Phycoerythrocyanin and Phycoerythrin: Properties and Occurrence in Cyanobacteria

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A number of cyanobacterial phycoerythrocyanins were isolated and characterized with respect to spectroscopic properties and chromophore content. All were similar to the phycoerythrocyanin of *Anabaena* PCC 6411 and cross-reacted strongly with an antiserum prepared against that protein. The synthesis of phycoerythrocyanin was not controlled by a complementary chromatic adaptation mechanism in nine strains tested, but its synthesis appeared to be affected by light intensity. A survey of 240 strains of cyanobacteria revealed that no strain synthesized both phycoerythrocyanin and phycoerythrin and that phycoerythrocyanin was largely confined to those strains which can form heterocysts. Phycoerythrocyanin may be a taxonomically useful marker for certain strain clusters of this cyanobacterial grouping. Spectroscopic properties and chromophore compositions of several cyanobacterial phycoerythrins were also determined. At present, the ability to synthesize phycoerythrin and the capacity to exhibit complementary chromatic adaptation responses are widely distributed traits of limited taxonomic usefulness.

INTRODUCTION

Phycobiliproteins are the major photosynthetic accessory pigments of cyanobacteria and red algae. These proteins are assembled into supramolecular complexes, phycobilisomes, which form regular arrays on the stromal surfaces of the thylakoid membranes of both groups of organisms (Gantt, 1980). On the basis of their long-wavelength visible absorption properties, the phycobiliproteins have been classified into several groups (Table 1).

Spectroscopic studies performed with isolated phycobilisomes have demonstrated that the phycobiliproteins constitute an energy transfer chain (Ley *et al.*, 1977; Searle *et al.*, 1978):

Within the cell, light energy trapped by the phycobiliproteins is transferred to chlorophyll *a* with an efficiency approaching 100% (Porter *et al.*, 1978).

Phycocyanin and allophycocyanin are universally present in cyanobacteria, whilst allophycocyanin B apparently occurs in most cyanobacteria (Glazer & Bryant, 1975; Ley *et al.*, 1977) but was not detected in the unicellular cyanobacterium *Gloeobacter violaceus* (Bryant *et al.*, 1981). Phycoerythrins are widely distributed among all taxonomic groupings and form the most spectroscopically variable class of phycobiliproteins (Stanier & Cohen-Bazire, 1977; Table 1). Many, but not all, cyanobacteria containing phycoerythrin undergo an adaptive phenomenon known as complementary chromatic adaptation (Bogorad,

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Table 1. *Distribution and properties of biliproteins*

Data were taken from Glazer (1977), Muckle & Rüdiger (1977), Glazer & Hixson (1977) and Nies & Wehrmeyer (1980). C and R indicate that the protein occurred in cyanobacteria and red algae, respectively. The exact positions of the absorption and fluorescence emission maxima vary depending upon the aggregation state of the protein and upon the organism which serves as the source of the protein. Abbreviations for the phycobilin chromophores are: PCB, phycocyanobilin; PEB, phycoerythrobilin; PXB, phycobiliviolin-type chromophore of undetermined structure.

Biliprotein	Distribution	Visible absorption maxima (nm)*	Fluorescence emission maximum (nm)	Subunit structure of stable assembly forms	Chromophore content of the promoter ($\alpha\beta$)
Allophycocyanin B	C, R	671>618	675	($\alpha\beta$) ₃	2 PCB
Allophycocyanin	C, R	650	660	($\alpha\beta$) ₃	2 PCB
C-phycocyanin	C, R	620	640	($\alpha\beta$) ₃ ; ($\alpha\beta$) ₆	3 PCB
R-phycocyanin	R	617>555	636	($\alpha\beta$) ₃ ; ($\alpha\beta$) ₆	2 PCB; 1 PEB
Phycoerythrocyanin	C	570>595(s)	625	($\alpha\beta$) ₃ ; ($\alpha\beta$) ₆	2 PCB; 1 PXB
C-phycoerythrin	C	560	577	($\alpha\beta$) ₃ ; ($\alpha\beta$) ₆	5-6 PEB
b-phycoerythrin	R	545>563(s)	570	($\alpha\beta$) _n	6 PEB
B-phycoerythrin	R	545>563>498(s)	575	($\alpha\beta$) ₆ γ	6 PEB†
R-phycoerythrin	C, R	565>540>498	578	($\alpha\beta$) ₆ γ	ND‡

ND, Not determined.

