Biochimica et Biophysica Acta, 634 (1981) 237—248

BBA 47996

ENERGY TRANSFER FROM PHYCOBILIPROTEINS TO PHOTOSYSTEM I IN VEGETATIVE CELLS AND HETEROCYSTS OF *ANABAENA VARIABILIS* *

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(Received May 9th, 1980)
(Revised manuscript received September 26th, 1980)

Key words: Heterocyst; Photosystem I; Phycobiliprotein; Nitrogenase; Energy transfer; (Anabaena variabilis)

Summary

The presence of phycobilins in heterocysts of *Anabaena variabilis* is established on the basis of absorption and fluorescence spectroscopy. At 77 K heterocysts exhibit fluorescence emission bands at 645 and 661 nm indicative of phycocyanin and allophycocyanin, respectively. Both allophycocyanin levels and fluorescence emission at 695 nm were low in heterocysts relative to whole filaments. In situ fluorescence microscopy confirmed the presence of phycobilins in individual heterocysts, but the pigment levels varied considerably among cells.

Heterocysts exhibited Photosystem I activity, as evidenced by photooxidation of *P*-700, but no Photosystem II activity. The quantum efficiency of phycobilins in sensitizing *P*-700 photooxidation was 50—70% that of chlorophyll *a*. Phycobilins were also effective in promoting light-dependent reduction of acetylene to ethylene. The results are discussed in terms of the role of the heterocyst in nitrogen fixation and of the significance of energy transfer from phycobilins to Photosystem I in heterocysts.

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Abbreviations: PS I(II), Photosystem I (II); DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; Chl, chlorophyll; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulfonic acid; Pipes, piperazine-*N*,*N'*-bis(2-ethanesulfonic acid).

Introduction

Early investigations into the utilization of radiant energy in photosynthesis underscored the importance of accessory pigments, such as chlorophyll *b* in green algae and higher plants and phycobilins in red and blue-green algae, in promoting photosynthetic efficiency [1,2]. These accessory pigments perform this task by transferring energy of absorbed light quanta to chlorophyll *a*. The two-photosystem model for oxygenic photosynthesis [3] placed the phycobilins in close association with Photosystem II (PS II) and most of the chlorophyll *a* with Photosystem I (PS I). Nevertheless, these authors and others [3—6] have clearly demonstrated the ability of the phycobilins to activate Photosystem I.

Heterocysts of aerobically grown nitrogen-fixing cyanobacteria comprise about 7% of the filament cells. Since they lack PS II activity [7—9], their light-driven nitrogenase activity is presumably coupled to PS I [10—14]. The presence of recognized Photosystem I components, including *P*-700 [8,9,15], certain cytochromes, plastocyanin, iron-sulfur proteins and plastoquinone [9,16], have been documented in heterocysts of, e.g., *Anabaena cylindrica*. Classical PS I activities such as NADP-reduction [9,17] and photophosphorylation [9] have also been demonstrated. However, little is presently known concerning pigment composition and functional organization of the light-harvesting apparatus in mature heterocysts or the changes that occur in this apparatus upon differentiation of a vegetative cell into a heterocyst.

While occasionally being reported as absent or present at low concentrations in heterocysts [7,18,19], phycobilins do occur in easily detectable quantities [20,21], although their levels are lower on a chlorophyll basis compared to vegetative cells and appear to vary with growth conditions and age of culture [22,23]. Low phycobilin levels have generally been accepted as consistent with the lack of oxygen-evolving ability in heterocysts [7,22]. Fay [18] investigated the effect of light quality on nitrogenase activity in intact filaments of *A. cylindrica* and reported a positive correlation with absorption by chlorophyll *a*. The previously mentioned evidence for sensitization of PS I by phycobilins, however, invites renewed speculation as to the role of these pigments in photoreactions and nitrogen fixation in heterocysts.

We report here that when heterocysts isolated from *Anabaena variabilis* by a gentle procedure are compared to vegetative cells on the same chlorophyll basis they possess only about 1/3 to 1/2 as much phycocyanin and an even smaller quantity of allophycocyanin. This comparison is derived from absorption and fluorescence measurements in-situ as well as pigment isolation and determination. In addition, we find that both oxygen evolution and light-induced fluorescence-yield changes generally associated with PS II are negligible in these isolated heterocysts. Finally, the phycocyanin in intact heterocysts is shown to be almost as efficient as chlorophyll *a* in (1) both transferring energy of absorbed light quanta to fluorescent PS I chlorophyll as well as promoting photooxidation of *P*-700 and (2) supporting nitrogenase activity, as assayed by light-dependent reduction of acetylene to ethylene.

