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Characterization of the Photosynthetic Electron Transport Chain in Normal and Photobleached *Anabaena cylindrica* by Flash Spectroscopy

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Abstract

Electron transport of normal and photobleached *Anabaena cylindrica* was studied using spectral and kinetic analyses of absorbance transients induced by single turnover flashes. Between 500 and 600 nm two positive bands (~540 and ~566 nm) and two negative bands (~515 and ~554 nm) were found. Absorbance changes at 515 and 540 nm were partly characterized. None of these absorbance changes represent an electrochromic shift. Absorbance changes at 554 and 566 nm correspond to the oxidation of cytochrome *f* and the reduction of cytochrome b_{563}, respectively. We found a very slight 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) sensitivity of cytochrome *f* in normal cells, while DCMU was completely ineffective for cytochrome *f* reduction in photobleached cells. The absorbance change of cytochrome b_{563} increased, while the absorbance change of cytochrome *f* was smaller than in normal cells. The increased O_2 evolution in photobleached cells and the negligible electron transport via cytochrome *f* suggest the participation of other electron acceptor(s) in the electron-transport chain of photobleached *Anabaena cylindrica*.

Key Words: Electron transport; cytochrome *f*; *Anabaena cylindrica*.

Introduction

Detailed studies of electron-transport components between the two photosystems in blue-green algae emphasized that there is no essential difference compared to higher plants (Ho and Krogmann, 1982; Binder, 1982; Almon and Böhme, 1980). However, functional studies revealed some peculiarities. The two reaction centers do not always show a 1:1 stoichiometric relationship in cyanobacteria, but strongly depend on the cultivation conditions (Kawamura *et al.*, 1979; Meyers *et al.*, 1980; Melis and Brown, 1980). Moreover, no stoichiometric relationship was found among any electron

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carrier in the electron transport between the two photosystems (Aoki *et al.*, 1983). In a thermophilic blue-green alga (*Synechococcus*) the cytochrome *f* (cyt *f*)[2] reduction was DCMU insensitive (Hirano *et al.*, 1980). Organic or inorganic acid, as well as respiratory substrates, can serve as electron donor to Photosystem I (PS I) via cyt *f* instead of electrons from PS II (Murai and Katoh, 1975; Sörensen and Halldal, 1977; Padan, 1979).

In our earlier studies, the filamentous cyanobacterium *Anabaena cylindrica* grown at high light intensity ($100\,W\,m^{-2}$) evolved 2.5 times more oxygen per mg dry weight at saturating light intensity compared to cells grown at low light intensity ($15\,W\,m^{-2}$) (Laczkó and Barabás, 1981). This *in vivo* O_2 evolution could be completely inhibited in the presence of DCMU. The increased O_2 evolution rate, which indicates an increased PS II activity in photobleached cells, raised the question of whether or not there is an effective linear transport via cyt *f* in *Anabaena cylindrica* and of what kind of changes can be observed in photobleached cells.

Flash-induced absorbance changes were used to monitor single oxidation–reduction processes of cyt *f* and b_{563} both in intact filaments and in spheroplast preparations.

Materials and Methods

Anabaena cylindrica strain PCC7122 was cultivated in a modified Allen and Arnon's medium (Allen and Arnon, 1955), pH 7.4 (modification: $NaHCO_3$, 0.25 mM; Fe^{3+}-citrate, 0.02 mM) and gassed with air. The cultures were maintained at 22°C and continuously illuminated by Tungsram 20 WF 33 white fluorescent lamps with a light intensity of $15\,W\,m^{-2}$. For obtaining photobleached cells a light intensity of $100\,W\,m^{-2}$ was applied. Figure 1 shows the absorbance of normal and photobleached cells, calculated to the same dry weight. Note that photobleaching is an adaptation to high light intensity rather than a photodamage, since normal pigmentation reappears keeping the bleached, yellow cells under low light intensity (data not shown).

