Arch. Microbiol. 120, 155–159 (1979)

Archives of Microbiology

Isolation and Characterization of Phycocyanins from the Blue-Green Alga *Spirulina platensis*

Samy Boussiba and Amos E. Richmond

Institute for Desert Research, Sede Boqer, and Department of Biology, Ben-Gurion University of the Negev, P.O.B. 2053, Beersheva 8412, Israel

Abstract. Two main biliproteins, c-phycocyanin and allophycocyanin were identified and characterized in the blue-green alga *Spirulina platensis*. The specific absorbance, fluorescence maxima, sub-unit make-up and amino acid composition of the biliproteins in *Spirulina platensis* resemble those reported for other blue-green algae. However, the minimum molecular weights (44,000 for c-phycocyanin and 38,000 for the allophycocyanin) and the specific extinction coefficients (73, and 58 for c-phycocyanin and allophycocyanin respectively) of these biliproteins were different from these values in other blue-green algae.

Key words: Biliproteins – c-Phycocyanin – Allophycocyanin – Blue-green alga – *Spirulina platensis*.

Phycocyanins have been extensively studied due to their involvement in photosynthesis as major accessory pigments (Myers and Kratz, 1955; Bogorad, 1975; Glazer, 1976; Goedheer, 1976).

In this study, biliproteins from the blue-green alga *Spirulina platensis* were investigated in relation to their possible role as a reserve source of nitrogen (Allen and Smith 1969; Van Liere et al., 1977). This is of particular interest because the biliproteins may comprise a large part of the total algal protein which could be used as a source of nutrition for both humans and animals (Clement, 1975; Clement et al., 1967). For this purpose, c-phycocyanin and allophycocyanin of *Spirulina platensis* were purified and characterized with respect to their specific light absorbance, sub-units make up, minimum molecular weight and amino acid composition.

Abbreviation used. SDS = sodium dodecyl sulphate

Materials and Methods

Materials

DEAE Sephadex A-50 and Lyzosyme were obtained from Sigma, Hydroxylapatite (HTP) from Bio-rad and markers of known molecular weight from British Drug Houses Ltd. All reagents were of analytical grade.

Organism and Growth Conditions

Spirulina platensis (LB 1475/4a) was cultivated in a modified Zarouk medium (1966) as follows: $NaHCO_3$ 2.1 g/l instead of 16.9 g/l and K_2HPO_4 0.185 g/l instead of 0.5 g/l.

The algae were grown in a batch culture at 35° C, illuminated by cool white lamps and stirring was provided by bubbling with a mixture of air with 1.5% CO_2. The cells were allowed to grow to a density of 1.3–1.6 mg/ml and were harvested by centrifugation. The pellets were stored at −18° C.

Isolation and Purification of the Phycocyanins

Phycocyanins were isolated by one of two procedures. In the first, approximately 7–10 g (wet weight) of algae were suspended in 200 ml of 0.1 M Na-phosphate buffer pH 7.0 containing 100 µg/ml lyzozyme and 10 mM EDTA. The enzymatic disintegration of the cell-wall was brought about by placing the algae in a shaking bath at 30 C for 24 h. The slurry was then centrifuged for 1 h at 40,000 × g to remove cell debris, yeilding a clear blue supernatant.

In the second procedure, algae cells were disintegrated by a mechanical cell homogenizer, Brawn model MSK according to Kao et al. (1975) with the exception that total cell disruption was achieved after 3 min of homogenizer operation.

The crude phycocyanin obtained from either procedure was precipitated in 50% $(NH_4)_2\ SO_4$ and was then recovered by centrifugation at 10,000 × g for 10 min. The colourless, clear supernatant was discarded and the blue precipitate was dissolved in a small volume of 0.0025 M Na-phosphate buffer pH 7.0 and dialyzed against the same buffer.

The dialyzed phycocyanin was then placed in a 2.5 × 30 cm hydroxylapatite column and two main fractions were pooled following a stepwise elution with phosphate buffers of increasing ionic strength at pH 7.0, as follows:

The first fraction with the highest 620/280 ratio was eluted between 2.5 mM – 70 mM and represented c-phycocyanin. The

0302-8933/79/0120/0155/$01.00

second, which exhibited a ratio of 655/280 > 4 was eluted with 100 mM and contained the allophycocyanin.

