

# Presence of Both Hemidiscoidal and Hemiellipsoidal Phycobilisomes in a *Phormidium* Species (Cyanobacteria)

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Hemidiscoidal and hemiellipsoidal phycobilisomes have been determined in cells of the complementary chromatically adapting cyanobacterium *Phormidium* sp. C86. They could be isolated from red and green light-adapted cells, respectively. Hemidiscoidal red light phycobilisomes show molar pigment ratios of allophycocyanin:phycocyanin of 1:4.5 with phycoerythrin lacking. Hemiellipsoidal phycobilisomes induced by green light present allophycocyanin:phycocyanin:phycoerythrin ratios of 1:1:6.8. The differences between the two phycobilisome types could additionally be demonstrated by their ultrastructure and sedimentation values. Isolated red light phycobilisomes have six rods, show dimensions of  $70 \times 30 \times 15$  nm and a sedimentation value of 66 S whereas green light phycobilisomes are nearly twice larger. They contain ten rods and present dimensions of  $70 \times 40 \times 25$  nm and a sedimentation value of 98 S. The number of phycobilisomes in red light cells is almost twice as large as in green light cells. There is evidence that cells grown under white light contain both types as well as “intermediate” forms.

## Introduction

The light-harvesting antennae of red algae and cyanobacteria are organized in supramolecular protein complexes designated phycobilisomes [1]. Phycobilisomes are arranged in regular rows on the outer surface of the thylakoid membranes. Hemidiscoidal phycobilisomes with six peripheral rods radiating from a tricylindrical core are characteristic for most of the cyanobacteria and some red algae [2, 3]. Hemiellipsoidal phycobilisomes were found in *Porphyridium cruentum*, several higher red algae and in the cyanobacterium *Phormidium persicinum* [4–6]. The latter are made up of a triangular allophycocyanin core and a periphery of about ten rods in a close parallel arrangement.

Three groups of cyanobacteria can be differentiated according to their ability of adaptation to light quality [7]. *Phormidium persicinum* belongs to group I which does not adapt chromatically. Group II cyanobacteria regulate only their phycoerythrin levels whereas group III organisms exchange phycoerythrin and phycocyanin. Red light induces phycocyanin accumulation and phycoery-

thrin decrease, green light causes the antagonistic reaction. This “complementary chromatic adaptation” was preferentially studied on the phycobilisomes of *Fremyella diplosiphon* [8, 9]. Apart from light quality alterations in structure and number of phycobilisomes can also be induced by several other environmental factors [10–12]. This work presents spectroscopic, biochemical and ultrastructural analyses of phycobilisomes isolated from red and green light-adapted cells of *Phormidium* sp. The results demonstrate an extraordinary type of complementary chromatic adaptation characterized by two structurally different types of phycobilisomes in one cyanobacterial species.

## Materials and Methods

### Strain and culture conditions

*Phormidium* sp. C86 was obtained from the culture collection of the National Institute for Basic Biology, Okazaki, Japan, and grown in a modified ASP<sub>7</sub> medium [13]. The cell suspensions were grown in tubes and gassed with 2% CO<sub>2</sub>-enriched air in a light-dark regime of 16:8 h at 20 °C. After six to seven days of growth under white light, the cultures were diluted 1:5 with fresh medium and irradiated for 14 days with red or green light of  $15 \mu\text{E} \times \text{m}^{-2} \times \text{s}^{-1}$ . The red light cultures were transformed after seven days into ASP<sub>7</sub> medium without nitrogen. White light cultures were illumi-

**Abbreviations:** AP, allophycocyanin; PC, phycocyanin; PE, phycoerythrin.

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nated with Osram fluorescent lamps L 40 W/15-1, L 40 W/25-1 and L 40 W/35-1. Red and green light fields were generated by Philips fluorescent lamps TLD 36 W/15-red and TLD 36 W/17-green with a maximum of emission at 657 nm and 525 nm, respectively. The growth of the cells was determined by measuring the culture turbidity at 740 nm.

