all sedimentation-velocity studies was asymmetric, and measurements demonstrated that in addition to a 17.30S peak, there was a peak between 19.7 and 19.2S (Fig. 7).

Fig. 4. Isoelectric focusing of C-phycoerythrin from *C. caldarium*

----, pH of samples; —, E_{620} of samples. Numbers on peaks are pI values. (a) About 3 mg of C-phycoerythrin crude extract in sodium phosphate buffer, pH 6.0, $I_{0.1}$ mol/l. (b) About 6 mg of purified C-phycoerythrin, $E_{620}/E_{280} \geq 4$, in sodium phosphate buffer, pH 6.0, $I_{0.1}$ mol/l.

Fig. 5. Isoelectric focusing of *C-phyco*cyanin from *C. caldarium* after prolonged exposure at pH9.0

----, pH of sample; —, E_{620} of sample. The sample was dialysed in sodium carbonate buffer, pH9.0, 1.0 mol/l, for 2 h and then in sodium phosphate buffer, pH6.0, 1.0 mol/l, for 2.5 h. About 6 mg of the final sample in phosphate buffer, pH6.0, 1.0 mol/l, was put on an isoelectric-focusing column. Number on peak is pI value.

Fig. 6. Fluorescence polarization of *C-phyco*cyanin from *C. caldarium* (○) and *P. calothricoides* (Δ)

$P_{\text{nm(max.)}} = (I_{\text{vv}} - GI_{\text{vh}})/(I_{\text{vv}} + GI_{\text{vh}})$. P is the polarization at the wavelength of maximum extinction. I_{vv} and I_{vh} are the measured fluorescence intensity with the two analysers vertically and horizontally oriented. Correction factor G is defined as $I_{\text{hv}}/I_{\text{hh}}$. The concentration of the samples is in the range 0.038–0.04 mg/ml.

Sedimentation properties as a function of pH at high *C-phyco*cyanin concentrations are shown in Table 3. Even with the lengthy purification procedure, the faster-sedimenting aggregates (19S) of *C-phyco*cyanin from *C. caldarium* are extremely stable over the entire pH region examined. The relative amount of 6S aggregates increases with the increase of pH

Fig. 7. Sedimentation-velocity patterns of *C-phyco*cyanin from *C. caldarium* at 59780 rev./min

Sedimentation is from left to right. Concentration is approximately 10 mg/ml. The AND rotor was used at 23°C, and the ANH rotor at 50°C. (a) 23°C, after 24 min; (b) 23°C, after 32 min; (c) 50°C, after 24 min; (d) 50°C, after 28 min.

Table 3. Sedimentation properties of *C. caldarium C-phyco*cyanin at high concentrations at 23°C

A model E analytical ultracentrifuge with a schlieren optical system was used. Concentrations were approx. 10 mg/ml.

Buffer ($I = 0.1 \text{ mol/l}$)	Relative percentage of species present*		
	19S	11S	6S
Sodium acetate, pH3.9	15	85	—
pH4.7	64	36	—
Sodium phosphate, pH5.4	59	41	—
pH6.0	46	19	35
pH7.0	46	10	44
pH8.0	36	14	50

* Estimated from the area under the appropriate schlieren patterns.

value, and it eventually becomes the predominant species at pH8.0. Studies performed at low concentrations over a pH range are presented in Table 4. Even at 0.1 mg/ml a remarkable amount of the largest aggregates was retained at pH4.6, 6.0 and 7.0. The relative percentage of each species was estimated from the extinction of each migrating species. However, the higher aggregates (15.4–16.5S) species are not very stable at these low phycocyanin concentrations. Dissociation occurs when samples are left at room temperature (23°C) for 4–5 h or in a cold-room (4°C) for 24 h.

Table 4. *Sedimentation properties of C. caldarium C-phycocyanin at low concentrations at 23°C*

Concentrations were 0.1–0.15 mg/ml. Data were gathered by using the photoelectric scanner optics on the model E analytical ultracentrifuge, monitored at 620 nm.

pH	(S)	Relative percentage
3.9	9.3	66.5
	3.2	33.4
4.6	15.4	43
	11.7	57
6.0	15.5	33
	10.6	28
	5.0	39
7.0	16.5	33
	11	Trace
	5.93	67
8.0	6.3	>75

Table 5. *Concentration-dependence of C-phycocyanin aggregates from C. caldarium at 23°C*

Data were gathered by using the photoelectric-scanner optics on the model E analytical ultracentrifuge, monitored at 620 nm.