For spectroscopic measurements, cells were suspended (at 0.75 ± 0.07 mg dry wt. per ml) in 20 mM Tris-HCl buffer, pH 7.4, and, to avoid sedimentation of the cells, 7% Ficoll was added. Spheroplasts were prepared according to Spiller (1980). Cells were suspended in a medium containing 0.5 M sucrose, 10 mM MES-NaOH, 5 mM K_2HPO_4, 10 mM $MgCl_2$, and 2% (w/v) BSA, pH 6.9, and solid egg white lysozyme (Sigma) was added to give a final concentration of 0.1% (w/v). The suspension was incubated under

[2]Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, *N*-methylphenazonium methosulfate; MV, methylviologen; PQ, plastoquinone; cyt, cytochrome; PS, photosystem; P_{700}, reaction center of PS 1; PC, plastocyanin.

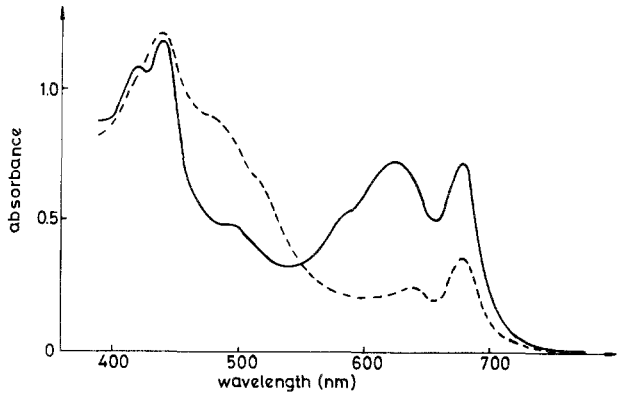


Fig. 1. Absorption spectra of *Anabaena cylindrica* cells suspended in 20 mM Tris-HCl buffer (pH 7.4). The cells were grown at low, 15 W m^{-2} (———) and high 100 W m^{-2} (— — —) light intensity. The absorbance is adjusted to the same dry weight.

illumination for 60 min at 35°C and then washed twice in the above buffer and resuspended in the same solution containing 0.5% (w/v) BSA.

The absorbance changes between 460 and 580 nm were induced by saturating Xe flashes fired at a frequency of 0.25 s^{-1} (> 630 nm, 3 μs duration at half peak emission). The measurements were carried out at room temperature in a single-beam kinetic spectrophotometer (Horváth *et al.*, 1979) on light-adapted samples. All traces, shown in Fig. 3, 5, and 6, are the averages of 40 flashes.

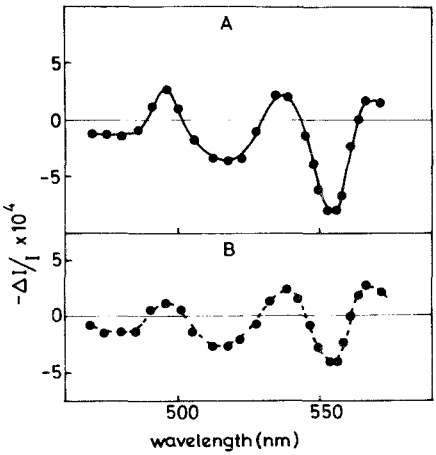


Fig. 2. Flash-induced difference spectra of normal (A) and photobleached (B) *Anabaena* cells. Kinetic traces were collected at the individual wavelengths, and the absorbance change relative to the initial dark level were measured at 1 ms after the flashes.

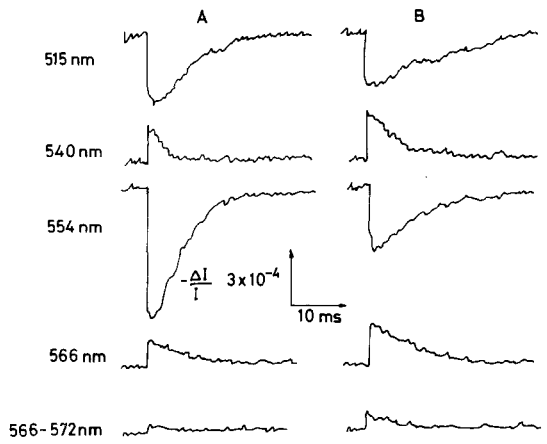


Fig. 3. Kinetic traces of flash-induced absorbance changes at different wavelengths in normal (A) and in photobleached (B) *Anabaena* cells.

