BBA Report

BBA 41196

Absence of Photosystem 2 in heterocysts of the blue-green alga Anabaena

M. DONZE, J. HAVEMAN and P. SCHIERECK
Biophysical Laboratory of the State University, Schelpenkade 14A, Leiden (The Netherlands)
(Received November 5th, 1971)

SUMMARY

Heterocysts of the filamentous blue-green alga *Anabaena cylindrica* have a high concentration of the System 1 reaction center P700 and are able to photooxidize a cytochrome. They have a low yield of chlorophyll *a* fluorescence, do not show light-induced changes in fluorescence yield, show a very low intensity of delayed light emission and do not show Hill-reaction activity. It is concluded that heterocysts contain Photosystem 1 only.

Many filamentous blue-green algae form two kinds of cells: "normal" vegetative cells and heterocysts. Normally about 5% of the cells are differentiated into heterocysts; in Anabaena these enlarged cells occur singly and rather regularly spaced along the filament. They contain two cellular inclusions of about 1 μm; the function of these so-called polar bodies is unknown. Heterocysts are an interesting subject for the study of cell differentiation[1]. Under aerobic conditions heterocysts are believed to be the site of N_2 fixation[2,3]. Pigment composition and several other indirect arguments[2,4] suggested that heterocysts do contain System 1 but not System 2 of the photosynthetic mechanism.

Here we present direct evidence which supports this hypothesis. *Anabaena cylindrica* (Cambridge Culture Collection No. 1403/2a) was grown in the medium of Allen and Arnon[5] in 1 l Roux bottles. During growth the culture was gassed with 5% CO_2 in air, and stirred vigorously by a "vibro-Mischer" (Chemap AG, Switzerland) to prevent clumping of the filaments. Absorption spectra were recorded with a Cary 14 spectrophotometer equipped with a scattered light transmission accessory. Changes in intracellular light absorption were measured with a split-beam difference spectrophotometer[6]. The Hill reaction was measured with an Aminco–Chance dual wavelength spectrophotometer which was provided with a side-illumination for one of the cuvettes. Fluorescence spectra were recorded with the instrument used by Vredenberg and Slooten[7]. Light-induced changes in

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-tris(hydroxymethyl)methylglycine.

