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Probing connection of PBS with the photosystems in intact cells of *Spirulina platensis* by temperature-induced fluorescence fluctuation

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Abstract

Temperature-dependent fluorescence for intact cells of cyanobacterium *Spirulina platensis* was detected to search for the connection of the phycobilisome (PBS) with Photosystem I (PSI) and Photosystem II (PSII). Some interesting results were obtained from the deconvoluted fluorescence components of C-phycocyanin (C-PC), allophycocyanin (APC), PSI and PSII as well as the fluorescence spectra of the intact cells at room temperature (RT = 25 °C) and 0 °C. It was observed that, compared to those at RT, both of the fluorescence components for PSI and APC increased, whereas those for PSII and C-PC decreased at 0 °C with excitation at 580 nm, that is, the fluorescence for C-PC is not synchronous with that for APC, and the fluorescence fluctuation for PSI is not synchronous with that for PSII. On the other hand, the decrease in C-PC fluorescence is synchronous with the increase in PSI fluorescence, and the increase in APC fluorescence is synchronous with the decrease in PSII fluorescence. Therefore, it can be readily deduced that PBS should be coupled not only with PSII through the terminal acceptors in the APC core but also with PSI through C-PC in PBS rods at physiological condition, while at 0 °C, a migration of a PBS makes the APC partially detached from PSII but the C-PC more efficiently coupled with PSI. The results provide good evidences for "mobile PBS" model and "parallel connection" model but not for the "spillover" model.

Keywords: C-phycocyanin; Allophycocyanin; Photosystem; Thylakoid membrane

1. Introduction

It is well known that phycobilisomes (PBSs) play the most important role of light harvesting in photosynthesis for a cyanobacterium, which is able to adapt its photosynthetic apparatus to not only light intensity, but also spectral composition, temperature, and availability of CO_2 or other essential nutrients [1]. It is commonly believed that the PBSs are principally coupled with Photosystem II (PSII) [2–5], but there are also some evidences that the excitation energy in PBSs can be transferred into the Photosystem I (PSI) directly [6,7]. For instance, in PSII-less mutants of some cyanobacteria, it was observed that PBSs could still stay bound to the photosynthetic membrane and transfer the excitation energy to PSI efficiently [8,9]. In addition, the energy transfer from C-phycocyanin (C-PC) to PSI was also observed in an apcA-defective mutant of *Synechocystis* sp. PCC 6803 [10]. Till now, it is still not clear how a PBS is connected with the two photosystems, though several models were proposed, such as "spillover" model, based on an assumption of permanent association of PBSs with PSII [11], and "mobile phycobilisome" model, based on that of a transient and unstable association of PBSs with the two photosystems [12]. Interestingly, it was observed that PBSs were mobile on the surface of the membrane with the mobility depending on size of a PBS, lipid composition of the membrane, temperature and so on [13]. Recently, an even more complicated model was proposed that the 20% of PBSs were linked to PSII dimers, the another 20% to PSI monomers and the rest (60%) to PSI trimers [14]. Temperature-induced decoupling of PBSs from reaction centers was observed in both intact algal cells and the PBS–thylakoid

Abbreviations: PBS, phycobilisome; PSI, Photosystem I; PSII, Photosystem II; RT, room temperature (25 °C); C-PC, C-phycocyanin; APC, allophycocyanin; Chl, chlorophyll; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid

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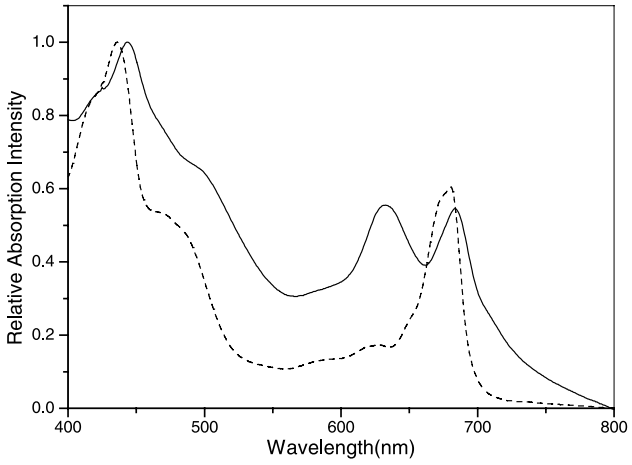

Fig. 1. Absorbance spectra, normalized at 436 nm, of the intact cells (solid line) and thylakoid membranes (dashed line) of *S. platensis* at room temperature.

