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Structural and functional alterations of cyanobacterial phycobilisomes induced by high-light stress

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ABSTRACT

Exposure of cyanobacterial or red algal cells to high light has been proposed to lead to excitonic decoupling of the phycobilisome antennae (PBSs) from the reaction centers. Here we show that excitonic decoupling of PBSs of *Synechocystis* sp. PCC 6803 is induced by strong light at wavelengths that excite either phycobilin or chlorophyll pigments. We further show that decoupling is generally followed by disassembly of the antenna complexes and/or their detachment from the thylakoid membrane. Based on a previously proposed mechanism, we suggest that local heat transients generated in the PBSs by non-radiative energy dissipation lead to alterations in thermo-labile elements, likely in certain rod and core linker polypeptides. These alterations disrupt the transfer of excitation energy within and from the PBSs and destabilize the antenna complexes and/or promote their dissociation from the reaction centers and from the thylakoid membranes. Possible implications of the aforementioned alterations to adaptation of cyanobacteria to light and other environmental stresses are discussed.

1. Introduction

Light harvesting in cyanobacteria and red algae is carried out primarily by phycobilisomes (PBSs; [1]) — large, soluble molecular assemblies that consist of phycobiliproteins and linker, mostly non-pigmented, polypeptides. The phycobiliproteins form tightly bound heterodimers that belong to one of four groups: allophycocyanin (APC, $\lambda_{max} \approx 650$ nm), phycocyanin (PC, $\lambda_{max} \approx 620$ nm), phycoerythrin (PE, $\lambda_{max} \sim 545–565$ nm), and phycoerythrocyanin (PEC, $\lambda_{max} \approx 575$ nm). The phycobiliprotein dimers assemble into trimeric and hexameric rings which, with the aid of linker polypeptides, congregate into cylindrical stacks that are typically organized into hemi-discoidal or hemi-ellipsoidal structures. These structures consist of a core complex, which is associated with the cytoplasmic face of the thylakoid membrane, and of peripheral rods that extend from the core towards the cytoplasm. Positioning of PC, PC-PE, or PC-PEC in the rods and of APC in the core ensures that the harvested excitation energy is efficiently funneled into the PBS core. From there, it is transferred, through dedicated terminal emitters, into the photosynthetic reaction centers within the thylakoid membranes [2–10].

Due to the dominant role of PBSs in photon capture, their function has to be continuously modulated to enable adaptation to variations in the environment, particularly in light quality and quantity. Such adaptations are especially critical during exposure to strong irradiance, which can rapidly saturate the photosynthetic electron transport chain. Under these conditions, accumulation of over-excited chlorophyll molecules within the reaction centers, particularly of photosystem II (PSII), could lead to generation of reactive oxygen species. These, in turn, can severely damage the photosynthetic apparatus and cellular milieu. It is believed that the primary strategy used by cyanobacteria to manage excessive excitation of PSII is a non-radiative energy dissipation mechanism, termed non-photochemical quenching (NPQ), which quenches PBS fluorescence in a process mediated by the orange carotenoid-binding protein OCP. Unlike the functionally equivalent but mechanistically distinct process in higher plants and green algae, OCP-dependent NPQ is induced only by (moderate and strong) blue light and is independent of trans-thylakoidal ΔpH and excitation pressure on PSII. The mechanism by which OCP functions is not fully understood, but appears to involve interaction of OCP with components of the PBS core, presumably with the terminal emitters L_{cm} or α^{AP-B} [11–13]. Other strategies employed for protection of the cyanobacterial photosynthetic apparatus against excess irradiance include state transitions, which regulate the distribution of excitation energy between the two PSs, and quenching of PSI chlorophylls by P700 cation radical or triplet state [14,15]. Notably, recent studies suggest that processes intrinsic to the PBS can also prevent

Abbreviations: APC, allophycocyanin; Chl, chlorophyll; NPQ, non-photochemical quenching; OCP, orange carotenoid protein; PBSs, phycobilisomes; PC, phycocyanin; PSI, photosystem I; PSII, photosystem II.

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over-excitation of the reaction centers under high light [16,17], possibly by excitonic decoupling of the antenna complexes from the latter.

In the current work, we studied the effects of strong irradiance on the energetic coupling, stability, and membrane association of PBSs in the model cyanobacterium *Synechocystis* sp. PCC 6803. We found that exposure of the cells to strong monochromatic or white light leads to electronic decoupling of the PBSs from the reaction centers. This decoupling can be triggered by over-excitation of either phycobilin or chlorophyll molecules and is accompanied by disassembly of the PBSs and/or their detachment from the thylakoid membranes. These processes are discussed in relation to the thermo-optic effect proposed by Cseh et al., [18] and Stoitchkova et al., [16] and to the role they may serve in the protection of cells against photo-oxidative damage under stress conditions.

2. Materials and methods

2.1. Strains and culture conditions

Wild type and PC-deficient (Olive) *Synechocystis* sp. PCC 6803 cells [19] were grown photoautotrophically in BG11 medium at 22 °C, under 25 μmol photons $m^{-2} \cdot s^{-1}$ of white light.

2.2. Light treatments

Cells were harvested at the mid-exponential growth phase and their density was adjusted by dilution or concentration (by centrifugation) in BG11. Actinic light was provided either by the laser beam of a confocal microscope (see below) or by 150/250 W halogen lamps. In the latter case, the light reaching the samples was both far-red- and UV-filtered. The temperature was maintained at ~22 °C. Photon flux densities were measured with a Coherent FieldMaster powermeter or a Li-Cor Li-189 light meter. The optical density (at 730 nm) of the cells treated under the microscope or in bulk was ~0.3. To ensure homogeneous illumination of the cells in the bulk experiments, the suspension was gently stirred and illuminated from above. With the exception of the experiments conducted in the presence of osmolytes, all light treatments were carried out in BG11. For microscopic examinations performed in the presence of osmolytes, cells were washed with and suspended in buffered solution (pH 7.0) containing 0.5 M potassium phosphate and 0.3 M sodium citrate.

