Carotenoid-induced quenching of the phycobilisome fluorescence in photosystem II-deficient mutant of *Synechocystis* sp.☆

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Abstract Brief – 10-second long – irradiation of a photosystem II-deficient mutant of cyanobacterium *Synechocystis* sp. PCC 6803 with intense blue or UV-B light causes an about 40% decrease of phycobilisome (PBS) fluorescence, slowly reversible in the dark. The registered action spectrum of PBS fluorescence quenching only shows bands at 500, 470 and 430 nm, typical of carotenoids, and an additional UV-B band; no peaks in the region of chlorophyll or PBS absorption have been found. We propose that quenching induced by carotenoids, possibly protein-bound or glycoside, reveals a new regulatory mechanism protecting photosynthetic apparatus of cyanobacteria against photodamage.

Keywords: Carotenoid; Fluorescence quenching; Phycobilisome; *Synechocystis* sp.

1. Introduction

Multiple photoprotective mechanisms involved in oxygenic photosynthesis allow a cell to survive under intense light. The main goal of photoprotection is to prevent excess energy of absorbed light from damaging the photosynthetic apparatus. The processes leading to dissipation of excess absorbed light energy in the photosynthetic antenna have been well documented for plants [1], but are less studied for pigment apparatus of cyanobacteria, and especially for phycobilisomes (PBSs). The PBSs, the primary cyanobacterial antenna, are large water-soluble phycobiliprotein complexes coupled to the cytoplasmic surface of the thylakoid membrane [2]. The light energy absorbed by PBSs is transferred with high efficiency to antenna chlorophylls of the photosystem II (PSII) and photosystem I (PSI) [3–5]. However, PBSs apparently lack the capacity to dissipate excess absorbed excitation energy without assistance; no quenchers have been found in isolated PBSs [6].

A decrease of fluorescence yield from antenna chlorophylls is indicative of utilization of absorbed energy as a result of photosynthetic electron transport and/or of non-photochemical quenching [7]. Wild-type cyanobacterial cells give little insight into energy dissipation in the PBSs due to a large overlap of the emission spectrum of the PBSs with that of PSII chlorophylls [8]. Besides, the variable fluorescence of PSII chlorophyll in wild-type cyanobacteria is normally excited via the PBSs. On the contrary, in mutant strains of cyanobacteria lacking PSII, the 293 K fluorescence emission in the red region of the spectrum is determined predominantly by membrane-associated PBSs [9–11]. In PSII-deficient cyanobacterial mutants, the PBSs display a fluorescence yield greater than that in the wild-type, but considerably smaller than observed in isolated systems [11,12]. This fact indicates that energy absorbed by PBSs in such mutant strains may dissipate via PSI [9] and other unknown mechanisms.

We have studied light-induced fluorescence changes in the PBSs of a PSII-deficient mutant of the cyanobacterium *Synechocystis* sp. PCC 6803. By using D2-less strain, we ruled out PSII light-induced fluorescence changes. We present spectral and kinetic evidence that cyanobacterial carotenoids activated by absorbed blue light induce reversible quenching of PBSs emission.

2. Materials and methods

2.1. Strains and growth conditions

The wild-type of the cyanobacterium *Synechocystis* sp. PCC 6803 as well as the *psbDI/C/DII*$^-$ strain lacking both gene copies of the D2 protein of PSII, originally produced by Dr. W.F.J. Vermaas [13], were obtained from the Culture Collection of the Department of Genetics, Faculty of Biology of the Moscow State University. Wild-type and *psbDI/C/DII*$^-$ cells were grown photoheterotrophically in BG-11 medium [14] supplemented with 5 mM glucose in 100 mL flasks at 30 °C under continuous cool white fluorescent light, at photon flux density 12 $\mu E\,m^{-2}\,s^{-1}$. Chloramphenicol and spectinomycin were added at 20 $\mu g\,ml^{-1}$ to the medium of *psbDI/C/DII*$^-$ cells as required. All experiments were performed with 3-day cultures in the log phase of growth.

