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Carotenoid-triggered energy dissipation in phycobilisomes of *Synechocystis* sp. PCC 6803 diverts excitation away from reaction centers of both photosystems

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ABSTRACT

Cyanobacteria are capable of using dissipation of phycobilisome-absorbed energy into heat as part of their photoprotective strategy. Non-photochemical quenching in cyanobacteria cells is triggered by absorption of blue-green light by the carotenoid-binding protein, and involves quenching of phycobilisome fluorescence. In this study, we find direct evidence that the quenching is accompanied by a considerable reduction of energy flow to the photosystems. We present light saturation curves of photosystems' activity in quenched and non-quenched states in the cyanobacterium *Synechocystis* sp. PCC 6803. In the quenched state, the quantum efficiency of light absorbed by phycobilisomes drops by about 30–40% for both photoreactions—P700 photooxidation in the photosystem II-less strain and photosystem II fluorescence induction in the photosystem I-less strain of *Synechocystis*. A similar decrease of the excitation pressure on both photosystems leads us to believe that the core–membrane linker allophycocyanin APC-L_{CM} is at or beyond the point of non-photochemical quenching. We analyze 77 K fluorescence spectra and suggest that the quenching center is formed at the level of the short-wavelength allophycocyanin trimers. It seems that both chlorophyll and APC-L_{CM} may dissipate excess energy via uphill energy transfer at physiological temperatures, but neither of the two is at the heart of the carotenoid-binding protein-dependent non-photochemical quenching mechanism.

1. Introduction

Under strong illumination, not all absorbed energy may be used for photosynthetic assimilation of CO_2. At saturating light intensity, most of the reaction centers of the photosystems are closed, and a mechanism that dissipates the excess absorbed energy into heat (non-photochemical quenching, or NPQ) is employed to divert part of the energy harvested by the antenna so that this energy does not arrive at the reaction centers. Difference in organization of the photosynthetic apparatus of cyanobacteria as compared with that of higher plants is accompanied by different pathways of protection against the damaging effects of intense light.

Light-harvesting in cyanobacteria is carried out by phycobilisomes (PBS), highly structured assemblies of water-soluble phycobiliproteins that are associated to the cytoplasmic surface of the thylakoid membranes. Acting as external antennae, PBSs transfer the absorbed excitation energy to PSII and PSI complexes embedded within the membrane [1]. Each phycobiliprotein has α and β subunits that are associated with linear tetrapyrrole chromophores designated as phycobilins; different phycobilin chromophores provide the complex with distinct spectral characteristics [2,3]. The hemi-discoidal PBSs in *Synechocystis* sp PCC 6803 (hereafter called *Synechocystis*) have a central tri-cylindrical core composed of allophycocyanins (APC) and specific linker polypeptides. Six rod cylinders composed of phycocyanin together with their associated linker polypeptides radiate out from the core [4,5]. Phycocyanin (PC) in PBSs emits at 650 nm, while the APC in the core emits at 660 nm. The PBS also contains terminal emitters – APC-B and APC-L_{CM} (L_{CM} or ApcE being the core–membrane linker protein [2,4,6,7]) – whose emission at 683 nm is detectable at 77 K. The APC and APC-B are characterized by the same β-subunits; the difference in α subunits determines the shift of the emission maximum to 680 nm for APC-B [8]. Each of the cylinders belonging to the core substructure is composed of four trimeric APC discs, and these discs have slightly different compositions (for new designation of cylinders composition see [5]). The upper cylinder is composed of two simple APC trimers $(\alpha\beta)_3$ called T, and two other

Abbreviations: APC, allophycocyanin; APC-B, APC that contain modified α subunit, called α^B; APC-L_{CM}, core–membrane linker allophycocyanin; BL, blue-green light 500 nm; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Fo, F'o, dark fluorescence in non-quenched and quenched state; Fmax, F'max, maximum fluorescence in non-quenched and quenched state; Fs, steady-state fluorescence; NPQ, non-photochemical quenching; OCP, orange carotenoid-binding protein; PBS, phycobilisome; PC, phycocyanin; P700, primary electron donor of the PSI reaction center; PSI (PSII), photosystem I (photosystem II); RL, red light 680 nm; Φ_{PSII}, effective quantum yield of PSII

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discs—T8 (T plus L_C, a linker of 8-kDa). The two basal cylinders are composed of one T disc, one T8 disc, one APC-B disc with L_C, a linker of 8-kDa designated B8, and one APC-L_{CM} disc designated M. The core–membrane linker L_{CM} or ApcE has an additional role in the core assembly [9], and may provide a flexible surface allowing interaction with multiple membrane components [10]. Special arrangement of different phycobiliproteins in the PBS facilitates an efficient, energetically downhill transfer of absorbed energy from the rods to core cylinders, i.e., from PC to APC and probably via APC-L_{CM} to PSII or PSI. Because of the low specificity of PBS binding to membrane components [10], PBSs may bind to a range of different membrane complexes. Energy distribution between PSII and PSI may change when the relative affinity of the PBS for the respective photosystems changes.

For a long time, cyanobacteria were not considered to be capable of performing NPQ as a photoprotective mechanism under light stress conditions. Instead, redistribution of the PBS's light-harvesting antenna between reaction centers in a process called state transitions was considered the major means of regulating the utilization of harvested excitation energy [11,12]. Recently, it was demonstrated that cyanobacteria are able to use NPQ as a component of their photoprotective strategies. A blue-green light-induced fluorescence quenching in the cyanobacterium *Synechocystis* sp. PCC 6803, reversible in the dark, was described; this fluorescence quenching was ascribed preliminarily to PBS emission [13]. In a PSII-less *Synechocystis* mutant, without variable fluorescence, illumination with intense 450–500 nm light was found to quench PBS fluorescence at 660 nm [14]. Similar fluorescence quenching was found in wild-type *Synechocystis* cells with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to inhibit PSII activity [15,16]. This quenching of PBS fluorescence in cyanobacteria induced by blue-green light is independent of the pH of the lumen and of treatment with uncouplers [17].