2.3. Absorption and fluorescence emission spectroscopy

Absorption spectroscopy measurements were carried out in 10-mm optical path-length cuvettes (Starna Scientific) with a JASCO V-7200 spectrophotometer. Fluorescence emission spectra were recorded on Fluorolog 3 (HORIBA Jobin Yvon) or SLM-Aminco 8100 spectrofluorometers, using 10- (Starna Scientific) or 1-mm (homemade) optical path-length cuvettes, for measurements conducted at room temperature and low temperature (77 K), respectively.

2.4. Confocal microscopy, spectral imaging, and FRAP measurements

Cells were adsorbed onto 1.5% low-melting point agarose suspended in BG11 or in potassium phosphate buffer (for the measurements carried out in the presence of osmolytes). Confocal fluorescence imaging, spectral imaging, and FRAP measurements were performed with an IX81-based Olympus FluoView 1000D laser confocal scanning microscope, equipped with a spectral scanning system (utilizing a galvanometer diffraction grating; 2 nm resolution) and two independent laser scanners. The latter allowed rapid simultaneous acquisition of images during photobleaching. Imaging, spectroscopy, and FRAP measurements were conducted using a 1.35-NA ×60 oil-immersed (UplansApo UIS2) objective.

2.4.1. Imaging

x–*y* scans were performed with the focal plane set to the mid-section of the cells; in some of the experiments, serial *z*-section images were also recorded. Images were acquired sequentially following excitation with 442- (max. power: 25 mW), 559- (20 mW), or 638-nm (20 mW) light, with the intensity adjusted by an acousto-optic tunable filter laser combiner (typically, 0.6% and 0.4% of the maximum power, when exciting at 442-nm and 638-nm, respectively). Emitted fluorescence was collected (by the spectral scanning unit) at 640–660 nm and 670–690 nm, upon excitation at 442 or 559-nm, and at 650–670 nm and 670–690 nm, upon excitation at 638 nm. Images were acquired at 512×512 pixels; the sampling speed was 40 and 20 μs/pixel for excitation at 442-nm and 559/638-nm, respectively. For the light treatments, a 200×200-pixel region containing the cell(s) was selected in order to minimize the scanning time; this procedure does not affect the resolution of the images. Scanning was performed continuously, unless otherwise stated.

2.4.2. Spectroscopy

Spectral images were acquired from the mid-plane of the cells, using an emission bandwidth of 3 nm and a step-size of 2 nm. Each spectrum consisted of 50 images collected between 600 and 701 nm, for excitation at 442 or 559 nm, or 35 images collected between 650 and 721 nm, when excited at 638 nm. Images were initially acquired at 256×256 pixels. To minimize light-induced effects during the recording, a small region of 100×100 pixels that contained the object of interest was selected and scanned at 12.5 μs/pixel.

2.4.3. FRAP measurements

FRAP measurements were performed by two laser scanners, one for imaging and one for bleaching, allowing for rapid (within ~30 ms) capture of images after photobleaching (tornado mode), which was applied for ~0.5 s over the entire cell with a 559-nm laser operating at 5 to 40% of its maximum power. Pre- and post-bleaching images (256×256 pixels) were collected at the range of 650–670 nm, following excitation with a 638-nm laser operating at 0.1% of its maximum power and attenuated further (~4 folds) by filter. In addition, sampling time was decreased to 2 μs per pixel, to prevent additional photobleaching and minimize light-induced effects during image acquisition. Following the recording of the first image after photobleaching, images were collected at 1 s intervals for the first 10 s, and at 10 s intervals thereafter (overall: 200 s). Prior to bleaching and after the recovery phase, spectral images were also recorded, using a 442-nm excitation light. Images were acquired at a sampling time of 2 μs/pixel and with a bandwidth and step-size of 3 nm.

3. Results

3.1. High-light-induced electronic and physical uncoupling of PBSs probed by confocal microscopy

Fig. 1 shows confocal fluorescence images of live *Synechocystis* sp. PCC 6803 cells. Following excitation at 442 nm (mainly exciting chlorophyll) or 638 nm (mainly exciting phycobilins), images were acquired in the emission range of 670–690 nm (panels A, D) or 640–660/650–670 nm (panels B, E and C, F), respectively, using an integrated spectral detection system. This setup allows separating the fluorescence emission of PSII (with possible contributions from the PBS terminal emitters, $\alpha^{AP\text{-}B}$ and L_{cm}) from that of the PC and APC subunits of the PBS. Note, however, that some bleed-through from the 640–660/650–670-nm emission to the 670–690 nm emission range had nevertheless occurred (see also Figs. 2 and S3). Panels A, B, D and E show images of the same cell upon excitation with a 442-nm laser. After a single scan, fluorescence of PSII-associated chlorophyll expectedly dominated the image (panel A), with only a very