#### Isolation of phycobilisomes

Cells of *Phormidium* sp. were harvested by low speed centrifugation and washed twice with 0.75 M potassium phosphate buffer, pH 7.0, resuspended and homogenized in the same buffer, with addition of 0.1 mM phenylmethanesulfonylfluoride, 2 mM ethylenediaminetetraacetic acid and 15% (wt/v) sucrose. The suspension was incubated for 20 min at 17 °C with 2% (v/v) N,N-dimethyldodecylamine-N-oxide (Fluka, Switzerland). To remove cell debris, the suspension was centrifuged for 20 min at  $48,000 \times g$  in a Sorvall RC-5B centrifuge (Du Pont-Sorvall, Bad Nauheim, F.R.G.). Aliquots of 2–3 ml of the supernatant were layered onto continuous sucrose gradients (15–45% wt/v sucrose in isolation buffer). Centrifugation was performed for 19 h at  $52,000 \times g$  at 17 °C (T 2060, TST 28.38, Kontron, Munich, F.R.G.). After centrifugation the phycobilisome bands were eluted and examined spectroscopically and electron microscopically.

#### Determination of the biliprotein stoichiometry

Cell suspensions with identical turbidity of 0.2 at 740 nm were harvested by low speed centrifugation and washed twice with 50 mM potassium phosphate buffer, pH 7.0. The cells were disrupted according to [6] and phycobilisomes were dissociated by three washing procedures in 50 mM potassium phosphate buffer, pH 7.0. The membranes were sedimented by centrifugation at  $106,000 \times g$  for 30 min (T 2060, TFT 65.38, Kontron, Munich, F.R.G.). Concentrations of biliproteins in whole cells as well as in isolated phycobilisomes were spectrophotometrically determined using the absorption coefficients from *Fremyella diplosiphon* [14]. The calculation of apparent molecular weight ( $M_r$ ) of the phycobilisomes was performed in reference to that of the biliprotein subunits of *Fremyella diplosiphon* [15].

#### Determination of sedimentation values

The sedimentation values of the phycobilisomes of *Phormidium* sp. were estimated according to [16], modified as described by [17]. The phycobilisomes of *Porphyridium cruentum* (120 S), *Phormidium persicinum* (98 S), *Mastigocladus laminosus* (52 S), and tripartite units of *Rhodella violacea* (22 S) were used as visible sedimentation markers [18–20].

#### Spectroscopic measurements

Absorption spectra and their second derivatives were recorded with a Hitachi U 3200 spectrophotometer (Colora, Lorch, F.R.G.) as previously described [21].

#### Electron and light microscopy

Electron microscopy was performed with a Philips 301 G electron microscope. Phycobilisomes were negatively stained according to [6]. Quick freezing and freeze substitution fixation were carried out in a freeze substitution unit FSU 010 (Balzers, Liechtenstein) following [22]. The cells were embedded according to [23] and ultrathin sections were made with an Ultratome III (LKB, Sweden).

Light and fluorescence microscopy were performed with an Ortholux II microscope (Leitz, Wetzlar, F.R.G.) with an epifluorescence illuminator Ploemopak (Leitz, Wetzlar, F.R.G.). Biliproteins were excited at 545 nm.

## Results

#### Chromatic adaptation of the cells

Growth and changes in the biliprotein ratios during chromatic adaptation were controlled each day by *in vivo* absorption spectra. First spectral differences could be observed after 24 h of growth. The ratio of phycocyanin to phycoerythrin increases in red light cultures and decreases under green light conditions. At white light conditions this ratio remains nearly constant. For a complete reduction of phycoerythrin, nitrogen deficiency must be applied after seven days of growth in red light. In red light cells cultured in full media, a small amount of phycoerythrin persists. The chlorophyll *a* content of the cells does not change significantly during the chromatic adaptation. After 14

days of growth in red and green light, the *in vivo* absorption spectra show no further variations.