The action spectrum of blue-green light-induced fluorescence quenching suggested the involvement of a carotenoid in sensitization of this process [14]. Deletion mutagenesis of Slr1963, the 35-kDa water-soluble orange carotenoid-binding protein (OCP) binding one molecule of 3′-hydroxyechinenone [18,19] and localized on the cytoplasmic side of thylakoid membranes in some cyanobacteria, showed that this protein was responsible for triggering of PBS fluorescence quenching upon blue-green light illumination [17,20]. Upon illumination with blue-green light, the isolated OCP undergoes a reversible transformation from its dark stable orange form to a red form [21]. The red form is considered to be essential for induction of the photoprotective mechanism. Light-triggered OCP conversion occurs with a very low quantum yield; this seems only natural because the OCP protein is involved in a photoprotective process that needs to be induced only under high light conditions [21].

Blue-green-light-induced quenching leads to energy dissipation, since oxygen evolution occurs at lower light intensities in wild-type control cells than in photoinhibited cells [17]. Only strains containing an intact OCP gene can operate the blue-green-light-induced photoprotective mechanism [17,22]. Quenching was not observed in *Synechocystis* mutants lacking PBSs [17] or the linker polypeptide ApcE [15].

The effect of glycerol on the yield and spectrum of fluorescence emission in the PSII-less mutant of *Synechocystis* indicates that only fluorescence of intact, membrane-bound PBS is quenched after exposure to blue-green light. The increase of protein hydration resulting from a high glycerol concentration causes the uncoupling of the PBS from the membrane and damages its structure, suppressing the blue-green-light-induced fluorescence quenching [23]. The temperature dependence of the rate of blue-green-light-induced PBS fluorescence quenching is similar to that of soluble protein folding [23]. The sensitivity of quenching to treatment with glutaraldehyde points at a reversible change in association and/or conformation of proteins that can be stabilized by cross-linking or freezing in either the high- or low-quenching state [16].

Nature and location of the quencher that converts excitation energy to heat are still unknown. Several mechanisms of OCP-induced quenching of PBS fluorescence have been proposed to account for the role of conformational changes in energy dissipation [24–27]: (i) the OCP binds to the PBS core and the carotenoid quenches excitation energy via direct interaction with the PBS, (ii) the OCP binds to the PBS core, but a quenching center forms through re-orientation of PBS pigments, and (iii) the OCP acts indirectly in the development of quenching via formation of a PBS quenching complex with other as yet unidentified components. Recently shown light-induced changes in the OCP [21] back with the first (i) scenario; the long wavelength shift of carotenoid absorption in the OCP red form probably allows quenching via its low-energy S1 excited level. Moreover, one of the options maybe charge transfer to a redox-active site of the protein like it was proposed for quenching by Trp acted as a very efficient trap of excitations on pigments [28]. All these scenarios will lead to fluorescence quenching at room temperature.

Even though it is clear that the OCP is the protein responsible for blue-green-light-induced fluorescence quenching, it is as yet unknown where in the PBS the OCP binds and hence where in the PBS the quenching takes place. In this study, we narrow down the PBS site of quenching center formation in the cyanobacterium *Synechocystis* sp. and show the significance of this point of excess energy dissipation for decreasing the excitation flow to PSII and PSI reaction centers.

2. Materials and methods

2.1. Strains and growth conditions

The wild type of *Synechocystis* sp. PCC 6803 was obtained from the Culture Collection of the Genetics Department of the Moscow State University. A PSII-less strain was created by insertional inactivation of the *psb*B gene (encoding the CP47 subunit of PSII) and of the *psb*DIC operon (encoding DI and CP43 subunits). Part of the *psbB* gene was deleted and replaced by a 2.0 kb streptomycin-resistance cartridge [29]. The deletion of *psb*DIC was originally introduced in *Synechocystis* by replacing the 2818-bp *Xmn*I/*Sfi*I fragment, which covered the entire *psb*DIC coding region from 212 bp upstream of *psb*DI to 172 bp downstream of *psb*C, with a 1.7 kb erythromycin resistance cassette (S. Ermakova-Gerdes and W. Vermaas, unpublished). The PSI-less strain was created by replacing a part of the *psaAB* operon coding for the core proteins of PSI with a chloramphenicol-resistance marker [30].

The wild-type strain was grown in liquid BG-11 medium [31] in 100 ml flasks at 30 °C under continuous white fluorescent light at a photon density of 40 μE m^{-2} s^{-1}. The PSII-less and PSI-less strains were grown at low irradiation (5 μE m^{-2} s^{-1}) in the presence of 10 mM glucose. The medium used to grow the mutants was routinely supplemented with 25 μg ml^{-1} streptomycin and 20 μg ml^{-1} erythromycin (for PSII-less cells) or with 25 μg ml^{-1} chloramphenicol (for PSI-less cells). However, in separate experiments, it was verified that the presence of the antibiotics in the growth media had no effect on fluorescence parameters. All experiments were performed with 4-day cultures of the wild type and of PSII-less strain, and with 7-day cultures of PSI-less strain, capturing both in the logarithmic growth phase.

2.2. Absorption spectra and pigment analysis

Room-temperature absorption spectra were recorded using a Cary-Bio 300 spectrophotometer (Varian); whole cell suspension samples were normalized to the optical density (OD) at 750 nm. Heat-bleaching of phycobiliproteins in *Synechocystis* strains suggested by [32] was performed in the spectrophotometer by transient heating of

cell cultures to 63 °C in a Peltier thermostatted cuvette holder with magnetic stirring; the cycle took around 4 min. Differential absorption spectra between the untreated cells and the heat-bleached cells were calculated. Under the experimental conditions, less than 5% of Chl absorption at 680 nm was bleached by this method and the difference spectra approximate the absorption spectra of PBSs *in vivo* [33]. The relative APC content was estimated from the absorption band at 650 nm in these difference spectra. The concentration of Chl *a* and the total carotenoid content were determined from 80% acetone extracts.