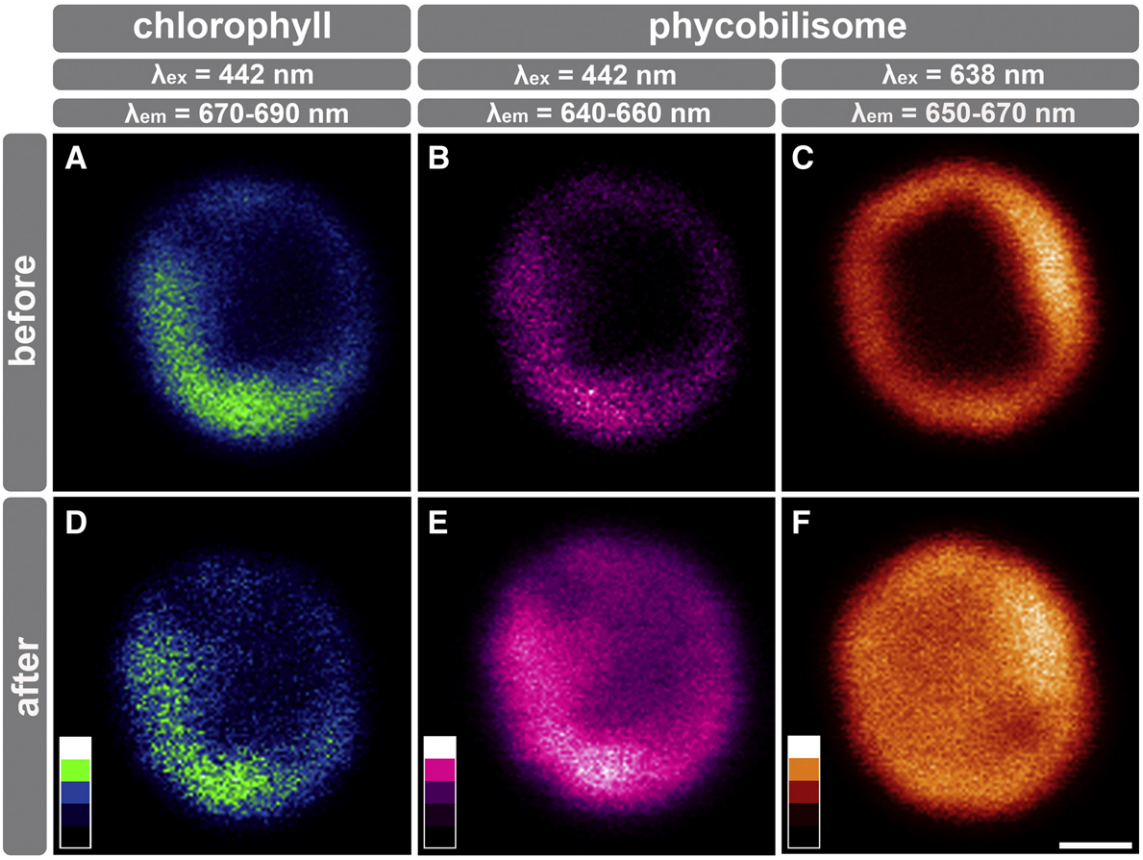

Fig. 1. Confocal fluorescence images of control and light-treated *Synechocystis* sp. PCC 6803 cells. Images were acquired before (A–C) and after (D–F) the cells were scanned repeatedly for 140 or 75 s, using a 442-nm (A, B, D, E) or 638-nm (C, F) laser excitation. The fluorescence is shown in pseudo-color from black (low) to white (high). In this and the following figure, images shown in A, B, D and E were recorded from the same cell, and those shown in C and F from another cell. Scale bar: 0.5 μm. Experiments with 559-nm laser excitation yielded similar results (data not shown). When higher laser intensities were used, the number of scans needed to elicit the changes in fluorescence intensity and distribution was significantly smaller. The data shown in all figures are representative of at least three independent experiments.

weak contribution from the antenna complexes (panel B). In each of the emission ranges collected (670–690 nm or 640–660 nm) the fluorescence formed a ring-shaped pattern, consistent with the distribution of the thylakoid membranes in this species, which are mostly confined to the cell periphery. Following acquisition of the initial images, the region of interest was scanned repeatedly for 140 s, using the same scanning parameters. Images obtained from the last scan (panels D and E) indicate that continuous exposure of the cell to the laser beam resulted in a decrease in chlorophyll fluorescence and a marked increase in PBS fluorescence. Such a rise in PBS fluorescence is clearly inconsistent with NPQ-related or other photoprotective mechanisms but can readily be explained by energetic decoupling of the antenna complexes from the chlorophyll bed of the reaction centers. Notably, the fluorescence emitted from the PBSs at the end of the scans was no longer confined to the periphery of the cell but extended to the central cytoplasmic region, which is generally devoid of thylakoids (panel E). Examination of confocal *z*-series revealed that the dispersion of PBS fluorescence was not limited to the mid-plane of the cells but extended throughout the entire cellular volume (not shown). Emission spectra recorded under the microscope showed that the fluorescence at the central cell region was indeed emitted from the PBSs (Fig. S1). The rise in PBS fluorescence at the center of the cell was not accompanied by a concomitant decrease at the periphery, precluding the possibility that intact, membrane-associated PBSs diffused into the cell center. The results described above indicate that excitonic uncoupling of the PBS from the reaction centers is accompanied by either detachment of phycobiliproteins from the PBS body or by dissociation of the PBS from the thylakoid membranes, or both (see Fig. 6); the free phycobiliproteins or the membrane-detached antennae are then able to diffuse away from the inter-thylakoid space to the center of the cell. Experiments conducted using a higher laser intensity (10% of the maximum power) yielded similar results, but the number of scans required to elicit the effects was smaller (data not shown).