#### *In vivo epifluorescence microscopy*

*Phormidium* sp. is a filamentous cyanobacterium which does not contain heterocysts. Excitation of the trichomes with 546 nm results in a visible biliprotein fluorescence. All cells of filaments grown in red light emit weak dark red fluorescence which is characteristic for phycocyanin. All cells of filaments irradiated with green light show the intensive orange fluorescence of phycoerythrin. The *in vivo* fluorescence of *Phormidium* sp. grown in white light is different from cell to cell. Parts of filaments of intensive orange fluorescence alter with parts of weak red fluorescence. By these observations it is evident that within one filament white light cells synthesize both types of phycobilisomes.

#### *Isolation of phycobilisomes*

Fig. 1 shows the banding pattern of red and green light phycobilisomes in the sucrose gradients. The green colored band 1 consists of hydrophobic membrane fragments with chlorophyll *a* and carotenoids. A weak band containing biliproteins of dissociated phycobilisomes is visible in the upper part of the gradients (band 2). The sucrose gradient of phycobilisomes from white

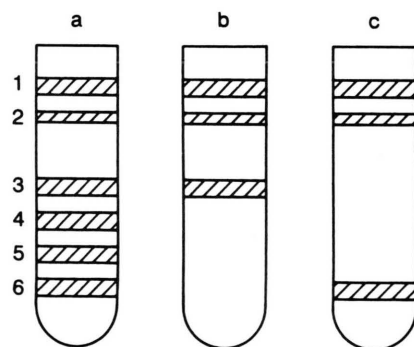


Fig. 1. Sucrose density gradients (15–45% wt/v sucrose in 0.75 M potassium phosphate buffer, pH 7.0 with 0.1 mM phenylmethanesulfonyl fluoride and 2 mM ethylenediaminetetraacetic acid) of phycobilisomes from *Phormidium* sp. grown in (a) white light, (b) red light and (c) green light after 19 h centrifugation at  $52,000 \times g$  and 17 °C. Band 1: hydrophobic phase with chlorophyll *a* and carotenoids; band 2: dissociated biliproteins; band 3–6: phycobilisomes.

light cells (Fig. 1a) shows four phycobilisome bands (band 3–6) in the region of 25–36% (wt/v) sucrose with sedimentation values of 66 S, 75 S, 88 S and 98 S. In cells exclusively irradiated with red or green light only one phycobilisome type remains. Phycobilisomes of red light cells can be separated as a deep blue band with 66 S (Fig. 1b). Green light phycobilisomes have an intensive red color and a sedimentation value of 98 S (Fig. 1c).

#### *Spectral properties of the phycobilisomes*

Absorption spectra and their 2nd derivatives of isolated phycobilisomes from *Phormidium* sp. grown in red and green light are presented in Fig. 2. The absence of chlorophyll *a* in the preparation can be demonstrated by the lack of minima

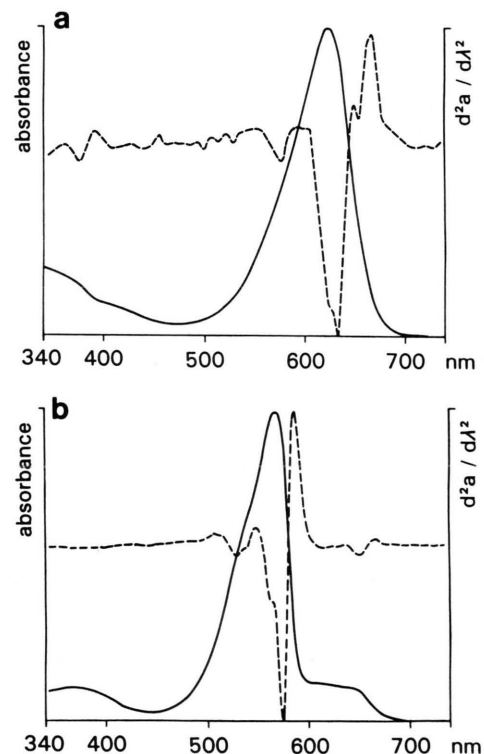


Fig. 2. Absorption spectra (solid line) and 2nd derivative (dashed line) of isolated phycobilisomes from *Phormidium* sp. grown in (a) red light and (b) green light in 0.75 M potassium phosphate buffer, pH 7.0 with 15% (wt/v) sucrose, 0.1 mM phenylmethanesulfonyl fluoride and 2 mM ethylenediaminetetraacetic acid.