* (s), shoulder.

† The γ subunit of *Porphyridium cruentum* B-phycoerythrin carries two phycoerythrobilins and two phycourobilin chromophores.

‡ *Cryptonemia obovata* R-phycoerythrin carries 31 phycoerythrobilin and 9 phycourobilin chromophores per ($\alpha\beta$)₆ γ .

1975). Tandeau de Marsac (1977) demonstrated that two responses can occur: either phycoerythrin synthesis alone, or phycoerythrin and phycocyanin synthesis is controlled in response to the light wavelength incident during growth.

Phycoerythrocyanin is a cyanobacterial phycobiliprotein functionally similar to phycoerythrin but bearing some structural similarities to phycocyanin (Bryant *et al.*, 1976, 1978; Fueglistaller *et al.*, 1980). The absorption properties of phycoerythrocyanin can effectively extend to shorter wavelengths the light-harvesting range of organisms containing this biliprotein (Table 1). In confirmation of its role as a light-harvesting accessory pigment, phycoerythrocyanin has been shown to be present in the phycobilisomes of *Anabaena* PCC 6411 (Bryant *et al.*, 1976) and *Fischerella* PCC 7603 (Nies & Wehrmeyer, 1980) [*Fischerella* PCC 7603 = *Mastigocladus laminosus* Cohn (Rippka *et al.*, 1979)].

Although no systematic search had been made, it was suggested that phycoerythrocyanin had a limited distribution among the cyanobacteria (Bryant *et al.*, 1976). This report describes the results of screening 240 strains of cyanobacteria for the presence of phycoerythrocyanin and/or phycoerythrin. The spectroscopic properties of several phycoerythrocyanins and phycoerythrins are described. The survey of Tandeau de Marsac (1977), concerning the distribution and pattern of chromatic adaptability among cyanobacteria which synthesize phycoerythrin, was extended to include recently isolated strains and some strains containing phycoerythrocyanin. The taxonomic significance of the occurrence of phycoerythrocyanin and phycoerythrin, and of chromatic adaptation, among cyanobacteria is discussed. Some preliminary results of this screening were reported by Rippka *et al.* (1979).

METHODS

Strains and culture conditions. Axenic strains of cyanobacteria were obtained from the Pasteur Culture Collection (PCC; Rippka *et al.*, 1979; Rippka & Stanier, 1979). A few strains contained unpigmented bacterial contaminants (Tables 2, 3 and 4). Unless specified, all cultures were grown photoautotrophically at 1.0 to 2.0×10^2

erg cm⁻² s⁻¹ under white fluorescent lamps (Osram Universal). Freshwater strains were grown in medium BG-11 (Stanier *et al.*, 1971) and strains of marine origin were grown in medium MN or ASN-III (Rippka *et al.*, 1979).

Materials. Electrophoresis-grade acrylamide and bisacrylamide were purchased from Serva Feinbiochemica, Heidelberg, F.R.G., and from Eastman Organic Chemicals, respectively; ampholytes (40% w/v; Bio-Lyte) from Bio-Rad; streptomycin sulphate from Merck; and pre-swollen Whatman microgranular DEAE-cellulose DE-52 from Whatman Biochemicals. All other chemicals were reagent grade.

Chromatic adaptation studies. Comparative studies of the effect of light wavelength on pigmentation were performed as described by Tandeau de Marsac (1977). Culture conditions, light intensities, pigment extractions and the optical properties of the filters used were the same as previously described (Tandeau de Marsac, 1977).

Spectroscopic measurements. Absorption spectra were measured with a Cary Model 17 recording spectrophotometer. Phycobiliprotein concentrations were estimated spectrophotometrically using previously published absorption coefficients (Bryant *et al.*, 1976, 1979).