The data described above indicate that antenna decoupling can be induced by direct excitation of the reaction center chlorophyll. To verify whether this can also be induced by direct excitation of the PBSs, we repeated the experiment using 559- or 638-nm light, which is preferentially absorbed by PC or APC, respectively. The results obtained from the measurements performed with 638-nm light are shown in panels C and F of Fig. 1. Here too, a series of repetitive laser scans of the cells for 75 s led to dispersion of the PBS-emitted fluorescence throughout the entire cellular volume without any concomitant decrease in the fluorescence intensity at the cell periphery. However, compared to the results obtained with the 442-nm light, the changes in the amplitude and distribution of the fluorescence signal occurred at significantly lower photon flux densities. This suggests that the sites which are modulated by the light and responsible for the observed changes in fluorescence emission are located within the PBS and/or its interface with the reaction centers. Exposing the cells to 559-nm light resulted in effects similar to those observed when the cells were exposed to a 638-nm light (not shown).

Energetic uncoupling and dispersion of PBS fluorescence were also observed when cells (mounted on a cover glass) were illuminated with strong white light (Fig. S2). In these experiments, however, the changes in fluorescence intensity and distribution occurred in the course of minutes, rather than seconds, as when the confocal lasers

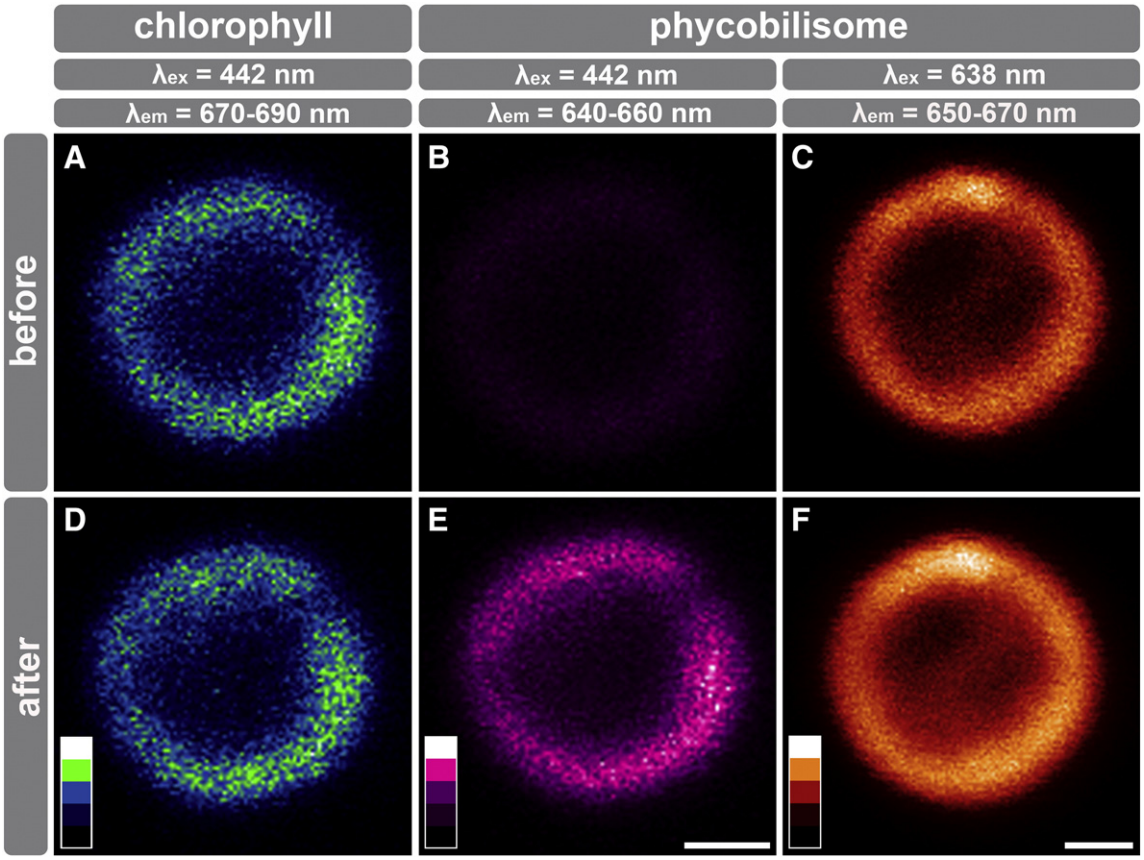

Fig. 2. Confocal fluorescence images of control and light-treated *Synechocystis* sp. PCC 6803 cells in the presence of 0.5 M potassium phosphate. The images were acquired and are presented as described in Fig. 1. The light treatments proceeded for 113 (442-nm light) or 85 (638-nm light) seconds. Scale bars: 0.5 μm.

were used for light stimulation. This is in accordance with the significantly lower irradiance power of the white light.

The fact that PBS fluorescence was observed in the central cell region following excitation with 638-nm light suggests that detachment or disassembly of the PBSs extends to their APC core subunits. To corroborate this notion, we used a strain that lacks PC (and, therefore, peripheral rods), yet contains intact cores [19,20]. Interestingly, the PBS fluorescence in this mutant was not distributed homogeneously within the cells but was concentrated at specific regions at or near the cell periphery, giving rise to highly fluorescing bright domains (Fig. S3). Regardless of this unusual distribution, repetitive scanning of the cells with 442-nm or 638-nm light resulted in a decrease of chlorophyll fluorescence accompanied by partial (442 nm) or complete (638 nm) dispersal of APC fluorescence throughout the intracellular space, as observed in the wild type.

