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Phycobilisome Variability in the Endocyanelles of *Cyanophora paradoxa*

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Abstract

Phycobilisomes of the endocyanelles of *Cyanophora paradoxa* were isolated and characterized. The isoelectric point of the phycobiliproteins and the spectral properties of their phycobilines are modified depending on the growth stage of the *Cyanophora* mass culture. The isoelectric points are shifted from pH 4.0 to pH 5.4. Moreover the ratio phycocyanin/allophycocyanin shifts from 0.8 : 1 to 4 : 1. The variability of the phycobilisomes is discussed in relation to the increased efficiency of energy transfer during cell development as well as to the modification of the intracellular environment of the endocyanelles and their dependence upon the nucleocytoplasm during growth of *Cyanophora* mass cultures.

Keywords: *Cyanophora paradoxa*, endocyanelles, phycobilisomes, symbiosis

1. Introduction

The endocyanelles of *Cyanophora paradoxa* contain phycobilisomes (Trench and Ronzio, 1978; Marten et al., 1982; Brandt et al., 1982), which serve as the primary light-gathering antennae as described generally for cyanobacteria (Zuber, 1985). These aggregates, composed primarily of phycobiliproteins, are particularly important under light-limiting conditions (Larkum and Barrett, 1983). The endocyanelles of *Cyanophora paradoxa* can adapt to light-limiting conditions by increasing the total phycobilisome content (Brandt et al., 1982). Light is known to regulate phycobiliprotein synthesis by enhancing their overall synthesis in all organisms, and in certain ones by determining the synthesis of a specific type of phycobiline (Larkum and

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Barrett, 1983). The aim of our investigation, however, is to show the variability of the phycobilisome species depending on the stage specific development of *Cyanophora paradoxa*.

2. Materials and Methods

Cyanophora paradoxa (Sammlung von Algenkulturen, Pfanzenphysiologisches Institut, Universität Göttingen) was cultivated according to Marten et al. (1981). The growth vessels of 5 cm in diameter were illuminated at two opposite sides and aerated vigorously from the bottom. The endocyanelles were isolated from *Cyanophora paradoxa* by the method of Herdman and Stanier (1977). Cyanelles (4 mg chlorophyll) were washed (0.33 M sorbitol, 1 mM $MgCl_2$, 2 mM EDTA, 4 mM mercaptoethanol, 50 mM Tricinine-KOH, pH 8.4) and then osmotically shocked in 2 ml medium comprising 25 mM Tricine, 12.5 mM KCl, pH 6.8. To solubilize the thylakoid proteins, the lysed cyanelles were incubated with Triton X-100 (final concentration 1%) for 30 min at 4°C. After addition of 1 vol. Tricine/KCl (pH 6.8) the solubilized thylakoid components were isolated by centrifugation (30 min, 8000× g). 1.5 ml of the blue-green supernatant of Triton X-100 solubilized thylakoid components was fractionated by gel filtration on AcA 34 (LKB, Bromma) (1.6× 100 cm active bed, preequilibrated with Tricine/KCl pH 6.8). Separation into one fraction containing only chlorophyll-protein complexes and another fraction containing only phycobilisomes was done by elution with Tricine/KCl (pH 6.8) comprising 0.5% Triton X-100. The phycobiliproteins were separated by polyacrylamide gel isoelectrofocusing according to the method of Siefermann-Harms and Ninnemann (1979), which was previously employed for the isolation of chlorophyll-protein complexes of chloroplasts. For isolation of phycobilisomes without detergent treatment the isolated cyanelles (20 g) were sonified for 10 sec (Branson Sonic Power, 40 D.C. Amperes). The cyanelle homogenate was centrifuged (10 min, 100,000× g). The red-blue supernatant was concentrated on Sartorius membrane filter SM 11306, dissociated with Na-phosphate buffer (2.5 mM, pH 7.0) and equal amounts of phycobiliproteins were loaded onto a DEAE-cellulose column (2.5× 100 cm active bed, pre-equilibrated with 2.5 mM Na-phosphate buffer, pH 7.0). The column was developed at 4°C in the dark first with 0.06 M Na-phosphate buffer, second with 0.1 M Na-phosphate buffer and third with 0.2 M Na-phosphate buffer, using a total volume of 350 ml. Fractions of 4 ml were collected and their absorption recorded at 578, 622 and 650 nm. Fractions with similar absorption characteristic were combined and their total absorption spectra were recorded between 440 nm and 700 nm. All procedures were done at 4°C. Proteolytic degradation during the isola-