2.3. Pulse amplitude modulated (PAM) kinetics

The light-induced fluorescence and absorption changes in *Synechocystis* cells were monitored with a PAM-101 fluorometer (Walz, Germany). PSI-less cell samples for PAM fluorescence measurements contained about 0.2 μg ml^{-1} Chl, whereas the PSII-less and wild-type cell suspensions were about 1 μg ml^{-1} Chl, corresponding to approximately the same number of cells as the PSI-less suspension. Cell suspensions were excited with modulated red light (650 nm), and fluorescence was detected at λ >700 nm. The cuvette system allowed for stirring and temperature control of the sample. To induce NPQ, cells were illuminated for 3 min with blue-green or, as a control, with red light with the intensity of 300 μE m^{-2} s^{-1} provided by a KL-1500 illuminator (Schott, Mainz, Germany) equipped with BPF500 (500 nm wavelength, 35 nm bandwidth) or BPF680 (680 nm wavelength, 60 nm bandwidth) glass band-pass filters (Photooptic, Russia). The cell suspensions were left in darkness for 1 min before PAM fluorometry measurements. Light saturation curves of the effective quantum yield of PSII reactions by PBS-absorbed actinic light before and after carotenoid-induced NPQ were made using a High Power LED-Lamp HPL-L620 with control unit HPL-C (Walz, Germany), with which the flash duration and intensity could be modified. The high power HPL-L620 lamp emitting at 620 nm was custom-built by Walz to achieve optimal selectivity of actinic light for absorption by PBSs. One-second, flash-induced fluorescence time courses were registered with 100 μs resolution through a PDA-100 instrument using the Win Control software (Walz, Germany). Measurement of each light intensity point on the light saturation curve took about 200 s, as 12 to 16 readings were taken for each point with 10 to 12 s between flashes. The temperature was kept at 20 °C, and under these conditions both the maximum (F'_m) and "dark" fluorescence yield (F'_0) did not change by more then 10% over this period. A fresh sample was used for each point on the light saturation curve. The final light saturation curve is an average of 4 independent series of experiments.

Flash-induced absorption changes of P700, the primary PSI electron donor, were measured using a dual-wavelength emitter detector unit ED-P700DW (Walz, Germany) at 810 and 870 nm. A cell suspension containing about 2 μg ml^{-1} Chl in a 10 mm cuvette was placed into the optical unit ED-101US/M connected to the instrument in remittance mode. A high-power 620-nm-emitting lamp HPL-L620 and a 730-nm-emitting lamp HPL-L730 were used for specific excitation of PBS and Chl, respectively.

2.4. Fluorescence emission spectra

Fluorescence spectra of cyanobacterial cells were recorded with a Shimadzu RF-5301PC instrument equipped with an R928-08 red photomultiplier. Cell suspensions were placed in a cuvette with a 3 or 1 mm optical path to measure fluorescence at 288 or 77 K, respectively. The Chl content was about 1 μg ml^{-1} for all samples except the PSI-less cells, in which Chl content was reduced to 0.1 μg ml^{-1} as these cells contain less Chl. The measurements were carried out upon 440 or 570 nm excitation; the bandwidths of excitation and emission monochromators were set to 3 nm. To determine the changes induced by actinic light, 288 K fluorescence spectra of every sample were recorded twice: before and immediately after a 3-min exposure to 500 nm or 670 nm light (photon flux of incident irradiation about 350 μE m^{-2} s^{-1}). Pre-illumination was provided by the excitation beam of the spectrofluorometer upon temporarily increasing the bandwidth of the excitation monochromator to 10 nm.

For 77 K fluorescence measurements, *Synechocystis* cells were pre-illuminated for 3 min with red or blue-green actinic light in a 15 °C thermostatted PAM cuvette. The photon flux of incident irradiation was about 300 μE m^{-2} s^{-1} for both red and blue-green light. As internal standard, dichlorofluorescein diacetate pre-treated with H_2O_2 was added to the cell suspensions to a final concentration of 1 μM before samples were frozen [15]. The 77 K fluorescence emission spectra were measured in each sample upon 440 and 570 nm excitation and were subsequently normalized to the intensity of the fluorescein emission maximum at 510 nm exited by 440 nm light. Then, the fluorescence spectra excited by 440 nm light were normalized to Chl content, and the fluorescence spectra excited by 570 nm were normalized to APC content relative to the pigment content in wild type.

3. Results

The use of strains with different photosystem compositions enables selective probing of specific energy transfer pathways. Here we will use strains lacking PSI or PSII but retaining essentially normal phycobilisome amounts to probe PBS quenching effects on energy transfer to the two photosystems.

3.1. Blue-green-light-triggered energy dissipation in the PSII-less mutant

Carotenoid-triggered NPQ in PSII-less cells is also induced by medium-intensity (300 μE m^{-2} s^{-1}) illumination with blue-green light (500 nm) for 3 min (70% quenching; Fig. 1, +BL) whereas no fluorescence quenching is observed upon a 3-min illumination with red actinic light (+RL) at 680 nm at the same intensity. Light-induced P700 absorption changes in PSII-less *Synechocystis* cells were measured with a PAM fluorometer for cells in non-quenched (+RL) and quenched (+BL) states to estimate the energy that NPQ diverts from energy flow to the PSI reaction center. No difference in rates of P700 oxidation by 730 nm (Chl-absorbed) light was detected between cells that had been illuminated for 3 min with red vs. blue-green light (Fig. 2A, black and gray lines, respectively). However, 620-nm light absorbed by PBSs showed a significantly decreased rate of P700 oxidation in cells pre-illuminated with blue-green light vs. in those pre-illuminated with red light (Fig. 2B, gray and black lines,

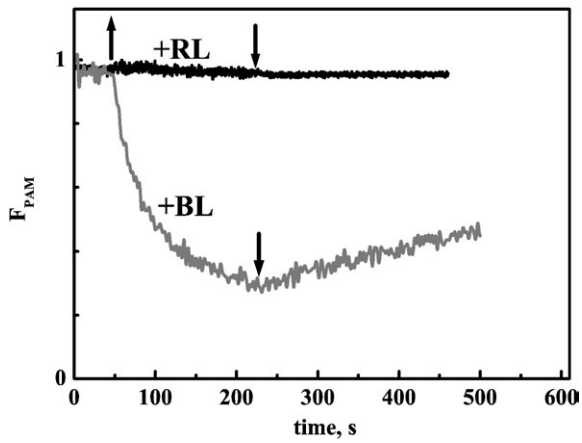

Fig. 1. Time course of PAM fluorescence in the PSII-less strain upon a 3-min exposure to actinic red (680 nm, 300 μE m^{-2} s^{-1}) or blue-green light (500 nm, 300 μE m^{-2} s^{-1}) followed by dark incubation. Arrow up: actinic light on; arrow down: actinic light off. For the results shown in Fig. 2 and selected other figures, strong actinic illumination was provided 1 min after the end of exposure to 500 or 680 nm light at 300 μE m^{-2} s^{-1}.