High osmotic strength solutions containing potassium phosphate or betaine are known to enhance subunit binding within the PBS and to stabilize the interaction between PBSs and the reaction centers and/or the thylakoid membrane [21–26]. We therefore repeated the experiments summarized in Fig. 1, but this time the cells were suspended in a buffered solution containing 0.5 M potassium phosphate. As observed in the absence of osmolytes, repeated scans of the cells resulted in enhancement of PBS fluorescence (Fig. 2). However, in this case, the fluorescence signal did not disperse significantly into the central cell region, but remained localized to the cell periphery. This indicates that energetic decoupling of PBSs can occur without subsequent dissociation of phycobiliproteins or detachment of the antenna complexes from the thylakoid membranes and likely precedes these steps. In addition, in the presence of osmolytes, chlorophyll fluorescence did not decrease after the light treatment (Fig. 2, panel D). This may be due in part to fluorescence bleed-through from the decoupled, yet membrane-associated PBSs to the chlorophyll channel, which would mask the decrease in chlorophyll fluorescence. Such a masking is probably also present in the absence of osmolytes, but to a lesser extent, as the PBSs are dispersed throughout the cell. However, spectral bleed-through alone cannot account for the difference in chlorophyll fluorescence between the untreated and osmolyte-treated cells. One possibility may be that that the osmolyte somehow protects the pigment bed of the reaction centers from photo-induced damage. Another possibility is that the osmolyte interferes with the activity of quenchers, *e.g.*, OCP, which, in its absence, reduce the amount of excitation energy delivered from the PBSs to the reaction centers.

3.2. Light-induced uncoupling of PBSs is also revealed by bulk spectroscopic measurements

In the next set of experiments, we studied the effect of strong irradiance on cell cultures by fluorescence spectroscopy at room temperature and at 77 K. In order to mimic the strong light conditions that were used under the confocal microscope, the culture was exposed to 13,000 μmol photons $m^{-2} \cdot s^{-1}$ of white light and emission spectra were recorded after various exposure times.

3.2.1. Room temperature fluorescence

When excited by 440-nm light, control cells exhibited an emission spectrum characterized by a broad peak at ~685 nm (Fig. 3A), corresponding to fluorescence emitted from PSII-associated chlorophyll and, possibly, from the PBS terminal emitters, mainly L_{cm}. Exposure of the cells to high light for 20 min caused a sharp decrease in the peak amplitude, following which it underwent only small fluctuations. This decrease may be due to deleterious alterations in the

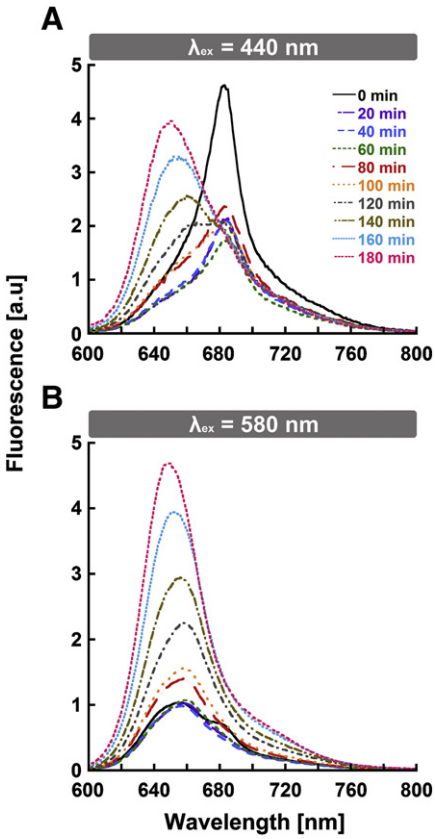

Fig. 3. Room temperature fluorescence emission spectra of *Synechocystis* sp. PCC 6803 cells subjected to strong irradiance. A cell culture was illuminated by strong (13,000 μmol photons $m^{-2}\cdot s^{-1}$) FR- and UV-filtered white light and emission spectra were recorded at various times, following excitation at 440 (A) or 580 (B) nm. The spectra were not normalized, as all bands within the emission ranges collected were affected by the light treatment.

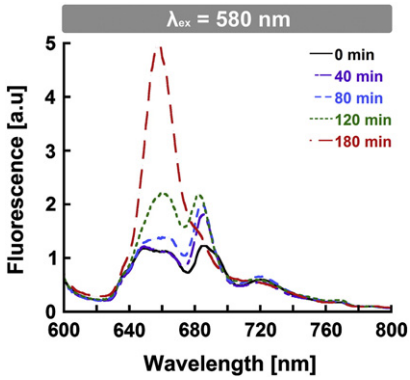

Fig. 4. 77 K fluorescence emission spectra of *Synechocystis* sp. PCC 6803 cells subjected to strong irradiance for different time periods. The experiment was carried out as described in Fig. 3. The spectra shown were recorded following excitation at 580 nm. The spectra were normalized to the 787-nm band, which is located well outside the PBS/Chl emission ranges.

reaction centers or OCP-mediated quenching of PBS fluorescence. After 80 min of illumination, a shoulder emerged at 657 nm, indicating that a considerable fraction of the PBSs had become uncoupled from the reaction centers. Upon further exposure, this shoulder had transformed into a broad peak (max: 655 nm) that subsequently intensified and blue-shifted to 650 nm. The progressive blue shift of this peak is consistent with the accumulation of electronic breaks between the rod and core subunits of the PBS. These breaks, which disrupt energy transfer from the PBSs to the reaction centers, likely protected the latter against further damage associated with excessively absorbed light. Fluorescence emission spectra recorded after excitation at 580 nm showed a similar pattern, namely a rapid (within 20 min) decrease in chlorophyll fluorescence [apparent as a shoulder at the pre-treatment spectrum (black line)], followed by an increase and blue shift of the PBS emission (Fig. 3B).