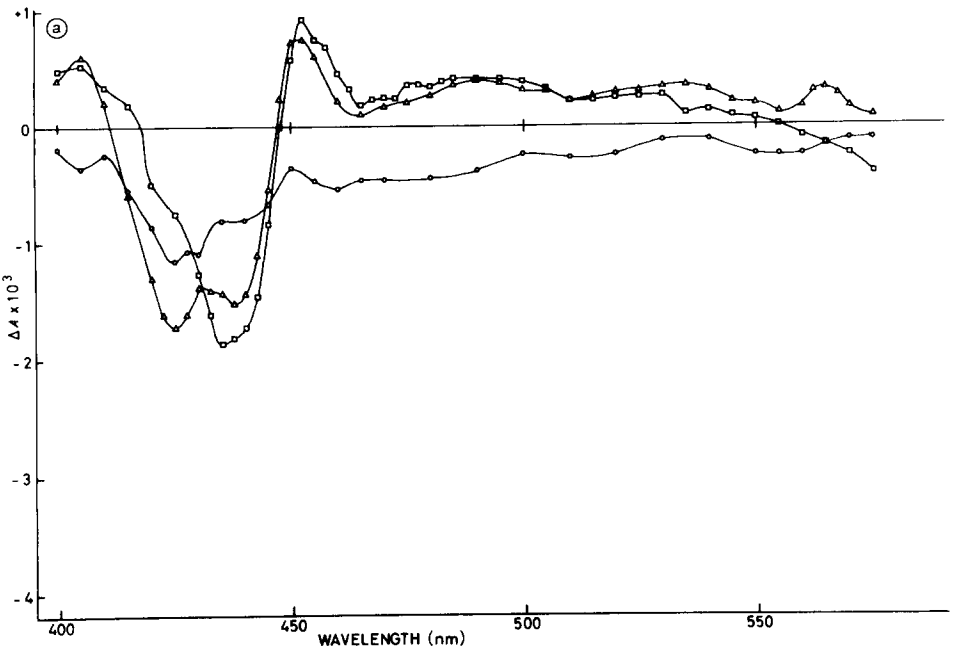
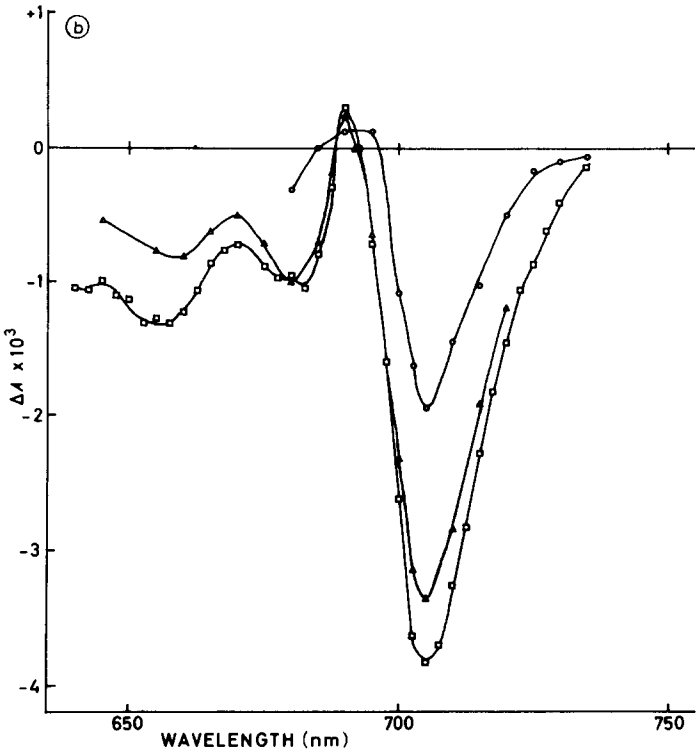


Fig. 1. Light *minus* dark absorption difference spectra. The absorbance of all samples at 680 nm, corrected for scattering was 0.30. (a) Saturating red actinic light (Schott RG 665-2 mm filter). (b) Saturating blue actinic light (Corning CS 4-96 and CS 5-61 filters). ○—○, intact filaments of Anabaena in growth medium with 0.5% agar to prevent movements of the organism. The initial rapid (2 sec) absorption change is plotted only. △—△, heterocysts isolated in growth medium with 0.25 M sucrose and 1 ‰ mercaptoethanol. Shortly before the measurement $2.5 \cdot 10^{-4}$ M ascorbate and 10^{-5} M DCIP were added to reduce P700 and cytochrome in the dark. □—□, heterocysts isolated in growth medium; ascorbate and DCIP added.

the fluorescence yield of chlorophyll were measured with an instrument analogous to the one used by Duysens and Sweers[8]. Fluorescence excitation light was filtered by a Corning CS 4-96 and a CS 5-61 filter, which transmit a broad band from about 400 to 500 nm. The emission was isolated with a Schott Al 683 interference filter together with a RG 5 cut-off filter. Delayed light from chlorophyll was measured with a Becquerel phosphoroscope at a chopping frequency of 570 Hz, with the same filter combinations as mentioned above. Chlorophyll fluorescence was also studied with a fluorescence microscope. A high-pressure mercury arc from which the blue lines were isolated with the same filter combination as mentioned above was used for excitation. The fluorescence image was observed through a Schott RG 695 cut-off filter. This filter transmitted only a negligible amount of phycocyanin fluorescence, as was checked by adding phycocyanin to the sample at such a concentration that the background fluorescence at 630 nm was much higher than that of the vegetative cells.