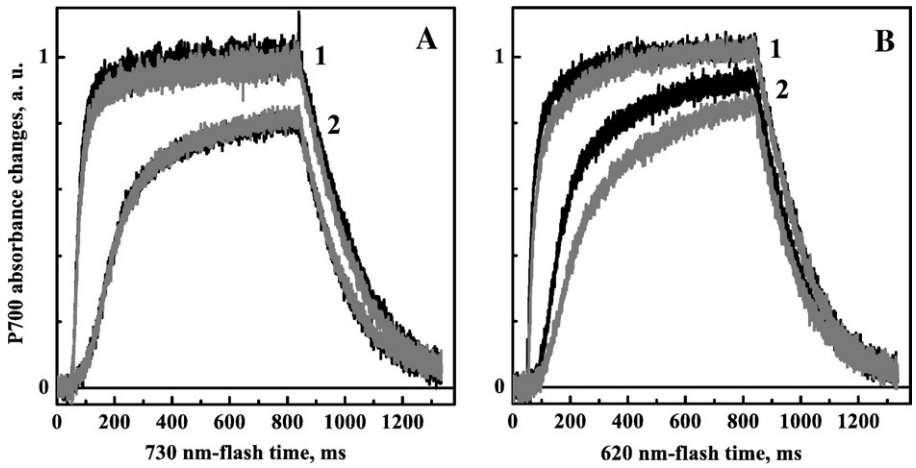

Fig. 2. Absorbance transitions of P700 (810 nm vs. 870 nm) measured by PAM in PSII-less mutant cells pre-illuminated with 3 min of 680 nm red light (black lines) or 500 nm blue-green light (gray lines), both at 300 μE m^{-2} s^{-1}. P700 redox state changes were induced with 730 nm actinic illumination absorbed by Chl (A) and 620 nm actinic illumination absorbed by PBSs (B). The P700 redox state transitions were monitored at two different actinic light intensities: (1) irradiation at 7 mE m^{-2} s^{-1} for 730 nm actinic illumination or 220 μE m^{-2} s^{-1} for 620 nm actinic illumination that fully oxidized P700 in 0.3 s, and (2) about 20% of these respective irradiation intensities.

respectively). The difference between the cells in non-quenched (+RL) vs. quenched (+BL) state was most visible at lower intensity of 620 nm illumination. This difference in rates of P700 oxidation reflects a decrease in energy flow from PBS to P700 after exposure to blue-green light.

To better quantify the difference in quantum yield of energy transfer between PBS and P700, P700 oxidation was determined in PSII-less cells (pre-illuminated with blue-green or red light) at different intensities of 620 nm illumination (Fig. 3). Cells in the non-quenched state (+RL) require less light for P700 oxidation saturation than cells in the quenched state (+BL). A quantitative analysis using the equation $\Delta A = \Delta A_{max} \times [1 - \exp(-k \times \text{flash intensity})]$ [34] shows k is 38×10^{-3} μE^{-1} m^2 s^1 for red light and 23×10^{-3} μE^{-1} m^2 s^1 for BL pre-illuminated cells. Thus, efficiency of PBS absorbed quanta in P700 photooxidation in the quenched state is 0.6 of that in the unquenched state.

3.2. Blue-green-light-triggered energy dissipation in the PSI-less mutant

Strong NPQ was induced with blue-green actinic light in the PSI-less strain like in the wild-type and PSII-less strains. As the PSI-less cells are quite photosensitive due to the lack of sufficient electron acceptor capacity at the level of the plastoquinone pool, we made sure that pre-illumination did not lead to photoinhibition. As shown in Fig. 4, a 3-min illumination with red light at 300 μE m^{-2} s^{-1} did not generate appreciable quenching. However, a 3 min-illumination with blue-green light (500 nm) at the same intensity caused major fluorescence quenching in PSI-less cells; variable fluorescence elicited by the 650-nm modulated light pulses was reduced by about five-fold, and F_o was reduced by two-fold (Fig. 4).

The results above suggest a major down-regulation of excitation energy transfer from PBS to PSII reaction centers upon illumination with blue-green light. In order to monitor this change quantitatively, we compared the quantum yield of photochemical energy conversion in PSII (Φ_{PSII}) upon PBS excitation in quenched and unquenched states of PSI-less *Synechocystis* cells by measuring induction of variable fluorescence upon exposure to 1-s, 620-nm illumination at different intensities (Fig. 5). Within this timeframe, BL pre-illuminated cells reached maximum fluorescence (F'_m) at maximum intensity of 620-nm illumination. As expected, BL-preilluminated cells required a longer 620-nm light exposure for the same fluorescence induction than RL-preilluminated cells. At actinic illumination at 75 μE m^{-2} s^{-1}, the time for reaching of 60% of F'_{max} was 0.4 s for cells illuminated

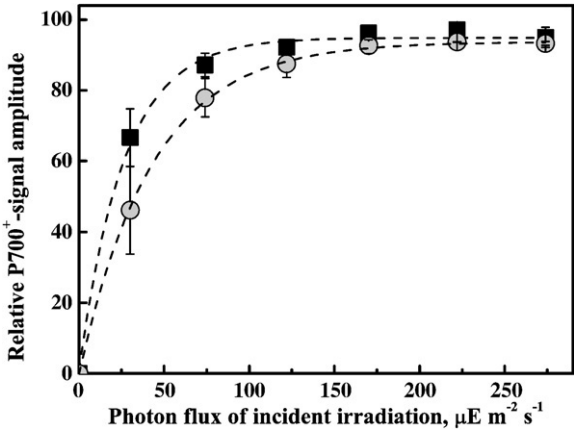

Fig. 3. Relative P700 absorbance change upon 620-nm illumination at different light intensities for 0.3 s. Cells of the PSII-less *Synechocystis* strain had been pre-illuminated for 3 min with red (black squares) or blue-green (gray circles) light 1 min before measurements.

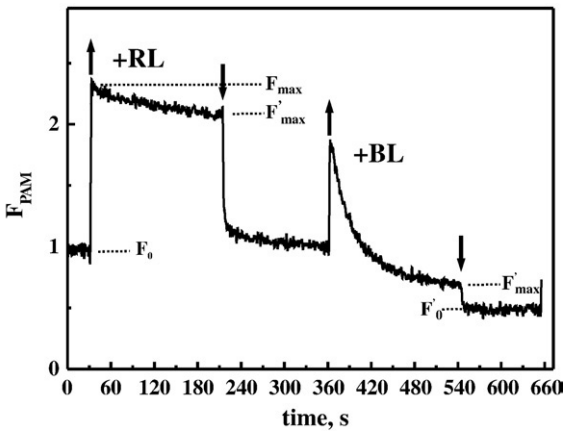

Fig. 4. Time course of fluorescence in the PSI-less strain using a PAM fluorometer. Little fluorescence quenching was observed upon illumination with 680 nm (300 μE m^{-2} s^{-1}) actinic light (+RL) but blue-green light at the same intensity (+BL) caused major NPQ. F_o and F_{max} indicate the initial ("dark") and the maximal fluorescence levels, F'_{max} is the maximal fluorescence after pre-illumination, and F'_o is the "dark" level in the quenched state.