membrane complex [15–17], which implies that a structural variation must occur when temperature changes from room temperature (RT) to 0 °C. In fact, some valuable information for structural connection and functional association of PBSs with the two photosystems can be derived from the temperature-induced changes on fluorescence yields of PBS, PSI and PSII. In the previous paper, it was proposed that PBS should be connected with the two photosystems parallelly and a position shift might occur during the temperature changes in the PBS–thylakoid membrane complex; however, it may be argued that the isolated complexes are not necessarily the same as that in the intact cells. In the current work, the temperature-dependent fluorescence fluctuations of PBS, PSI and PSII in the intact algal cells and the PBS-free thylakoid membranes were studied to search for how a PBS is connected with the two photosystems.

2. Materials and Methods

2.1. Culture and growth conditions

Spirulina platensis, a cyanobacterium, was cultured in a 5-l bottle at 28 °C, bubbled with air and irradiated with 40 W fluorescent lamps continuously. Ten-day-old cultures were used for the experiments.

2.2. Preparation of thylakoid membranes

The thylakoid membrane of *S. platensis* cells was prepared according to the reported method [18] with minor modifications. Suspended in 25 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES)–NaOH buffer (pH = 7.5) containing 20 mM NaCl, the intact cells were ultrasonically broken in ice bath for 30 min and then centrifuged at 9000 × *g* in a Ti-70 Beckman rotor for 5 min, the supernatant of which was further centrifuged at 50,000 × *g* in a Ti-70 Beckman rotor for 45 min. The pellet was resuspended in the HEPES–NaOH buffer (pH = 7.5) at a chlorophyll *a* (Chl *a*) concentration of about 1.2–1.5 mg/ml and was used immediately.

2.3. The spectra and deconvolution

Absorption spectra were measured with a UV-2001 ultra–vis spectrophotometer (Hitachi, Japan). Fluorescence emission spectra were obtained on a F4500 spectrofluorimeter (Hitachi). Samples were dark adapted either at 0 °C or RT for 30 min before fluorescence measurement. By using the nonlinear optimization approach in a computer, fluorescence spectra were deconvoluted into the components that were imitated with Gaussians, except for the longest wavelength one (Gaussian/Lorentzian mixture). The two restriction parameters for the deconvolution are the less than 3% relative error and the component number as less as possible. It should be noticed that the relative error ε is defined as

$$\varepsilon = 100\left(A_e - \sum_{i=1}^{N} A_i\right) \Big/ A_e$$

with A_e standing for the area under the experimental spectrum curve, A_i for that of the component i and N for the component number.

3. Results and discussion

3.1. Absorption and fluorescence emission spectra of the intact cells and PBS-free thylakoid membranes

Absorbance spectra for the intact cells and the PBS-free thylakoid membranes are shown in Fig. 1. For the intact

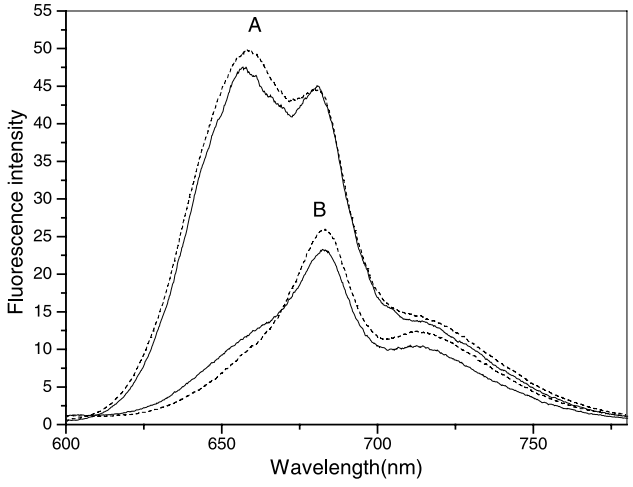

Fig. 2. Fluorescence emission spectra of the intact cell of *S. platensis*. (A) Excited at 580 nm at RT (solid line) and 0 °C (dashed line); (B) excited at 436 nm at RT (solid line) and 0 °C (dashed line).