2.2. Fluorescence measurements

Fluorescence emission spectra and time courses of emission at 293 K were recorded with a Shimadzu RF-5301PC spectrofluorimeter equipped with an R928-08 red photomultiplier. The fluorescence spectra were measured with the excitation wavelength of 580 nm. The bandwidth of the measuring monochromator was 5 nm; the bandwidth of the excitation monochromator was also 5 nm (photon flux density ca. 180 $\mu E\,m^{-1}\,s^{-1}$). Chlorophyll concentration of the sample in the quartz cuvette (0.3 cm optical path) was 2 $\mu g\,ml^{-1}$.

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☆ Dedicated to professor N. Murata.

Abbreviations: PBS, phycobilisome; PSII (PSI), photosystem II (photosystem I)

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Fluorescence quenching was induced by irradiation of the *psbDI/C/DII*$^-$ cells by the excitation monochromator in the range 270–700 nm. All experiments were performed in the single beam mode. The beam intensity in the range 10–900 $\mu E\,m^{-1}\,s^{-1}$ was controlled by both the slit width (10–15 nm) and by neutral glass filters, and was evaluated with an LM2 calorimeter. The decrease of fluorescence emission was registered under two conditions. In the first case, the actinic light inducing the quenching also served as the excitation light for the sample. In the second case, the actinic light was separated in time from subsequent illumination of the cells with excitation light at 580 nm. The emission spectrum of the sample upon excitation at 580 nm was measured twice: once prior to actinic illumination (the highest fluorescence level, F_{dark}) and then immediately after that (F_{light}). The degree of quenching was estimated as the relative change of emission $(F_{dark} - F_{light})/F_{dark}$. The action spectrum of quenching was adjusted for the actinic light intensity in all 30 points covering the range 270–700 nm. The intensity of actinic irradiation employed for registration of the action spectrum was not less than 200 $\mu E\,m^{-1}\,s^{-1}$. A fresh sample was used for each measurement. Each point was taken as the mean of 3–5 independent measurements.

3. Results

The *psbDI/C/DII*$^-$ cells of *Synechocystis* sp. PCC 6803 did not emit variable fluorescence and did not reveal oxygen evolving activity (data not shown), although the mutant strain and the wild-type had nearly equal relative amounts of PBSs and chlorophyll. According to the absorption measurements, the A_{625}/A_{680} ratio for the mutant strain was 10% higher than that for the wild-type. Fig. 1 shows the fluorescence emission spectra of wild-type and *psbDI/C/DII*$^-$ cells excited at 580 nm. The emission spectrum of this mutant strain differed from the spectrum of the wild-type by a much higher PBSs peak at 660 nm. As was expected, the 685-nm band corresponding to chlorophyll emission was not found in spectrum of the mutant cells (Fig. 1).

When the excitation light intensity was enhanced to 600 $\mu E\,m^{-2}\,s^{-1}$, the emission of PBSs in the mutant decreased to about 55–60% of the initial value within several tens of seconds (Fig. 1). Light differing from 580 nm also induced a decrease of fluorescence emission. The highest extent of quenching was registered upon excitation of the sample at 500 nm, although absorption of the PBSs at this wavelength was much lower than at 580 nm. Therefore, the fluorescence emission by PBSs and its quenching are two distinct light-dependent processes bound to different photosensitizers.

The half-time of the PBSs fluorescence quenching was equal to 40 s upon excitation with 500 nm light at 180 $\mu E\,m^{-2}\,s^{-1}$ and it dropped to 10 s at 670 $\mu E\,m^{-2}\,s^{-1}$ (Fig. 2). The quenching was observed even at photon flux density of 50 $\mu E\,m^{-2}\,s^{-1}$ upon excitation at 500 nm, although in this case it was significantly slower. The quenching was slowly reversible in the dark and appeared again after turning on the actinic light (Fig. 2). Half-time of reversal was about 30 min. No visible changes in the absorption spectrum (400–1100 nm) were observed for the *psbDI/C/DII*$^-$ cells at the end of the quenching process (data not shown).