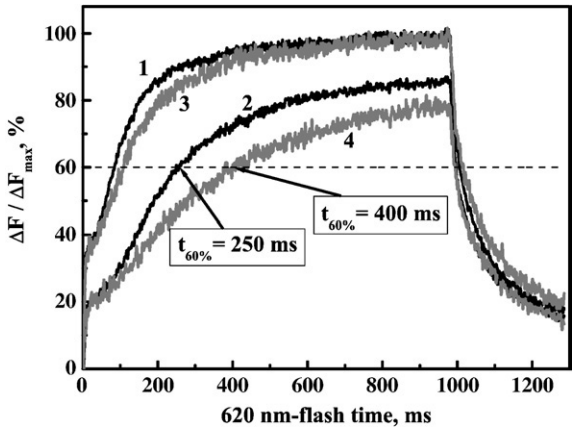

Fig. 5. Time course of the relative variable fluorescence ($\Delta F_t / \Delta F_{max} = (F_t - F'_0) / (F'_{max} - F'_0)$) of PSI-less mutant cells induced with 620 nm actinic light at two different intensities after pre-illumination with red (1, 2) or blue-green light (3, 4). The photon flux of the actinic light is 275 μE m^{-2} s^{-1} (curves 1, 3) and 75 μE m^{-2} s^{-1} (curves 2, 4). The time to reach 60% of the maximum level is marked as $t_{60\%}$.

with BL compared to 0.25 s for RL pre-illuminated cells. Therefore, less PBS-absorbed energy reaches PSII with blue-green-light-activated NPQ than without.

The effective quantum yield of PSII photochemistry [35,36] was calculated using the equation $\Phi_{PSII} = (F'_{max} - F_s) / \Delta F'_{max}$, where Φ_{PSII} is the effective quantum yield of PSII, $\Delta F'_{max} = F'_{max} - F'_0$ is the maximal flash-induced variable fluorescence measured upon a brief saturating illumination, and F_s is the steady-state fluorescence level reached after 0.3 s of illumination. Fig. 6 compares Φ_{PSII} in PSI-less cells as a function of the light intensity; cells have been pre-illuminated with BL or RL. Both data sets could be fitted to a simple exponential decay $\Phi_{PSII} = \Phi_{PSII,\ max} \times e^{-k \times E}$, where $\Phi_{PSII,\ max}$ is the effective quantum yield at theoretical zero irradiance, k is a constant, and E is the actinic light intensity. k is 13×10^{-3} μE^{-1} m^2 s^1 for RL and 9×10^{-3} μE^{-1} m^2 s^1 for BL pre-illuminated cells. Thus, efficiency of PBS absorbed quanta in PSII photoreaction in the quenched state is 0.7 of that in the unquenched state.

3.3. Spectral characteristics of mutants

3.3.1. Pigment composition

Table 1 shows similar content of Chl, carotenoids, PC, and APC in wild type and in the PSII-less mutant. Under the conditions grown, PSI-less cells contained only 11% of Chl and 19% of the carotenoids found in the wild type whereas the APC and PC content in the PSI-less mutant was 1.–-2 times that of wild type. The absorption spectra of PBS of wild type and both mutants measured as difference spectra of thermo-bleaching were similar (data not shown).

3.3.2. Fluorescence quenching spectra

77 K Fluorescence emission spectra of the three strains (wild type, PSI-less and PSII-less) upon excitation at 440 and 570 nm and after a 3-min exposure to blue-green light vs. red light are shown in Fig. 7. Whereas BL did not induce quenching upon excitation of Chl (confirming earlier results obtained on the wild type [15]), upon excitation of PBSs at 570 nm BL induced quenching of every emission component with the exception of PC emission at 650 nm. This implies that excitations at the site of carotenoid-induced NPQ cannot move back to PC at 77 K but can move to other pigments in the energy transfer pathway at this temperature. Also, the fact that no BL-induced quenching is observed upon Chl excitation indicates that at 77 K energy cannot move back from Chl to the site that is quenched. PBS quenching percentage at 77 K is noticeably lower than at room temperature and reaches about 25–30% of the main fluorescence components for the PSI-less strain, 15–20% for the wild type, and only about 10–15% for the PSII-less strain.

At room temperature, "uphill" energy equilibration can occur, and therefore quenching should occur regardless of whether Chl or PBSs are excited. To determine whether this indeed is the case, similar experiments were carried out with the PSI-less strain at room temperature (288 K). Fluorescence emission spectra of BL-treated cells of the PSI-less strain upon excitation of PBS at 580 nm (Fig. 8A) and of Chl at 440 nm (Fig. 8B) showed maximal quenching at 680 nm, which is also the fluorescence emission maximum at room temperature. The RL treatment used in our experiments did not result in significant changes of either the fluorescence level (see PAM measurements in Fig. 4) or the fluorescence emission spectra upon Chl or PBS excitation relative to dark-adapted cells (not shown). In PSII-less cells, however, quenching is shifted to 660 nm (Fig. 8C), as was reported in Rakhimberdieva et al. [14] and as may be expected from less efficient energy transfer interactions between PBSs and PSI. However, the second derivative of the difference spectrum (not shown), as well as its decomposition into three Gaussian bands (Fig. 8C), does show any additional components at 680 and 720 nm, indicative of an energy equilibration between PSI and PBSs at room temperature.