For ultrathin sections, cells were fixed at 20°C with 2.5% glutaraldehyde in phosphate buffer and postfixed in 1% OsO_4. After dehydration, the samples were embedded in epoxy resin (Spurr, 1969). The electron micrographs were taken with JEOL JEM 100 B electron microscope.

Results

We measured the wavelength dependence of the flash-induced absorbance changes both in normal and photobleached *Anabaena cylindrica*. Between 500 and 580 nm, we found two positive bands with peaks at ~ 540 and 566 nm and two negative bands at 515 and 554 nm (Fig. 2). The kinetic traces of the above peaks can be seen in Fig. 3 both in normal and photobleached cells.

The origin of absorbance changes measured at 515 and 540 nm is under debate. Absorbance changes measured at 554 and 566 nm are near to the α bands of the oxidation of cyt f_{556} and cyt c_{553} and the reduction of cyt b_{563}, respectively.

In our measurements cyt f and cyt c_{553} could not be resolved, but it is known that in some cyanobacteria plastocyanin and cyt c_{553} occur interchangeably, depending on the copper concentration of the culture medium (Sandmann and Böger, 1980). The copper concentration of the algal culture was 5×10^{-7} M in our experiment. According to Sandmann and Böger (1980) the amount of c_{553} can be neglected at this copper level. Therefore, the negative absorption change with a minimum at 554 nm corresponds to cyt f.

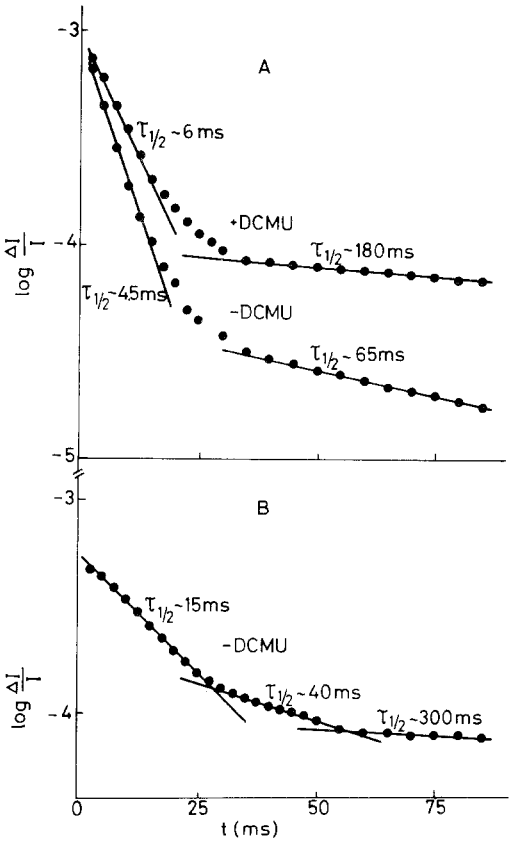


Fig. 4. Decay of the absorbance change at 554 nm of normal (A) and photobleached (B) *Anabaena* cells. DCMU, 20 μM.

Figure 2 shows that the ratio of amplitudes measured at 554 and 566 nm changed markedly during photobleaching. In normal cells, this ratio is 5.4 $\pm$ 1.1, and in photobleached, 1.5 $\pm$ 0.4 (the average of eight independent experiments). The above ratios are much higher ($\sim$ 17 and 4.5, respectively) if we take into account that cyt b_{563} has an isosbestic point at 572 nm and the amplitude which corresponds to cyt b_{563} is the difference of the amplitudes measured at 566 and 572 nm. Not only did the amplitude of cyt *f* decrease during photobleaching, but the amplitude of cyt b_{563} also increased concurrently. The increased absorbance change at 566 nm can be attributed solely to the increase of the cyt b_{563} signal, since the absorption change of cyt *f* has an isosbestic point at 562.5 nm (Böhme *et al.*, 1980); therefore it hardly contributes to the ΔA_{566}.