Materials and Methods

Cell harvest and heterocyst isolation. Anabaena variabilis (ATCC 29413) was grown in 5-gallon carboys illuminated by a combination of 'Cool White' (Sylvania) and 'Grow Lux' lamps. Other conditions were the same as described by Peterson and Wolk [24]. The cell doubling time was about 30 h. For experiments not directly dealing with nitrogenase activity the heterocysts were isolated aerobically [24].

Acetylene reduction. For measuring action spectra of acetylene reduction, 15-ml vials, 1.5 cm in diameter, were wrapped with aluminum foil except for the flat bottoms and sealed with rubber serum stoppers. The vials were twice evacuated and flushed with H_2 before being filled with a 7% acetylene-93% H_2 mixture. One-ml aliquots of a heterocyst suspension, maintained at about 15°C in the dark and containing 5 μg Chl/ml, were dispended into the vials and set on an array of glass neutral-density filters. Monochromatic illumination was supplied from below via a slide projector and a narrow-band interference filter of the appropriate wavelength. The samples were illuminated at room temperature for 30 min. Light intensities employed were within the linear range of activity vs. intensity. At the end of 30 min, the samples were inactivated by injection of 1 ml of 2.5 M H_2SO_2. Controls were periodically run under saturating white light for estimating any loss of nitrogenase activity over the course of the experiment. Dark controls yielded zero activity. Ethylene analyses were performed on a Carle model 9500 gas chromatograph equipped with a flame-ionization detector and a Poropak-N column.

Isolation of phycobiliproteins. Phycobilins were separated from *A. variabilis* by ion-exchange chromatography. Samples either of intact filaments or heterocysts suspended in buffer A (0.03 M Hepes, 0.03 M Pipes, 1 mM $MgCl_2$, pH 7.2 (KOH); see Ref. 24) were twice passed through a pre-chilled French pressure cell at 20 000 lb/inch^2, dialyzed against two or three changes of 2.5 mM sodium phosphate, pH 7 (starting buffer), and centrifuged at 120 000 $\times g$ for 2 h to remove chlorophyll-containing fragments. The phycobilins were then precipitated by slow addition of $(NH_4)_2SO_4$ to 60% saturation. The precipitate was collected by centrifugation at 20 000 $\times g$ for 30 min, resuspended in the starting buffer and finally dialyzed against this buffer plus 1 mM NaN_3 and 1 mM 2-mercaptoethanol. After a column of Whatman DE52 (34 cm $\times$ 2 cm) was equilibrated with the starting buffer, the sample was applied and washed in with 100 ml of the same buffer. A linear salt gradient was then developed at a flow rate of 0.5—0.9 ml/min by appropriate mixing of the starting buffer with 0.2 M sodium phosphate, pH 7, each buffer 1 mM in NaN_3 and 2-mercaptoethanol. Individual fractions were collected until all blue bands were eluted.

Optical. Absorption spectra were taken on a Cary model-14 recording spectrophotometer equipped with a scattered transmission accessory for analyzing turbid samples. Fluorescence measurements were made with a double-beam fluorometer chamber (Cary accesory No. 56—231) [25]. The fluorescence caused by one beam was analyzed by a JY model H-10 monochromator equipped with a Hamamatsu R928 photomultiplier, while a reference signal was obtained from the other beam using a silicon photodiode (model PIN-10, United Detector Technology). For low-temperature experiments, the sample was

placed in a metal cuvette with a pathlength of 2 mm. Reported emission spectra are uncorrected for efficiencies of the emission monochromator or the photomultiplier.

The same fluorescence apparatus in modified form was also used for measuring fluorescence excitation spectra. To further eliminate extraneous light, narrow-band interference filters (690 or 730 nm) were mounted at the entrance slit of the monochromator. For fluorescence-excitation spectra. light from a 150-W xenon-arc lamp (Osram XBO-150W/S) was passed first through two inches of a 1% $CuSO_4$ solution and then a motor-driven 'filter monochromator' (Schott wedge filter Veril S-200, half-bandwidth about 10 nm) at the entrance port of the fluorometer chamber.

Fluorescence microscopy. Filaments were first concentrated by centrifugation and resuspended in a small volume of used medium. A few drops of suspension were placed between a glass slide and a cover slip, the edges then sealed with wax. The slide was mounted on a Leitz Ortholux-II microscope equipped with an epifluorescence attachment. The 546-nm excitation light was separated from fluorescence emission by a red filter passing only wavelengths above 580 nm. Fluorescence emission was passed through the 'filter monochromator' described above, and then directly to the photomultiplier tube whose output was amplified and recorded. The motor drive for the filter monochromator was synchronized with the time base of the recorder. A 400-μm aperture was mounted on the microscope eyepiece and used in conjunction with the 100X objective to produce an effective field diameter of 4 μm.

