Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1767 (2007) 750–756
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbabio

Heat- and light-induced reorganizations in the phycobilisome antenna of *Synechocystis* sp. PCC 6803. Thermo-optic effect

Katerina Stoitchkova [a,c,1], Ottó Zsiros [a,1], Tamás Jávorfi [a], Tibor Páli [b], Atanaska Andreeva [c], Zoltán Gombos [a], Győző Garab [a,*]

[a] *Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary*
[b] *Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary*
[c] *Sofia University, Faculty of Physics, Department of Condensed Matter Physics, 5, J. Bourchier Blvd., 1164 Sofia, Bulgaria*

Received 31 October 2006; received in revised form 15 February 2007; accepted 2 March 2007
Available online 14 March 2007

Abstract

By using absorption and fluorescence spectroscopy, we compared the effects of heat and light treatments on the phycobilisome (PBS) antenna of *Synechocystis* sp. PCC 6803 cells. Fluorescence emission spectra obtained upon exciting predominantly PBS, recorded at 25 °C and 77 K, revealed characteristic changes upon heat treatment of the cells. A 5-min incubation at 50 °C, which completely inactivated the activity of photosystem II, led to a small but statistically significant decrease in the F_{680}/F_{655} fluorescence intensity ratio. In contrast, heat treatment at 60 °C resulted in a much larger decrease in the same ratio and was accompanied by a blue-shift of the main PBS emission band at around 655 nm (F_{655}), indicating an energetic decoupling of PBS from chlorophylls and reorganizations in its internal structure. (Upon exciting PBS, F_{680} originates from photosystem II and from the terminal emitter of PBS.). Very similar changes were obtained upon exposing the cells to high light (600–7500 μmol photons m^{-2} s^{-1}) for different time periods (10 min to 3 h). In cells with heat-inactivated photosystem II, the variations caused by light treatment could clearly be assigned to a similar energetic decoupling of the PBS from the membrane and internal reorganizations as induced at around 60 °C. These data can be explained within the frameworks of thermo-optic mechanism [Cseh et al. 2000, Biochemistry 39, 15250]: in high light the heat packages originating from dissipation might lead to elementary structural changes in the close vicinity of dissipation in heat-sensitive structural elements, e.g. around the site where PBS is anchored to the membrane. This, in turn, brings about a diminishment in the energy supply from PBS to the photosystems and reorganization in the molecular architecture of PBS.

Keywords: Energetic decoupling of phycobilisome from photosystems; Fluorescence spectroscopy; Structural changes in phycobilisomes; *Synechocystis* sp. PCC 6803; Thermo-optic effect

1. Introduction

In cyanobacteria, a large part of the light harvesting capacity is provided by the peripheral antenna system, the phycobilisomes (PBSs). These multimeric protein complexes are attached to the outer side of the thylakoid membrane. They are arranged regularly on the membrane surface and supply energy mainly to photosystem II (PSII) in a manner that every PBS acts as an antenna for several reaction centers. PBSs are comprised of both chromophore-containing and colorless polypeptides. The main building blocks of PBSs are disc shaped aggregates of phycobiliproteins. In *Synechocystis* sp. PCC 6803, PBSs are constructed from two main structural elements: a core substructure and peripheral rods that are arranged in a hemidiscoidal fashion around the core. The rods and cores are composed of phycocyanin (PC) and allophycocyanin (APC), respectively. PC and APC carry the same blue chromophore called phycocyanobilin, the properties of each phycocyanobilin being strongly influenced by its protein environment. Colorless linker polypeptides join the rods to the core and the discs to each other, as well as anchor the entire structure to the thylakoid membrane [1–4].

Abbreviations: APC, allophycocyanin; chl, chlorophyll; LHCII, light harvesting complex II; PBS, phycobilisome; PC, phycocyanin; PFD, photon flux density; PS, photosystem

* Corresponding author. Tel.: +36 62 433131; fax: +36 62 433434.
E-mail address: Gyozo@brc.hu (G. Garab).
[1] The first and the second author contributed equally.

0005-2728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2007.03.002

PBSs absorb light in the wavelength range of 500–650 nm. The excitation energy from PBS is transferred to the thylakoid membrane via a cascade of directed down-hill energy transfer. A single PBS may contain up to 2000 bilin chromophores; these are arranged in such a structure that the high energy, i.e., short wavelength absorbing forms are located at the peripheral positions and the low energy, i.e., long wavelength absorbing pigments are closer to the center of the hemisphere, i.e. close to the membrane surface [5].

Cyanobacteria, like all oxygenic photosynthetic organisms, have the ability to maintain an efficient light-energy conversion under a variety of environmental conditions. Similarly to higher plants [6], cyanobacteria are also capable of adjusting their light harvesting antenna functions in response to changes in the environment, e.g. in the spectral composition and intensity of illumination and in the ambient temperature. In most of these regulatory functions PBS plays major roles [7]. Several independent models have been proposed for the participation of PBS in this type of regulatory processes. The energy supply to PSII can be regulated via PBS-associated non-photochemical quenching. Recent data show that this process is governed by the so-called orange carotenoid protein [8,9]. According to the 'mobile PBS model', redistribution of the excitation energy can be achieved by the movement of PBS between PSII and PSI [7,10]. Energetic uncoupling between the peripheral antenna and PSII, facilitated by light at low temperatures [11] or by heat [12] has been proposed to originate from the detachment of PBS from the membrane. These latter two models, i.e., the mobile PBS and the detachment model, assume substantial flexibility in the association of PBS with the membrane. Evidently, this type of movement can only be accomplished if the anchoring of PBS to the membrane is not tight, and allows substantial degree of motional freedom to PBS. As FRAP (fluorescence recovery after photobleaching) experiments demonstrated, PBSs can rapidly diffuse on the membrane surface [13,14]. Hence, this condition appears to be satisfied at least after photobleaching. However, the physical mechanism(s) underlying the 'release' of PBS from PSII reaction centers, and the loosening of the tight association with the membrane, has (have) remain to be elucidated.