at 436 or 680 nm in the 2nd derivative. Red light phycobilisomes (Fig. 2a) have an absorption maximum at 623 nm and a shoulder at 580 attributed to C-PC. AP can be detected by a shoulder at 652 nm, enhanced as a minimum in the 2nd derivative. The lack of C-PE in red light cells is indicated by the absence of any absorption maximum at 566 nm.

Green light phycobilisomes exhibit an absorption maximum at 566 nm and a shoulder at 530 nm due to the high content of C-PE whereas the presence of C-PC and AP is indicated by shoulders at 623 nm and 652 nm, respectively (Fig. 2b). The molar ratios of biliproteins from red and green light phycobilisomes are shown in Table I.

Table I. Characteristics of the biliprotein antenna from *Phormidium* sp. C86 grown in red and green light.

| Properties                                        | Light condition |                 |
|---------------------------------------------------|-----------------|-----------------|
|                                                   | Red             | Green           |
| Phycobilisomes/<br>$\mu\text{m}^2$ thylakoid area | 1240            | 600             |
| AP:PC:PE<br>(molar ratios)                        | 1.0:4.5:0       | 1.0:1.0:6.8     |
| Chromophores/<br>phycobilisome                    | 500             | 1400            |
| $S_{20,w}$                                        | 66              | 98              |
| Width [nm]                                        | 65–70           | 65–70           |
| Height [nm]                                       | 25–30           | 35–40           |
| Thickness [nm]                                    | 10–15           | 20–25           |
| Rods                                              | 6               | 10              |
| Structure                                         | hemidisoidal    | hemiellipsoidal |

#### Structure of negatively stained phycobilisomes

Negatively stained phycobilisomes of *Phormidium* sp. grown in red and green light are shown in Fig. 3. The phycobilisomes in micrograph 1–3 are seen in face view, those in 4 and 5 in profile view from top. Red light phycobilisomes (Fig. 3a) with a height of 25–30 nm are 65–70 nm wide at the base. In profile view a thickness of 10–15 nm can be measured. The 6 peripheral rods are arranged

on both sides of the core similar to those of well-known hemidisoidal phycobilisomes [2, 3]. The rods are made up of 4–5 “hexameric” biliprotein aggregates. In some cases it is possible to detect the division of the “hexameric” discs into two “trimeric” discs. The upper cylinder of the core with a thickness of 15 nm is sometimes visible in top view (Fig. 3a). The diameter of single discs dissociated from the rods was determined to be 10–11 nm.