Polyacrylamide gel electrophoresis of these two major fractions showed that only the allophycocyanin was not contaminated by other proteins. The fraction containing c-phycocyanin, however, had to be further purified by chromatography on a 1.5 × 20 cm DEAE Sephadex A-50, according to Binder et al. (1972). In early experiments, this purification step was carried out by a second run on a hydroxylapatite column, yeilding the same results. The fractions showing a ration of 620/280 > 4 were pooled and dialyzed against a Na-phosphate buffer (pH 7.0, 0.1 M). Electrophoresis of this fraction did not reveal the presence of additional proteins.

Polyacrylamide Gel Electrophoresis

Disc electrophoresis on 7% polyacrylamide gel was carried out as described by Davis (1964). To effect separation of the sub-units in c-phycocyanin and allophycocyanin, 10 – 50 µg of the biliprotein dialyzate was dissolved in a craking buffer (0.25 M tris HCl buffer pH 7.0) which contained 2% SDS + 1% β mercaptoethanol. This mixture was then boiled for 5 min.

For molecular weight determination, 10 – 50 µg of protein markers with a range of molecular weight from 14,300 – 71,500 were dissolved and boiled in the same manner. SDS gel electrophoresis was performed in 12 cm long slab gels (10%) that included a stacking gel (4.8%). Electrophoresis was run at 100 V, 12.5 mA, for 4 h. The gel was stained with a Coomassie Brilliant Blue (R 250) and the protein sub-units were located at 590 nm using a Zeiss chromatogram spectrophotometer. The mobility of phycocyanin sub-units was determined by referenee to the standard protein markers of known molecular weight.

Spectroscopic Measurements

All visible and UV spectra were measured on a Beckman model 24 spectrophotometer. Fluorescence was measured using a Hitachi Perkin-Elmer MPF2A speetrofluorimeter.

Amino Acids Analysis

Analyses were performed as deseribed by Moore and Stein (1963) on a Beckmanspinco automatic analyser, model 121. Samples were hydrolyzed in 6N HCl at 110° C under vacuum for 48 h.

Ultracentrifuge Studies

Phycocyanin solution of about 5 mg/ml were used to determine the sedimentation velocity in the Beckman model E. ultracentrifuge that was run at 56,000 rpm and operated at 20° C.

Results and Discussion

As shown in Fig. 1, phycocyanins from the blue-green alga *Spirulina platensis* are separated in a hydroxylapatite column into two biliprotein fractions. Judged by their specific absorbance, the first is c-phycocyanin and the second represents allophycocyanin (Fig. 2). Sufficient purification of allophycocyanin was already achieved after one run in hydroxylapatite column, since elution with 100 mM buffer phosphate in one steep yielded allophycocyanin with a 655/280 ratio greater

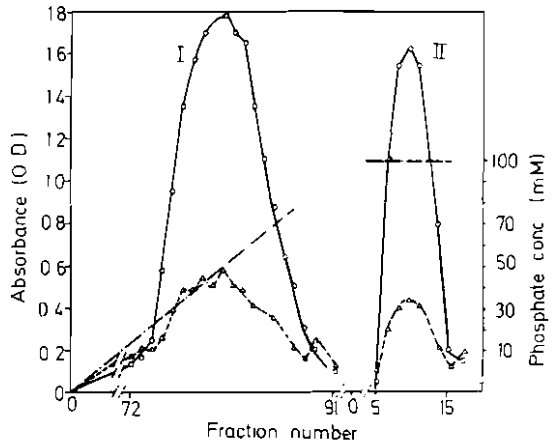


Fig. 1. Chromatography of the 50% $(NH_4)_2SO_4$ fraction (extracted from 7 – 10 g wet weight of the algae), on hydroxylapatite. A 2.5 × 30 cm column and a gradient of 2.5 – 100 mM phosphate buffer at pH 7.0 was used to collect 2.5 ml fractions. A620(I), A655(II) (———), A280 (– – – –), concentration of phosphate buffer (– · – · –)