Fluorescence-yield changes. Fluorescence yield at 680—690 nm was produced by a weak excitation beam at 610 nm (10 nm bandwidth, intensity about 10 erg $\cdot$ cm^{-2} $\cdot$ s^{-1}) modulated at 25 kHz by an acousto-optic modulator [26]. Actinic excitation was provided by an intense beam at 620 nm ($5 \cdot 10^4$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1}) modulated at 100 Hz by a phosphoroscopic device. Modulation of the excitation light eliminated unwanted transients completely.

Action spectra for P-700 photooxidation. With minor modifications the same apparatus employed in fluorescence-yield measurements was used to measure light-induced absorption changes. The 704-nm measuring beam modulated at 25 kHz was passed through the sample and on to the photomultiplier. Monochromatic excitation at various intensities was provided by a 1000-W tungsten-iodine lamp and appropriate narrow-band interference filters. The excitation beam, modulated at 100 Hz, impinged upon the sample 90° from the measuring beam. The phosphoroscopic device insured that the photomultiplier was unperturbed by the excitation beam during the measurement period. The light-induced absorption-change signal was detected by a PAR model 220 lock-in amplifier and recorded on a Fabri-tek model 1062 signal averager. Depending on the noise content of the signal, 8 to 64 sweeps were averaged. In all cases, the extent of *P*-700 photooxidation by monochromatic light was estimated relative to the signal obtained with saturating white light at $(2—3) \cdot 10^5$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1}. *P*-700-photooxidation signals in monochromatic light ranged from 6 to 78% of that observed in white light. All light intensities were measured with a Kettering model-68 Radiometer.

Results

Absorption and fluorescence spectra

Fig. 1 shows absorption spectra of intact filaments and heterocysts measured at 295 and 77 K. The spectrum for intact filaments closely resembles those reported previously for cyanobacteria [27]. Heterocysts closely resembled filaments in absorption characteristics, most notably in the 550—650 nm region of phycobilin absorption. The ratio of absorption at 620 to that at 680 nm varied somewhat among heterocyst preparations.

Intact filaments at 77 K, when excited by light absorbed predominantly by phycocyanin (570 nm), produced a fluorescence emission spectrum (not shown) similar to that described by Murata et al. [28]. A peak at 661 nm (*F*-661) and shoulder at 648 nm (*F*-648) correspond to allophycocyanin and phycocyanin, respectively [29]. A peak at 692 nm probably arises from Photosystem II chlorophyll and probably includes the shoulder at 684 nm reported previously [28]. A peak at 730 nm (*F*-730) is characteristic of PS I chlorophyll.

When intact, isolated heterocysts at 77 K were excited at 570 nm, either of two types of fluorescence emission spectra resulted. This variability encountered between different preparations is illustrated in Fig. 2. The first (Fig. 2, top) exhibited a strong phycocyanin emission band at 645 nm and a weak allophycocyanin shoulder around 658 nm, plus a small peak at approx. 689 nm. The second (Fig. 2, middle) showed the allophycocyanin peak at 658 nm, with phycocyanin emission present only as a shoulder; in addition, an emission band at 684 nm appeared. Both showed low emission in the 695-nm region. *F*-730 was prominent in all preparations. Variability among different preparations may reflect differences in inherent efficiencies of quantum transfer to chlorophyll and ultimately to PS I.

Room-temperature spectra of filaments and heterocysts excited at 570 nm revealed single, broad phycobilin emissions centered at 660 nm and 645 nm, respectively (Fig. 2, bottom). When heterocysts were excited at 77 K by 430-nm light, absorbed primarily by chlorophyll *a*, the ratio of emission intensity in the 670—700 nm region to that of the 730-nm band was greatly reduced compared to filaments (Fig. 3).

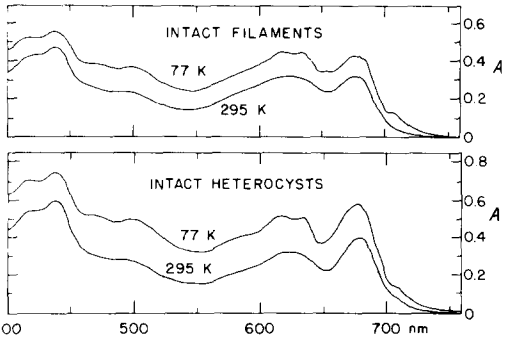

Fig. 1. Absorption spectra of intact filaments and isolated heterocysts from *Anabaena varibilis* at room temperature and at 77 K. Sample pathlength was 2 mm. Filaments were suspended in the growth medium at 25 μg Chl/ml and heterocysts were suspended in buffer A at 20 μg Chl/ml.