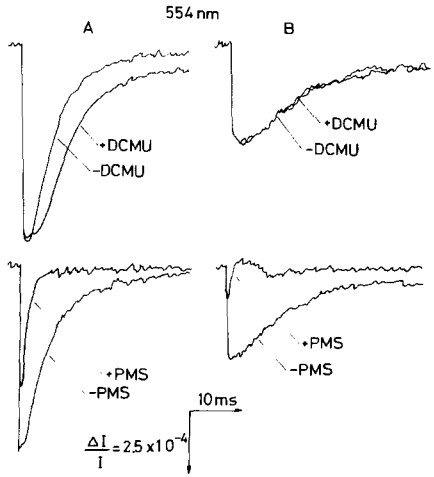


Fig. 5. Effect of DCMU (20 μM) and PMS (20 μM) on the absorbance changes of cytochrome *f* (554 nm) in normal (A) and in photobleached (B) *Anabaena* cells.

Analyzing the decay of the absorbance change at 554 nm, we found two exponentials (Fig. 4) with half times of $\sim$4.5 and $\sim$65 ms for normal and three exponentials of $\sim$15, $\sim$40, and $>$200 ms for photobleached cells (we cannot resolve signals below 1 ms). The different half times show that cyt *f* can be reduced by different processes. Houchins and Hind (1983a) measured 0.25, 2.8, and 45 ms half times for cyt *f* reduction in isolated heterocyst of

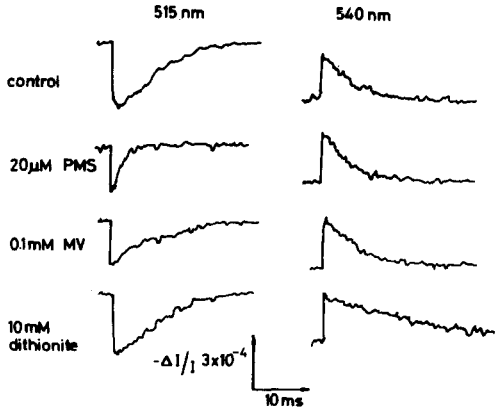


Fig. 6. Effect of PMS (20 μM), MV (0.1 mM), and dithionite (10 mM) on absorbance changes measured at 515 and 540 nm in normal *Anabaena* cells.

Anabaena cylindrica and found that the process with 2.8 ms half-time (which may possibly corresponds to the 4.5 ms half time process seen here) correlates to the cyt b_{563} oxidation both in amplitude and in time. Under our conditions only a small amount of flash-induced cyt b_{563} reduction is visible, which did not allow for detailed kinetic analyses. Houchins and Hind (1983b) supposed that reoxidation of cyt b_{563} occurs simultaneously with its reduction so that full cyt b_{563} turnover cannot be seen.

In the presence of dithionite, all cyt *f* is reduced and hence the total cyt *f* turnover can be estimated (Wasserman, 1980). We did not find dithionite (10 mM) sensitivity of photobleached cells, and a maximum increase of 10% in the amplitude of normal cells can be detected at 554 nm. This means that practically the total cyt *f* turnover was detected.

Figure 5 shows the effect of DCMU (20 μM) and *N*-methylphenazonium methosulfate, PMS (20 μM), on the absorbance changes measured at 554 nm both in normal and photobleached cells. The absorbance changes of normal cells are sensitive to DCMU, but this was not observed in the case of photobleached cells. Kinetic analyses of DCMU sensitivity of normal cells show that the slow decay of absorbance change measured at 554 nm was affected markedly (~ 65 ms $\rightarrow$ > 150 ms, Fig. 4) in the presence of DCMU; however, no considerable change in the faster component was observed (~ 4.5 ms $\rightarrow$ $\sim$ 6 ms). In Fig. 5 the average of absorbance transients between 40–80 flashes can be seen after the addition of DCMU. We found that the absorbance change for the first 40 flashes usually showed higher DCMU sensitivity (15–20% decrease in the amplitude) in normal cells than the responses for the second and third 40 flash series, which gave the same amplitude. (In a very young normal algal culture we could detect a 30-40% decrease of amplitude in the presence of DCMU.) The above results show that electron transport from PS II blocked by DCMU can be partly substituted by other processes.