The slow reversal of quenching permitted to separate the irradiation of the mutant cells with actinic monochromatic light of different intensities from the subsequent registration of the PBS emission upon excitation at 580 nm. It was found that regardless of the wavelength of actinic illumination, the relative decrease of emission $(F_{dark} - F_{light})/F_{dark}$ was maximal at 676 nm (data not shown). Therefore, the degree of quenching

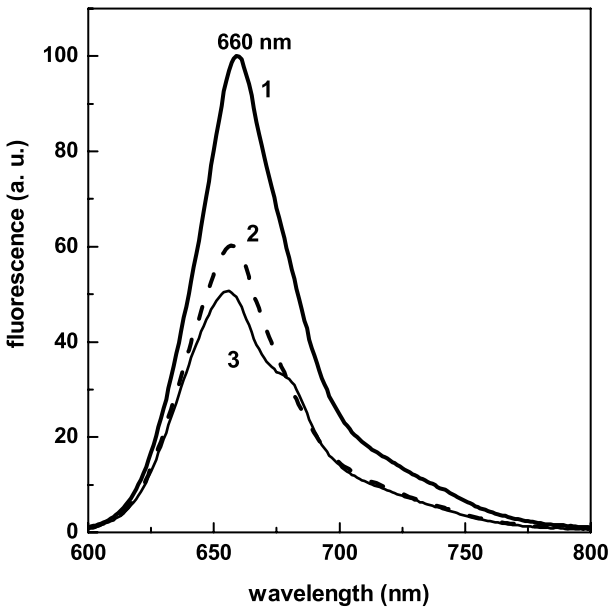

Fig. 1. 293 K fluorescence emission spectra of mutant (1,2) and wild-type (3) cells, dark-adapted (1,3) or preilluminated (2) for 120 s at 580 nm, 600 $\mu E\,m^{-2}\,s^{-1}$. Excitation wavelength 580 nm; spectra are normalized to phycocyanin absorption at 625 nm.

was always evaluated at 676 nm. With 30 s actinic illumination at 500 nm, the quenching reached its maximum at the photon flux density of about 400 $\mu E\,m^{-2}\,s^{-1}$ (Fig. 3). But in case of 10 s illumination, quenching was not saturated even at the intensity of 900 $\mu E\,m^{-2}\,s^{-1}$. Since at 10 s illumination the decrease of the PBSs emission was linearly dependent on the

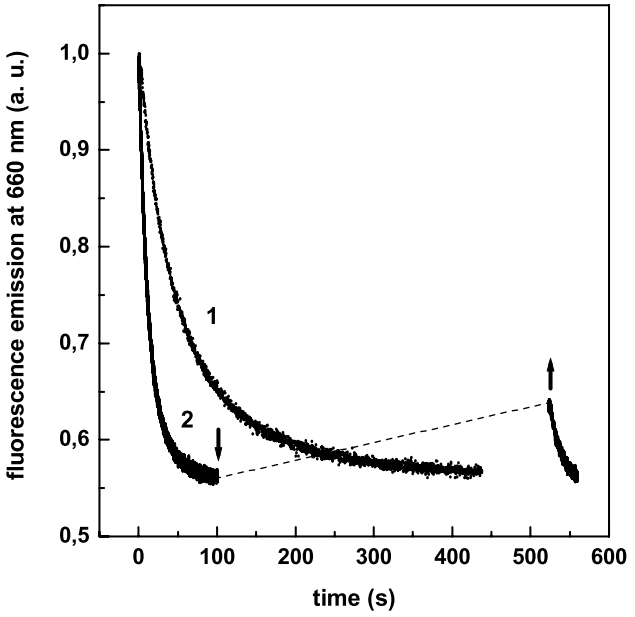

Fig. 2. Time course of fluorescence emission at 660 nm of mutant cells excited at 500 nm, 180 $\mu E\,m^{-2}\,s^{-1}$ (1), and 600 $\mu E\,m^{-2}\,s^{-1}$ (2). The kinetic curves were normalized at the highest level of fluorescence emission. Arrows indicate the excitation light (600 $\mu E\,m^{-2}\,s^{-1}$) turned off (↓) and resumed (↑). The dotted line indicates the reversibility of quenching.

intensity of preliminary irradiation (Fig. 3), these conditions were applied to register the adjustable action spectrum of quenching.