Polyacrylamide gel electrophoresis and isoelectric focusing. Electrophoresis at pH 7.5 on 5% (w/v) polyacrylamide gels (0.6 × 10 to 12 cm) was performed as described by Cohen-Bazire *et al.* (1977). Isoelectric focusing was performed on photopolymerized 7.0% (w/v) polyacrylamide gels in the pH range 4.0 to 6.0, according to the procedure of Bio-Rad Laboratories (Technical Bulletin 1030, April, 1975). For some experiments, samples were mixed with the acrylamide solution before photopolymerization.

Immunological studies. Rabbit antiserum against *Anabaena* PCC 6411 phycoerythrocyanin and *Synechocystis* PCC 6808 phycoerythrin were obtained as described by Glazer *et al.* (1971) and Bryant *et al.* (1976). Immunodiffusion experiments were performed as described by Glazer & Bryant (1975).

Preparation of cell extracts. All buffers contained 2 mM-azide and, unless specified, all operations were performed at room temperature.

Cells were washed and suspended in 10 mM-sodium acetate buffer pH 5.5 (about 0.3 to 0.5 g wet wt cells ml⁻¹) and disrupted by two passages through an Aminco French pressure cell operated at 140 MPa at 4 °C. Cell extracts were adjusted to contain 1% (w/v) streptomycin sulphate. The suspensions were incubated 15 min at 0 °C and precipitated membranes removed by centrifugation at 27000 g for 15 min. The supernatants were decanted and the pellets discarded. The supernatant was applied to a small column (1.5 × 3.0 cm) of DEAE-cellulose which had been pre-equilibrated with 10 mM-sodium acetate buffer pH 5.5. After washing the column with two column volumes of starting buffer, the phycobiliproteins were frontally eluted with 0.30 M-sodium acetate buffer pH 5.5. Solid ammonium sulphate was added to the eluate to 65% of saturation. The precipitates were stored at 4 °C for no more than 1 month before analysis.

Ammonium sulphate precipitates were collected by centrifugation for 10 min at 27000 g, resuspended in small volumes of 0.01 M-potassium phosphate buffer, pH 7.0, to yield total phycobiliprotein concentrations of 1 to 2 mg ml⁻¹, and exhaustively dialysed against the same buffer. The resulting protein solutions were suitable for analysis by isoelectric focusing or polyacrylamide gel electrophoresis at pH 7.5.

Purification and the detection of phycoerythrocyanin and phycoerythrin. Preparative polyacrylamide gel electrophoresis and preparative isoelectric focusing were effective methods for the purification of small amounts of phycoerythrocyanin and phycoerythrin. Larger amounts of *Chroococcidiopsis* PCC 7203 and *Fischerella* PCC 7603 phycoerythrocyanins were purified by chromatography of cell extracts on DEAE-cellulose and hydroxyapatite as previously described (Bryant *et al.*, 1976).

Isoelectric focusing allowed rapid and sensitive screening for the presence of phycoerythrocyanin and/or phycoerythrin. Samples (100 to 400 µg phycobiliprotein) of cell extract were subjected to isoelectric focusing in the pH range 4.0 to 6.0. Phycoerythrocyanin was easily distinguishable from phycoerythrin by several criteria. (1) Phycoerythrocyanin is reddish-purple in colour, whereas phycoerythrins are reddish-orange. (2) When excited by long-wavelength u.v. illumination, phycoerythrocyanin fluorescence is reddish-orange (approx. 610 to 625 nm; Bryant *et al.*, 1976; Nies & Wehrmeyer, 1980) whereas phycoerythrins fluoresce golden-yellow (approx. 575 to 580 nm; Glazer, 1977). (3) The isoelectric points of phycoerythrocyanins detected fell into a narrow pH range of about 5.2 to 5.6, as previously reported for two phycoerythrocyanins (Bryant *et al.*, 1976); phycoerythrins generally had more acidic isoelectric points (pH 4.5 to 5.1). (4) The reddish-coloured α subunit of phycoerythrocyanin was also frequently detected on the gels; this more basic subunit (pI approx. 6.0) was also a useful distinguishing feature. No phycoerythrins or phycoerythrin subunits were observed with isoelectric points greater than pH 5.5.