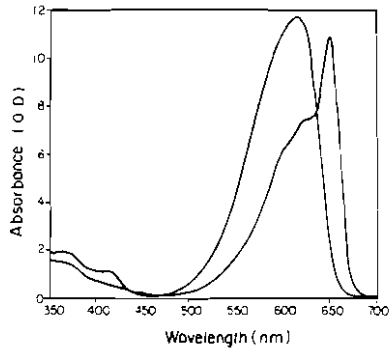


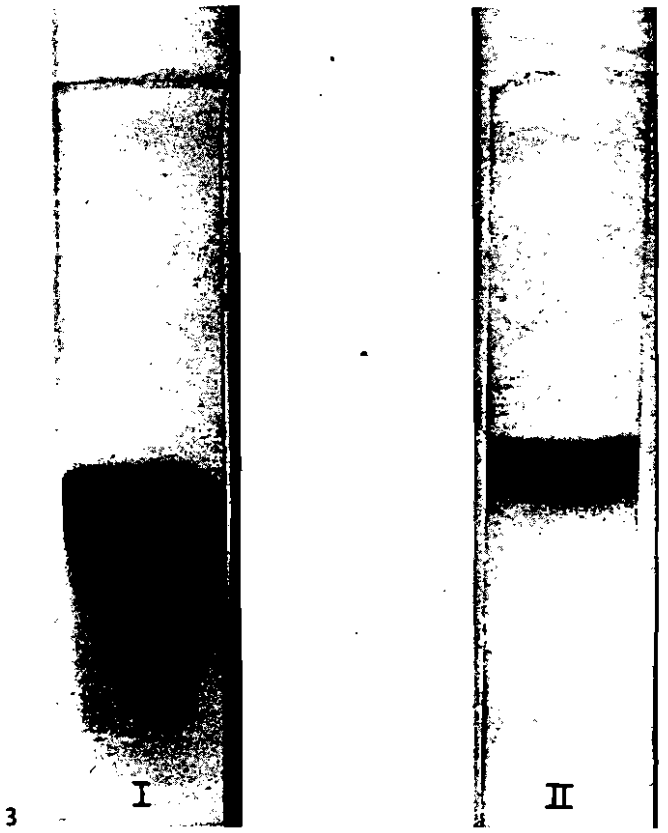
Fig. 2. Absorption spectra of the two major phycobiliproteins of *Spirulina platensis* in 0.1 M phosphate buffer pH 7.0

Table 1. Data from the purification of the biliproteins obtained from the blue-green alga *Spirulina platensis*

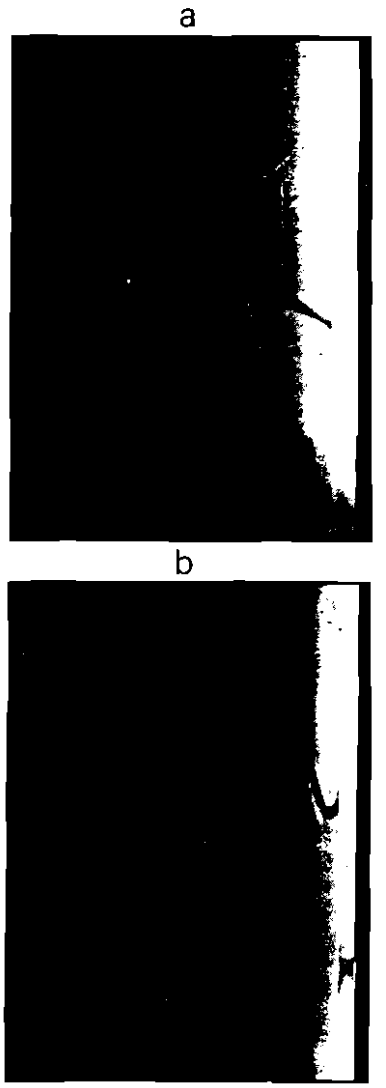
Purification step	Absorbance ratios (nm)	
	620/280	655/280
Crude extract	0.905	0.36
50% $(NH_4)_2SO_4$ precipitate	1.26	0.52
Hydroxylapatite column	2.8	4.12
DEAE Sephadex A-50 column	4.15	

Table 2. General properties of the biliproteins from the blue-green alga *Spirulina platensis*