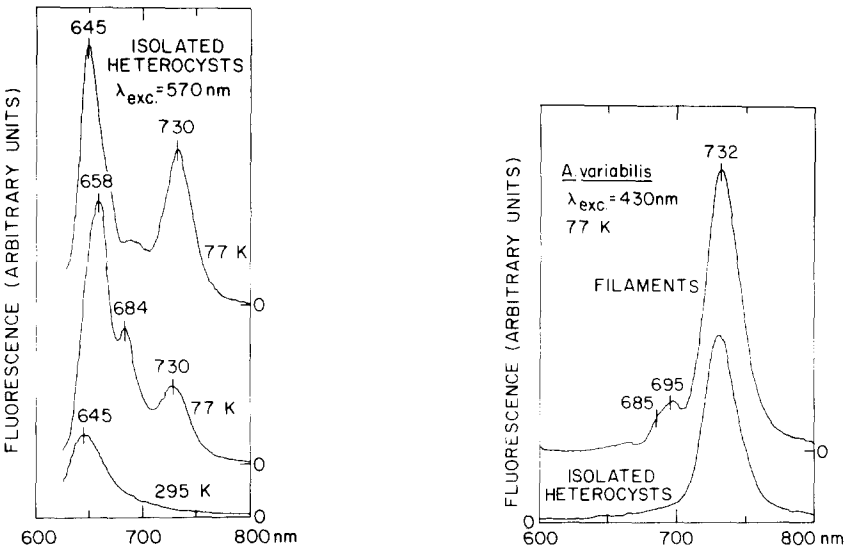

Fig. 2. Fluorescence-emission spectra of intact heterocysts excited at 570 nm. Top: 2 μg Chl/ml; middle: 3 μg Chl/ml; bottom spectrum same as the middle but at room temperature. Spectra displaced vertically for clarity.

Fig. 3. Fluorescence-emission spectra (77 K) of intact filaments and isolated heterocysts excited at 430 nm. Sample pathlength 2 mm; Chl concentration, 2 μg/ml. Spectra displaced vertically for clarity.

The evidence presented thus far, without additional supporting data, could be explained by contamination of heterocyst preparations by vegetative cells or by soluble materials released from these cells during the isolation of heterocysts. Thomas [22] has previously shown that heterocysts in older cultures (15-dayl-old) contain, on the average, more phycocyanin than those in young cultures (2-day-old). Therefore, heterocyst cells were examined individually in situ for fluorescence emission at 294 K (Fig. 4). When filaments from a 4-day-old cul-

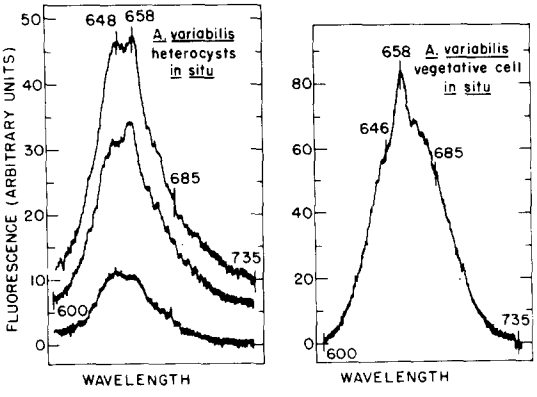

Fig. 4. Typical in situ fluorescence-emission spectra of individual cells of *A. variabilis* at 294 K. Excitation wavelength was 546 nm. Spectra displaced vertically by five units for clarity. Details of the measuring system discussed in Materials and Methods. See Results for other details.

ture were excited with green light, vegetative cells exhibited an intense red fluorescence. Heterocyst cells, on the other hand, exhibited a wide range of fluorescence emission intensities, some appearing even brighter than vegetative cells. Emission spectra of three representative mature heterocysts are presented in Fig. 4. About 20 spectra of mature heterocysts were recorded and all are dominated by phycobilin emission bands at 648 and 658 nm. Vegetative cells appeared to emit more fluorescence in the 685 nm region than heterocysts.

Separation of pigments

Aqueous extracts of filaments were subjected to gradient elution on DE52 cellulose using a method similar to that described by Bryant et al. [29]. Filament extracts showed three peaks, the second being phycocyanin and the third, allophycocyanin. Heterocyst extracts revealed the presence of phycocyanin which was spectrally indistinguishable from the filament phycocyanin, but allophycocyanin was hardly detectable. Phycobilin moieties eluting in the region of the first band in these experiments remain unidentified and no counterparts for these have been reported in the literature. Spectral properties of these components are summarized in Table I.

Heterocysts and absence of fluorescence-yield changes related to Photosystem II

Light-induced changes in fluorescence yield are assumed to arise solely from PS II [30], and can thus be used as a probe for PS II function. Fig. 5 illustrates the comparison between filaments and isolated heterocysts in this regard. In filaments, the PS II inhibitor DCMU (10 μM), either alone or in conjunction with the electron donor NH_2OH (0.1 mM), increases the fluorescence yield in the presence of strong 620-nm light. No such light-induced fluorescence-yield changes occur in heterocysts. In addition, when heterocysts of *A. variabilis* were exposed to intense white light, no oxygen evolution could be detected by an oxygen electrode (experiments performed in the absence of added Hill oxidant).