Heterocysts were isolated after destruction of the vegetative cells in a French press[9] Cell debris was removed by filtration through a 2-μm Nuclepore filter (General Electric). Polar bodies were prepared by sonication of isolated heterocysts and separated from chlorophyll-containing membrane fragments by centrifugation in 25% fycoll (Pharmacia,



Sweden) solution. The polar bodies were identified under a polarization microscope by means of their optical anisotropy.

Light *minus* dark absorption difference spectra were measured for intact filaments and isolated heterocysts of Anabaena (Fig. 1). Illumination of isolated heterocysts caused an oxidation of the System 1 reaction center P700 with main bands at 705 nm and 437 nm and of a cytochrome with bands at about 420 nm and 560 nm. Isolated heterocysts do not reduce P700 and cytochrome in the dark, so an artificial reducing couple (ascorbate and DCIP) was added in our experiments. Cytochrome oxidation was observed only if the heterocysts were isolated in growth medium with sucrose and mercaptoethanol; washing in medium without these additions abolished this reaction. The time dependence of the light-induced absorption changes in intact filaments was rather complicated, only the first rapid (2 sec) change is plotted in the difference spectrum.

Assuming that the molar extinction of P700 at 705 nm is the same as that for chlorophyll *a in vivo*, we found a P700 concentration of 1 molecule to 90 chlorophyll molecules in heterocysts and in isolated polar bodies. The concentration in intact filaments was 1 to 170 chlorophylls. The same value was obtained by repeating the measurement in the presence of 10^{-6} M DCMU, 10^{-4} M ascorbate and 10^{-5} M DCIP.

These observations show that heterocysts contain an active Photosystem 1. The absence of Photosystem 2 is indicated by the high concentration of P700, which is about the same as found by Ogawa *et al.*[10] in System 1 particles isolated from *Anabaena variabilis*. This conclusion is also supported by the observations that in isolated heterocysts

photoreduction of P700 did not occur for any wavelength of actinic light between 400 and 750 nm and the absence of any effect of DCMU on the rate of photooxidation of P700.

The absence of Photosystem 2 was also supported by measurements of the Hill reaction (Table I). Semicarbazide[11] and hydrazobenzene[12] were used as electron donor,

TABLE I

HILL REACTION OF SONICATED ANABAENA AND HETEROCYSTS

Intact Anabaena and isolated heterocysts were sonicated for 3 min in 0.05 M Tricine buffer (pH 7.5) with 0.01 M $MgCl_2$. The reaction mixture contained in addition $2 \cdot 10^{-5}$ M DCIP and $5 \cdot 10^{-3}$ M semicarbazide or 10^{-4} M hydrazobenzene. DCIP reduction was measured with saturating red light.

	DCIP reduction (μmoles/h per mg chlorophyll)		
	With semicarbazide	*With hydrazobenzene*	*No electron donor*
Sonicated Anabaena	23.0	37.4	0.0
Sonicated heterocysts	0.0	5.8	0.0

DCIP as electron acceptor. For these experiments both heterocysts and intact filaments were sonicated for 3 min in Tricine buffer solution. With semicarbazide as electron donor no Hill reaction was observed with the heterocyst preparation. With hydrazobenzene the rate of reduction with sonicated heterocysts was only one-seventh of that of the sonified filaments. This (low) rate may be due to a System 1 rather than a System 2 reaction with hydrazobenzene (J. Haveman, unpublished experiments). Without electron donor no DCIP reduction was observed in either case.

Delayed light from chlorophyll has been shown to arise mainly in Photosystem 2[13] In agreement with this we found that delayed light from heterocysts was 0.2 ± 0.1% from the delayed light emitted by intact filaments for the same intensity of absorbed light.

In oxygen-evolving photosynthetic organisms the fluorescence yield of chlorophyll *a* belonging to System 2 is several times higher than the yield of System 1 chlorophyll *a*[14]. Changes in the fluorescence yield that occur upon illumination are generally ascribed to variations in the state of System 2[8]. Changes in the fluorescence of System 1 are considered small or nonexistent[7,14]

Fluorescence emission spectra of intact filaments and isolated heterocysts are shown in Fig. 2. The fluorescence from heterocysts was several times lower than from vegetative cells and especially the emission in the region 670–700 nm was strongly reduced. The low yield of chlorophyll fluorescence was not an artifact due to the isolation procedure. Observation of a preparation containing isolated heterocysts and intact filaments with a fluorescence microscope showed no observable difference between the fluorescence yield of isolated heterocysts and heterocysts in intact filaments.