Biliprotein	Absorption maxima nm	Fluorescence maxima nm	$E^{1\%}_{1\,cm}$
c-Phycocyanin	620	640	73
Allophycocyanin	655	660	58



3



4

Fig. 3. Polyacrylamide gel electrophoresis of the purified phycobiliproteins, usually 50 – 100 µg of protein samples were applied to each gel tube. *I* c-phycocyanin; *II* allophycocyanin

Fig. 4a and b. Sedimentation velocity of the purified biliproteins (pH 7.0, 0.1 M) performed at 56,000 rpm, 20° C. The frames shown were taken 24 min after reaching full speed. **a** c-Phycocyanin; *short arrow* designates the 7S (trimer) with $S_{20} = 5.56$. *long arrow* designates the 11S (hexamer) with $S_{20} = 10.26$. **b** Allophycocyanin – the $S_{20} = 5.00$

than 4. In contrast, purification of c-phycocyanin required passage of the hydroxylapatite eluate on a second column of DEAE-Sephadex A-50. This procedure resulted in a c-phycocyanin with an absorbance ratio of 620/280 >4. The purification steps are summarized in Table 1.

General characterization of the biliproteins from the blue-green alga *Spirulina platensis* are shown in Table 2. The absorption and fluorescence maxima resemble those reported for other blue-green algae, e.g. *Synechococcus sp.*, *Aphanocapsa* sp. and *Anabeana* sp. (Glazer and Cohen Bazire, 1971; Bryant, et al., 1976). The molar extinction coefficient ($E|^{\%}_{cm}$) that we calculated for both biliproteins, i.e. 73 for c-phycocyanin and 58 for allophycocyanin, is either lower or higher than those cited in the literature. Thus, the molar extinction coefficient for c-phycocyanin and allophycocyanin were reported to be 77, 65 respectively (Chapman, 1973), and 60 for c-phycocyanin obtained from the halotolerant blue-green *Coccochloris elabens* (Kao et al., 1973). According to Craig and Carr (1968) however, the molar extinction coefficient of c-phycocyanin from the blue-green alga *Anacystis nidulans* was 73, as observed by us. These disagreements may be due to variation in organisms or perhaps to the methods used for extraction and purification.