Buffer	Concentration (mg/ml)	Relative percentage of species present			
		19S	11S	6S	3S
Sodium acetate	0.15	—	67	—	33
(pH 3.9,	0.11	—	81	—	19
10.1 mol/l)	0.03	—	76	—	25
Sodium phosphate	0.12	33	28	39	—
(pH 6.0,	0.11	27	33	40	—
10.1 mol/l)	0.08	24	34	42	—
	0.06	23	34	43	—
	0.04	—	30	70	—
	0.02	—	—	>85	—

Two pH values, 3.9 and 6.0, were selected to study the concentration-dependence of the sedimentation-velocity patterns. At pH 6.0 (Table 5) the relative percentages of 19, 11 and 6S species remained essentially constant from 0.12 to 0.06 mg/ml. There was no monomeric species, even at 0.02 mg/ml. At pH 3.9, studies at concentrations below 0.1 mg/ml were extremely difficult to perform reproducibly (Table 5). Disaggregation to the monomer form was not complete even at 0.03 mg/ml, and the concentration of 3S species did not appear to change from 0.15 to 0.03 mg/ml.

Sedimentation-velocity studies were performed at high protein concentrations, pH 6.0, at 23° and 50°C (Table 6). The only apparent changes were a negligible increase in 19S, a 10% increase in 11S and a 14% decrease in 6S species. Similar studies at 0.14 mg/ml

Table 6. *Sedimentation of C. caldarium phycocyanin at two temperatures*

Data were gathered by using the schlieren optical system. Sample concentrations were approx. 10 mg/ml and the percentage composition was calculated from the relative area under the schlieren pattern. The buffer was sodium phosphate, pH 6.0, 10.1 mol/l.

Temperature (°C)	Relative percentage of species present		
	19S	11S	6S
23	46	19	35
50	50	29	21

demonstrated little if any detectable change in the distribution of sedimenting species.

Discussion

Many characteristics of C-phycocyanin isolated from *C. caldarium*, a eukaryotic alga, appear identical with those of the several phycocyanins already studied from prokaryotic sources. It has two non-identical polypeptide chains, similar to those reported for other C-phycocyanins (Glazer & Cohen-Bazire, 1971; Bennett & Bogorad, 1971; O'Carra & Killilea, 1971; Torjesen & Sletten, 1972; Binder *et al.*, 1972). Recent studies (Troxler *et al.*, 1974; Harris & Bernis, 1975) indicate that the N-terminal sequences of the α - and β -polypeptide chains are in close agreement with those of the two chains isolated from other C-phycocyanins. The absorption and fluorescence properties are also quite similar to those of other C-phycocyanins (Chapman, 1973).

The c.d. behaviour of *C. caldarium* C-phycocyanin (Fig. 3) suggests that the longer-wavelength band is associated with larger aggregates (19S and perhaps 11S) and that the shorter-wavelength band is enhanced by the presence of 6S species. Pecci & Fujimori (1969) observed the same shift for mesophilic phycocyanin and suggested that two separate chromophores are responsible for the two optically active bands. When the dependence of the c.d. spectra of *C. caldarium* C-phycocyanin on concentration was studied over the protein range 0.02–0.14 mg/ml at pH 6.0 in phosphate buffer, ellipticity at 626 and 570 nm decreased with decreasing concentration, and no isosbestic point was formed. This was consistent with our ultracentrifugation results (Table 5), since the aggregation states at these concentrations were predominantly 11S and 19S. This is very different from the c.d. spectrum of *Synechococcus* sp. (*Anacystis nidulans*) phycocyanin reported by Glazer *et al.* (1973), in which an isosbestic point was reported at pH 7.0. Present evidence suggests that the chromophore environment of *C. caldarium* C-phycocyanin is affected by the state of aggregation.

The ability of C-phycocyanin to exist *in vitro* as a number of different aggregates has been well studied for several mesophilic algae (Berns, 1971; Neufeld & Riggs, 1969; MacColl *et al.*, 1971; MacColl & Berns, 1973). The equilibrium between 6S and 11S aggregates under defined conditions of temperature, ionic strength and pH appears to be reasonably constant for all phycocyanins tested. However, when the same protein is isolated from an alga that grows at an environmental extreme, the aggregation properties are modified, in some cases in a manner identifiable with the stress. To maintain a state of aggregation similar to that of C-phycocyanins from mesophiles, C-phycocyanin from a halophilic alga requires a high NaCl concentration (Kao *et al.*, 1973), and C-phycocyanin isolated from one strain of *Synechococcus lividus*, a thermophilic alga, requires a high temperature (Berns & Scott, 1966).

Several studies have characterized C-phycocyanin as an extrinsic membrane protein found in large aggregates called phycobilisomes, which exist in thermophiles as well as in mesophiles (Gantt & Conti, 1966; Edwards *et al.*, 1968; Gray *et al.*, 1973). Since aggregating properties appear to be essential for phycobilisome assembly, studies of natural perturbations of the aggregation process in *C. caldarium* may produce valuable information for understanding the intrinsic forces stabilizing the thermophilic protein.