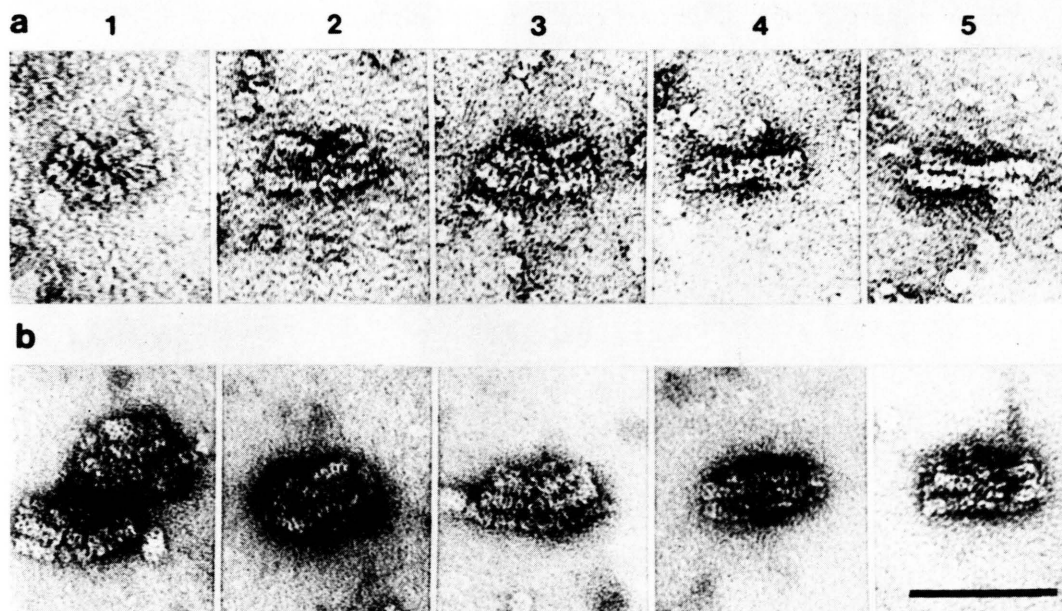


Fig. 3. Electron micrographs of negatively stained phycobilisomes from *Phormidium* sp. prefixed with 0.2% (v/v) glutaraldehyde and stained with 2% (wt/v) uranyl acetate, grown in (a) red light and (b) green light. 1–3: phycobilisomes in face view; 4–5: phycobilisomes in profile view (bar: 100 nm).

The micrographs of isolated green light phycobilisomes of *Phormidium* sp. show a tight parallel package of ten peripheral rods (Fig. 3b). They are superimposed, so that in face view only 3 or 4 are visible on both sides of the core. The dimensions of green light phycobilisomes in face view are 65–70 nm in width and 35–40 nm in height. In profile view, two levels of rods are visible increasing the thickness of green light phycobilisomes to 20–25 nm. They are approximately 10–15 nm larger in height and thickness than red light phycobilisomes. In contrast to the different structure of the phycobilisome periphery the tricylindrical cores of red and green light phycobilisomes with a width of 20–24 nm at the base have an identical ultrastructure.

#### *Thin sections of fixed cells*

Ultrathin sections of *Phormidium* sp. cut tangentially to the thylakoid surface, show the rows of phycobilisomes in top view (Fig. 4). Both red and green light cells exhibit phycobilisome rows which are regularly arranged in an angle of 45° to the longitudinal cell axis. In both cases the rows are 60–75 nm apart.

Confirming the measurements made on isolated phycobilisomes, red light phycobilisomes are less thick than green light phycobilisomes. Consequently, the center-to-center distance of the phycobilisomes within one row in red light cells was determined to be 12 to 13 nm (Fig. 4a), in green light cells 23 to 26 nm (Fig. 4b). Only in tangential

cuts this significant differences between red and green light cells can be shown unequivocally. Basing on this data, the number of phycobilisomes/ $\mu\text{m}^2$  thylakoid area in red light cells was calculated twice as large as in green light cells (Table I).

#### Discussion

*Phormidium* sp. is a complementary chromatically adapting cyanobacterium exchanging both PE and PC [7]. All recently examined chromatically adapting cyanobacteria remain constant in their hemidiscoidal phycobilisome type. Only the composition and sometimes the length of the peripheral rods is altered [24]. The present study is the first description of a light quality induced alteration of the number of peripheral rods and thereby change between hemidiscoidal and hemiellipsoidal phycobilisomes.

The absorption spectrum of red light phycobilisomes from *Phormidium* sp. shows the absence of PE and a molar ratio of AP:PC of 1:4.5. Assuming that the phycobilisome core is comprised of 12 trimeric AP complexes, 4–5 hexameric PC complexes must be arranged in 6 peripheral rods. This data agree with the electron micrographs of negatively stained phycobilisomes from red light cells. Using the molecular weights of AP, PC and PE from *Fremyella diplosiphon* [15] the molecular mass of red light phycobilisomes from *Phormidium* sp. can be calculated with  $M_r$  7–7.5  $\times 10^6$ . Molecular mass and sedimentation value of the red light