In the present article we compare the effects of high temperature and excess light on the energy supply from PBS and on the organization of PBS in *Synechocystis* sp. PCC 6803 in order to investigate whether or not the two effects can be correlated. It is hypothesized that high light induces antenna reorganizations via thermo-optic effect. According to the thermo-optic mechanism, fast local thermal transients, arising from the dissipation of excess (photosynthetically not used) excitation energy, induce elementary structural changes due to "built-in" thermal instabilities of the given structural units [15,16]. Thermo-optically induced reorganizations have earlier been identified in LHCII-containing macroassemblies, in thylakoid membranes as well as in isolated LHCII (lamellar aggregates and trimers). (LHCII, the main chlorophyll a/b light harvesting antenna complex of PSII.) In granal thylakoid membranes, thermo-optically inducible changes involve the (i) unstacking of the membranes, followed by (ii) a lateral disorganization of the macrodomains, and (iii) with strong light and prolonged illumination by (iii) monomerization of the LHCII trimers [17]. Light-induced effects of similar origin have been reported by other authors. It has been shown that phosphorylation of LHCII, both in thylakoid membranes and in isolated complexes, can be regulated by light at the substrate level [18,19]. Light-induced isomerization and fluorescence quenching in LHCII can also be explained in terms of local heat due to dissipation of the excitation energy [20,21].

Data presented in this paper are fully consistent with the assumption that the antenna functions of PBS can be regulated thermo-optically. In particular, we show that the energetic coupling between PBS and the membrane pigments can be altered both by heat and light treatments, and the effects are very similar to each other. Further we show that the changes are accompanied by reorganizations in the molecular architecture of PBS.

2. Materials and methods

2.1. Strain and growth conditions

Synechocystis sp. PCC 6803 cells were grown photoautotrophically in BG-11 supplemented with 20 mM HEPES–NaOH (pH 7.5). The liquid cultures were maintained in a temperature- and light-regulated box at 25 °C in white light of 70 μmol photons m^{-2} s^{-1} photon flux density (PFD). Cultures from the logarithmic phase were used for heat and light treatments and the spectroscopic measurements.

2.2. Heat and light treatments of the cells

Before the treatments the samples were diluted with BG-11 buffer to $OD_{730}=0.03$ or concentrated by centrifugation to $OD_{730}=1$ according to the demands of the different measurements. Heat treatments were performed with a block heater in a test tube for 5 min at temperatures between 28 °C and 75 °C. Light treatments of the cells were performed in a thermostated beaker (inner diameter, 25 mm; 4 ml) with heat filtered white light, and monochromatic lights of 436 nm and 546 nm, using tungsten lamp and a high pressure mercury arc lamp, respectively. The UV component of the light sources was filtered out by the glass optics. The PFDs used were between 600 and 7500 μmol photons m^{-2} s^{-1}. The illumination period was varied between 10 min and 3 h. Moderate and high light intensities were used to mimick natural conditions. The very strong light intensities (above 2000 μmol photons m^{-2} s^{-1} PFD), which were applied only for short periods of time, were used in order to minimize possible side effects (for the rationale of using extremely strong light pulses, see [22]). The PFDs of the photosynthetically active radiation were measured with a Li-Cor LI 250 light meter.

2.3. Spectroscopic measurements

Absorption measurements were carried out on UV-Visible spectrophotometer Nicolet Evolution 500 (Thermo Electron Corporation) in a cuvette with an optical pathlength of 10 mm. All absorption spectra were recorded at room temperature.

The fluorescence spectra at 25 °C, on samples at $OD_{730}=0.03$, were measured in a Jobin Yvon-Spex Fluorolog 13–22 fluorescence spectrofluorimeter using a 10 mm × 10 mm optical cell. The excitation was performed at 564 and 436 nm with slit widths of 5 and 7 nm, respectively. For 77 K fluorescence measurements, before the treatments, the cultures were concentrated by centrifugation to chlorophyll (chl) content of about 5 μg/ml. A homemade sample holder was used with a sample thickness of 1 mm. Prior to the measurements, the samples were quickly frozen by immersion in liquid N_2. The excitation was performed at 564 and 436 nm with slit widths of 7 and 14 nm, respectively. Both the 25 °C and 77 K emission spectra were recorded between 600 and 750 nm with a slit width of 4 nm. The spectra were not corrected for the spectral sensitivity of the equipment.

3. Results and discussion

The room temperature absorption spectra of *Synechocystis* sp. PCC 6803 cells exhibited four distinct regions with maxima at around: 437, 490, 623 and 678 nm, originating predominantly from chl *a* (B_X), carotenoids, PBS and chl *a* (Q_Y), respectively. It is known that heat treatment of the cells causes decrease in the PBS absorption band [23–25]. Indeed, a sharp decrease of this band was observed at around 64 °C, while virtually no change could be observed below 61 °C (Fig. 1A). A small, 2 nm, red shift of the PBS absorption was also observed following the 5 min heat treatment at 75 °C. This was similar to that observed in *Spirulina platensis* incubated for 30 min at 60 °C [25]. In contrast to PBS, the absorption of carotenoids and chl *a* did not exhibit any considerable change even upon a 5 min 75 °C treatment (Fig. 1A). High thermal stability of chls and carotenoids, contrasting the heat-instability of PBS, has earlier been observed in *S. platensis* [24,25]. These data clearly show that PBS is considerably more vulnerable to heat than thylakoid membranes [12].