tion of phycobilisomes or their subunits was prevented by the addition (Gray, 1982; Bartlett et al., 1982) of proteinase inhibitors (5 mM $\mathcal{E}$-amino-caproic-acid/1 mM phenylmethylsulfonylfluoride/1 mM benzamidine) to all buffers.

For calculation of the the relative phycobiliprotein content phycobilisomes were isolated and dissociated with Na-phosphate buffer (2.5 mM, pH 7.0). The ratio phycocyanin/allophycocyanin was determined by the method of Yamanaka and Glazer (1981). Light conditions within the mass culture of *Cyanophora paradoxa* were estimated by measuring the transmission at 617 nm, which is indicative for phycobiliproteins. The sample cuvette had a diameter of 2 cm.

3. Results

Under continuous light of 32 Wm^{-2} *Cyanophora* mass cultures show a typical sigmoid growth characteristic with a lag, a logarithmic and a stationary phase (Fig. 1). The light intensity within the *Cyanophora* mass culture decreases obviously by self shading concomitantly with the increase of the cell number/ml. We have previously shown that there is an increase of the ratio phycobiliprotein/chlorophyll of *Cyanophora* cells from 1:1.1 at the lag growth phase to 1:2.5 at the stationary growth stage (Brandt et al., 1982). This increase is accompanied with the modification of the phycobilisomes. With the view to demonstrate this phenomenon, the pigment-proteins of *Cyanophora* endocyanelles were solubilized with Triton X-100 and were separated into one fraction of chlorophyll-protein complexes and another fraction of phycobilisomes (Fig. 2). Their biliproteins were separated by isoelectrofocusing. The isoelectric point of the phycobiliproteins isolated by the Triton X-100 treatment changes from the acid to the neurtral pH range (Table 1). Always two bands (I and II) consisting of phycobilines were observed. Band I, however, cannot be distinguished from band II by its pigment composition. In connection with the isoelectric point shift, the spectral properties of the phycobilisomes in the endocyanelles also change during cultivation. The spectral analysis was done with dissociated phycobilisomes. After sonification of isolated endocyanelles and separation of their phycobilisomes, the treatment with a low ionic strength buffer yields a homogenate of phycobilisome subunits. The elution pattern of this homogenate is dependent on the growth stage of *Cyanophora paradoxa* (Fig. 3). At the lag and the logarithmic growth stage there are three subunit fractions and at the stationary growth stage two subunit fractions identified by different elution buffers and by their extinction at 622 nm. The second fraction C3 at the stationary growth phase, however, is divided into two fractions $C3_1$ and $C3_2$ as shown by their extinction at 650 and 578 nm (Fig. 4). The always present fraction C2 predominant at the

logarithmic and the stationary growth stage contains phycocyanin (maxima: 612, 628 or 624 nm) (Fig. 5). The fractions C3a and C3b predominant at the lag growth stage, the fraction C3 at the logarithmic growth stage and the fraction $C3_2$ at the stationary growth stage contain phycocyanin (maxima: 612, 618, 624 or 632 nm) and allophycocyanin (maxima: 650, 652, 655 or 660 nm) Fig. 5). The fraction C1 at the logarithmic growth stage and the fraction $C3_1$ at the stationary growth stage consist of phycocyanin (maxima: 630 and 620 nm) and perhaps phycoerythrobilin components (shoulders: 582, 558 or 528 nm) (Chapman, 1966; Goodwin, 1974) (Fig. 5). The homogenate of phycobilisome subunits were also used for estimation of the ratio phycocyanin/allophycocyanin at the three different growth stages of *Cyanophora paradoxa*. This ratio was 0.8:1 at the lag growth stage, shifted to 3.8:1 at the logarithmic growth stage and reached 4:1 at the stationary growth phase.