4. Discussion

All three strains studied here have normal PBS content (Table 1) and show effective BL-induced NPQ (Figs. 1 and 4) indicating that carotenoid-induced NPQ affects energy transfer to both photosystems (Figs. 2B and 5). This is in line with the observations that photosynthetic oxygen evolution rates in the wild-type saturate at a higher intensity in the quenched state than in the non-quenched state [17], and that the excitation spectra for PSI Chl *a* emission at 725 nm indicate reduced energy transfer from the PBS to PSI in samples where carotenoid-induced NPQ has been activated [16]. Our measurements enable a quantitative estimate of the energy that carotenoid-triggered dissipation diverts from the energy transferred

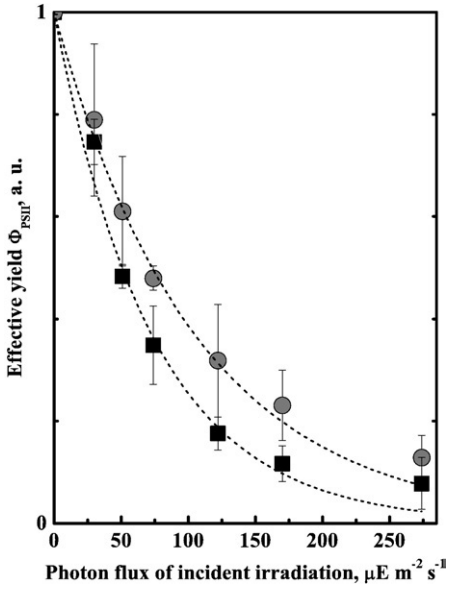

Fig. 6. Effective quantum yield of PSII ($\Phi_{PSII} = (F'_{max} - F_s) / \Delta F'_{max}$) as a function of the intensity of the 0.3-s, 620 nm actinic light illumination. Cells of the PSI-less mutant of *Synechocystis* were pre-illuminated for 3 min with red (squares) or blue-green (circles) light 1 min before the measurements.

Table 1
Pigment content and ratios in wild-type and mutant strains of *Synechocystis* sp. PCC 6803.

	Chl/cell (%)	Car/cell (%)	PC/cell (%)	APC/cell (%)	Car/Chl	PC/Chl (%)	APC/Chl (%)
Wild type	100	100	100	100	0.46	100	100
PSII-less	84 ± 13	70 ± 10	118 ± 15	106 ± 14	0.38	140	130
PSI-less	11 ± 3	19 ± 3	199 ± 26	164 ± 21	0.79	180	150

to photosynthetic reaction centers. For both photosystems, about 30–40% of the energy absorbed by PBSs does not reach the reaction centers when the carotenoid-induced NPQ is active. This is demonstrated by P700 oxidation kinetics (Figs. 2 and 3), as well as by fluorescence induction kinetics (Figs. 5 and 6). The rate of P700 photooxidation in the PSII-less strain demonstrates 40% decrease in 620 nm excitation energy transfer to the PSI reaction centers upon blue-light-induced fluorescence quenching. However, there is no change if Chl-absorbed 730 nm excitation energy is used for transfer to the PSI reaction center (Fig. 2), indicating that there is no noticeable transition of 730 nm excitation back to the PBS at room temperature.

The fact that our data were obtained with PSI-less and PSII-less mutants suggests independent pathways of energy migration from PBSs to PSII and PSI. This is in line with most other observations [10,37–40], but does not support the theory by Shimada et al. [41], who argue for energy transfer from PBS to PSI via PSII. This theory rests on interpretation of fluorescence spectra at 77 K without analysis of PSI activity at physiological temperature. In addition, it was recently found that energy transfer from PBS to PSI in *Synechococcus*