In accordance with the notion that cyanobacteria are tolerant to high light [26], we also found that prolonged illumination with intense light had virtually no effect on the absorption of the cells, neither on the PBSs nor on the pigments embedded in the thylakoid membrane (Fig. 1B). This held true even for very strong illumination, e.g. with white light of 7500 μmol photons

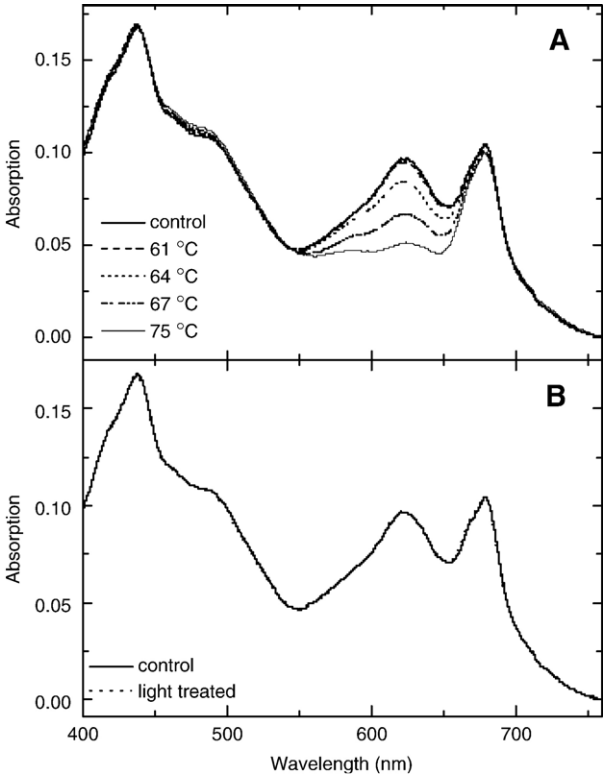

Fig. 1. Effect of heat and light treatments (A and B, respectively) on the absorption spectra of *Synechocystis* sp. PCC 6803 cells. The heat treatments were performed for 5 min at the indicated temperatures. The light treatment (1500 μmol photons m^{-2} s^{-1} PFD white light, 1 h) was carried out at the growth temperature of the cells, at 25 °C.

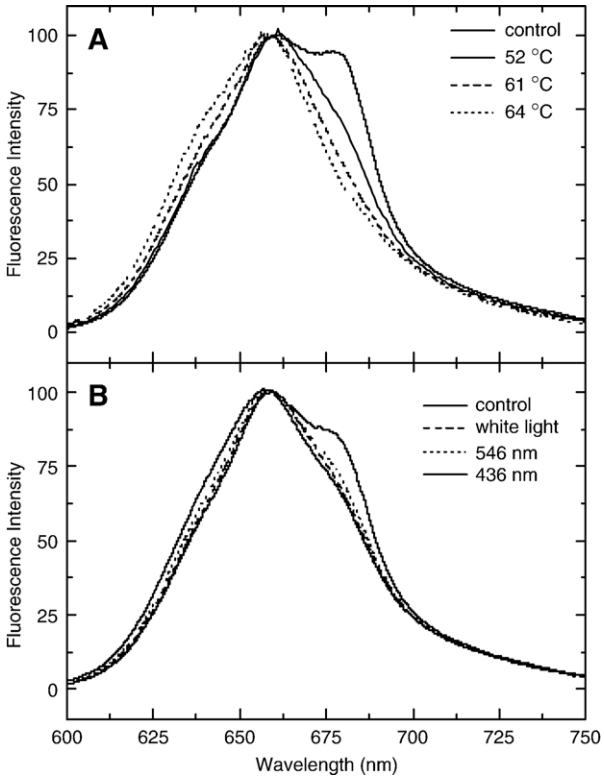

Fig. 2. Effect of heat and light treatments (A and B, respectively) on the fluorescence spectra of *Synechocystis* sp. PCC 6803 cells. The heat treatments were performed for 5 min at the indicated temperatures. The light treatments (3 h, 600 μmol photons m^{-2} s^{-1} PFD) were performed at the growth temperature of the cells at 25 °C, with heat filtered white light and monochromatic light at the indicated wavelengths. The spectra were recorded at 25 °C, the excitation wavelength was 564 nm. The spectra are normalized to the emission maxima.

m^{-2} s^{-1} PFD, 1 h (data not shown). These data also show that the antenna system of cyanobacteria is largely immune to photodestruction. Experiments with circular dichroism spectroscopy were in good agreement with absorbance data: we found virtually no effect up to 60 °C; also, 30 min preillumination of whole cells with white light of 7500 μmol photons m^{-2} s^{-1} PFD induced no discernible change in either the PBS region or in the chl *a* region (data not shown).

As shown in Fig. 2A exposure of the cells to elevated temperatures leads to a gradual loss of the long wavelength room temperature fluorescence emission shoulder at around 678 nm (F_{680}), which originates from PSII and the terminal emitter of APC [12,27]. This decrease of F_{680} relative to the main emission band (F_{655}) occurs gradually, but most sharply between 50 °C and 60 °C. Above 60 °C we can also observe gradual increase in the intensity of the short wavelength shoulder at around 640 nm. Similar observations were made by Mohanty and coworkers on *Anacystis nidulans* and *Porphyridium cruentum* cells and were accounted for by disruption of energy transfer from PBS to PSII and reorganizations in the PBS [12,23]. The occurrence of structural changes in the PBS is also indicated by a blue-shift in the APC emission peak at around 658 nm (F_{655}). This will be analyzed for the 77 K spectra.

