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BRIEF COMMUNICATION

Spectroscopic study of phycocyanobilin from the cyanobacterium *Spirulina platensis*

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Abstract

Spectroscopic characterization of phycobiliprotein (PBP) isolated from *Spirulina platensis* indicated that it had a molecular formula of $C_{33}H_{40}N_4O_6$ with molecular ion peak at m/e 586. The PBP was rich in aliphatic and acidic amino acid residues. The specific ultra-violet absorbance, infrared transmittance, specific absorbance coefficient, nuclear magnetic resonance and mass spectral analysis revealed that *S. platensis* PBP was composed mainly of C-phycocyanin and allophycocyanin.

Key words: amino acids, infrared transmittance, NMR, UV absorbance.

Spirulina platensis was isolated from the water of the estuary where the Nile water interferes with the Mediterranean sea water at Gamasa city, Damietta governorate, Egypt. The cultures were grown at temperature 35 ± 1 °C and irradiance 6.5 W m^{-2}, in the nutrient medium of Zarrouk *et al.* (1966) and kept in suspension by bubbling 1 % CO_2 enriched air. To start a culture, a sample containing 10^4 hormogonia (vegetative cyanobacteria fragments) per 1 cm^3 of culture medium [calculated using haemocytometer, 0.1 mm depth, *Thoma* (*Weber*)] was prepared (El-Malky 1982).

Phycobiliprotein (PBP) was purified according to Kremer (1988). Algal cells were harvested from the liquid culture by centrifugation at 2 000 *g* for 10 min. The freeze-dried cyanobacterium (5 g) was homogenized by the *Bronson* probe sonifier *LS-75*, in 5 cm^3 of 0.10 M phosphate buffer (pH 6.8). The homogenate was extracted by continuous stirring the suspension for 12 - 17 h at 5 °C. The resulting suspension was filtered through a multiple cheese cloth and filter paper. The filtrate was centrifuged at 2000 *g* for 5 min to remove any particulate material that passed through the filters. The phycobiliprotein was purified by precipitation with $(NH_4)_2SO_4$ [1.65 mg m^{-3} (pigment extract)] in portions of about 33 mg s^{-1} to give 30 % saturation. Continuous

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stirring was applied for 30 min at 5 °C. Greenish precipitate was discarded by centrifugation at 2000 *g* for 5 - 10 min. Then another 20 g of $(NH_4)_2SO_4$ was added in portions (67 mg s^{-1}) to bring about 60 % saturation. Phycobiliproteins of blue, reddish or even violet colours were precipitated in the saturation range between 30 - 60 % $(NH_4)_2SO_4$. These pigments were collected by centrifugation at 2000 *g* for 5 - 10 min and the supernatant was discarded. The precipitated pigments were dissolved by suspending in 1 - 3 cm^3 of the buffer (used for extraction) and insoluble materials were removed by centrifugation. Finally, the extracted pigments were deionized by dialysis tube against distiled water at 4 °C overnight and then freeze-dried. The freeze dried material was the pure PBP.

Using a *Shimadzu* UV-visible recording spectrophotometer and an infra-red spectrometer *Perkin-Elmer* 983, spectral properties of the PBP were determined. The Nuclear Magnetic Resonance study (NMR) and the Mass Spectra (MS) analyses were done at the National Taiwan Normal University, Republic of China. The amino acids composition of the purified PBP was analyzed by *Beckman* Amino Acid Analyzer *Model 118/119 Cl* according to Moore and Stein (1963). The PBP from *S. platensis* had absorbance maxima at 620 and 655 nm, molar absorbance coefficient ($A^{1\%}_{1\ cm}$) = 73 and 58 and the absorbance ratio 620/280 nm was 0.902, similar to those reported for other cyanobacteria, *e.g. Synechococcum* sp, *Aphanocapsa* sp. and *Anabaena* sp. (Glazer and Cohen-Bazire 1971, Bryant *et al.* 1976). The $A^{1\%}_{1\ cm}$ we used for calculation were 73 and 58 for C-phycocyanin and allophycocyanin, respectively (Boussiba and Richmond 1979). According to Craig and Carr (1968) the molar absorbance coefficient of C-phycocyanin from *Anacystis nidulans* is also 73, which agrees with the present results. The infra-red spectra (KBr) of *S. platensis* PBP (Fig. 1) showed a broad peak at 3470 cm^{-1} that indicated the presence of (OH) group, and further peaks: that at 1692 cm^{-1} indicated the