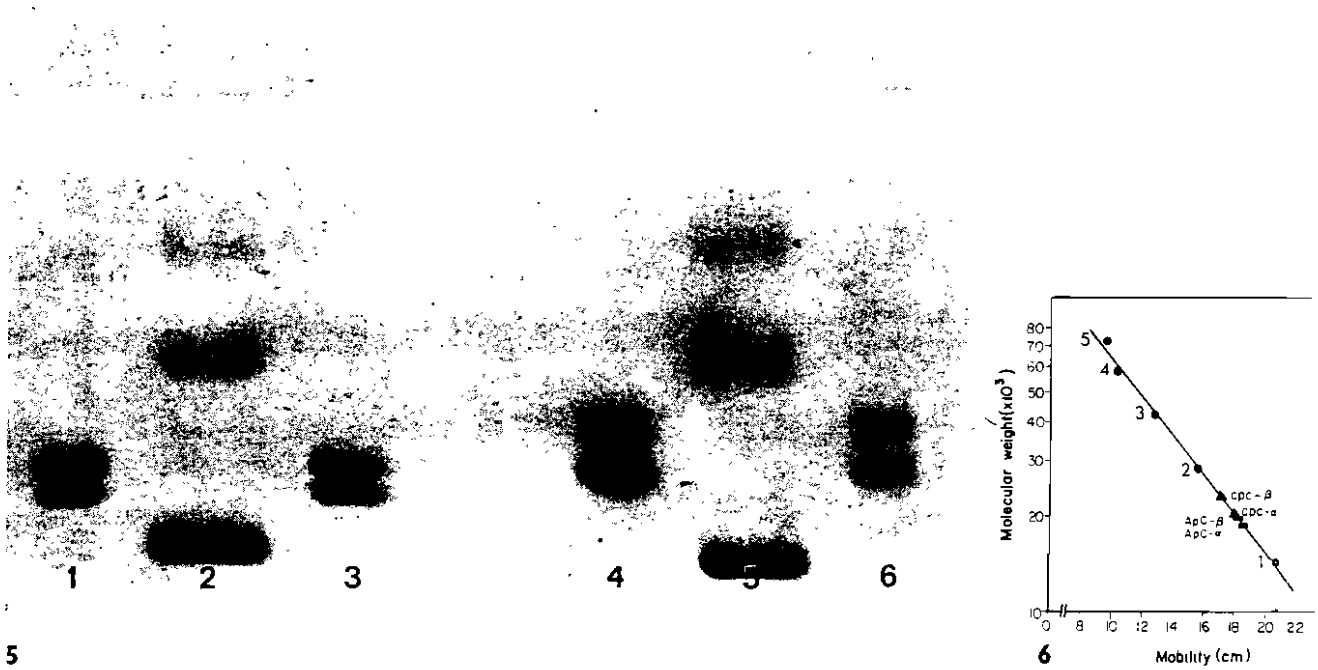


Fig. 5. SDS gel electrophoresis of the purified c-phycocyanin and allophycocyanin. *1, 3* α, β sub-units of allophycocyanin (30, 15 µg); *4, 6* α, β sub-units of c-phycocyanin (30, 15 µg): *2, 5* standard protein markers of known molecular weight (25 µg)

Fig. 6. Calibration line for the molecular weights of the phycocyanin sub-units and markers. The molecular weight of the sub-units were as follows: c-phycocyanin α sub-unit 20,500, c-phycocyanin β sub-unit 23,500, allophycocyanin α sub-unit 18,000, allophycocyanin β sub-unit 20,000. The molecular weight of the known markers were: *1* monomer 14,300, *2* dimer 28,600, *3* trimer 42,900, *4* tetramer 57,200, *5* pentamer 71,500

For further detailed characterization, the two biliproteins were subjected to gel electrophoresis (Fig. 3). The results indicate that while allophycocyanin is resolved in one clear band, c-phycocyanin under our experimental conditions may separate into two fractions. Evidence for this possibility came from ultracentrifugation showing that the c-phycocyanin separated into two aggregates with sedimentation values of 5.56 and 10.26 (Fig. 4a). This supports the possibility of the existence of a trimer and a hexamer in c-phycocyanin, as already reported by Berns (1970) and Kao et al. (1973). Allophycocyanin exhibited one peak in ultracentrifugation, with sedimentation value of 5.00 (Fig. 4b). The same value was obtained by Bryant et al. (1976) for the allophycocyanin from the blue-green alga *Anabaena* sp.

Electrophoresis of the two biliproteins after treatment with SDS indicated that each included two subunits (Fig. 5). Markers of known molecular weight indicated that the molecular weight for the α and β subunits of c-phycocyanin were 20,500 and 23,500 respectively and 18,000, 20,000 for these respective sub-

Table 3. Amino acid composition of phycocyanins expressed as a number of residues

Amino acid	Phycocyanin	
	c-Phycocyanin	Allophycocyanin
Lysine	13	16
Histidine	1	0
Arginine	18	20
Aspartic acid	31	30
Threonine	18	20
Serine	24	21
Glutamic acid	30	32
Proline	8	6
Glycine	27	35
Alanine	56	47
Half cystein	4	n.d.[a]
Valine	18	23
Methionine	9.0	7
Isoleucine	19	23
Leucine	29	28
Tyrosine	16	20
Phenylalanine	12	5
Tryptophan	n.d.	n.d.

[a] not determined

units in allophycocyanin (Fig. 6). Thus the minimum molecular weight for c-phycocyanin and allophycocyanin were 44,000 and 38,000 respectively. These values were higher than those found in other blue-green algae, e.g. 36,000, 28,000 for c-phycocyanin in *Anabaena* sp. and *Mastigocladus laminosus* (Glazer and Fang, 1973; Binder et al., 1972) and 29,600, 33,000 for allophycocyanin in *Anabaena* and *Synechococcus* (Bryant et al., 1976; Cohen-Bazire et al., 1977).

The amino acid composition of the native c-phycocyanin and allophycocyanin are given in Table 3, indicating a marked similarity in their amino acid content, both biliproteins being relatively rich in aliphatic and acidic residues. The amino acid composition shown in Table 3 is similar to that reported by Glazer (1976) for c-phycocyanin in *Phormidium luridum*, *Spirulina maxima* and *Oscillatoria aghardii* and to the amino acid composition of allophycocyanin in *Synechocystis* sp. according to Cohen-Bazire et al. (1977)

Acknowledgements. We wish to thank Ms. Varda Shoshan for helpful advice.