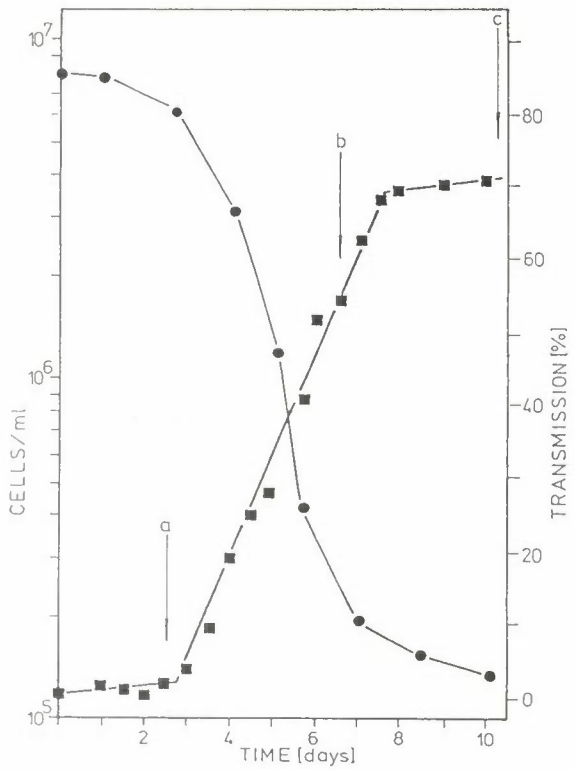

Figure 1. Increase of cells/ml (-■—■-) of *Cyanophora* mass culture under continuous light of 32 Wm^{-2} and decrease of the transmission at 617 nm (-•—•-). Sample of the lag growth phase (a), of the logarithmic growth phase (b) or the stationary growth phase (c).

Table 1. Isoelectric points of the phycobilines isolated by Triton X-100 treatment (1%; 30 min) from endocyanelles of *Cyanophora paradoxa* at the lag, logarithmic or stationary growth stage.

	Isoelectric point (pH)		
	Lag	Logarithmic	Stationary
Band I	4.1	5.1	5.5
Band II	3.9	4.9	5.3

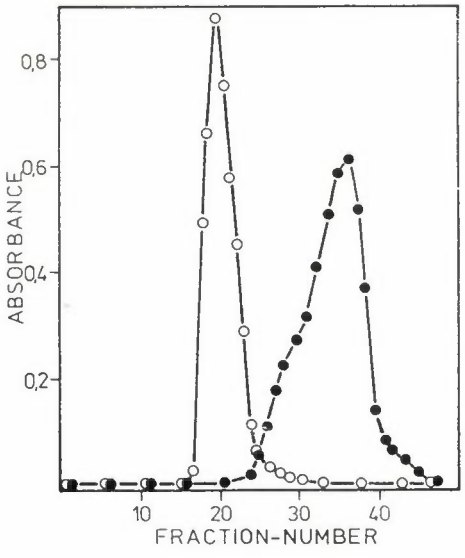

Figure 2. Fractionation of Triton X-100 solubilized pigment-protein complexes from endocyanelles by gelfiltration on AcA 34 (LKB, Bromma). Endocyanelles were isolated at the logarithmic growth stage of a *Cyanophora* mass culture. –o—o– absorbance at 678 nm, –•—•–absorbance at 622 nm.

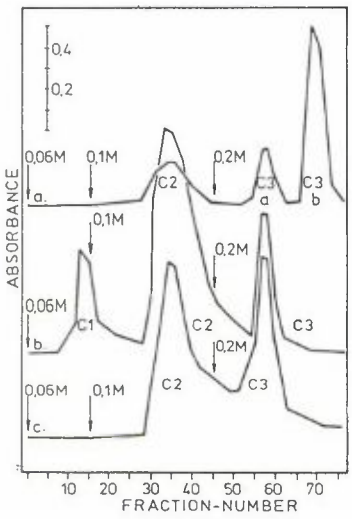

Figure 3. Elution diagram of DEAE-cellulose adsorption chromatography of phycobilisome subunits isolated from endocyanelles of *Cyanophora paradoxa* at the lag (a), logarithmic (b) and stationary growth stage (c). Absorbance at 622 nm.

