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

Eyal Tamary [a,b], Vladimir Kiss [a], Reinat Nevo [a], Zach Adam [b], Gábor Bernát [c], Sascha Rexroth [c], Matthias Rögner [c], Ziv Reich [a,*]

[a] Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel
[b] The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Hebrew University of Jerusalem, Rehovot 76100, Israel
[c] Plant Biochemistry, Faculty of Biology & Biotechnology, Ruhr University Bochum, D-44780 Bochum, Germany

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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.

* Corresponding author. Tel.: +972 8 934 2982; fax: +972 8 934 6010.
E-mail address: ziv.reich@weizmann.ac.il (Z. Reich).

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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