Further confirmation of the presence of phycoerythrocyanin or phycoerythrin was obtained by excising the coloured bands, eluting the proteins from the acrylamide gel and examining the absorption spectra of the eluted proteins. Eluted proteins were also examined with antibodies specific for phycoerythrocyanin and phycoerythrin. Antisera prepared against phycoerythrocyanins do not cross-react with phycoerythrins, and similarly, antisera prepared against phycoerythrins do not cross-react with phycoerythrocyanins (Bryant *et al.*, 1976; Bryant, 1977). For this study all identifications of phycoerythrocyanin-positive strains were confirmed by double-diffusion studies against rabbit antiserum to *Anabaena* PCC 6411 phycoerythrocyanin. Strong cross-reactions with this antiserum were observed for all phycoerythrocyanins.

Fig. 1. Absorption spectra (in 50 mM-sodium acetate buffer pH 5.5) of cyanobacterial phycoerythrocyanins and phycoerythrins, and the effect of light intensity on the phycoerythrocyanin content of *Chroococcidiopsis* PCC 7203. (a) Absorption spectra of *Chlorogloeopsis* PCC 6718 (—) and PCC strain 7702 (generic assignment unknown; ----) phycoerythrocyanins. (b) Absorption spectra of *Scytonema* PCC 7110 (—) and *Calothrix* PCC 7810 (----) phycoerythrocyanins. (c) Absorption spectra of whole cell extracts of *Chroococcidiopsis* PCC 7203 grown under high light intensity (4.0×10^3 erg cm⁻² s⁻¹; —) or low light intensity (1 to 2×10^2 erg cm⁻² s⁻¹; ----). At low light intensity the content of phycoerythrocyanin is approximately 12-fold higher (relative to the allophycocyanin content). (d) Absorption spectra of *Synechococcus* PCC 7822 phycoerythrin (—) and of the typical C-phycoerythrin of *Pseudanabaena* PCC 7409 (----). (e) Absorption spectra of *Gloeotheca* PCC 6909 (—) and *Oscillatoria* PCC 7823 (----) phycoerythrins. (f) Absorption spectra of *Pseudanabaena* PCC 7409 (—) and *Gloeotheca* PCC 6909 (.....) phycoerythrins in 0.8 M-urea at pH 3.0. Identical spectra, typical of phycoerythrobilin under these conditions (Glazer & Hixson, 1975), were also obtained with *Oscillatoria* PCC 7823 and *Synechococcus* PCC 7822 phycoerythrins.

RESULTS AND DISCUSSION

The absorption spectra of phycoerythrocyanins isolated from a wide range of cyanobacteria were remarkably similar (Figs 1 and 2). The long-wavelength absorption maxima of

Fig. 2. Absorption spectra of phycoerythrocyanins under non-denaturing and denaturing conditions. (a) Absorption spectra of *Chroococcidiopsis* PCC 7203 (—) and *Fischerella* PCC 7603 (= *Mastigocladus laminosus* Cohn; filled circles) phycoerythrocyanins in 50 mM-sodium acetate buffer pH 5.5. (b) Absorption spectra of *Chroococcidiopsis* PCC 7203 (—), *Fischerella* PCC 7603 (open circles) and *Calothrix* PCC 7810 (filled circles) phycoerythrocyanins in 8.0 M-urea at pH 3.0. The absorption spectra of these phycoerythrocyanins were identical to that obtained with *Anabaena* PCC 6411 phycoerythrocyanin under the same conditions (Bryant *et al.*, 1976) and indicate a relative chromophore content of two phycocyanobilins per phycobiliviolin-type chromophore.

these biliproteins varied within the narrow range 568 to 577 nm; shoulders were observed at 515 to 530 nm and 595 to 600 nm for all phycoerythrocyanins examined. However, the ratio of the absorbance at the maximum to that at the 595 to 600 nm shoulder varied significantly (Figs 1 and 2). As frequently only small amounts of protein were isolated, this variation may have been due to differences in the aggregation state caused by the low protein concentrations of some of the solutions. The absorption properties of biliproteins are known to vary considerably as a function of protein concentration and aggregation state (Glazer *et al.*, 1973; Glazer & Bryant, 1975; Glazer, 1976).