The action spectrum of *psbDI/C/DII*$^-$ cells of *Synechocystis* sp. PCC 6803 did not correspond to the absorption spectrum of PBSs or chlorophylls. Two peaks at 500 and 470 nm and a shoulder at 430 nm were revealed in the visible region of action spectrum (Fig. 4). Overall, the action spectrum in the visible region corresponded well to the typical three-band absorption spectrum of carotenoids. The positions of the three bands and an additional peak at 290 nm found in the UV-B region suggested that the carotenoid could be classified as myxoxanthophyll, one of the major carotenoids in *Synechocystis* sp. PCC 6803 [15,16]. However, we cannot rule out zeaxanthine or another cyanobacterial carotenoid, especially if it is bound to apoprotein or represented by its *cis* isomer, as both may result in the UV-B band. Summing up our results, we conclude that carotenoids as natural regulators may be involved in transition of the PBS antenna from an unquenched to a quenched state.

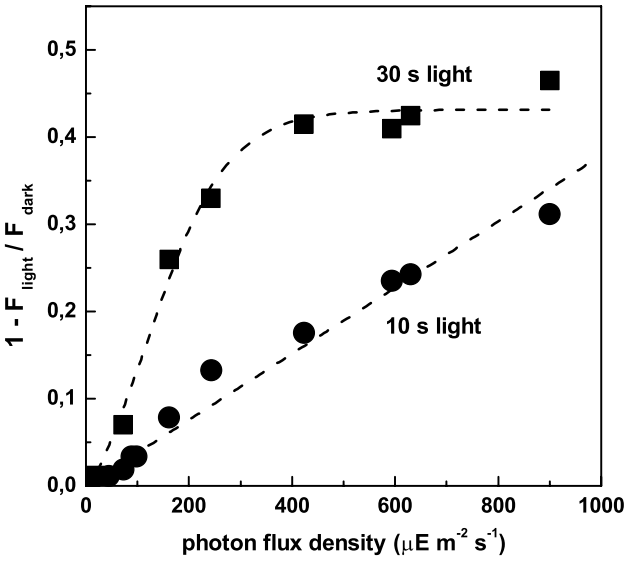

Fig. 3. Dependence of relative quenching of fluorescence at 676 nm in mutant cells on intensity of 500 nm actinic light at 10 and 30 s preillumination. Excitation wavelength 580 nm. Lines are approximations from measurement data.

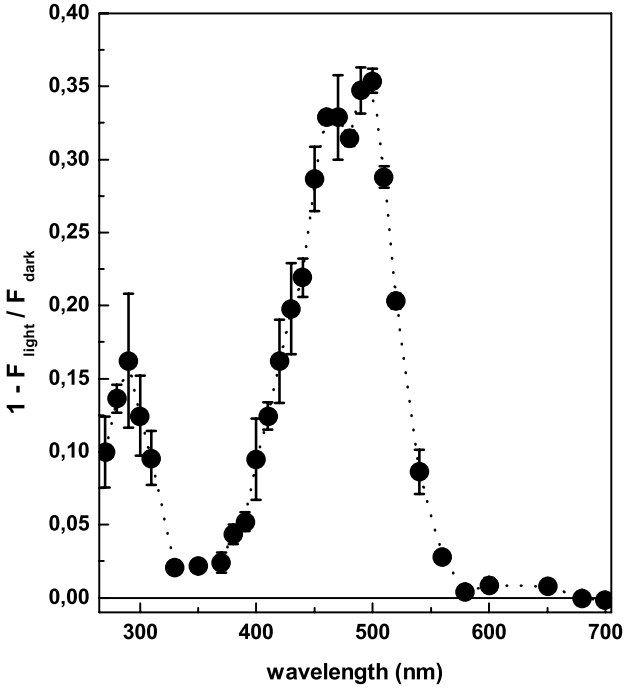

Fig. 4. Action spectrum of fluorescence quenching in mutant cells. Values are averages of 3–5 measurements, bars show deviations.