It can be seen in Fig. 2B that prolonged (3 h) illumination of the cells with moderate PFD (600 μmol photons m^{-2} s^{-1}) light leads to very similar changes as seen above for elevated temperatures. In broad terms, this is in reasonable agreement with the notion that excess light, via heat packages produced upon dissipation, leads to elementary structural changes in the close vicinity of the site of dissipation [15,16]. This type of light-induced changes in the antennae were first reported in LHCII-containing systems and were accounted for by a thermo-optic mechanism (see Introduction). Hence, it is reasonable to assume that similar heat packages can be held responsible for the elementary structural changes beyond the observed variations in the fluorescence emission of *Synechocystis* PCC 6803 cells. It appears that these structural changes, induced by excess light, affect the energy transfer between PBS and the membrane in the same manner as the structural changes induced by elevated temperatures. It must however be noted that the decrease of F_{680} relative to F_{655} in heat treated cells was manifested in somewhat different manner than in the light treated samples. With heat treatment, we observed an approximately 20–30% increase in F_{655} between 25 °C and 52 °C, and an additional 20–30% increase upon rising the temperature from 52 °C to 61 °C, followed by a 20–40% decrease between 61 and 64 °C (these values were estimated from 3 to 5 independent series of temperature treatments on different batches). In contrast, with light treatments, performed at 25 °C, we observed the tendency of an approximately 10–30% decrease of the overall fluorescence, depending on the light intensity and duration, and also on the culture. The origin of this decrease is not clear. It might be caused by secondary effects of light on the partially disassembled PBS–membrane system. The similarity of the effects obtained with white, blue and green lights suggests that the orange carotenoid protein is unlikely to play a major role in this decrease of the fluorescence intensities [8,9]. Further, we also note that albeit the extent of the spectral changes varied somewhat between treatments with different light qualities, they all induced a marked decrease in the F_{680}/F_{655} and a blue-shift in the main emission band. The fact that the 436 nm and the 546 nm preilluminations induced similar effects suggests that the heat packages, which are thought to provoke the elementary structural changes, are produced in the membrane. The nature of reorganizations in the PBS antenna and variations in the energetic coupling between PBS and chl *a* in heat treated and light treated cells can most probably be elucidated by using time resolved fluorescence emission spectroscopy [28–30].

As shown in Fig. 3A, the temperature dependence of the changes in the emission intensity ratio F_{680}/F_{655} exhibits two well-discernible phases. The first phase, between 25 and about 50 °C probably can be accounted for by the thermal inactivation of PSII in the cells. In unreported experiments, by using thermoluminescence, we verified that a 5-min incubation of the cells at around 50 °C completely inactivated PSII activity. This is in good accordance with literature data [31,32]. Thus, the changes of F_{680}/F_{655} in the second phase cannot be explained with variations in PSII activities. Also, the decline in the F_{680}/F_{655} between 52 °C and 61 °C cannot be accounted for by PBS denaturation, because in this temperature range no change was observed in the PBS absorption (see above). The heat stability of chl *a* and carotenoids is even higher in this range. In this context, it is also interesting to note that under the same conditions the fluorescence intensity at 680 nm excited with 436 nm decreased by about 30% between 25 and 52 °C; in contrast, the fluorescence intensity hardly changed between 52 and 61 °C, and the intensity of the chlorophyll fluorescence remained virtually the same even at 75 °C (data not shown). These observations strongly suggest that the sharp decline in the F_{680}/F_{655} ratio above 52 °C originates from an energetic decoupling of PBS from PSII. Again, it is interesting to observe that the excess light treatment closely mimics the moderate heat treatment of the cells which leads to energetic decoupling of PBS from the membrane (Fig. 3A).

In order to discriminate more clearly between the inhibitory effect of strong light on PSII and on the energetic decoupling of PBS from the membrane, we carried out the light treatment also

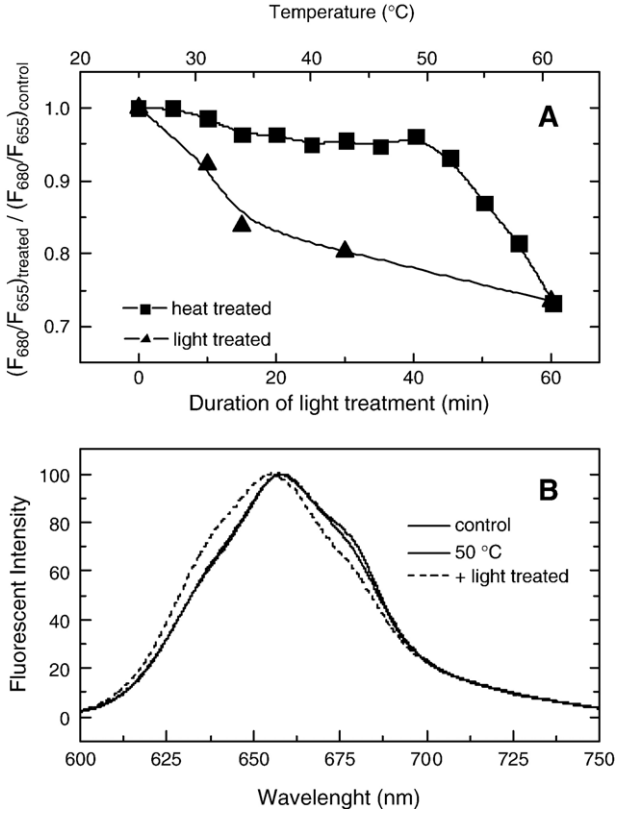

Fig. 3. Typical dependence of the ratio of the fluorescence emission intensities at 680 and 655 nm on the temperature of the heat treatment and on the duration of the light treatment (white light of 7500 μmol photons m^{-2} s^{-1} PFD) (A), and a comparison of the effects of PSII inactivation, at 50 °C, and the additional effect of 10 min light treatment (white light of 1500 μmol photons m^{-2} s^{-1} PFD) on the same cells; the spectra are normalized to the emission maxima (B). The heat treatments were performed for 5 min at the given temperature. The light treatments were carried out at the growth temperature of the cells, at 25 °C. Since, in panel A, the ratio of F_{685}/F_{660} also varied from batch to batch the variations were normalized to the corresponding F_{685}/F_{660} ratios of the controls; the mean values for the 61 °C treated, and the light treated (1 h) cells were 0.78 ± 0.047 and 0.73 ± 0.048, respectively.