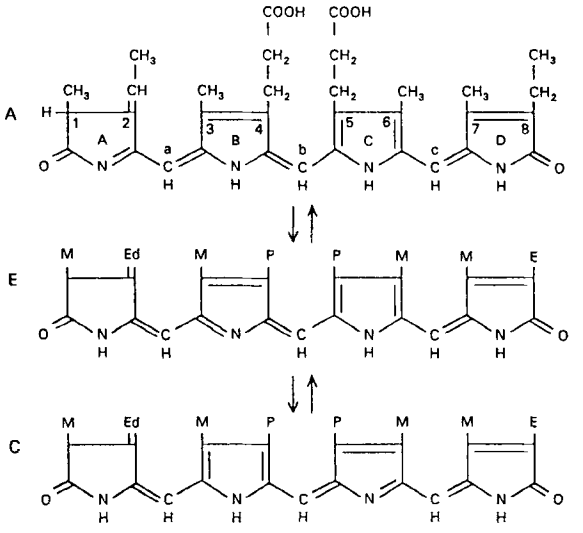

Fig. 1. Three possible structures proposed for phycocyanobilin. M - methyl group, E - ethyl group, Ed - ethylene group, P - propyl group.

presence of carbonyl group (C=O), peak at 1461 cm^{-1} indicated the presence banding isopropyl group, and the strong peak at 850 cm^{-1} indicated the plane of (C-H) of the aromatic or heterocyclic system. Small peaks appeared at 1157, 1116, 1032, 602 and 190 nm.

NMR, MS and amino acid analyses showed that the PBP was best fit by the structure *A* (Fig. 1). The base catalyzed exchange of the ethylidene methyl group could be explained in terms of the ionic form. In addition to the high-resolution, mass spectral (FAB) analysis indicated the molecular formula $C_{33}H_{40}N_4O_6$. The molecular ion peak at m/e 586 had about 40 % of the intensity of m/e 588.

Table 1. Amino acid composition of phycocyanobilin from *Spirulina platensis*.

Amino acid	[mg g^{-1}(protein)]	Amino acid	[mg g^{-1}(protein)]
Aspartic acid	14.220	Isoleucine	24.377
Threonine	0.929	Leucine	2.906
Serine	0.988	Tyrosine	0.245
Glutamic acid	0.625	Phenylalanine	0.894
Glycine	1.323	Histidine	0.222
Alanine	1.725	Lysine	1.027
Valine	2.999	Arginine	3.483
Methionine	0.170		

The amino acid composition of the native PCB (Table 1) indicated a high content of aliphatic and acidic residues. The amino acid composition was similar to that reported by Glazer (1976) for phycocyanin in *Phormidium luridum, Spirulina maxima* and *Oscillatoria aghardii.*

References

Boussiba, S., Richmond, A.E.: Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*. - Arch. Microbiol. **120**: 155-159, 1979.

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, 1979.

Craig, I.W., Carr, N.G.: Ribosomes from the blue-green alga, *Anabaena variabilis*. - Arch. Microbiol. **26**: 167-177, 1968.

El-Malky, W.A.: Studies on Blue-green Algae as Food Source. - Ph.D.Thesis. Al-Azhar University, Cairo 1982.

Glazer, A.N.: Phycocyanins; structure and function. - Photochem. Photobiol. Rev. **1**: 71-115, 1976.

Glazer, A.N., Cohen-Bazire, G.: Subunit structure of the phycobiliproteins of the blue-green algae. - Proc. nat. Acad. Sci. USA **68**: 1398-1401, 1971.

Kremer, B.P.: Electrophoretic separation an spectral characterization of algal phycobiliproteins. - In: Lobban, C.S., Chapman, D.J., Kremer, B.P. (ed.): Experimental Phycology. Laboratory Manual. Pp. 113-116. Cambridge University Press, Cambridge - New York - New Rochelle - Melbourne - Sydney 1988.

Zarrouk, C.: Contribution à l'étude d'une influence de divers facteurs physiques et chimiques sur la croissance et photosynthèse de *Spirulina maxima*. - Ph.D. Thesis. University Paris, Paris 1966.