3.2.2. 77 K fluorescence spectroscopy

Untreated cells showed a characteristic emission spectrum with peaks at 647, 663, 685 and 723 nm when excited at 580-nm (Fig. 4, solid line). The first two peaks represent emission from PC and APC, respectively. After 40 min, the amplitude of the 685-nm peak increased by ~50%. Following an additional 40 min of illumination, the 663-nm peak, but not the 647-nm peak, also intensified, indicating obstruction of energy transfer from the PBS core to the reaction centers. At 120 min post-illumination, the amplitude of both the 663-nm and the 685-nm peaks continued to increase, indicating additional decoupling of the antenna system. As mentioned before, the 685-nm peak includes contributions from both PSII as well as from the PBS terminal emitters $\alpha^{AP\text{-}B}$ and L_{cm}, with the latter being the major router of excitation energy from the PBS [27–35]. Based on the microscopic observations and the room temperature fluorescence measurements, increased emission from PSII following the light treatment is unlikely. It is also inconsistent with the substantial increase in the PC/APC fluorescence seen in the 77 K spectra. Moreover, the room temperature emission spectra recorded after direct excitation of the chlorophyll molecules (Fig. 3A) show that chlorophyll fluorescence had actually decreased during the light treatment. This was also observed in the 77 K spectra recorded following excitation at 440 nm (not shown). We therefore interpret the increase in the 685-nm peak as enhanced emission from the PBS L_{cm} terminal emitter, due to uncoupling of the PBSs from the reaction centers. Additional exposure to the light (180 min) resulted in further and substantial enhancement of the 663-nm peak but the fluorescence emitted from L_{cm} (685-nm peak) decreased markedly and was blue-shifted. This likely reflects excitonic uncoupling of L_{cm} from the PBS core and/or disassembly of the PBS complex.

3.3. Facilitated uncoupling of PBSs revealed by FRAP measurements

To follow the dynamics of energetic decoupling of PBSs to high light conditions, cells were exposed to high photon density fluxes for short durations, using protocols employed in FRAP measurements. Unlike conventional FRAP experiments in which only a selected region of the cell is bleached, here the entire cell was subjected to the bleaching light, as was done previously with cyanobacterial [36] and red algal [37] cells. The cells were photobleached, throughout their entire thickness, with 559-nm laser light, and images were acquired in the range of 650–670 nm, following excitation at 638 nm. Prior to the bleaching and after image recording, emission spectra were acquired (under the microscope), using 442-nm excitation light. Fig. 5 shows images obtained before (panel A) and after (panels B–D) a cell was exposed to the light provided by the 559-nm laser (operating at 1 mW) for ~0.5 s. As can be seen in panel B, the exposure resulted in a large (~70%) decrease of the PBS fluorescence. Notably, however, the fluorescence signal was largely and rapidly regained throughout the whole cell (panels C, D, and F), even though the entire cell was exposed to the bleaching light. Here too, the recovered fluorescence spread into the central, thylakoid-free region of the cell (panel D), indicating that the PBSs had disassembled and/or detached from the thylakoid membranes. Experiments employing *x–y–z* scans of the cells also revealed that the fluorescence dispersed through the entire cellular volume (not shown). Emission spectra recorded before bleaching and after the recovery phase revealed that the bleaching light caused only a moderate decrease in PSII fluorescence (panel

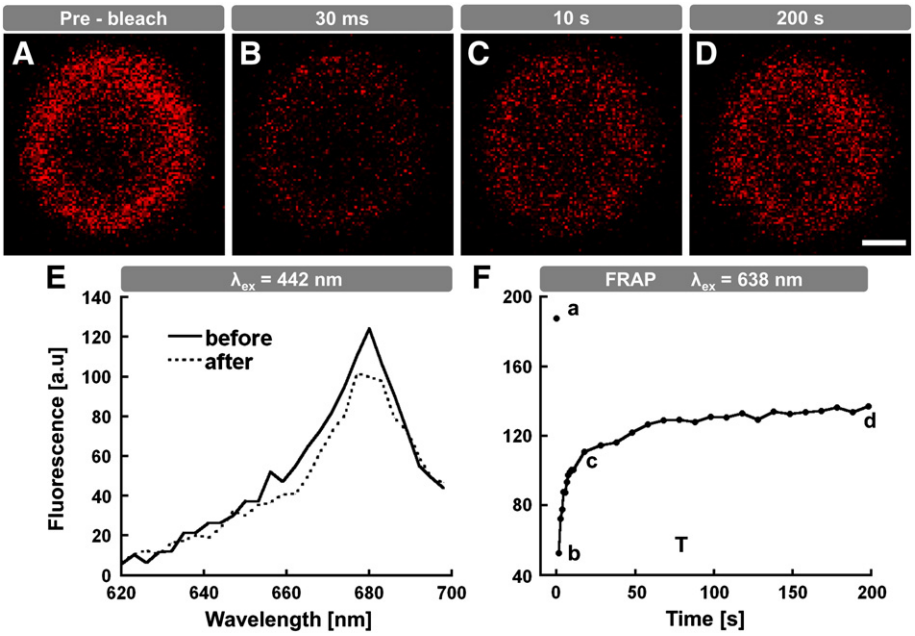

Fig. 5. Photobleaching of whole *Synechocystis* sp. PCC 6803 cells. A single cell was subjected to an intense 559-nm laser light for ~0.5 s throughout its entire cross-section. Images were acquired at 650–670 nm, following excitation at 638 nm. (A–D) Selected images recorded before (A) and after (B–D) photobleaching. Scale bar: 0.5 μm. (E) Fluorescence emission spectra of the cell recorded under the microscope before photobleaching and after recovery. (F) Integrated fluorescence intensity of the cell shown in panels A–D as a function of time. Measurements conducted at higher laser intensities yielded similar results but the extent of fluorescence recovery was lower.