References

Allen, M. M., Smith, A. J.: Nitrogen chlorosis in blue-green algae. Arch. Microbiol. **69**, 114–120 (1969)

Berns, D. S.: Protein aggregation in phycocyanin-osmotic pressure studies. Biochem. Biophys. Res. Comm. **38**, 65–73 (1970)

Binder, A., Wilson, K., Zuber, H.: C-phycocyanin from the thermophilic blue-green alga *Mastigocladus laminousus*. FEBS Lett. **20**, 111–116 (1972)

Bogorad, L.: Phycobiliproteins and complementary chromatic adaptation. Ann. Rev. Plant Physiol. **26**. 369–401 (1975)

Bryant, D. A., Glazer, A. N., Eiserling, F. A.: Characterization and structural properties of the major biliproteins of *Anabaena* sp. Arch. Microbiol. **110**. 61–75 (1976)

Chapman, D. J.: Biliproteins and bile pigments. In: The biology of blue-green algae (N. G. Carr, B. A. Whitton, eds.), pp. 162–185. Oxford: Blackwell 1973

Clement, G.: Production et constituants characteristiques des algues *Spirulina platensis* and *maxima*. Ann. Nutr. Alim. **29**, 477–488 (1975)

Clement, G., Giddey, C., Menzie, R.: Amino acid composition and nutritive value of the alga *Spirulina maxima*. J. Sci. Fd. Agric. **18**, 497–501 (1967)

Cohen-Bazire, G., Beguin, D. M., Rimon, S., Glazer, A. N. Brown, D. M.: Physicochemical and immunological properties of allophycocyanins. Arch. Microbiol. **111**, 225–238 (1977)

Craig, I. W., Carr, N. G.: C-Phycocyanin and allophycocyanin in two species of blue-green algae. Biochem. J. **106**, 361–366 (1968)

Davis, B. J.: Disc electrophoresis – II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. **121**, 404–427 (1964)

Glazer, A. N.: Phycocyanins: structure and function. Photochem. Photobiol. Rev. **1**, 71–115 (1976)

Glazer, A. N., Cohen-Bazire, G.: Sub-unit structure of phycobiliproteins of blue-green algae. Proc. Nat. Acad. Sci. U.S.A. **68**, 1398–1401 (1971)

Glazer, A. N., Fang, S.: Chromophore content of blue-green algal phycobiliproteins. J. Biol. Chem. **248**, 659–662 (1973).

Goedheer, J. C.: Spectral properties of the blue-green alga *Anacystis nidulans* grown under different environmental conditions. Photosynthetica **10**, 411–422 (1976)

Kao, O. H. W., Berns, D. S., Town, W. R.: The characterization of c-phycocyanin from an extremely halotolerant blue-green alga *Coccochloris elabens*. Biochem. J. **131**, 39–50 (1973)

Kao, O. H. W., Edwards, M. R., Berns, D. S.: The physical-chemical properties of c-phycocyanin isolated from an acido-thermophilic eukaryote *Cyanidium caldarium*. Biochem. J. **147**, 63–70 (1975)

Moore, S., Stein, W. H.: Chromatographic determination of amino acids by the use of automatic recording equipment. In: Methods of enzymology, Vo. VI (S. P. Colowick, N. O. Kaplan, eds.), pp. 819–829. New York, N.Y.: Academic Press 1963

Myers, J., Kratz, K. A.: Relation between pigment content and photosynthetic characteristics in a blue-green alga. J. Gen. Physiol. **39**, 11–22 (1955)

Van Liere, L., Zavenboom, W., Mur, L. R.: Nitrogen as a limiting factor for the growth of the blue-green alga *Oscillatoria Agardhi*. Prog. Nat. Tech. **8**, 312–321 (1977)

Zarouk, C.: Contribution a l'etude d'une cyanophycee influence de divers facteurs physiques et chimiques sur la croissance et photosynthese de *Spirulina maxima*. Ceitler. Ph. D. Thesis, pp. 4–5 (1966)

Received June 10, 1978