TABLE I

PHYCOBILIN COMPOSITION OF FILAMENTS AND HETEROCYSTS FROM *ANABAENA VARIABILIS*

Aqueous extracts were prepared and subjected to gradient elution on DE52 as described in Methods. The three major pigment bands eluted from filament extracts were assigned numerals according to Bryant et al., Fig. 1 [29]. Peak fractions in Bands II and III were pooled and absorption spectra recorded. Identification of the phycobilin component(s) in each band was based on the reported λ_{max} and in comparison with published spectra. Minor component (a) was found at the leading edge of Band I while component (b) was comprised of the rest of the band.

	Band I (minor component) λ_{max} (nm)	Band II (phycocyanin) λ_{max} = 617 nm	Band III (allophycocyanin) λ_{max} = 651 nm
Filaments	(a) 620 (650) (b) 635	present	present
Heterocysts	610	present	absent

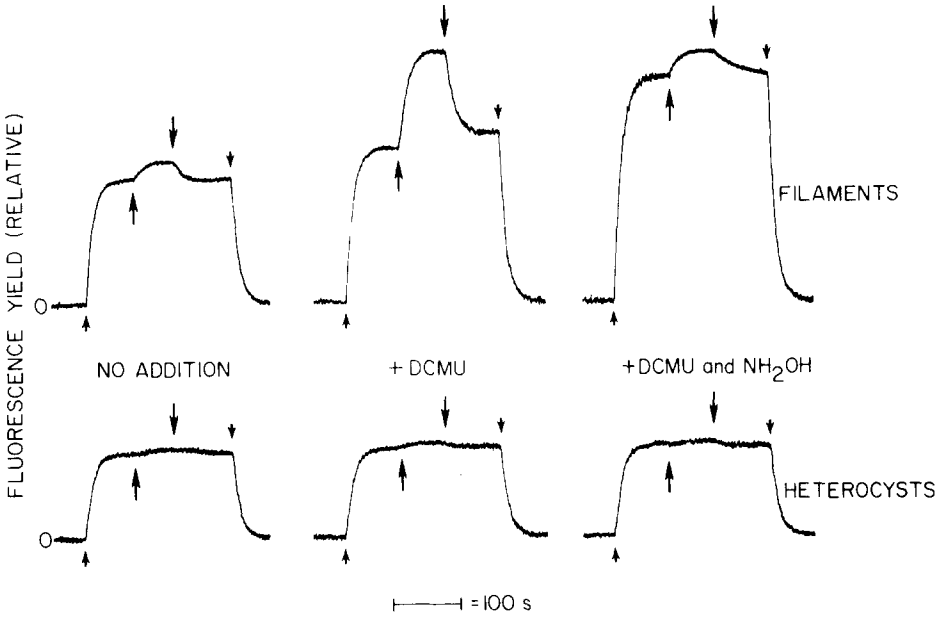

Fig. 5. Light-induced fluorescence-yield changes in whole filaments and heterocysts. Upward and downward arrows represent light on and off, respectively. Short arrows for the weak probing beam, and the larger arrows for the strong actinic beam. Samples contained 3 μg Chl/ml. DCMU and NH_2OH, when present, were 10 and 100 μM, respectively. The weak probing beam was at 610 nm and the strong actinic beam at 620 nm. Fluorescence in the 680-690 nm region was isolated by a combination of filters (Corning 2-64; Schott RG-5 and RG-8). See Materials and Methods for a description of the measuring system.

Fluorescence excitation spectra, action spectra, and evidence for energy transfer from phycobilins to Photosystem I

Fluorescence emission spectra described earlier suggested that light absorbed by phycobilins could be transferred to PS I chlorophyll *a*. Fluorescence excita-

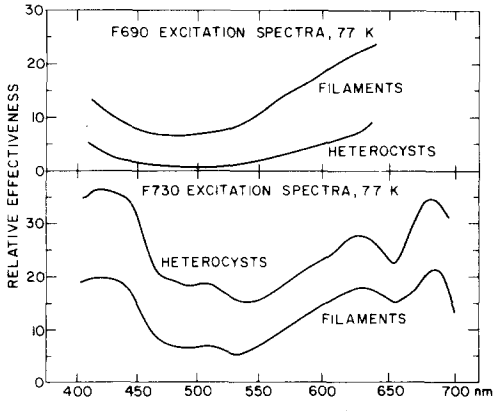

Fig. 6. Fluorescence-excitation spectra of *F*-690 and *F*-730 (at 77 K) for filaments and heterocysts of *A. variabilis*. Samples containing 2 μg Chl/ml were frozen in the metal cuvette and mounted in the fluorometer chamber described in Materials and Methods. The upper spectra in each half of the figure are displaced upward for clarity. See text for other details of the measurements.