The spectroscopic properties of the chromophores of biliproteins can be studied free from the influence of the polypeptide chains when the proteins are denatured with acidic 8.0 M-urea (Glazer & Fang, 1973; Glazer & Hixson, 1975, 1977; Bryant *et al.*, 1976). The chromophore compositions of the phycoerythrocyanins of three organisms (*Fischerella* PCC 7603, *Calothrix* PCC 7810, and *Chroococcidiopsis* PCC 7203) were examined under these denaturing conditions. As shown in Fig. 2 (b), the absorption properties of these three proteins were identical when dissolved in 8.0 M-urea at pH 3.0. Moreover, these absorption spectra, with maxima at 333, 605 and 664 nm, were identical to that of *Anabaena* PCC 6411 phycoerythrocyanin under the same conditions (Bryant *et al.*, 1976). The phycoerythrocyanin of *Anabaena* PCC 6411 was shown to carry two phycocyanobilin chromophores on the β subunit and a phycobiliviolin-type chromophore of unknown structure on the α subunit (Bryant *et al.*, 1976). This chromophore distribution presumably applies to the phycoerythrocyanins described in this study as well.

All phycoerythrocyanins cross-reacted strongly with an antiserum prepared against *Anabaena* PCC 6411 phycoerythrocyanin (not shown). On the other hand, no phycoerythrocyanin tested gave any detectable cross-reaction with an antiserum prepared against *Synechocystis* PCC 6808 phycoerythrin. Thus, as found previously for other spectroscopic classes of biliproteins (Berns, 1967; Glazer *et al.*, 1971; Bryant, 1977), the antigenic properties of phycoerythrocyanin are strongly conserved.

A wide variety of cyanobacteria (*Anabaena* PCC 6411, *Nostoc* PCC 6720, *Aphanizomenon* spp., *Chlorogloeopsis* PCC 6718, and *Chroococcidiopsis* PCC strains 7203, 7431, 7432, 7433, 7436 and 7439) were tested for the ability to photocontrol phycoerythrocyanin synthesis through a complementary chromatic adaptation mechanism. No strain tested

Table 2. *Cyanobacterial strains which do not synthesize either phycoerythrin or phycoerythrocyanin*

The section and generic assignments are those of Rippka *et al.* (1979).

Generic assignment	Pasteur Culture Collection no.
	Section I
<i>Synechococcus</i>	6301, 6307, 6311, 6312, 6603, 6710, 6713, 6715, 6716, 6717, 6904, 6907, 6908, 6910, 6911, 7001, 7002, 7003, 7009, 7117, 7202, 73109, 7418, 7425, 7502, 7511, 7809
<i>Gloethece</i>	73017
<i>Synechocystis</i>	6308, 6702, 6714, 6803, 6804, 6805, 6806, 6902, 6905, 6906, 7005, 7008, 7201, 7509, 7719, 7813
<i>Microcystis</i>	7806, 7820
<i>Gloeocapsa</i>	7428, 7701
	Section II
<i>Chroococcidiopsis</i>	7434
	Section III
<i>Spirulina</i>	6313, 7344*, 7345, 'Oswald'*
<i>Oscillatoria</i>	6304, 6401, 6407, 6412, 6506, 7412, 7805, 7811, 'limnetica'
<i>Pseudanabaena</i>	6901, 6903, 7402, 7403, 7429, 7704
<i>Lyngbya-Plectonema-</i> <i>Phormidium</i> group	6306, 6402, 7004, 7104, 7105, 7114, 7123, 7124, 73110, 7404, 7406, 7407, 7410, 7411, 7419, 7420, 7427, 7505, 7602, 7703, 7705
	Section IV
<i>Nodularia</i>	73104, 7804
<i>Nostoc</i>	7413, 7423
<i>Calothrix</i>	7102, 7111, 7711

* Strains were unialgal but not axenic.

showed any significant increase in phycoerythrocyanin synthesis during growth in green light. This result differs from that of Fueglistaller *et al.* (1980), who recently reported that synthesis of phycoerythrocyanin in *Fischerella* PCC 7603 (*Mastigocladus laminosus* Cohn) was subject to control by a complementary chromatic adaptation mechanism.