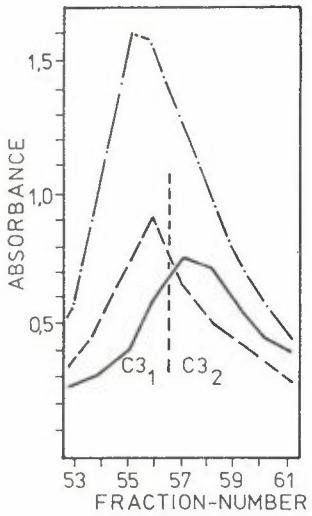

Figure 4. Elution diagram of DEAE-cellulose adsorption chromatography of the third phycobilisome subunit fraction C3 isolated from endocyanelles of *Cyanophora paradoxa* at the stationary growth stage. Absorbance at 622 nm (– · — · —·), at 650 nm (————— , at 578 nm (– – – – –).

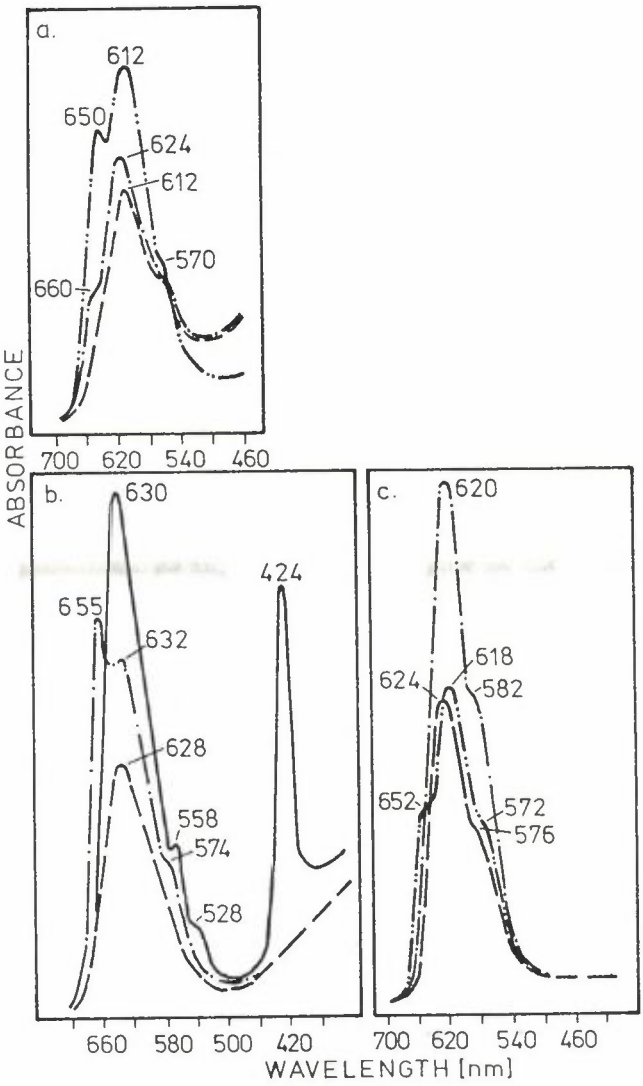

Figure 5. Absorption spectra of the phycobilisome subunit fractions from endocyanelles of *Cyanophora paradoxa* isolated at the lag (a), logarithmic (b) or stationary (c) growth stage. (a) - - - - - = C2, — · — · — = C3a, — · · — · · — = C3b, (b) ———— = C1, - - - - - = C2, — · — · — = C3, (c) - - - - - = C2, — · — · — = $C3_1$, — · · — · · — = $C3_2$.