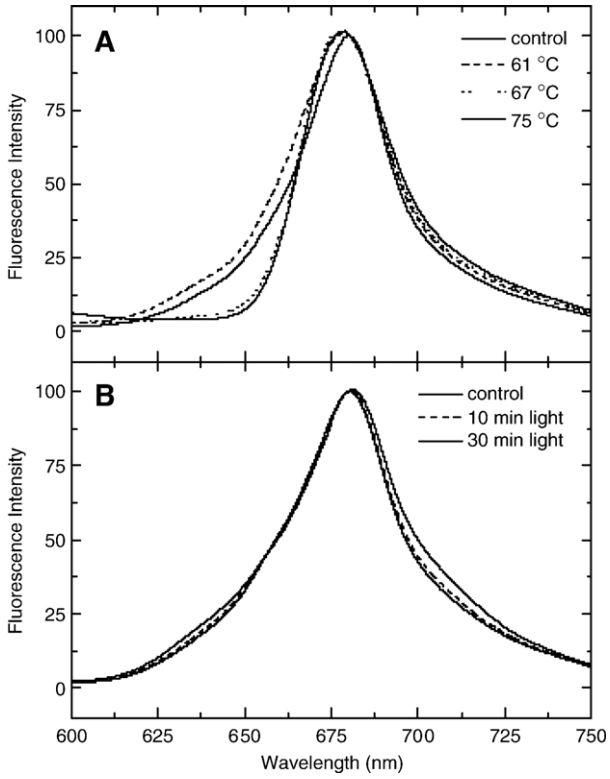

Fig. 4. Effects of heat and light treatments (A and B, respectively) on the fluorescence spectra of *Synechocystis* sp. PCC 6803 cells. The heat treatment was performed for 5 min at the indicated temperatures. The light treatment was carried out at the growth temperature of the cells, at 25 °C, with 7500 μmol photons m^{-2} s^{-1} PFD of white light. The spectra were recorded at 25 °C, the excitation wavelength was 436 nm. The spectra are normalized to the emission maxima.

on samples with heat-inactivated PSII. It can be seen in Fig. 3B that heat treatment at 50 °C induced only a minor change in the spectral distribution of the fluorescence emission; in particular, we observed only a minor decrease at around 680 nm, and virtually no change in the main emission band. When the same cells, i.e., with heat-inactivated PSII, were exposed to excess light at 25 °C the spectra exhibited the same characteristic features which were obtained only after heat treatment at 61 °C. We observed the pronounced decrease in the F_{680}/F_{655} ratio and a few nm blue shift of the main PBS emission band. We also observed that heat pretreatment appeared to increase the susceptibility of cells to light treatments. The characteristic spectral changes could readily be induced after 10 min preillumination with 1000 μmol photons m^{-2} s^{-1} PFD white light. This might be due to the fact that while heat inactivation of PSII was complete in 5 min at 50 °C, this could hardly be achieved even with very high intensity light (data not shown). Cyanobacteria are known to endure high light, much better than higher plants [33]. Even after illumination of the cells for 1 hr with white light of 2500 μmol photons m^{-2} s^{-1} 30% of the PSII activity was retained [33]. We cannot exclude, however, that heat pretreatment of the cells loosened the anchoring of PBSs to the membrane, and thus making them more vulnerable for energetic decoupling.

When comparing the effect of heat and light treatments on the fluorescence emission excited with 436 nm it can be seen that, again, preillumination-induced changes which resembled those induced at 61 °C (Fig. 4). In both cases, the spectra exhibited broadening both on the short and long wavelength sides. At higher temperatures, it can also be observed that the PBS emission is eliminated but this does not occur in the light-treated samples, an observation that is in perfect agreement with absorption spectroscopic data (see Fig. 1).

Heat treatment also induced considerable changes in the spectral distribution of the 77 K fluorescence emission (Fig. 5A). At around 50 °C, F_{685} and F_{695} were most prominently affected; these bands decreased substantially relative to F_{720}. Similar to the room temperature data (Figs. 2 and 3), this can be accounted for by the heat-inactivation of PSII. At higher temperatures, at around 60 °C, the PBS emission appears to increase, as evident in the PC and APC emission bands at around 646 and 662 nm, respectively. The increase of the band at around 685 nm at 61 °C relative to the 51 °C sample probably originates from an increase of the emission from the terminal emitter. Since PSII is inactivated already at 51 °C the additional decrease in the PSII emission between 51 °C and 61 °C must originate from a partial energetic decoupling of PBS from PSII (see also the room temperature fluorescence