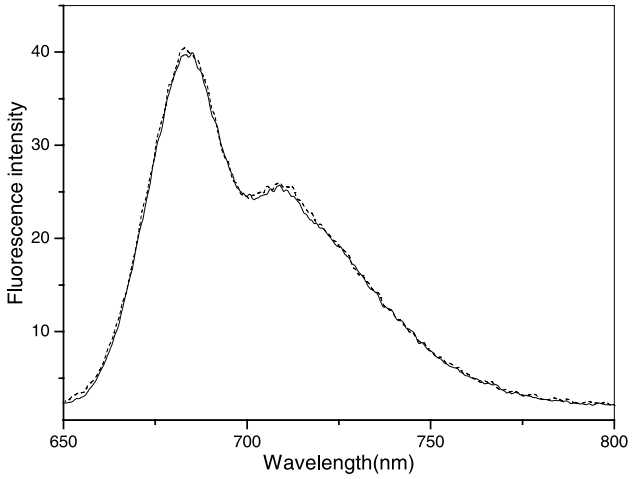

Fig. 3. Fluorescence emission spectra of PBS-free thylakoid membranes from *S. platensis* at 0 °C (dashed line) and RT (solid line) with excitation at 436 nm.

cells, the absorbance peaks for Chl *a* appear at 418, 436, and 678 nm, whereas for carotenoids and PBSs, they appear at 490 and 630 nm, respectively. Compared to those, the peak for PBSs is absent for the PBS-free membranes, whereas the observable absorbance at 585 and 624 nm could be assigned to the Chl *a* Q_x (0,0) transition and the Q_y (0,1) transition [19].

The fluorescence spectra of the intact cells at RT and 0 °C are shown in Fig. 2. The fluorescence emission peaks at 665, 683 and 715 nm can be reasonably assigned to PBS, PSII and PSI, respectively. Compared to those at RT, the fluorescence intensities increased at 665 and 715 nm but decreased at 683 nm at 0 °C with excitation at 580 nm, while it decreased at 665 nm but increased at 683 and 715 nm with excitation at 436 nm. Qualitatively, the temperature-induced fluorescence fluctuations are quite similar to those for PBS–thylakoid membrane complexes [17]. Excited at 436 nm, the fluorescence of PBS should originate from the so-called back transfer of the excitation energy in the photosystems [17,20]. The fluorescence spectra of PBS-free thylakoid membranes at RT and 0 °C are shown in Fig. 3. The recognizable fluorescence emission peaks at 683 and 715 nm should originate from PSII and PSI, respectively. It can be seen that the fluorescence spectra at RT and 0 °C are nearly the same, which means that the photosystem themselves will not be remarkably affected by the temperature change. According to these results, it can be logically suggested that it is the association of a PBS with the photosystems, instead of individual structure of PBS, PSI or PSII, that is affected by the temperature change.

The fluorescence spectrum of intact cells is almost completely reproduced after the temperature raise from 0 °C to RT (figure not shown). This is consistent with the results in previous papers [15,17,20].

3.2. Deconvolution of fluorescence spectra of the intact cell

The fluorescence intensities for each spectrum were normalized before deconvolution. It can be seen that the peak wavelength for each component remains constant while the integral area is variable. The ratio of sub-band area to the total area of the experimentally measured spectrum can be considered to be the fluorescence yield of a component. For the intact cells, the deconvoluted parameters are listed in Table 1 and the spectral profiles for the four components, C-PC, allophycocyanin (APC), PSII and PSI, are shown in Fig. 4.

In Table 2, the percentage of increase or decrease in fluorescence yield is defined as $100(F_0 - F_R)/F_R$, in which F_0 and F_R stand for the fluorescence yields at 0 °C and at RT, respectively.

From Table 2, it can be clearly seen that the decrease in C-PC fluorescence is synchronous with the increase in PSI fluorescence, whereas the decrease in the APC fluorescence is synchronous with the increase in the PSII fluorescence. According to the common point of view, the fluorescence yields for C-PC and APC should have changed synchronously, so should those for PSI and PSII. However, the results in Table 2 did not support for the commonly believed model.