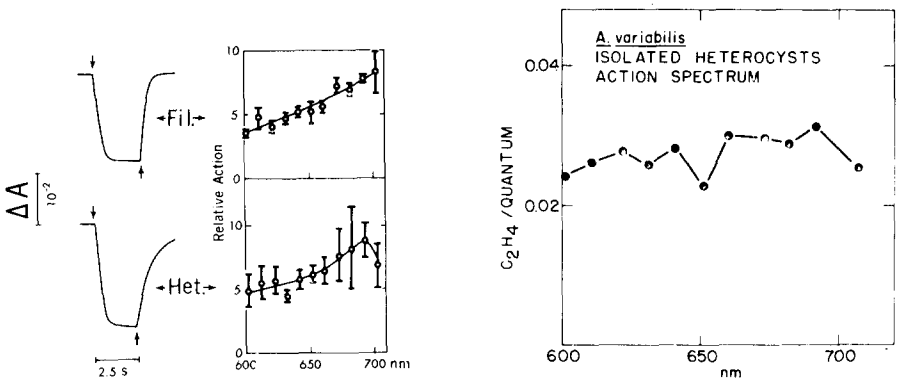

Fig. 7. (Left) Typical *P*-700 absorption-change transients at 704 nm induced by saturating white light in nitrogen-fixing filaments and isolated heterocysts of *A. variabilis*. First arrow indicates light on, and the second arrow light off. Intensity of the measuring beam was 25 erg · cm^{-2} · s^{-1}. Samples were prepared by first sonicating whole filaments for 2 min in a Cole-Palmer model 8845-50 3 qt. Sonic Bath followed by washing and resuspending in buffer A. This procedure merely fragmented the filaments and produced a more homogeneous suspension. Final samples of filaments or intact, isolated heterocysts contained 10 μg Chl/ml in buffer A (final volume was 2.75 ml), 1.8 mM sodium ascorbate, 27 μM DCIP, 9 μM DCMU, 9 μM methyl viologen, and 10% Ludox (DuPont) to retard sample setting. (Right) Action spectra for *P*-700 photooxidation. *P*-700 photooxidation in heterocysts was corrected in each case to 30% development as described in the text, i.e., relative values shown are proportional to the inverse of the absorbed quantum flux necessary to produce 30% photooxidation of *P*-700. *P*-700-photooxidation data from replicate experiments were normalized relative to the average quantum effectiveness of all wavelengths tested. Results shown are combined values of four experiments for heterocysts and three experiments for filaments. Bars indicate standard deviation.

Fig. 8. Action spectrum for acetylene reduction by isolated heterocysts. Experimental procedures as described in Materials and Methods. The nitrogenase activity of these heterocysts under saturating white light was 1.0 nmol/μg Chl in 20 min.

tion spectra at 77 K for *F*-690 and *F*-730 (Fig. 6) are quite similar for filaments and heterocysts. Light absorbed by either chlorophyll *a*, carotenoids, or phycobilins is effective in exciting *F*-730, while *F*-690 appears to be sensitized only by phycobilins. Excitation spectra for *F*-690 and *F*-730 were the same for both types of heterocyst emission spectra shown in Fig. 2. Careful determinations were made at 77 K of the relative effectiveness of 600 nm (ϕ_{600}) and of 440 nm (ϕ_{440}) light in exciting *F*-730. After correction for differences in total absorption at the two wavelengths, the average ϕ_{600}/ϕ_{440} for filaments is 1.20 (standard deviation for five experiments is 0.18) and 1.35 (standard deviation for four experiments is 0.17) for heterocysts.

A direct means of assessing whether light quanta absorbed by the phycobilins can be transferred to PS I is to compare the relative effectiveness of nonsaturating intensities of monochromatic light at various wavelengths in bringing about photooxidation of *P*-700. Absorption-change transients shown in Fig. 7 indicate that steady-state levels of *P*-700$^+$ were established rapidly. The steady-state signal of *P*-700 photooxidation was taken as a relative measure of effectiveness at the particular wavelength used. *P*-700$^+$ development was linear with monochromatic light intensity in filament preparations, but with heterocysts the *P*-700$^+$ level vs. light intensity relationship was hyperbolic. To facilitate comparisons, all points were corrected to 30% of the full *P*-700$^+$ signal in satu-

rating white light using $P\text{-}700^+$ level vs. light intensity curves developed at 623 and 683 nm. The results indicate that for both filaments and heterocysts, in the presence of DCMU, light quanta absorbed by the phycobilins are about 60% as effective as light quanta absorbed directly by chlorophyll *a*. Also shown in Fig. 8 is an effectiveness spectrum for reduction of acetylene to ethylene by isolated heterocysts. Again, the phycobilins are found to be nearly as effective as chlorophyll *a*.