E). It thus appears that the energetic decoupling of the PBSs from the reaction centers, at least from PSII, under the strong irradiance used in these experiments occurs sufficiently fast to prevent severe photodamage. Panel F shows the recovery curve derived from the experiment presented in panels A–D. The data could not be fitted with a simple first-order kinetic model, which would be expected if a simple diffusion process were involved. Recovery of the fluorescence proceeded with an apparent $t_{1/2}$ of ~7 s and finally reached ~70% of the initial, pre-bleaching value (point *A*). Analysis of recovery traces from other experiments yielded similar results. In all cases, the integrated fluorescence intensity at the central region of the cells was higher (up to two folds) than the corresponding pre-bleaching values, due to dispersion of membrane-detached PBSs and/or disassembled phycobiliproteins inside the cells. Measurements performed with higher laser intensities yielded similar recovery rates, but the extent of recovery was lower (not shown).

4. Discussion

In this work, we show that exposure of *Synechocystis* PCC 6803 cells to strong monochromatic or white light leads to electronic uncoupling of PBSs from the reaction centers, at least from those of PSII, as well as to disruption of excitation energy transfer within the PBS antennae themselves. The energetic decoupling could have been induced by either over-excitation of phycobilin or chlorophyll molecules. This was followed by detachment of the PBSs from the thylakoid membranes and/or dissociation of phycobiliproteins from the PBS body. Notably, energetic decoupling was also observed in the presence of osmolytes. This suggests that the interactions that maintain PBS integrity and its association with the thylakoid membranes are distinct, at least partially, from those that are necessary for excitonic coupling. Finally, using FRAP measurements conducted on whole cells, we show that the dynamics of energetic decoupling can have a strong impact on the extent of photodamage inflicted on the reaction centers by excess irradiance.

The exact nature of the photo-induced processes that lead to energetic decoupling of PBSs and their subsequent detachment from the thylakoid membrane surface and/or disassembly is not clear. A plausible option is that they are driven by locally induced thermal fluctuations. This idea was originally proposed by Stoitchkova et al., [16] who observed that exposure of *Synechocystis* PCC 6803 cells to high light or to short heat treatments led to similar changes in their fluorescence emission spectra, both at room temperature and 77 K, which were consistent with energetic uncoupling of PBSs from the reaction centers. Based on this and on a previous study [18], they suggested that excess light energy absorbed by PBSs is dissipated locally as heat. They further proposed that the resulting heat transients lead to structural alterations in the PBS at certain sites characterized by comparatively low thermal stability. The presence of such labile sites is supported by results obtained from single-molecule fluorescence spectroscopy measurements, which showed that light-induced energetic decoupling in isolated *P. cruentum* PBSs occurs at the interaction sites between two PE isoforms (b-PE and B-PE) found at the peripheral rod endings [38]. The effected component was proposed to be a pigmented linker polypeptide (the γ subunit of B-PE) that connects the two phycobiliproteins.

Combining our results and those obtained in previous studies, the following picture emerges: strong illumination leads to over-excitation of phycobilin and chlorophyll molecules within the PBSs and photosystems, respectively. Saturation of the electron transport chain by the strong irradiance may also lead to equilibration of PSII chlorophyll excitation energy with that of APC, resulting in backward energy transfer from the reaction centers to the PBS core [39]. [This latter process likely underlies energetic decoupling and the accompanying dissociation events induced upon illumination with 442-nm light.] In each case, the resulting excessive excitation pressure leads to the generation of local heat transients within the antenna complexes, due to non-radiative relaxations. These in turn lead to modifications in the structure of specific thermolabile elements in the PBS, resulting in energetic decoupling and disassembly of the antenna complexes and/or their dissociation from the reaction centers/thylakoid membranes. These events are schematically illustrated in Fig. 6. We suggest that the heat-sensitive elements correspond to PBS linker proteins. The strategic roles of these linkers in connecting the different parts of the PBS and in coupling them to the reaction centers render them ideal candidates for safeguarding the photosynthetic

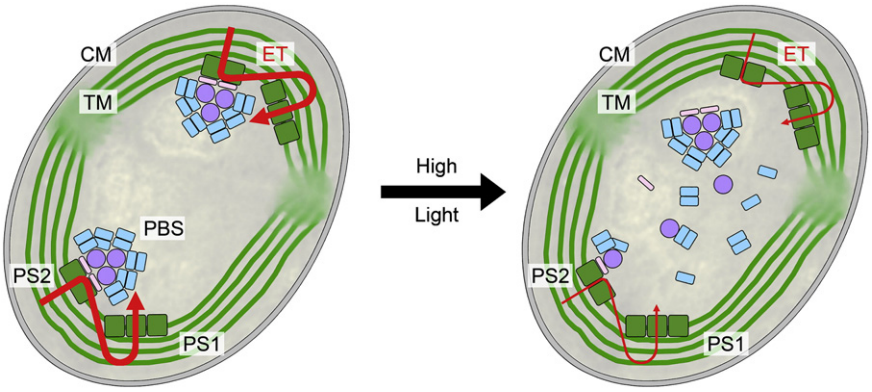

Fig. 6. Schematic representation of the final events that occur in the PBS following exposure to high light. The internal reorganizations that precede these events are discussed in the text.