Light-induced changes in the yield of chlorophyll fluorescence in intact filaments showed the normal pattern for blue-green algae as discussed by Duysens and Talens[15]. In isolated heterocysts no light-induced changes in the fluorescence yield exceeding 0.5% of the total fluorescence were observed. Addition of DCMU or reduction by dithionite did not affect the fluorescence yield, within the same error of measurement.

Both the low fluorescence yield and the absence of changes in fluorescence indicate

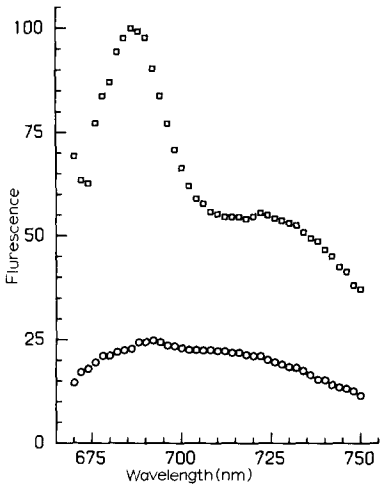


Fig. 2. Fluorescence emission spectra, plotted as relative number of quanta per wavelength interval. The same intensity of blue excitation light was absorbed by each sample. □□□, intact filaments of Anabaena in growth medium with 0.5% agar and 10^{-7} M DCMU. ○○○, isolated heterocysts in growth medium with agar.

the absence of Photosystem 2 in heterocysts. Vredenberg and Slooten[7] demonstrated with System 1 particles, prepared from spinach chloroplasts, that the fluorescence of System 1 is independent of the redox state of P700. This is confirmed by our experiments for the intact photosynthetic membranes in the heterocysts. This point has been discussed in detail by Duysens[14].

This work was supported in part by the Netherlands Foundation for Biophysics and that for Chemical Research (S.O.N.). These foundations are financed by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

REFERENCES

1 P. Fay and N.J. Lang, *Proc. R. Soc. London, Ser. B*, 178 (1971) 185.
2 P. Fay, W.D.P. Stewart, A.E. Walsby and G.E. Fogg, *Nature*, 220 (1968) 810.
3 W.D.P. Stewart and M. Lex, *Arch. Mikrobiol.*, 73 (1970) 250.
4 J. Thomas, *Nature*, 228 (1970) 181.
5 M.B. Allen and D.I. Arnon, *Physiol. Plant.*, 8 (1955) 652.
6 J. Amesz, Thesis, University of Leiden, 1964.
7 W.J. Vredenberg and L. Slooten, *Biochim. Biophys. Acta*, 143 (1967) 583.
8 L.N.M. Duysens and H.E. Sweers, in S. Miyachi, *Studies on Microalgae and Photosynthetic Bacteria*, Special Issue of *Plant Cell Physiol.*, Tokyo, 1963, p. 353.
9 P. Fay and A.E. Walsby, *Nature*, 209 (1966) 94.
10 T. Ogawa, L.P. Vernon and H.H. Mollenhauer, *Biochim. Biophys. Acta*, 172 (1969) 216.
11 T. Yamashita and W.J. Butler, *Plant Physiol.*, 44 (1969) 435.
12 J. Haveman and M. Donze, *Proc. 2nd Int. Congr. Photosynth. Res., Stresa, 1971*, in the press.
13 J.C. Goedheer, *Biochim. Biophys. Acta*, 64 (1962) 294.
14 L.N.M. Duysens, *Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symp. Biol.*, 19 (1966) 71.
15 L.N.M. Duysens and A. Talens, in H. Metzner, *Progress in Photosynthesis Research*, Vol. II, Tübingen, 1969, p. 1073.