ELSEVIER

Process Biochemistry 41 (2006) 2017–2023

Process Biochemistry

www.elsevier.com/locate/procbio

Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata*—Isolated from the rocky shores of Bet-Dwarka, Gujarat, India

Badrish Soni [a], Beena Kalavadia [b], Ujjval Trivedi [a], Datta Madamwar [a,*]

[a] *Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, 388120 Gujarat, India*
[b] *Shree M. and N. Virani Science Collage, Rajkot, Gujarat, India*

Received 21 January 2006; received in revised form 26 April 2006; accepted 27 April 2006

Abstract

Phycocyanin is a major phycobiliprotein produced by cyanobacteria, but only few strains for its efficient purification have been reported until now. In the present study, we discussed the extraction, purification and characterization of C-phycocyanin from a novel isolate *Oscillatoria quadripunctulata*. The phycocyanin was extracted by repeated freeze–thaw cycles and purified by a three-step process: ammonium sulfate fractionation, Sephadex G-150 size exclusion chromatography and DEAE cellulose ion exchange chromatography. Purified phycocyanin showed absorbance maxima at 620 nm. A purity ratio (*R*) of 3.31 was achieved. The phycocyanin to phycoerythrin and phycocyanin to allophycocyanin ratio were 4.90 and 3.92, respectively. The recovery efficiency of C-phycocyanin from crude extract was above 68%. Twenty milligram pure phycocyanin was obtained from 10 g of dried cell mass. The purified protein showed p*I* of 5.0. The purity was checked by gel electrophoresis and UV-vis spectroscopy. High performance liquid chromatography (HPLC) determined the molecular weight of intact phycocyanin to be 215 kDa, whereas denaturing gel electrophoresis showed the presence of two bands of 19 and 20 kDa molecular mass, indicating the characteristic $(\alpha\beta)_6$ subunit assembly of phycocyanin. Zinc-assisted fluorescence enhancement further confirmed that both subunits are bilin-linked polypeptides.

Keywords: C-phycocyanin; Purification; *Oscillatoria quadripunctulata*; HPLC; $(\alpha\beta)_6$ subunit assembly; Zinc-assisted fluorescence enhancement

1. Introduction

Cyanobacteria are a valuable ubiquitous component of marine picophytoplankton that contributes significantly to the total carbon biomass and primary productivity of the oceans [1]. Phycobilisomes are large supramolecular aggregates attached to the thylakoid membrane of cyanobacteria (blue–green algae) and rhodophyta (red algae) that function in light harvesting and energy migration [2–5]. These phycobilisomes are composed of phycobiliproteins: a family of hydrophilic, brilliantly coloured and stable fluorescent pigment proteins, covalently linked with linear tetrapyrrole prosthetic group (bilins) that, in their functional state, are covalently linked to specific cysteine residues of the proteins [5,6]. Cyanobacterial phycobiliproteins are classified into three main groups: phycocyanin (C-PC), phycoerythrin (C-PE) and allophycocyanin (C-APC) depending on inherent colour and absorbance properties. Here prefix ‘‘C’’ to the abbreviation of each phycobiliprotein indicates its cyanobacterial origin. The absorption maxima for C-PC, C-PE and C-APC are between 610 and 620, 540 and 570, and 650 and 655 nm, respectively [7,8]. Phycobiliproteins participate in an extremely efficient energy transfer chain in photosynthesis [2]. The energy transfer occurs from PC to APC to chlorophyll *a* (in the case of cyanobacteria) and is so effective that the phycobiliproteins in intact phycobilisomes are only weakly fluorescent [1,3,9]. However, when phycobiliproteins released outside the cells they become highly fluorescent in a region of the spectrum that is well separated from the autofluorescence of other biological cell matter [1].

Because of the protein nature, unique colour, fluorescence and antioxidant properties a wide range of promising applications of phycobiliproteins in diagnostics, biomedical research and therapeutics are possible [2,9,10]. The main application of phycobiliproteins is as fluorescent markers of cells and macromolecules in biomedical research and in highly

* Corresponding author. Tel.: +91 2692 234402; fax: +91 2692 226865.
E-mail address: datta_madamwar@yahoo.com (D. Madamwar).

1359-5113/$ – see front matter
doi:10.1016/j.procbio.2006.04.018

sensitive fluorescent techniques [8]. Recent studies have shown their applicability in immunomodulating and anticarcinogenic activities as well as natural dyes in food and cosmetics, replacing the synthetic colourants [2].

Various methods have been reported for the purification of phycobiliproteins involving a combination of several techniques such as centrifugation, ammonium sulfate precipitation, ion exchange chromatography, gel filtration chromatography, chromatography on hydroxyapatite and expanded bed adsorption chromatography [1,2,6–8,10–12]. In the present study, we report a combination of ammonium sulfate precipitation, gel filtration and ion exchange chromatography techniques for the purification of phycocyanin from *Oscillatoria quadripunctulata*. Although *Oscillatoria* sp. are the most commonly found cyanobacteria in salt water bodies, no reports are available on the purification of C-PC from it. We are reporting here for the first time the purification of phycocyanin from *O. quadripunctulata*. The strain was isolated from the unexplored rocky shores of Bet-Dwarka, Gujarat, India. The purity of C-PC was checked at each stage of purification by various methods. The results obtained are consistent with the frequently reported characteristic α, β subunit composition of C-PC [13–18]. We could recover 20 mg pure phycocyanin from 10 g (dry cell mass) of *Oscillatoria quadripuntulata* with a purity ratio (*R*) of 3.31 that is in the range required by food and cosmetic industries [6,7].