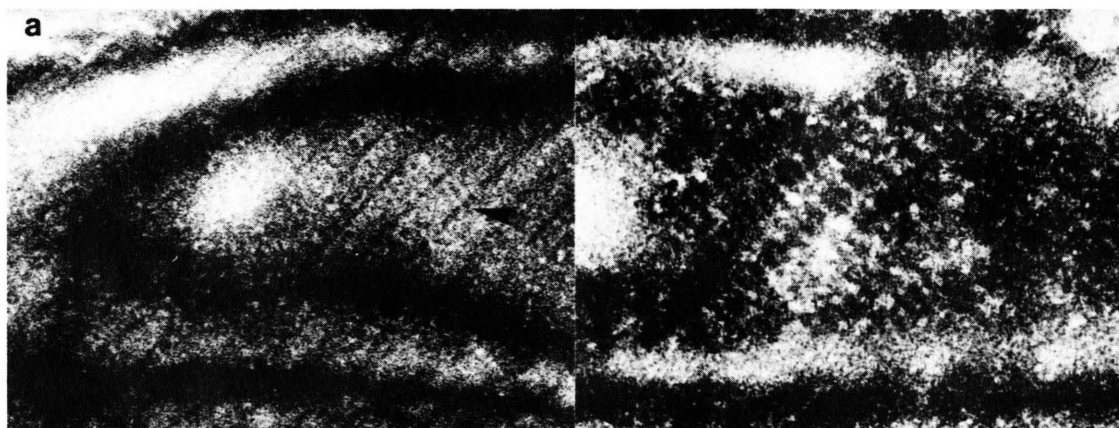


Fig. 4. Electron micrographs of *Phormidium* sp. cells with phycobilisomes seen in top view in sections cut tangentially to the thylakoid surface, grown in (a) red light and (b) green light. Arrowheads: phycobilisomes (bar: 100 nm).

phycobilisomes correspond to those of large hemidiscoidal phycobilisomes [15].

The molecular mass of green light phycobilisomes from *Phormidium* sp. can be calculated with  $M_r$  14–14.5 × 10<sup>6</sup> which is similar to that of hemiellipsoidal phycobilisomes from red algae [4]. Green light phycobilisomes of *Phormidium* sp. are in molar pigment ratio, sedimentation value, spectral and ultrastructural properties identical to those of *Phormidium persicinum*. They are consistent with the model of a new hemiellipsoidal cyanobacterial phycobilisome which was exemplified first in *Phormidium persicinum* [6]. This model postulates a periphery of 10–12 rods in a close parallel package with each rod being composed of 4–5 biliprotein hexamers. SDS gel electrophoresis of green light phycobilisomes from *Phormidium* sp. in comparison to phycobilisomes from *Phormidium persicinum* showed an identical polypeptide composition [25].

Ultrathin sections of *Phormidium* sp. cells reveal that the number of phycobilisomes per µm<sup>2</sup> thylakoid area is in red light cells twice as high as in green light cells. These data agree with the values calculated for other hemidiscoidal [5] and hemiellipsoidal phycobilisome containing organisms [26].

Isolation of white light phycobilisomes in sucrose density gradients revealed four phycobilisome bands of different size. The epifluorescence of white light filaments showed different color and intensity from cell to cell. Similar epifluorescence

properties could be observed on white light-grown filaments of *Fremyella diplosiphon*. These results implicate that white light-grown filamentous chromatically adapting cyanobacteria contain various types of phycobilisomes in individual cells. At white light conditions the simultaneously applied red and green light stimuli probably induce the differentiation of red and green light phycobilisomes as well as “intermediate forms”.

The different number of rods in red and green light phycobilisomes should also cause variations of the rod-core linkerpolypeptides and their association to the cores. The changes of the phycobilisome density and number of chromophores per phycobilisome demand alterations of energy distribution within the cores and variations of the energy transfer to the associated photosystem II particles. So it is evident that the alterations of the phycobilisome periphery during chromatic adaptation of *Phormidium* sp. are accompanied by changes in the polypeptide composition of the cores and their coupling to the photosystem II particles.

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