Discussion

Our results agree with those of Fay [18,21] in that we find lower phycocyanin levels in heterocysts compared to vegetative cells, but we come to different conclusions as to the role of phycobiliproteins in heterocyst photosynthesis (see below). Low-temperature fluorescence-emission spectra (see also Ref. 31) and absorption spectra provide support for the presence of phycobilins in heterocysts. Yet an active PS II appears to be absent [8,9] (see above). Thomas [22,23], using microspectrophotometry to examine heterocysts of a species of *Anabaena*, observed that phycocyanin levels were low in heterocysts of 2-day-old cultures, but that this pigment returned to heterocysts of cultures in the later linear phase of growth.

Our results, obtained by fluorescence microscopy, reveal typical phycocyanin and allophycocyanin emission bands in heterocysts. Variable fluorescence intensities may reflect heterocysts at different stages of differentiation, ranging from very young to very old.

Extraction and separation by ion-exchange chromatography of phycobilins revealed some interesting differences between heterocysts and vegetative cells. The very low allophycocyanin content in heterocysts suggests a possible modification of the phycobilisomes during heterocyst differentiation. Allophycocyanin B, a minor component of the phycobilisome which may directly transfer excitation energy to chlorophyll [29,32,33], was not evident in any of our fractions, presumably owing to the limited quantity of cell extract used in our studies. We note that the 77 K fluorescence emission at about 685 nm may arise from allophycocyanin B as well as PS II chlorophyll (cf. Refs. 32, 33). The minor phycobilins may be precursors or breakdown products of the previously described phycobilins. These minor components each exhibit preferential association with either heterocysts or vegetative cells.

We sought evidence for an association of the phycobilins with PS I. PS I fluorescence-yield studies with cyanobacteria and red algae point out that on a quantum basis light absorbed by the phycobilins is about equally as effective as light absorbed by chlorophyll *a* [28,30,34,37]. Action spectra for the rate-limiting PS I reaction measured as net oxygen evolution against a background of strong PS II illumination clearly showed a strong involvement of the phycobilins and chlorophyll *a* [3,5]. Pullin et al. [35] have recently reported the presence of bound allophycocyanin in PS I particles isolated from *Chlorogloea fritschii*. Fluorescence excitation spectra for both filaments and heterocysts of *A. variabilis* presented herein point out the constant high yield of quantum transfer from the phycobilins to PS I (F-730). Likewise, the excitation spectra for F-690 are also similar for the two preparations.

On the basis of fluorescence studies at 77 K with spinach chloroplasts, Butler has presented models for energy distribution of PS II and PS I [36]. When the models were extended to the red alga *Porphyridium cruentum*, it was concluded that the phycobilisomes transfer essentially all of the energy of their absorbed light quanta to very small PS II units while almost all of the chlorophyll *a* is associated with PS I. High photosynthetic efficiency in green light is attributed to energy transfer from PS II to PS I possibly at the level of allophycocyanin B by 'spillover' [30]. Fluorescence yield [37] and *P*-700 photooxidation [38] experiments suggest that the state of the PS II traps determines energy partitioning. The yield of energy transfer from PS II to PS I increases by 80% when the PS II traps are closed (maximum PS II fluorescence). Although lacking phycoerythrin, the photosynthetic apparatus of *A. variabilis* can be assumed to be functionally similar to that in *P. cruentum*.

Considerable loss of PS II related properties occurs upon differentiation of a vegetative cell into a heterocyst. Nevertheless, convincing evidence for energy transfer from the phycobilins to an active PS I can be found in the action spectra of *P*-700 photooxidation. The phycobilins are about 50—70% as effective as chlorophyll *a* in sensitizing this PS I reaction in both vegetative cells and isolated heterocysts. Consideration of the effects of DCMU on variable fluorescence yield and the similarity of shapes of the *P*-700-photooxidation action spectra suggests that the model for quantum utilization in heterocysts functionally resembles that found in vegetative cells when all of the PS II traps are closed (i.e., in the presence of DCMU and under illumination). Probably a minimum of reorganization occurs within PS I upon heterocyst differentiation, although some enrichment of PS I reaction centers results.

The action spectrum of H_2-supported light-dependent reduction of acetylene by heterocysts clearly demonstrates a high relative quantum yield in the phycobilin region of the spectrum consistent with the *P*-700-photooxidation action spectrum. Fay [18], working with whole filaments of *A. cylindrica*, also concluded that PS I was responsible for photostimulation of nitrogen fixation. Monochromatic light in the 550—650 nm region absorbed by phycocyanin was nearly equal in effectiveness when compared to light absorbed by chlorophyll, especially if the data are replotted as activity per absorbed quantum. PS I in heterocysts may drive nitrogenase activity via cyclic photophosphorylation [7,8,11,39] or by a non-cyclic electron transfer possibly coupled to ATP synthesis culminating in photoreduction of nitrogenase [14,40].