machinery against excessively absorbed energy. Moreover, impairments in these proteins may readily cause dissociation of phycobiliproteins from the PBSs that, according to our observations, may follow excitonic uncoupling. As mentioned above, a chromophore-bearing linker protein has been implicated in energetic uncoupling of PBSs in the red alga *P. cruentum* [38]. The involvement of the pigmented linker polypeptide, L_{cm}, in this process is suggested by the 77 K fluorescence emission spectra we obtained as well as by experiments with L_{cm}-deficient *Synechocystis* PCC 6803 [19]. Besides being the primary mediator of excitation energy transfer from the PBS, L_{cm} is essential for the assembly of the PBS core and its association with the reaction centers and/or thylakoid membranes [5,27–35,40]. Therefore, it is expected that structural alterations in L_{cm} would strongly interfere with energy transfer from the PBSs to the reaction centers and would lead to destabilization of the antenna complexes and/or their dissociation from the thylakoid membranes, which indeed takes place.

The data acquired from the FRAP measurements revealed that, following quenching by the bleaching light, the fluorescence rapidly rises to about 70% of the initial, pre-bleaching value. This can be ascribed to enhanced fluorescence by PBSs that have escaped irreversible bleaching by the intense light but were excitonically uncoupled, detached, and/or disassembled from the reaction centers. Alternatively, it may reflect a transient quenched state induced during the thermal relaxations discussed above. Transient quenching of PBS fluorescence following exposure to high light was previously reported for wholly bleached *P. cruentum* and *Thermosynechococcus elongatus* cells [36,37]. These studies also revealed quenched fluorescence transients in isolated PBSs and in cells treated with glutaraldehyde or with betaine, in both cases membrane detachment and disassembly of PBSs is unlikely. Photodynamic processes, possibly corresponding to reversible reorganizations of phycobilins or changes in their surrounding associated with heat dissipation within the PBS, may therefore contribute to or underlie the transient decrease of its fluorescence.

An intriguing point is how components of the PBSs, following disassembly or detachment from the thylakoid membranes, reach the cell center. This is because the thylakoid membranes of *Synechocystis* PCC 6803, as well as of many other cyanobacteria, are arranged in multiple, seemingly continuous, concentric shells, which follow the contour of the cell [41]. Examination of several cyanobacterial species, by us and others, using electron microscope tomography, revealed that their thylakoid membranes contain multiple perforations, which likely evolved to enable unperturbed traffic throughout the cell volume [42,43]. We propose that it is through such perforations, which are large enough to accommodate even an entire antenna complex, that components of the PBS reach the cell center following the light treatments.

Based on FRAP measurements, it has been proposed that PBS rapidly diffuse on the surface of the thylakoid membranes, making only weak, transient interactions with the two PSs [44–46]. Our data clearly support weak interactions between PBSs and PSs and/or thylakoid membranes. However, the various effects exerted by high light on PBS electronic and structural characteristics (see also [37]), make it problematic to use the results of FRAP experiments as a measure for PBS diffusion, as these effects include changes in PBS fluorescence intensity and distribution. Elucidation of the role of PBS mobility in light adaptation responses, particularly state transitions, may therefore require additional experimental means (see [46]).

The light-dependent effects reported in this work, as well as in the single-molecule studies described above, were observed at overly high light intensities. However, as energetic decoupling of PBSs is photon dose-dependent, it should also occur under lower light intensities (above some critical threshold), provided the exposure time is sufficiently long. Indeed, excitonic decoupling of PBSs was observed in *Synechocystis* PCC 6803 cells after their exposure to 600 μmol photons $m^{-2}\cdot s^{-1}$ for 3 h [16]. Energetic decoupling is expected to be even more pronounced in cyanobacteria (or red algae) that are more sensitive to light than *Synechocystis* PCC 6803, which is highly tolerant to strong irradiance [16]. Consistent with this, preliminary data we obtained on *Gloeobacter violaceus* indicate that similar effects can be exerted by exposure of the cells to substantially lower light intensities (*i.e.*, 500 μmol photons $m^{-2}\cdot s^{-1}$) for similar time periods. It also appears that the light-dependent effects described in this work can also be induced or accentuated by other environmental stresses. As mentioned above, short heat treatments of *Synechocystis* PCC 6803 cells led to significant decoupling of PBSs from the reaction centers [16]. Similar observations were made on heat-stressed *Anacystis nidulans* [47] and *Spirulina platensis* cells [48]. PBS fluorescence likewise increased significantly when *A. nidulans* cells were exposed to temperatures below 10 °C [49]. Notably, the fluorescence emitted from the PBSs was enhanced by exposure of the cells to low light. Decoupling of PBS antennae was also reported for *Trichodesmium* cells exposed to high oxygen levels [50]. In this non-heterocystous diazotroph, decoupling/coupling of PBSs from/to PSII likely plays a key role in the ability of this organism to carry out nitrogen fixation and oxygenic photosynthesis concurrently ([17]; see also [50,51]). It thus seems that antenna decoupling is a general mechanism induced under stress or other conditions that require down-regulation of PSII activity. As far as protection against photo-oxidative damage is concerned, decoupling of PBSs may serve as an emergency valve when the protective capacity of all other light-adaptation responses has been exhausted. This could be particularly important under sustained stress conditions, when maintaining the photosynthetic machinery in a state optimized for efficient light harvesting and energy transfer is both unnecessary and hazardous. Disassembly of the PBS antennae

under such conditions may result in their degradation, possibly similarly to their degradation during nitrogen/phosphate starvation [7]. This, in turn, should lead to a sustained decrease in light absorption, reducing chlorophyll over-excitation and photodamage.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bbabio.2011.11.008.

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