In cyanobacteria the photosynthetic and respiratory electron transport chain share common components (Binder, 1982; Hirano *et al.*, 1980; Lockau, 1981). Two paths are available for electrons from cyt *f*: (i) to P_{700} (photosynthetic pathway) and (ii) to cytochrome oxidase (respiratory pathway) (Peschek, 1983). In order to ascertain that the measured cyt *f* oxidation feeds electrons exclusively to P_{700}, we added PMS (20 μM) to the sample. PMS can act as electron donor for P_{700} (Marsho and Kok, 1980). The O_2 uptake in the dark was not affected in the presence of PMS (data not shown), so we supposed that PMS does not give electrons directly to the cytochrome oxidase. It can be seen in Fig. 5 that electrons from PMS competed with the electrons from cyt *f* (cyt *f* oxidation is smaller in the presence of PMS), both in normal and photobleached cells. This significant acceleration of cyt *f* reduction in the presence of PMS was also observed by other authors (Hirano

and Katoh, 1981). They interpreted this acceleration as the occurrence of a backward electron transfer from the reducing site to electron donors of PS I. Respiration can be completely supressed in the presence of KCN (Hirano *et al.*, 1980). The cyt *f* signal was not affected by the addition of 10 mM KCN (data not shown).

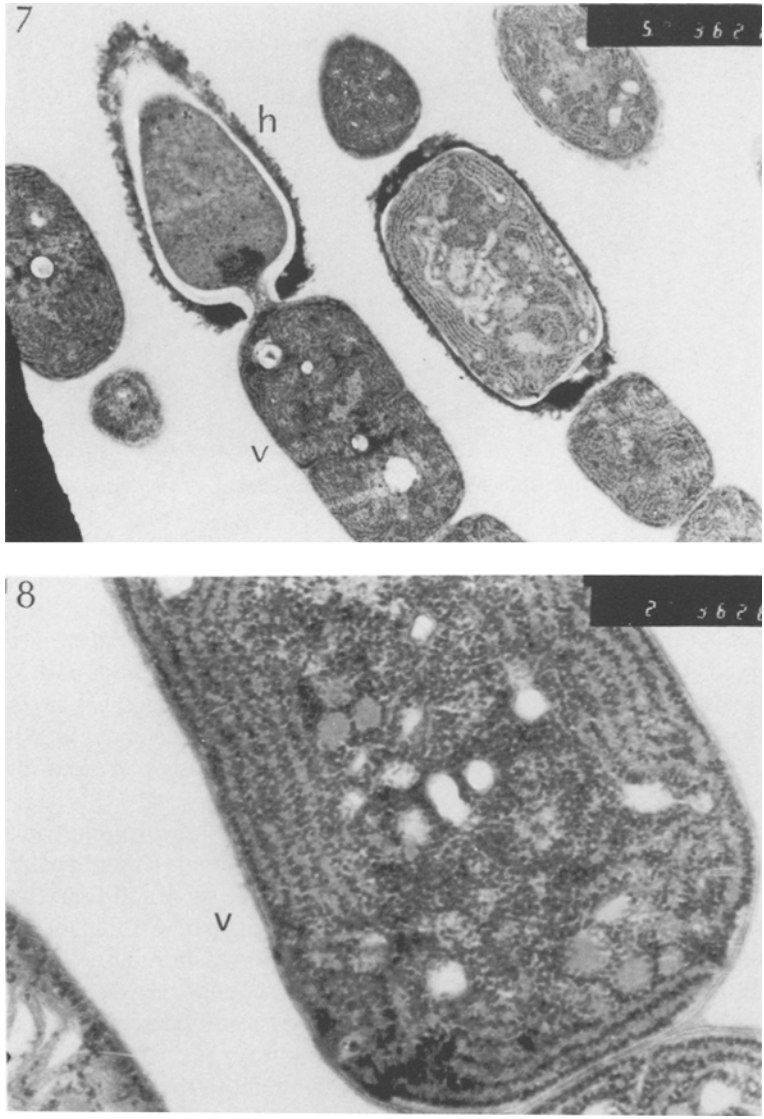
The above measurements were also performed on spheroplasts, and similar results were obtained. The only difference was a higher sensitivity of normal spheroplasts to dithionite (15–35%, depending on the preparation) than of normal cells. Photobleached spheroplasts have neither dithionite nor DCMU sensitivity.