4. Discussion

The intensity of light that induced the quenching of PBSs fluorescence was sub-saturating for the oxygen-evolving activity of the wild-type *Synechocystis* sp. PCC 6803 [17]. The light intensities inducing non-photochemical quenching of chlorophyll fluorescence are at least two or three orders of magnitude higher than that of growth conditions for cyanobacteria [18]. The non-photochemical PSII quenching in the wild-type *Synechocystis* sp. PCC 6803 induced by strong blue light (400–1000 $\mu E\,m^{-2}\,s^{-1}$) was previously shown to be unrelated to state transitions or photoinhibition, pointing towards an unknown mechanism of quenching [17]. Our data presented for mutant cells as well as our preliminary data on the wild-type *Synechocystis* sp. PCC 6803 allow to state that such PSII-quenching could correspond to the carotenoid-dependent quenching of PBSs.

Low quantum yield of chlorophyll emission in the *psbDI/C/DII*$^-$ cells of *Synechocystis* sp. PCC 6803 at 293 K made it possible to study the PBS fluorescence in vivo. Our results indicate that, indeed, intense blue light induces a decrease of the PBS fluorescence emission; UV-B acts alongside visible light. The highest degree of quenching at 676 nm indicates that the terminal PBS emitters are the preferred quenching point. The action spectrum for the PBS quenching peaking at 500, 470, 430, and 290 nm resembles the absorption spectrum of cyanobacterial carotenoid(s), which permit to propose a new photoprotective role of carotenoids in dissipation of energy absorbed by PBSs. The mechanisms involved in quenching remain unclear. Since cyanobacteria are unable to synthesize epoxy carotenoids, the xanthophyll cycle found in eukaryotes [19] is absent in cyanobacterial cells [20] and cannot be involved in quenching of the PBS emission. On the other hand, the photoprotective process in the *Synechocystis* sp. could be driven by the *cis* to *trans* photoisomerization of xanthophylls [21].

Carotenoids play a key role in deactivation of excited antenna chlorophylls under conditions of excess excitation [22,23]. The protective function of carotenoids within the pigment–protein complexes is due to their proximity to chlorophylls. An intriguing aspect of our data is the effect of hydrophobic carotenoids on the water-soluble PBS supercomplex. The quenching may arise from direct interaction between carotenoids and phycobilin chromophores, or from carotenoid-mediated alterations of the PBS structure. The major carotenoids found in *Synechocystis* are zeaxanthine, β-carotene, echinenone and myxoxanthophyll [16]. According to the registered action spectrum, myxoxanthophyll could be the

most probable carotenoid to come in contact with PBSs. Myxoxanthophyll is a myxol glycoside commonly found in cyanobacteria, both in the cellular and in the thylakoid membranes [24]. Polar functional groups obviously may alter the polarity of carotenoids and affect their interactions with other molecules. The glycoside nature of this molecule may be important for the known highly efficient protection by myxol glycosides, which builds up under intense light or UV irradiation [24]. We suggest that the position of the myxoxanthophyll molecule piercing the membrane due to polar interactions [25] may be more pronounced in its *trans* form. These properties of myxoxanthophyll correspond well with our data and with the proposed docking of the PBSs to lipid head groups at the membrane surface in the model of a mobile PBS antenna [4]. The same type of functioning of polar zeaxanthine, known as an efficient quencher of chlorophyll fluorescence through singlet–singlet interaction [7], cannot be excluded.

Carotenoids may be involved as well in dissipation of energy absorbed by PBSs in the *psbDI/C/DII*$^-$ mutant via PSI and probably via inactive PSII centers formed in the absence of D2-protein [26]. The high-light-inducible proteins of cyanobacteria could also well deal with the dissipation of the energy absorbed by the PBSs [27]. Future studies should elucidate the molecular mechanism that causes the quenching of excess light energy absorbed by PBSs in cyanobacteria following light activation and possible photochemical alterations of carotenoids, and thus protects the photosynthetic apparatus.

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