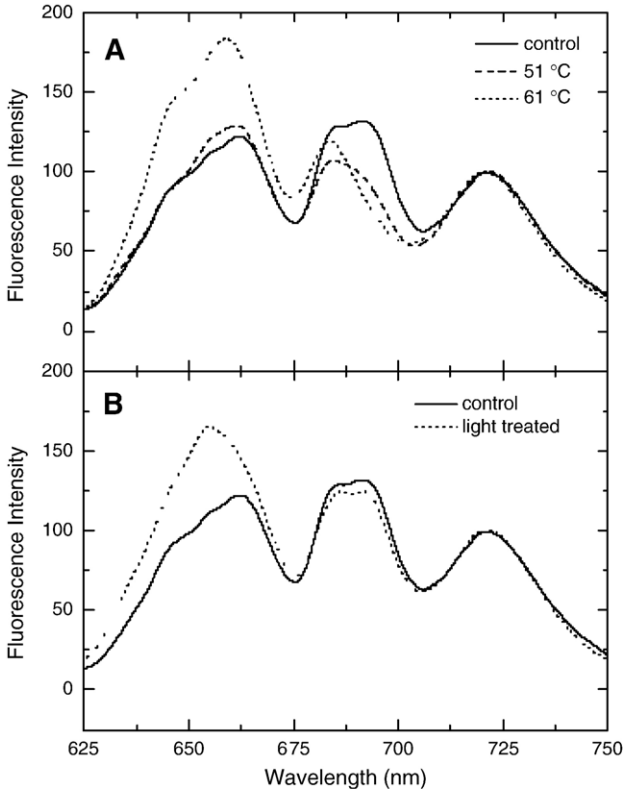

Fig. 5. Effects of heat and light treatments (A and B, respectively) on the 77 K fluorescence spectra of *Synechocystis* sp. PCC 6803 cells. The heat treatments were performed for 5 min at the indicated temperatures. The light-treatment (1 h) was performed at the growth temperature of the cells, at 25 °C, with 1500 μmol photons m^{-2} s^{-1} PFD of white light. The excitation wavelength was 564 nm. The spectra are normalized to the long wavelength maxima.

data). Similar changes occurred upon high light treatment (1500 μmol photons m^{-2} s^{-1}, 1 h) of the cells both without (Fig. 5B) and with heat pretreatment at 50 °C. Similar to the 61 °C treated cells, the relative intensity of the PBS emission increased and its main, composite emission band shifted to the blue.

In Fig. 6, variations in the fluorescence emission ratios are plotted against the temperature of the heat treatment and the duration of the light treatment. It is again evident that heat and light treatments led to very similar alterations in the fluorescence parameters. As argued above for the room temperature data, the decrease in F_{685}/F_{660} between 25 °C and 51 °C can mainly be accounted for by the inactivation of PSII. In contrast, the sharp decrease in the same ratio at around 60 °C suggests that it originates from a partial energetic decoupling of PBS from PSII (Fig. 6A). The fact that at the same temperature the energy supply of PSI from PBS also decreases provides support to the suggestion of decoupling (Fig. 6B).

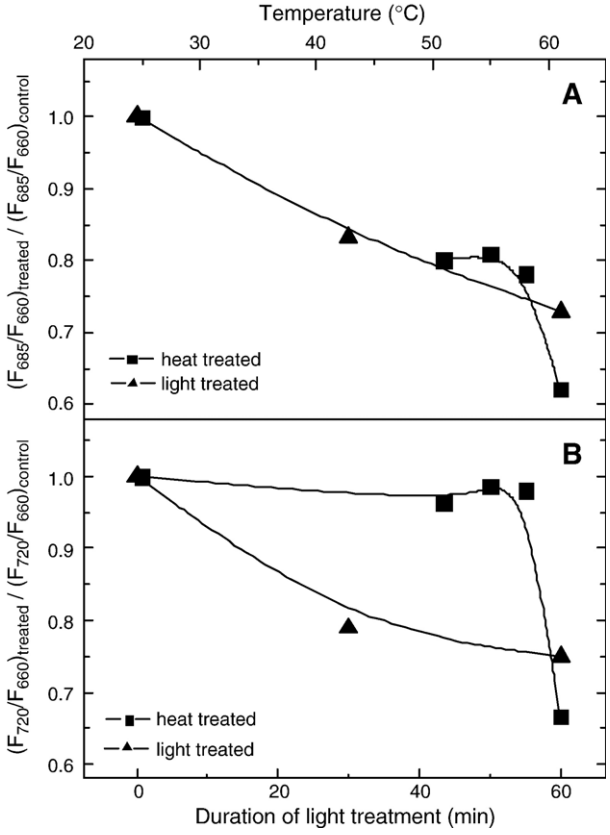

Fig. 6. Dependence of the ratio of the 77 K fluorescence emission at around 685 and 660 nm (A), and 720 and 660 nm (B) on the temperature of treatment (■) and on the duration of the light treatment (▲). As in Fig. 3, variations in the ratio were compared to the corresponding values of the control. The heat treatments were performed for 5 min at the indicated temperatures. The light treatments were carried out with white light of 1500 μmol photons m^{-2} s^{-1} PFD at the growth temperature of the cells, at 25 °C. Data points were obtained from one series. The mean values±SD at the 61 °C heat treatment and the 1-h light treatment were 0.67±0.06 (A) and 0.76±0.09 (B), and 0.77±0.03 (A) and 0.080±0.04 (B), respectively. (For the heat treatment, the curve is not drawn between 25 and 51 °C.)