2. Materials and methods

2.1. Chemicals

Sephadex G-150 powdered matrix (bead diameter 20–300 μm and fractionation range 5–300 kDa) was from Pharmacia Fine Chemicals (Uppsala, Sweden), Bio-Sil SEC 125-5 (300 mm × 7.8 mm) and gel filtration standard were purchased from BioRad Laboratories (Hercules, USA), cellulose membrane dialysis tubing D9652 (33 mm × 21 mm) MWCO 12.4 kDa and DEAE cellulose weak anion exchange resin were purchased from Sigma (St. Louis, USA), protein molecular mass standard Electran 44264 2L, bis-acrylamide and sodium dodecyl sulfate were from BDH Laboratories (UK). Electrophoresis grade acrylamide was from Merck (Darmstadt, Germany). Centriplus YM-30 regenerated cellulose 30 kDa MWCO centrifugal devices were purchased from Amicon Bioseparations, Milipore Corporation (Bedford, USA). All other chemicals were ultrapure or molecular biology grade and were used without further purification.

2.2. Organism isolation, identification and laboratory growth conditions

Enrichment, isolation, purification and identification of cyanobacterial culture were carried out using procedures described elsewhere [19]. Identification of the taxa was done by observing under Karl Zeiss Image Analyzer System equipped with Axioplan MPM-400 microscope (Germany), using KS 300 software at 1282× magnification and compared with monographs [20]. The organism was isolated from the rocky shores of Bet-Dwarka, Gujarat, India (located between 20′ and 25′ latitude and 65′ and 70′ longitude) and was identified as *O. quadripunctulata*. The microscopic morphology of *O. quadripunctulata* is shown in Fig. 1. The culture was cultivated in ASN III medium [21] in 12-h light/12-h dark cycles under 36 W white fluorescent lamp illumination (130 μmol photon m^{-2} s^{-1}) at 27 ± 2 °C.

2.3. Spectrophtometric estimation of phycobiliproteins

The UV-vis absorbance spectra (250–820 nm) were recorded on Hewlett Packard 8452A UV-vis Diode Array Spectrophotometer (Washington, USA).

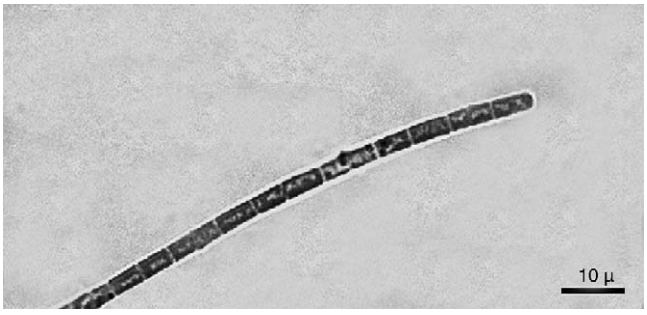

Fig. 1. Bright field morphograph of *Oscillatoria quadripunctulata* (magnification 1282×). Pair of large granules on the either side of wall—a distinct characteristic of *Oscillatoria quadripunctulata* is clearly visible.

The amount of C-PC, C-PE and C-APC in the sample was calculated using simultaneous equations of Bennet and Bogorad (1973) and the extinction coefficients from Bryant et al. (1979) as follows:

$$\text{C-PC}(\text{mg ml}^{-1}) = \frac{(\text{OD}_{620} - 0.7\text{OD}_{650})}{7.38} \quad (1)$$

$$\text{C-APC}(\text{mg ml}^{-1}) = \frac{(\text{OD}_{650} - 0.19\text{OD}_{620})}{5.65} \quad (2)$$

$$\text{C-PE}(\text{mg ml}^{-1}) = \frac{(\text{OD}_{540} - 2.8[\text{C-PC}] - 1.34[\text{C-APC}]}{12.7} \quad (3)$$

The recovery efficiency of C-PC was calculated using following equation:

$$\text{Recovery efficiency}(\%) = \frac{\text{Stage IV C-PC(mg)}}{\text{Stage I C-PC(mg)}} \times 100 \quad (4)$$

2.4. Chlorophyll a estimation

Growth was measured by extracting Chlorophyll *a* from the cell mass according to procedure described by Marsac and Houmard [22] and measured spectrophotometrically at 665 nm (Spectronic 20 D^+, Spectronic Instruments Inc., New York, USA).

2.5. Extraction of phycobiliproteins

Thirty-four day grown cyanobacterial cells were harvested by centrifugation at 3000 × *g* for 5 min (Eppendorf, Hamburg, Germany, rotor F-34-6-38 07168) at 20 °C. Cell pellet was washed with 1 M Tris–Cl buffer (pH 8.1). One volume of washed cell mass was resuspended in five volumes of the same buffer and subjected to repeated freeze–thaw cycles of −25 and 4 °C temperature shocks for the release of phycobiliproteins. The cell debris was removed by centrifugation at 17,000 × *g*. The supernatant was pooled and labeled as crude extract.

2.6. Purification methods

The entire purification procedure was carried out in dark at 4 °C unless specified. All buffers and solutions were prepared in milli-Q water supplemented with 0.01% sodium azide. The complete purification protocol is shown in Fig. 2.

2.6.1. Ammonium sulfate fractionation

Finely powdered ammonium sulfate was gradually added into the crude extract to obtain 20% saturation with continuous stirring for 1 h. The resulting solution was kept overnight and centrifuged at 17,000 × *g* for 20 min. The supernatant was pooled and subjected to 70% ammonium sulfate saturation in a manner similar to that of 20% saturation. After overnight incubation, the solution was centrifuged at 17,000 × *g* for 20 min. The pellets were resuspended in a small quantity of 20 mM Tris–Cl buffer (pH 8.1) and subjected to