C-Phycocyanin from *C. caldarium* has remarkably large amounts of large aggregates (19S) at both high and low concentrations and a wide range of pH values (Tables 3 and 4). This is confirmed by the unusually low fluorescence-polarization values (Fig. 6). Its disaggregation properties are distinctly different from those reported for mesophilic C-phycocyanin (Berns, 1971; MacColl *et al.*, 1971) and a halophilic C-phycocyanin (Kao *et al.*, 1973). At pH 6.0 it has not been possible to disaggregate this protein completely to the 3S monomer at the lowest concentrations investigated. The temperature-dependence of the aggregation property is also distinctive, although it is similar to that for C-phycocyanin from one strain (Sy I) of the thermophile *S. lividus* (R. MacColl, unpublished work). The isoelectric point, 5.11, of the purified C-phycocyanin is higher than any reported to date (MacColl *et al.*, 1974; Kao *et al.*, 1973; Glazer & Fang, 1973). This high isoelectric point may be related to the protein's different charge characteristics.

Both of the amino acid analyses of *C. caldarium* C-phycocyanin listed in Table 2 indicate a higher glutamic acid plus glutamine content than for certain mesophiles. However, its amino acid contents are very similar to those of C-phycocyanin from the thermophilic *S. lividus* and the halophilic *C. elabens*. The aggregation properties of C-phycocyanin from *S. lividus* (Sy I; MacColl *et al.*, 1974) are similar in

many respects to those reported in the present study. It would be tempting to assert, as we did for *S. lividus*, the possible importance of glutamic acid substitutions, using as analogies the work on enolase (Barnes & Stellwagen, 1973) and ferredoxin (Tanaka *et al.*, 1971) from thermophilic sources. There is, however, an important difference: the very large deviation in the isoelectric point with *C. caldarium* C-phycocyanin, an increase of almost 0.4 unit over other reported values for the native protein (MacColl *et al.*, 1974; Kao *et al.*, 1973; Glazer & Fang, 1973). A change of this magnitude can be explained either by a significant increase in the number of basic residues or by a decrease in the number of acid residues. The amino acid data are not consistent with the former explanation, since the total number of basic residues is essentially the same as that for other C-phycocyanins.

Although the total glutamic acid plus glutamine and aspartic acid plus asparagine content does not decrease by virtue of a simple substitution, the increase in the glutamine and/or asparagine content may be at the expense of a decrease in glutamic acid and/or aspartic acid. There could also be a greater inclusion of aspartic acid and/or glutamic acid residues in the protein-protein contact areas that are effectively shielded from the water phase. The pI decrease from 5.11 to 4.69 after partial disaggregation owing to prolonged exposure at pH 9.0 indicates that the latter explanation is more likely. The sedimentation behaviour on dilution or increase in temperature indicates the existence of stronger interactions, which may be more polar than in the mesophilic phycocyanins. The fact that at pH 6.0, 10.1 mol/l, the monomeric species is not seen at concentrations as low as 0.02 mg/ml is also consistent with the presence of greater charge forces stabilizing the aggregates.

It is important to consider that apparent inconsistencies between pH and proportion of 19 and 11S components as shown in Table 3 when the pH is raised from 3.9 to 4.7 could be related to the proportion of undissociated acetic acid in the buffer (Chiancone & Gilbert, 1965). The alteration in protein configuration after penetration by acetic acid may explain inconsistencies in other measurements.

The stabilized higher state of aggregation in C-phycocyanin from *C. caldarium* and its similarity to that of C-phycocyanin from *S. lividus* (Sy I) may reflect a specific adaptation mechanism. The study by MacColl *et al.* (1974) substantiates the possibility of two general modes of adaptation for thermophily: (a) a requirement for high temperature, as for the thermophilic *S. lividus* (Sy III; Berns & Scott, 1966); or (b) an increase in protein stability to changes in pH, temperature and concentration, thus discouraging denaturation of the protein. The adaptation of *C. caldarium* phycocyanin to high temperature seems to be more consistent with the latter mechanism.

We acknowledge with thanks the excellent technical assistance of Mrs. Martha Mulks and Miss Olga Gibbons. We also thank Dr. Robert MacColl for his valuable comments on the manuscript. This work was supported in part by grants from the National Science Foundation (GB 24315) and the National Institutes of Health (AI 08119-05).