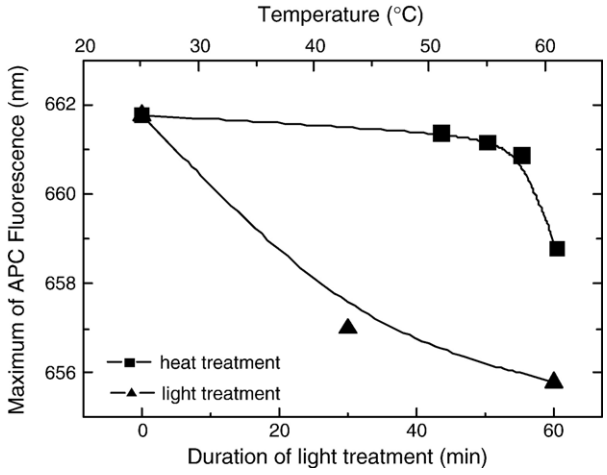

Fig. 7. Changes in the maximum of the predominant APC fluorescence emission at 77 K after heat and light treatments. The excitation wavelength was 564 nm. The light treatments were performed at the growth temperature of the cells, at 25 °C, with 1500 μmol photons m^{-2} s^{-1} PFD white light. Data points were obtained from one series. The mean values±SD for the control, 61 °C treated and light treated (1 h) were 661±1 nm 658±1 nm, and 656±0.5 nm, respectively.

Both light and heat treatments also induce changes in the fluorescence maxima of APC emission at around 660 nm (Fig. 7). It is important to note that the shift occurs in the same temperature range where decoupling of PBS from the thylakoid membrane appears to occur. It is also interesting to note that with the light treatment the magnitude of the shift depended on the duration of preillumination. The occurrence of the shift shows that the energetic decoupling of PBS from the photosystems is accompanied by or brings about a reorganization in the molecular architecture of PBS. As discussed above, this might be caused e.g. by a loosening the anchoring of PBS and/or its detachment from the thylakoid membrane. Again, the fact that PBS can diffuse along the membrane sheet [13,14] shows that these macrocomplexes are not anchored tightly to the membrane.

In summary, the data presented in this work show that heat and light induced changes closely resemble each other. The observed changes are consistent with the notion that the reorganizations induced by excess light can be explained in terms of a thermo-optic mechanism. Further experiments on the possible sites of dissipation and on the anchoring of PBS to the membrane might elucidate the nature and mechanism of the regulation of the antenna functions of phycobilisomes.

Acknowledgments

The authors are indebted to Profs. A. R. Holzwarth, N. Karapetyan and D. Kirilovsky for helpful discussions. This work was supported by Marie Curie grant MCRTN-CT-2003-505069 (INTRO2) to G.G., grants from the Hungarian Fund for Basic Research (T043425, K06109 and K63252 to T.P., Z.G. and G.G., respectively) and by grant from Bulgarian National Fund for Scientific Research, Program “Support for the research activities at the universities” (Project N(11/2005) to A.A.).

References

[1] H. Scheer, Phycobiliproteins: molecular aspects of photosynthetic antenna system, in: F.K. Fong (Ed.), Light Reaction Path of Photosynthesis, Mol. Biol. Biochem. Biophys., vol. 35, Springer-Verlag, Berlin, 1982, pp. 7–45.

[2] H. Zuber, The structure of light-harvesting pigment–protein complexes, in: J. Barber (Ed.), Topics in Photosynthesis, The Light Reactions, vol. 8, Elsevier, Amsterdam, 1987, pp. 197–260.

[3] E. Gantt, Supramolecular membrane organization, in: D.A. Bryant (Ed.), Advances in Photosynthesis, The Molecular Biology of Cyanobacteria, vol. 1, Kluwer Academic, Dordrecht, 1994, pp. 119–138.

[4] L.-N. Liu, X.L. Chen, Y.Z. Zhang, B.C. Zhou, Characterization, structure and function of linker polypeptides in phycobilisomes of cyanobacteria and red algae: an overview, Biochim. Biophys. Acta 1708 (2005) 133–142.

[5] A.R. Holzwarth, Structure–function relationships and energy transfer in phycobiliprotein antennae, Physiol. Plant. 83 (1991) 518–528.

[6] P. Horton, M. Wentworth, A. Ruban, Control of the light harvesting function of chloroplast membranes: the LHCII-aggregation model for non-photochemical quenching, FEBS Lett. 579 (2005) 4201–4206.

[7] J. Biggins, D. Bruce, Regulation of excitation energy transfer in organisms containing phycobilins, Photosynth. Res. 20 (1989) 1–34.

[8] M.G. Rakhimberdieva, I.N. Stadnichuk, I.V. Elanskaya, N.V. Karapetyan, Carotenoid-induced quenching of the phycobilisome fluorescence in photosystem II-deficient mutant of Synechocystis sp. FEBS Lett. 574 (2004) 85–88.

[9] A. Wilson, G. Ajlani, J.M. Verbavatz, I. Vass, C.A. Kerfeld, D. Kirilovsky, A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria, Plant Cell 18 (2006) 992–1007.

[10] J.F. Allen, Protein phosphorylation in regulation of photosynthesis, Biochim. Biophys. Acta 1098 (1992) 275–335.

[11] U. Schreiber, Reversible uncoupling of energy transfer between phycobilins and chlorophyll in *Anacystis nidulans*: light stimulation of cold-induced phycobilisome detachment, Biochim. Biophys. Acta 591 (1980) 361–371.

[12] P. Mohanty, S. Hoshina, D.C. Fork, Energy transfer from phycobilins to chlorophyll a in heat-stressed cells of *Anacystis nidulans*: characterization of the low temperature 683(nm fluorescence emission band, Photochem. Photobiol. 41 (1985) 589–596.

[13] C.W. Mullineaux, M.J. Tobin, G.R. Jones, Mobility of photosynthetic complexes in thylakoid membranes, Nature 390 (1997) 421–424.