The absorbance change measured at 515 nm was also observed in other cyanobacteria (Hirano and Katoh, 1981; Amesz and Visser, 1971). This absorbance change in chloroplasts corresponds to an electrochromic band-shift in carotenoids (Witt, 1979). The absorbance change in chloroplasts disappears in the presence of valinomycin which collapses $\Delta\Psi$. No effect of valinomycin (7 μM + 7 mM KCl) was detected at either 515 or 540 nm in spheroplasts. Figure 6 shows the effect of PMS (20 μM), methyl viologen (MV, 0.1 mM), and dithionite (1 mM) on the absorbance changes measured at 515 and 540 nm in normal spheroplasts. The absorbance change at 515 nm was affected by PMS which decreased the amplitude by about 40% and resulted in about a threefold faster decay. MV also decreased the amplitude of the 515 nm peak by about 35%. A similar effect of MV was found for the absorbance change at 515 nm in isolated heterocysts of *Anabaena cylindrica* (Houchins and Hind, 1983b). The absorbance change at 540 nm was very sensitive to dithionite, which slowed down considerably the decay of the signal.

It is well known that the filamentous cyanobacterium *Anabaena cylindrica* has two different types of cells, vegetative cells and heterocysts. In vegetative cells, both photosystems are present, while in heterocysts (5–7% of the whole cell number) only PS I operates (Haselkorn, 1978). Moreover, vegetative cells have the capabilities to be transformed into heterocysts (Bradley and Carr, 1976). Figs. 7–9 show micrographs of thin-sectioned normal and photobleached cells. The cytoplasmic membrane and the thylakoids of normal vegetative cells running parallel to the cell wall can clearly be distinguished. This typical structure of vegetative cells disappears in photobleached cells and a granular structure of cytoplasm can be observed similar to that of heterocysts.

Discussion

The valinomycin insensitivity of absorbance changes measured at 515 and 540 nm indicates that absorbance changes are not of electrochromic



Figs. 7-8. Micrographs of thin-sectioned cells of normal *Anabaena cylindrica*. (v and h denote vegetative cells and heterocyst, respectively.) Magnification: Fig. 7, 5,000 ×; Fig. 8, 20,000 ×.

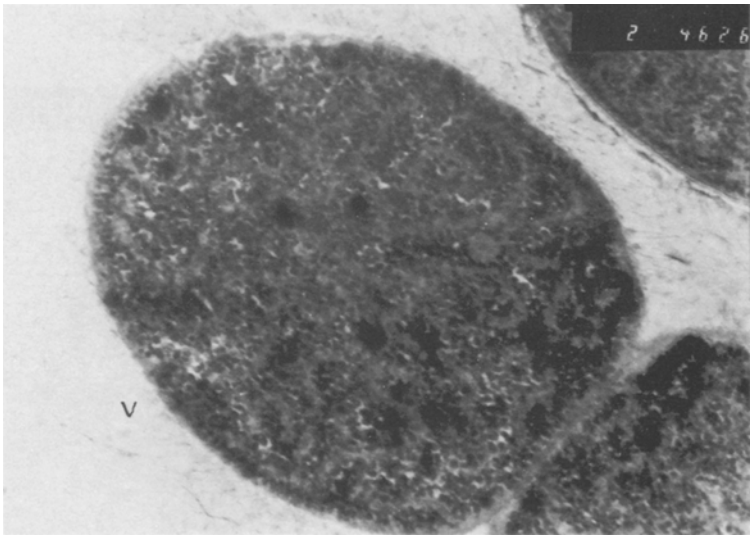


Fig. 9. Micrograph of thin-sectioned cells of photobleached *Anabaena cylindrica*. Magnification: 20,000 ×.