Although phycoerythrocyanin synthesis did not appear to be photocontrolled by the wavelength of the incident light during growth, phycoerythrocyanin synthesis did appear to be dramatically affected by light intensity. As shown in Fig. 1(c), when *Chroococcidiopsis* PCC 7203 was grown under low light intensity, up to 40 to 50% of the biliprotein of this strain was phycoerythrocyanin. When this strain was grown under high light intensity, only small amounts of phycoerythrocyanin were synthesized. Similar but somewhat less dramatic differences were observed when other strains were grown under high and low light intensities. Thus, extracts of cells grown under low light intensity were used in screening for the synthesis of phycoerythrocyanin.

During the course of these studies, phycoerythrins with a variety of spectroscopic characteristics were detected. The most common cyanobacterial phycoerythrin spectral-form possessed a single, rather narrow absorption band with its maximum at 555 to 565 nm (e.g. *Pseudanabaena* PCC 7409 phycoerythrin, Fig. 1d). This phycoerythrin class is the typical cyanobacterial (C-type) phycoerythrin; a number of phycoerythrins of this type have been described in detail (Bennett & Bogorad, 1971; Glazer & Hixson, 1975; Muckle & Rüdiger, 1977; Zickendraht-Wendelstadt *et al.*, 1980). Other phycoerythrin spectral forms detected included those with broadened absorption bands and maxima at 550 to 565 nm (e.g. *Synechococcus* PCC 7822 phycoerythrin; Fig. 1d), and those possessing two absorption maxima or a maximum and a shoulder in the 550 to 570 nm region (e.g. *Oscillatoria* PCC 7823 and *Gloethece* PCC 6909; Fig. 1e). These spectroscopic differences apparently arise from chromophore-chromophore and chromophore-protein interactions and do not reflect differences in chromophore composition. When these spectroscopically variable

Table 3. *Cyanobacterial strains which synthesize phycoerythrocyanin*The section and generic assignments are those of Rippka *et al.* (1979).

Generic assignment	Pasteur Culture Collection no.
	Section II
<i>Chroococcidiopsis</i>	7203, 7431, 7432, 7433, 7436, 7439
	Section III
<i>Pseudanabaena</i>	6406
	Section IV
<i>Anabaena</i>	6309, 6411*, 7108, 7118*, 7119, 7120, 7122
<i>Cylindrospermum</i>	73101, 7417, 7604
<i>Nostoc</i>	6314, 6705, 6719, 6720, 7107, 7416, 7524
<i>Aphanizomenon</i>	No strain number†
<i>Scytonema</i>	7110, 7814
<i>Calothrix</i>	7116, 7204, 7426, 7507, 7810, 7815
	Section V
<i>Fischerella</i>	7115, 73103, 7414, 7520, 7521, 7522, 7523, 7603‡
<i>Chlorogloeopsis</i>	6718, 6912
Section and generic assignment unknown	7517, 7518, 7519, 7702

* Phycoerythrocyanin purified and characterized (Bryant *et al.*, 1976).

† Strain was unialgal but not axenic.

‡ Phycoerythrocyanin purified and characterized (Fueglistaller *et al.*, 1980; Nies & Wehrmeyer, 1980).

phycoerythrins were denatured with 8.0 M-urea at pH 3.0, phycoerythrobilin was the only chromophore detected (Fig. 1*f*). Quite different results were obtained with *Gloeobacter violaceus* PCC 7421 phycoerythrin. The *G. violaceus* phycoerythrin, which has absorption maxima at 501 and 564 nm and which carries phycoerythrobilin and phycoerythrobilin chromophores in the ratio 1:6, was recently characterized in considerable detail (Bryant *et al.*, 1981).