4. Discussion

Our investigations demonstrate that the spectroscopic properties of the endocyanelle phycobilisomes of *Cyanophora paradoxa* are modified in two aspects. Depending on the growth stage of the mass culture the type and number of bilin prosthetic groups is changed and the characteristics of the protein ensemble related to the phycobilisomes are modified. The spectral properties of biliproteins depend very much on their state of aggregation (Glazer, 1976; Zuber, 1985). The shift to higher wavelength, demonstrated for phycocyanin, indicates different aggregation forms, monomers and hexamers at the lag growth stage but higher aggregates at the logarithmic and stationary growth stage. The shift to lower wavelengths, demonstrated for allophycocyanin, must be interpreted vice versa. Moreover the amount of allophycocyanin in relation to the amount of phycocyanin is diminished during the growth of *Cyanophora paradoxa* under continuous light. The increasing efficiency of energy transfer corresponding to the developmental stage of the *Cyanophora* cells (Brandt et al., 1982) is therefore affected by stabilization of higher organized phycocyanin aggregates caused by additional assembly of unpigmented proteins (Müller, 1982) with basic isoelectric points (Cohen-Bazire and Bryant, 1981). It is reasonable to assume that for an optimal energy transfer within the phycobilisomes (Glazer, 1976; Grabowski and Gantt, 1978) from phycocyanin to allophycocyanin the number of chromophores should be diminished from outside to the inside. But this is obviously not realized in the phycobilisomes of the *Cyanophora* endocyanelles. The increase of the phycocyanin content in the endocyanelles of *Cyanophora paradoxa* looks like the stimulated phycocyanin synthesis in the free-living blue-green alga *Synechococcus 6301*, which results in the typical organization of the phycobilisomes with longer rod substructures, but with unaltered cores (Yamanaka and Glazer, 1980). Furthermore this re-organization of the phycobilisome substructure at the stationary growth stage of *Cyanophora paradoxa* cannot be explained by nitrogen-limiting conditions for the endocyanelles within the *Cyanophora* cells, because their phycobilisomes do not serve as a nitrogen sink as described for *Syncechococcus* species (Gantt, 1980; Yamanaka and Glazer, 1980) and their phycobilisome content increases (Brandt et al., 1982).

The increase of the phycocyanin amount in the cyanelles of *Cyanophora paradoxa* is possibly regulated at the transcriptional level. The α and β subunits of both phycocyanin and allophycocyanin are encoded in dicistronic transcripts (Lemaux and Grossman, 1985). Thus the two subunits can be synthesized in equimolar amounts. Levels of the phycocyanin transcripts, however, are approximately five times that of the allophycocyanin tran-

scripts. Although nothing is known about message stability in *Cyanophora paradoxa*, the assumption of a light-regulated synthesis of the endocyanelle-encoded light-harvesting system in the endocyanelles of *Cyanophora paradoxa* coincides with the light-regulated synthesis of the nucleus-encoded light-harvesting system of higher plant chloroplasts, both regulated at the transcriptional level (Lemaux and Grossman, 1985; Apel, 1979). The encoding of the subunits of both phycocyanin and allophycocyanin in the endocyanelles of *Cyanophora paradoxa*, however, does not exclude the dependence of the phycobilisome differentiation upon the nucleocytoplasm of the 'host cell'. The inhibition of the translation in 80S ribosomes by cycloheximide treatment (Müller, 1982) prevents the increase of the phycocyanin content at the logarithmic growth stage. This is consistent with findings of Trench (1979), who demonstrated the inhibition of ^{14}C incorporation into the small subunit of the phycocyanin by cycloheximide treatment. This phenomenon aligns with our earlier investigations (Marten et al., 1982). We speculate that this dependence of the phycobilisome differentiation upon the 'host-cell' is caused by unpigmented proteins of the phycobilisomes, which may be encoded in the nucleus of *Cyanophora paradoxa*. Their inhibited availability may prevent the assembly of the endocyanelle-encoded phycocyanin.

We evaluate the coincidence of the onset of phycobilisome modification at the beginning of the logarithmic growth stage with the drastic decrease of the light intensity within the *Cyanophora* mass culture as physiological response of the endocyanelles, because this special phycobilisome modification in the endocyanelles is independent of the further growth behaviour of the eukaryotic 'host cell' *Cyanophora paradoxa*, i.e. the rate of cell division.

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