nature in *Anabaena cylindrica*. A similar conclusion has been drawn for the 515 nm absorbance change in isolated heterocysts of *Anabaena cylindrica* (Houchins and Hind, 1983b), where an absorbance change at 540 nm was not observed. Other authors (Houchins and Hind, 1983a; Amesz and Visser, 1971) also failed to detect electrochromic absorbance changes in cyanobacteria. However, an ionophore-sensitive absorbance change at 530 nm, attributed to an electrochromic shift of carotenoids, was described in *Synechococcus* (Hirano and Katoh, 1981).

The different half times of the decay of the absorption transients measured at 515 and 540 nm indicate separate origins for these peaks. The different behavior of these absorbance changes to the same additions (see Fig. 6) confirm the above statement. The dithionite sensitivity of the absorbance change at 540 nm may reflect a process near to the plastoquinone (PQ) pool. The PMS and MV sensitivity of the absorbance change measured at 515 nm suggests that part of this absorbance change may result from reactions on the reducing side of PS I.

The lack of a 1:1 stoichiometric ratio among both the two photosystems and any other electron carriers suggest that, between compartments of PS I and PS II, mobile electron carriers redistribute the charges (Aoki *et al.*, 1983; Hirano *et al.*, 1981). There is evidence that the PQ pool can function as a mobile electron carrier between PS II and cyt *b–f* complex in chloroplast of higher plants (Anderson, 1981) as well as in a thermophilic blue-green alga

(*Synechococcus*) (Hirano *et al.*, 1981). Plastocyanin or cyt c_{553} is possibly a mobile component of the linear electron transport chain between cyt *b–f* complex and PS I (Nolan and Bishop, 1975). The small DCMU sensitivity of normal cells of *Anabaena cylindrica* agrees with the earlier results of several authors (Murai and Katoh, 1975) who emphasized that not only PS II can give electrons to PS I via cyt *f*, but so can accumulated organic compounds. Accordingly, in younger cells, cyt *f* showed stronger DCMU sensitivity than in older cells, where the decrease in the amplitude of cyt *f* in the presence of DCMU was partly compensated within a few minutes.

In photobleached cells the reduction of cyt *f* was DCMU insensitive. There are several explanations available for this phenomenon: (i) Let us assume that PQ pool is a mobile electron carrier. In a thermophilic alga (Hirano *et al.*, 1981) the decrease of temperature, which can "freeze" the mobility of PQ molecules embedded in lipid molecules, decelerates the reduction of cyt *c* (and reduction ceases below 10°C). In our photobleached alga, a considerable accumulation of carotenoids, mainly xanthophylls, can be observed (Laczkó and Barabás, 1981). Carotenoids may increase the rigidity of lipids. The slower reduction of cyt *f* can be explained in this way. If electrons from PS II are not faster than electrons from other sources, then the electrons blocked by DCMU can be compensated for unnoticeably. (ii) The electrons of PS II reach PS I, but not via cyt *f*. (iii) The electrons of PS II do not reach PS I, but feed other electron acceptor(s).

The decreased ratio of cyt *f* and b_{563} in photobleached cells also reflects a change of electron transport inside the cyt *b–f* complex or between the complex and PS I. Dithionite insensitivity suggests that cyt *f* works optimally inside the complex.

In conclusion, it is very difficult to suppose that the increased PS II activity in photobleached cells is able to increase the electron transport between the two photosystems without bypassing cyt *f*. We feel it is more probable that in photobleached cells other electron acceptor(s) enter(s) the electron transport chain.

Comparison of micrographs of vegetative cells in normal and photobleached algae (see Figs. 7–9) also shows a dramatic difference. The curious fact that the vegetative cells of photobleached alga have an increased PS II activity, but show structural similarity with heterocysts, where only PS I works, suggests the possibility that vegetative cells of photobleached algae are in an intermediary state where both photosystems have the ability to work independently from each other, i.e., they are "uncoupled" to some extent.

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