[14] M. Sarcina, M.J. Tobin, C.W. Mullineaux, Diffusion of phycobilisomes on the thylakoid membranes of the cyanobacterium Synechococcus 7942. Effects of phycobilisome size, temperature, and membrane lipid composition, J. Biol. Chem. 276 (2001) 46830–46834.

[15] Z. Cseh, S. Rajagopal, T. Tsonev, M. Busheva, E. Papp, G. Garab, Thermooptic effect in chloroplast thylakoid membranes. Thermal and light stability of pigment arrays with different levels of structural complexity, Biochemistry 39 (2000) 15250–15257.

[16] G. Garab, Z. Cseh, L. Kovács, S. Rajagopal, Z. Várkonyi, M. Wentworth, L. Mustárdy, A. Dér, A. Ruban, E. Papp, A. Holzenburg, P. Horton, Light-induced trimer to monomer transition in the main light-harvesting antenna complex of plants: thermo-optic mechanism, Biochemistry 41 (2002) 15121–15129.

[17] A.G. Dobrikova, Z. Várkonyi, S.B. Krumova, L. Kovács, G.K. Kostov, S.J. Todinova, M.C. Busheva, S.G. Taneva, G. Garab, Structural rearrangements in chloroplast thylakoid membranes revealed by differential scanning calorimetry and circular dichroism spectroscopy. Thermo-optic effect, Biochemistry 42 (2003) 11272–11280.

[18] H. Zer, M. Vink, N. Keren, H.G. Dilly-Hartwig, H. Paulsen, R.G. Herrmann, B. Andersson, I. Ohad, Regulation of thylakoid protein phosphorylation at the substrate level: reversible light-induced conformational changes expose the phosphorylation site of the light-harvesting complex II, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 8277–8282.

[19] H. Zer, M. Vink, S. Shochat, R.G. Herrmann, B. Andersson, I. Ohad, Light affects the accessibility of the thylakoid light harvesting complex II, (LHCII) phosphorylation site to the membrane protein kinase(s), Biochemistry 42 (2003) 728–738.

[20] W. Grudzinski, M. Matula, J. Sielewiesiuk, P. Kernen, Z. Krupa, W.I. Gruszecki, Effect of 13-cis violaxanthin on organization of light harvesting complex II in monomolecular layers, Biochim. Biophys. Acta 1503 (2001) 2091–2302.

[21] W. Grudzinski, Z. Krupa, M. Garstka, W. Maksymiec, T.E. Swartz, W.I. Gruszecki, Conformational rearrangements in light-harvesting complex II accompanying light-induced chlorophyll a fluorescence quenching, Biochim. Biophys. Acta 1554 (2002) 108–117.

[22] O. Zsiros, S.I. Allakhverdiev, S. Higashi, M. Watanabe, Y. Nishiyama, N. Murata, Very strong UV-A light temporally separates the photoinhibition of photosystem II into light-induced inactivation and repair, Biochim. Biophys. Acta 1757 (2006) 123–129.

[23] G.S. Singhai, P. Mohanty, Govindjee, Effect of preheating intact algal cells on pigments revealed by absorption and fluorescence spectra, Z. Pflanzenphysiol. 103 (1981) 217–228.

[24] T.S. Babu, S.C. Sabat, P. Mohanty, Heat induced alternations in the photosynthetic electron transport and emission properties of the cyanobacterium *Spirulina platensis*, J. Photochem. Photobiol., B Biol. 12 (1992) 161–171.

[25] S.D.S. Murthy, V.V. Ramanaiah, P. Sudhir, High temperature induced alternations in energy transfer in phycobilisomes of the cyanobacterium *Spirulina platensis*, Photosynthetica 42 (2004) 615–617.

[26] L. Zolla, M. Bianchetti, S. Rinalducci, Functional studies of the Synechocystis phycobilisomes organization by high performance liquid chromatography on line with a mass spectrometer, Eur. J. Biochem. 269 (2002) 1534–1542.

[27] A.N. Glazer, D.A. Bryant, A new cyanobacterial phycobiliprotein, Arch. Microbiol. 104 (1975) 15–22.

[28] A.R. Holzwarth, Picosecond fluorescence spectroscopy and energy transfer in photosynthetic antenna pigments, in: J. Barber (Ed.), Topics in Photosynthesis, The Light Reactions, vol. 8, Elsevier, Amsterdam, 1987, pp. 95–157.

[29] A.U. Gulbinas, V. Karpicz, G. Garab, L. Valkunas, Nonequilibrium heating in LHCII complexes monitored by ultrafast absorbance transients, Biochemistry 45 (2006) 9559–9565.

[30] K. Kondo, Y. Ochiai, M. Katayama, M. Ikeuchi, Reconstitution of phycobilisome subcomplexes associated with CpcG1 or CpcG2, Plant Cell Physiol. 47 (2006) s86.

[31] Z. Gombos, H. Wada, N. Murata, Direct evaluation of effects of fatty-acid unsaturation on the thermal-properties of photosynthetic activities, as studied by mutation and transformation of Synechocystis PCC6803, Plant Cell Physiol. 32 (1991) 205–211.

[32] N. Inoue, Y. Taira, T. Emi, Y. Yamane, Y. Kashino, H. Koike, K. Satoh, Acclimation to the growth temperature and the high-temperature effects on photosystem II and plasma membranes in a mesophilic cyanobacterium, Synechocystis sp. PCC6803, Plant Cell Physiol. 42 (2001) 1140–1148.

[33] S.I. Allakhverdiev, N. Tsvetkova, P. Mohanty, B. Szalontai, B.Y. Moon, M. Debreczeny, N. Murata, Irreversible photoinhibition of photosystem II is caused by exposure of Synechocystis cells to strong light for a prolonged period, Biochim. Biophys. Acta 1708 (2005) 342–351.