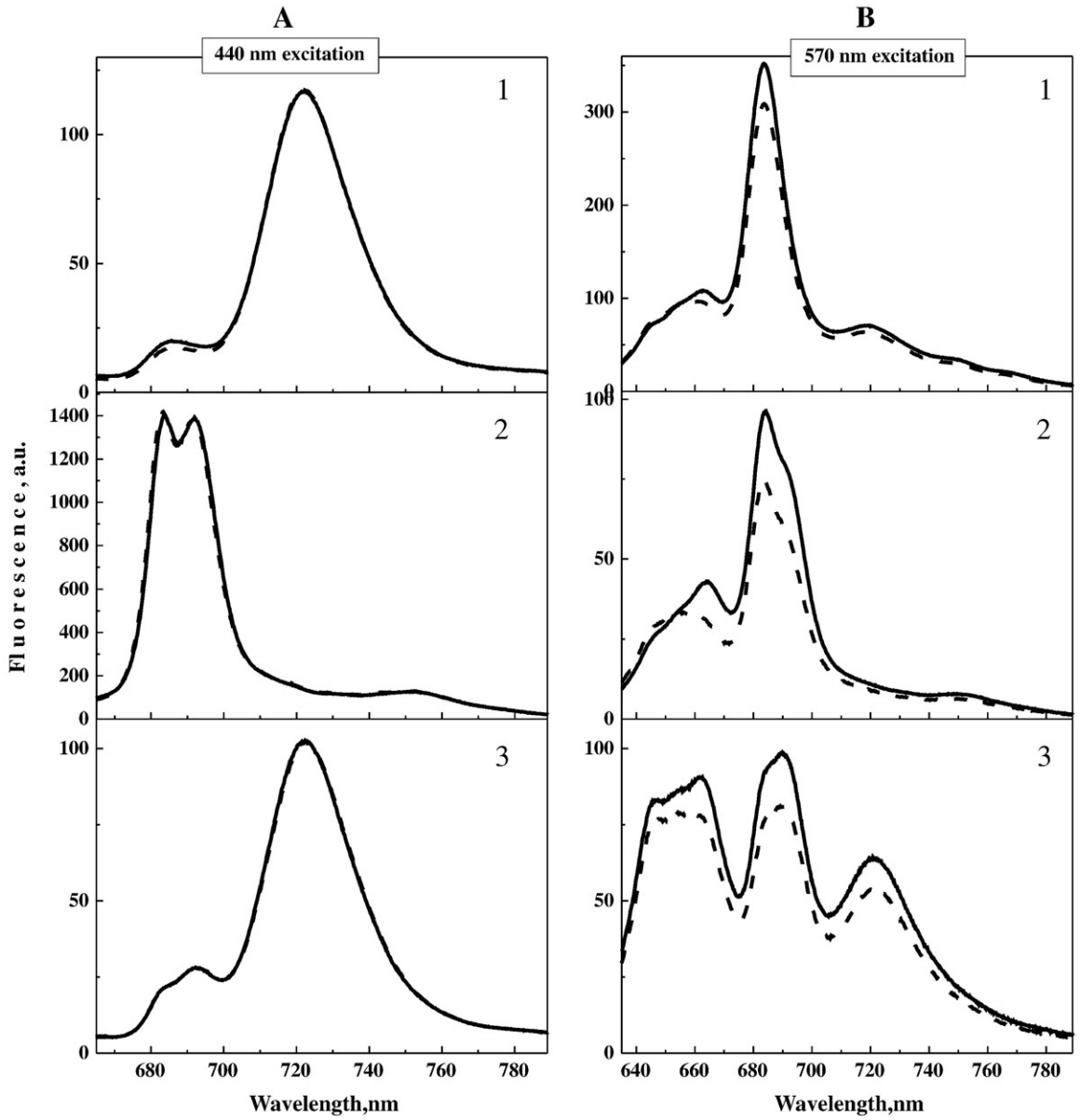

Fig. 7. 77 K fluorescence emission spectra in quenched (dashed line) and unquenched (solid line) states of PSII-less (1), PSI-less (2), and wild-type (3) *Synechocystis* cells upon Chl-absorbed 440 nm excitation (A) and PBS-absorbed 570 nm excitation (B). Spectra are normalized to Chl content (A) and to APC content (B) calculated from PBS absorption spectra as described in Materials and methods.

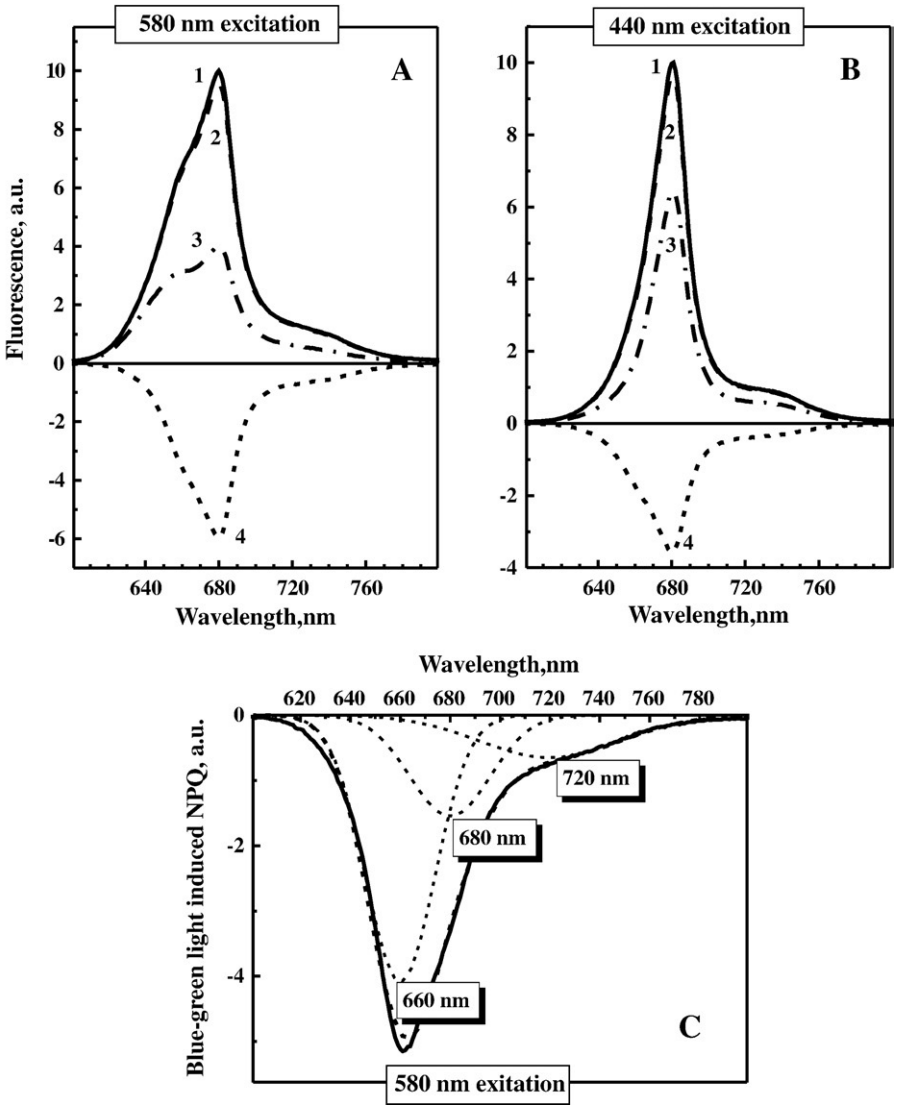

Fig. 8. Fluorescence and NPQ spectra of PSI-less (A, B) and PSII-less (C) *Synechocystis* cells measured at 288 K upon 580-nm (PBS) (A, C) or 440-nm (Chl) (B) excitation. The fluorescence spectra were measured before (1) and after red (2) or blue-green (3) light exposure for 3 min. NPQ spectra of PSI-less cells (4) were obtained by subtracting the unquenched state spectrum (2) from the quenched state spectrum (3). The NPQ spectrum of the PSII-less strain (C, solid line) was fitted as a superposition of three Gaussian peaks at 660, 680, and 720 nm (C, dotted lines).

sp. PCC 7002 is extremely efficient and occurred directly; efficient energy transfer was dependent upon the APC-B subunit [42].

Of course, chlorophyll excitation at 730 nm cannot go back into the PBS, but 680 nm light absorbed by PSII should do so at room temperature. It may seem possible to calculate fairly accurately the wavelength of the PBS component where quenching occurs by comparing the degree of quenching we get in those cases and the 30% quenching we get when exciting at 620 nm (Fig. 5). Unfortunately, the PAM-fluorometer does not allow saturating illumination at 680 nm for fluorescence measurements. This limits our options to comparing fluorescence spectra to elicit the contribution of the different PBS components to formation of the quenching center.

At room temperature, illumination with BL effectively quenches the Chl PSII excitation in the PSI-less strain (Fig. 8B), as we have earlier seen in the DCMU-poisoned wild type. Our data, obtained for the PSI-less mutant, support the concept of phycobilin/Chl excitation equilibration upon quenching [15]. There is no BL-induced quenching of Chl excitation (440 nm) in any strain at 77 K (Fig. 7). This makes us think that Chl is not involved in the quenching mechanism. Upon excitation of the PBS, 580 nm light is absorbed mostly by PC and APC, and excitation energy is transferred to APC-B and APC-L_{CM} [43]. Therefore, the increased quenching of excitation energy by APC results in decreased fluorescence of the APC, as well as of terminal emitters and of Chl. This is consistent with the spectra shown in Fig. 7B—the APC form with emission at 660 nm, the terminal APC species with emission at 680–685 nm, and Chl forms are evenly quenched at 77 K upon PBS excitation of samples in the quenched state. Only PC emission at 650 nm is not quenched, but on the contrary, it sometimes shows an increase.