Tables 2, 3 and 4 summarize a survey of the pigmentation patterns of 240 strains of cyanobacteria. In these tables, the cyanobacterial genera have been divided into five major sub-groups: Section I, unicellular cyanobacteria; Section II, baeocyte-forming cyanobacteria; Section III, filamentous, non-heterocystous cyanobacteria; Section IV, filamentous, heterocystous cyanobacteria; and Section V, heterocystous cyanobacteria which exhibit true branching (Rippka *et al.*, 1979). The tentative generic assignments are those of Rippka *et al.*, (1979) and Rippka & Stanier (1979).

Several important observations can be drawn from the data of Tables 2, 3 and 4. Firstly, it should be noted that strains synthesized either phycoerythrocyanin or phycoerythrin but not both. Phycoerythrocyanin was largely found to be limited to two sub-groups of cyanobacteria, those which form heterocysts (Sections IV and V). Phycoerythrocyanin was not detected in any unicellular cyanobacterium (Section I, 66 strains examined), and only one strain, *Pseudanabaena* PCC 6406, of Section III (65 strains examined) contained this accessory pigment. In contrast, phycoerythrocyanin was found in 26 of the 63 strains of Section IV examined and in all 10 strains of Section V examined. Additionally, phycoerythrocyanin was detected in four rather similar strains whose generic assignment is uncertain. Three of these strains were received as species of *Mastigocladus* (synonymous with *Fischerella*, Section V; Rippka *et al.*, 1979) and, although they no longer form heterocysts, may have been heterocystous at one time.

Among the strains of Section II, phycoerythrocyanin was confined to members of the genus *Chroococcidiopsis*, in which it occurred in six of eight strains. This finding might signal a phylogenetic relationship of these organisms to the heterocystous cyanobacteria of Sections

Table 4. *Cyanobacterial strains which synthesize phycoerythrin*

The section and generic assignments are those of Rippka *et al.* (1979). This table includes the data on the patterns of chromatic adaptation described by Tandeau de Marsac (1977). Symbols in parentheses following strain numbers denote the pattern of chromatic adaptation observed: (I), does not chromatically adapt; (II), chromatically adapts by photocontrolling phycoerythrin synthesis alone; (III), chromatically adapts by photocontrolling the synthesis of both phycoerythrin and phycocyanin; (ND), pattern of chromatic adaptation not determined.

Generic assignment	Pasteur Culture Collection no. and chromatic adaptation pattern
Section I	
<i>Synechococcus</i>	7335 (III), 7336 (III), 7424 (I), 7822 (I)
<i>Gloeotheca</i>	6501 (I), 6909 (I), 7109 (I), 73108 (I)
<i>Gloeobacter</i>	7421 (I)
<i>Synechocystis</i>	6701 (II), 6711 (II), 6808 (II)
<i>Gloeocapsa</i>	7007 (I), 73106 (I), 7501 (I), 7512 (I)
<i>Chamaesiphon</i>	6605 (I), 7430 (I)
Section II	
<i>Dermocarpa</i>	7301 (I), 7302 (III), 7303 (III), 7304 (III), 7437 (III), 7438 (III)
<i>Xenococcus</i>	7305 (III), 7306 (III), 7307 (III)
<i>Dermocarpella</i>	7326 (III)
<i>Myxosarcina</i>	7312 (III), 7325 (III)
<i>Chroococcidiopsis</i>	6712 (III)
<i>Pleurocapsa</i> group	7310 (I), 7314 (III), 7317 (I), 7319 (III), 7320 (III), 7321 (I), 7322 (III), 7324 (I), 7327 (I), 7440 (III), 7506 (ND), 7516 (III)
Section III	
<i>Spirulina</i> *	7340 (ND), 7341 (ND), 7342 (ND), 7343 (ND)
<i>Oscillatoria</i>	6602 (I), 7112 (I), 7515 (I), 7801 (I), 7821 (I), 7823 (I)
<i>Pseudanabaena</i>	6802 (III), 7367 (I), 7408 (III), 7409 (III)
<i>Lyngbya-Plectonema-</i> <i>Phormidium</i> group	6409 (III), 6703 (III), 7113 (II), 7375 (I), 7376 (I), 7816 (I), 7817 (I), 7818 (I), 7819 (I), 'sordida' (I)
Section IV	
<i>Nostoc</i>	6302 (I), 6310 (II), 7121 (I), 73102 (II), 7422 (I), 7706 (II), 7707 (I), 7717 (III), 7718 (III), 7803 (II), 7807 (I), 7808 (I), 7812 (I),
<i>Calothrix</i>	6303 (II), 6305 (III)†, 6601 (III)†, 7101 (III), 7103 (III), 7415 (III), 7504 (III), 7601 (III), 7708 (II), 7709 (II), 7710 (III), 7712 (III), 7713 (III), 7714 (I), 7715 (II), 7716 (II)
Section and generic assignment unknown	7126 (III)