References

- Ascione, R., Southwick, W. & Fresco, J. R. (1966) *Science* **153**, 752-754
- Barnes, L. D. & Stellwagen, E. (1973) *Biochemistry* **12**, 1559-1565
- Bennett, A. & Bogorad, L. (1971) *Biochemistry* **10**, 3625-3634
- Bernis, D. S. (1971) in *Biological Macromolecules* (Timasheff, S. N. & Fasman, G., eds.), vol. 5A, pp. 105-148, Marcel Dekker, New York
- Bernis, D. S. & Morgenstern, A. (1966) *Biochemistry* **5**, 2985-2990
- Bernis, D. S. & Scott, E. (1966) *Biochemistry* **5**, 1528-1533
- Bernis, D. S., Scott, E. & O'Reilly, K. T. (1964) *Science* **145**, 1054-1056
- Binder, A., Wilson, K. & Zuber, H. (1972) *FEBS Lett.* **20**, 111-116
- Brock, T. D. & Brock, M. L. (1966) *Nature (London)* **209**, 733-734
- Castenholz, R. W. (1969) *Bacteriol. Rev.* **33**, 476-504
- Chapman, D. J. (1973) in *The Biology of Blue-Green Algae* (Carr, N. G. & Whitton, B. A., eds.), vol. 9, p. 162, Botanical Monographs, University of California Press, Berkeley, California
- Chen, R. F. & Bowman, R. L. (1965) *Science* **147**, 729-732
- Chiancone, E. & Gilbert, G. A. (1965) *J. Biol. Chem.* **240**, 3866-3867
- Cohn, E. J. & Edsall, J. T. (1943) in *Proteins, Amino Acids and Peptides* (Cohn, E. J. & Edsall, J. T., eds.), p. 370, Reinhold Publishing Co., New York
- Edwards, M. R. & Gantt, E. (1971) *J. Cell Biol.* **50**, 896-900
- Edwards, M. R., Bernis, D. S., Ghiorse, W. C. & Holt, S. C. (1968) *J. Phycol.* **4**, 283-298
- Gantt, E. & Conti, S. F. (1966) *Brookhaven Symp. Biol.* **19**, 393-405
- Glazer, A. N. & Cohen-Bazire, G. (1971) *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1398-1401
- Glazer, A. N. & Fang, S. (1973) *J. Biol. Chem.* **248**, 663-671
- Glazer, A. N., Fang, S. & Brown, D. M. (1973) *J. Biol. Chem.* **248**, 5679-5685
- Goedheer, J. C. & Birnie, F. (1965) *Biochim. Biophys. Acta* **94**, 579-581
- Gray, B. H., Lipschultz, C. A. & Gantt, E. (1973) *J. Bacteriol.* **116**, 471-478
- Haglund, H. (1971) *Methods Biochem. Anal.* **19**, 1-104
- Harris, J. & Bernis, D. S. (1975) *J. Mol. Evolution* in the press
- Ilani, A. & Bernis, D. S. (1972) *J. Membrane Biol.* **8**, 333-356
- Kao, O., Bernis, D. S. & MacColl, R. (1971) *Eur. J. Biochem.* **19**, 595-599
- Kao, O. H. W., Bernis, D. S. & Town, W. R. (1973) *Biochem. J.* **131**, 39-50
- MacColl, R. & Bernis, D. S. (1973) *Arch. Biochem. Biophys.* **156**, 161-167
- MacColl, R., Lee, J. J. & Bernis, D. S. (1971) *Biochem. J.* **122**, 421-426
- MacColl, R., Edwards, M. R., Mulks, M. H. & Bernis, D. S. (1974) *Biochem. J.* **141**, 419-425
- Myers, J. & Kratz, W. A. (1955) *J. Gen. Physiol.* **39**, 11-22
- Neufeld, G. J. & Riggs, A. F. (1969) *Biochim. Biophys. Acta* **181**, 234-243
- O'Carra, P. & Killilea, S. D. (1971) *Biochem. Biophys. Res. Commun.* **45**, 1192-1197
- Pecci, J. & Fujimori, E. (1969) *Biochim. Biophys. Acta* **188**, 230-236
- Singleton, R., Jr. & Amelunxen, R. E. (1973) *Bacteriol. Rev.* **37**, 320-342
- Spencer, R. L. & Wold, F. (1969) *Anal. Biochem.* **32**, 185-190
- Tanaka, M., Haniu, M., Matsueda, G., Yasunobu, K. T., Himes, R. H., Akagi, J. M., Barnes, E. M. & Devanathan, T. (1971) *J. Biol. Chem.* **246**, 3953-3960
- Teale, F. W. J. & Dale, R. E. (1970) *Biochem. J.* **116**, 161-169
- Torjesen, P. A. & Sletten, K. (1972) *Biochim. Biophys. Acta* **263**, 258-271
- Troxler, R. F., Brown, A., Foster, J. A. & Franzblau, C. (1974) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **33**, 1446
- Vernotte, C. (1971) *Photochem. Photobiol.* **14**, 163-173
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406-4412