We feel that these results further our knowledge of the utilization of radiant energy by heterocysts, cells specialized for nitrogen fixation. Furthermore, as heterocysts show considerably diminished PS II related properties, they may afford a unique opportunity to study directly the relationship between cyanobacterial PS I and the light-harvesting phycobiliproteins.

Acknowledgement

The authors express gratitude to M. Lalonde, J.C. Meeks, G.A. Peters, V.V.S. Tyagi and C.P. Wolk for helpful discussions.

References

1 Emerson, R. and Lewis, C.M. (1942) J. Gen. Physiol. 25, 579-595
2 French, C.S. and Young, V.K. (1952) J. Gen. Physiol. 35, 873—890
3 Duysens, L.N.M. and Amesz, J. (1962) Biochim. Biophys. Acta 64, 243—260, 261—278
4 Kok, B. and Gott, W. (1960) Plant Physiol. 35, 802—808
5 Jones, L.W. and Myers, J. (1964) Plant Physiol. 39, 938—946
6 Wang, R.Y., Stevens, C.L.R. and Myers, J. (1977) Photochem. Photobiol. 25, 103—108
7 Haselkorn, R. (1978) Annu. Rev. Plant Physiol. 29, 319—344
8 Donze, M., Haveman, J. and Schiereck, P. (1972) Biochim. Biophys. Acta 256, 157—161
9 Tel-Or, E. and Stewart, W.D.P. (1977) Proc. R. Soc. Lond. B 198, 61—86
10 Giddings, T.H. and Staehelin, L.A. (1979) Biochim. Biophys. Acta 546, 373—382
11 Wolk, C.P. and Wojciuch, E. (1971) Planta (Berl.) 97, 126—134
12 Bothe, H. and Loos, E. (1972) Arch. Mikrobiol. 86, 241—254
13 Peterson, R.B. and Burris, R.H. (1976) Arch. Microbiol. 108, 35—40
14 Lockau, W., Peterson, R.B., Wolk, C.P. and Burris, R.H. (1978) Biochim. Biophys. Acta 502, 298—308
15 Alberte, R.S. and Tel-Or, E. (1977) Plant Physiol. 59, 129
16 Cammack, R., Tel-Or, E. and Stewart, W.D.P. (1977) FEBS Lett. 70, 241—244
17 Wolk, C.P. and Simon, R.D. (1969) Planta (Berl.) 86, 92—97
18 Fay, P. (1970) Biochim. Biophys. Acta 216, 353—356
19 Stewart, W.D.P. (1973) Annu. Rev. Microbiol. 27, 283—316
20 Stewart, W.D.P., Haystead, A. and Pearson, H.W. (1969) Nature 224, 226—228
21 Fay, P. (1969) Arch. Mikrobiol. 67, 62—70
22 Thomas, J. (1970) Nature 228, 181—183
23 Thomas, J. (1972) J. Bacteriol. 110, 92—95
24 Peterson, R.B. and Wolk, C.P. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 6271—6275
25 Ke, B. and Vernon, L.P. (1967) Biochemistry 6, 2221—2226
26 Breeze, R.H. and Ke, B. (1972) Anal. Biochem. 50, 281—303
27 Cho, F. and Govindjee (1970) Biochim. Biophys. Acta 216, 151—161
28 Murata, N., Nishimura, M. and Takamiya, A. (1966) Biochim. Biophys. Acta 126, 234—243
29 Bryant, D.A., Glazer, A.N. Eiserling, F.A. (1976) Arch. Microbiol. 110, 61—75
30 Ley, A.C. and Butler, W.L. (1977) Photosynthetic Organelles (Miyachi, S., Katoh, K., Fujita, Y. and Shibata, K., eds.), pp. 33—46, University of Tokyo, Tokyo
31 Thomas, J. (1977) Nucl. Ind. 15, 2—5
32 Ley, A.C., Butler, W.L., Bryant, D.A. and Glazer, A.N. (1977) Plant Physiol. 59, 974—980
33 Katoh, K. and Gantt, E. (1979) Biochim. Biophys. Acta 546, 383—393
34 Bergeron, J.A. and Olson, J.M. (1967) Biochim. Biophys. Acta 131, 401—404
35 Pullin, C.A., Brown, R.G. and Evans, E.H. (1979) FEBS Lett. 101, 110—112
36 Butler, W.L. (1978) Annu. Rev. Plant Physiol. 29, 345—378
37 Ley, A.C. and Butler, W.L. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 3957—3960
38 Ley, A.C. and Butler, W.L. (1977) Biochim. Biophys. Acta 462, 290—294
39 Cox, R.M. and Fay, P. (1969) Proc. R. Soc. Lond. B 172, 357—366
40 Smith, R.V., Noy, R.J. and Evans, M.C.W. (1971) Biochim. Biophys. Acta 253, 104—109