* These *Spirulina* strains were unialgal but not axenic.

† Originally reported to control phycoerythrin synthesis only, by Tandeau de Marsac (1977).

IV and V. In particular, *Chroococcidiopsis* strains resemble the strains of Section V in a number of ways: production of phycoerythrocyanin; mean DNA base composition (Herdman *et al.*, 1979b); genome size (Herdman *et al.*, 1979a); thylakoid structure (Waterbury & Stanier, 1978); formation of an additional outer wall layer (Waterbury & Stanier, 1978); photoheterotrophic behaviour (Rippka *et al.*, 1979); and, finally, ability to synthesize nitrogenase under anaerobic or microaerophilic conditions (Rippka *et al.*, 1979).

In contrast to the rather limited distribution of the accessory pigment phycoerythrocyanin, phycoerythrin was widely distributed among all cyanobacteria of Sections I–IV; at present, no phycoerythrin-containing strains exhibiting true branching exist in the Pasteur Culture Collection. Some clustering of phycoerythrin-containing strains was observed (e.g. among the genera *Gloeotheca*, *Gloeocapsa*, *Nostoc* and *Calothrix*); nearly half of the strains of Section IV synthesize phycoerythrin. One important correlation of pigmentation with habitat was evident. About 80% of the strains of marine origin (Rippka *et al.*, 1979), a grouping which includes most of the strains of Section II, synthesize either phycoerythrin or phycoerythrocyanin. These accessory pigments would be expected to confer an advantage upon those strains in natural habitats, as light of wavelengths other than those of the region

450 to 550 nm are severely attenuated by columns of sea water (Ramus *et al.*, 1976; Glazer, 1977).

The limited occurrence of the accessory pigment phycoerythrocyanin suggests that this protein might be a useful taxonomic marker. This view is supported by the results of a study of the relatedness, determined by the technique of DNA-DNA hybridization, of the cyanobacteria of Sections IV and V (Lachance, 1979, 1981). These studies indicated that the DNAs of organisms containing phycoerythrocyanin were not closely related to those of organisms containing phycoerythrin. Although the absence of both accessory pigments obviously precludes the formulation of conclusions concerning relatedness, the ability to synthesize phycoerythrin or phycoerythrocyanin might be useful as an exclusionary character in the identification of certain strain clusters of heterocystous cyanobacteria.

Table 4 includes the results of the present study, as well as those of Tandeau de Marsac (1977), concerning the patterns of chromatic adaptation exhibited by the cyanobacteria. As found for phycoerythrin synthesis, ability to photocontrol phycoerythrin (or phycoerythrin and phycocyanin) synthesis is a widely distributed trait. For example, among the closely related strains of a single *Calothrix* cluster (Lachance, 1979, 1981), all possible patterns of chromatic adaptation were found (phycoerythrin absent; phycoerythrin present but not photocontrolled; phycoerythrin present and photocontrolled; phycoerythrin and phycocyanin photocontrolled). At present, the ability to exhibit chromatic adaptation does not appear to be a very useful marker for cyanobacterial taxonomy.

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