At room temperature, many PBS components are in energy equilibrium, so it is logical that the 660 nm component of the PSII-less strain's fluorescence (Fig. 8C) is bleached even if it is the terminal emitter with the 680 nm component where the quenching occurs. Nevertheless, there are good reasons to interpret fluorescence quenching as originating from the short-wavelength APC with 660 nm emission rather than from APC-L_{CM} with 683 nm emission. Firstly, the fluorescence yield of APC (660 nm) was decreased, whereas uphill energy transfer from terminal emitters to APC would be very much retarded at low temperature. Secondly, the 77 K fluorescence yield of Chl would have decrease if the terminal emitter were involved in quenching, since most of the PSII pigments are essentially isoenergetic with the terminal emitter.

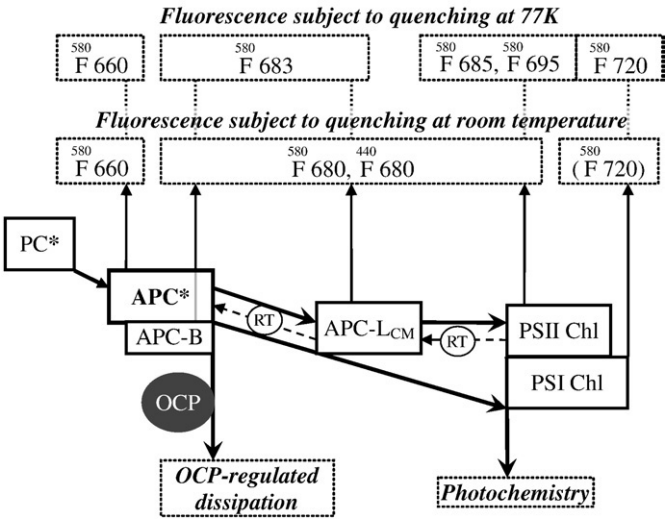

Fig. 9. Competitive reactions of PBS de-excitation in *Synechocystis*. A comparable decrease of PBS absorbed energy flow aimed at both photosystems suggests that APC-L_{CM} is not the site of quenching formation in the PBS core. Most probably, APC or/and APCB are put into the quenched state with blue-green light treatment. APC* marks APC in the excited state; PC* marks phycocyanin in the excited state, F660 (F683, etc.) marks fluorescence peaking at 660 (683) nm; 440 and 580 indicate the excitation wavelength.

A similar impact of NPQ on the excitation energy transfer from the PBS to the reaction centers of both photosystems (Figs. 3 and 6) supports the statement that quenching does not come from the APC-L_{CM} terminal emitter. We can estimate ratios of APC de-excitation rate constants by NPQ efficiency in two photoreactions:

$0.7 = k_{PII} / (k_{PII} + k_{NPQ})$, and
$0.6 = k_P / (k_{PI} + k_{NPQ})$,

where k_{PII} and k_{PI} are rate constants of APC de-excitation in photoreactions II and I, and k_{NPQ} is the rate constant of APC de-excitation in carotene-induced dissipation.

$k_{PII} = 2k_{NPQ} = 1.6k_{PI}$

In other words, PBS-absorbed energy is passed at a similar rate from an APC component in charge of quenching onto both PSII and PSI Chls. APC-L_{CM} cannot be ruled out, but is not a very likely candidate for the role of this component because of its very dissimilar connections with PSII and PSI [38,42].

This view is supported by the work of Scott et al. [16], who showed that BL-induced quenching acted through decrease of the excited-state population, which probably reflects the equilibrium between short- and long-wavelength APC within the PBS core.

There is less certainty about the other terminal emitter – APC-B, which is not the last link in energy transfer – at least to the PSII. At room temperature, fluorescence quenching spectra show a long-wavelength APC component – at about 680 nm in the PSII-less strain (Fig. 8C) and at about 677 nm in a strain lacking both photosystems (Rakhimberdieva, unpublished) – which may belong to APC-B. The possibility of uphill energy transfer from this level to short-wavelength APC cannot be completely ruled out. Moreover, quenching may well occur randomly at APC-B or APC, as T8 and B8 (the APC or the APC-B trimer plus a linker of 8-kDa) are positioned almost identically on the bases of the PBS core basal cylinders [5].

Fig. 9 illustrates the possible scheme of BL-induced de-excitation of PBS in *Synechocystis* cells. We show the APC-L_{CM} terminal emitter outside the site of OCP-triggered quenching, as we believe that the decreased quantum yields of APC-excitons trapped by APC-L_{CM} are responsible for the comparable decrease of PBS absorbed energy flow aimed at both photosystems. Equilibrium of Chl excitons with the quenching center in APC ensures that long-lived excitation energy in the Chl antenna (i.e., energy not efficiently trapped by open reaction centers) can also be quenched. Such equilibration requires a somewhat uphill energy transfer that can be easily achieved at room temperature. A similar situation probably also takes place for APC-L_{CM} excitation equilibration with short-wavelength APCs.

Our data suggest that all PBSs attached to the thylakoid membrane are subject to carotene-triggered dissipation of absorbed energy regardless of what they are bound to—whether they are "uncoupled", coupled to PSI, or coupled to open or closed PSII centers. It seems that a quenching center forms at the level of short-wavelength APCs—not longer than APC-B. Excess energy harvested by PC in the PBS rods would not excite the reaction centers once NPQ becomes prevalent. It would be dissipated as heat in the energetically higher APCs by a mechanism dependent on OCP, reducing by a third the excitation energy that reaches the APC-L_{CM} and the next Chl-based antenna of PSII or PSI. Furthermore, conditions that induce PSII reaction center closure, i.e., stop photochemical reaction, and induce NPQ would allow energy that is directly absorbed by Chl to be transferred into the PBS cores, where it would be dissipated as heat. As PBSs coupled to a closed PSII or uncoupled from either reaction center lack a photochemical de-excitation pathway, the excited Chl and/or APC terminal emitter state is long-lived, which allows for migration of the excitation energy to short-wavelength APCs. Uphill energy transfer is unlikely at low temperature.

Acknowledgements

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