With regard to the increase in APC fluorescence and the decrease in the PSII fluorescence, it can be explained by energetic decoupling of APC from PSII at 0 °C. On the other hand, the increase in PSI fluorescence and the decrease in the C-PC fluorescence can be explained by direct excitation energy transfer from C-PC to PSI. According to the "parallel connection" model [16,17], the excitation energy could be directly transferred from C-PC into PSI at RT [10]; therefore, a remarkable increase in PSI fluorescence yield at 0 °C may be an indication of more

Table 1
Percentages of the fluorescence for each component at RT and 0 °C with a series of excitation wavelengths

Component	↖Ex=540 nm		↖Ex=550 nm		↖Ex=560 nm		↖Ex=570 nm		↖Ex=580 nm	
	RT	0°C	RT	0°C	RT	0°C	RT	0°C	RT	0°C
C-PC (647 nm)	26.9	20.6	29.9	24.1	39.0	35.8	41.0	34.6	26.6	19.6
APC (660 nm)	29.2	38.3	25.4	31.9	12.7	16.1	11.8	17.6	26.1	36.1
PSII (683 nm)	19.6	13.9	19.3	16.9	23.0	20.6	21.9	20.0	21.0	16.0
PSI (715 nm)	24.3	27.2	25.4	27.1	25.3	27.5	25.3	27.8	26.3	28.3
Relative error (%)	2.7	2.6	1.9	1.9	2.1	2.4	2.8	2.7	2.4	2.3

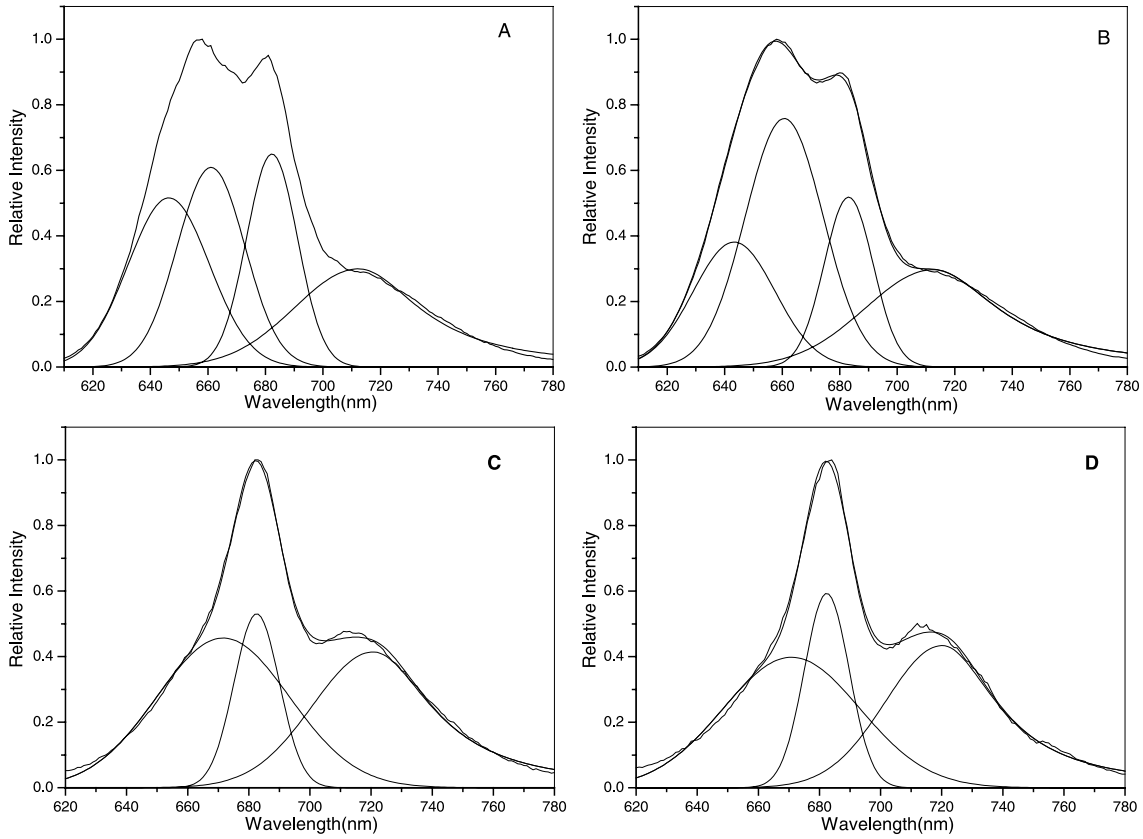

Fig. 4. Deconvolution of the fluorescence spectra of the intact cells excited at 580 nm at RT (A) and 0 °C (B), and excited at 436 nm at RT (C) and 0 °C (D).

efficient coupling of C-PC with PSI. At the moment, it may be proposed that a position shift of a PBS makes the distance and mutual orientation more suitable for the energy transfer from C-PC to PSI at 0 °C than at RT. On the other hand, excited at 436 nm, the APC fluorescence yield decreased by 7.6%, but those of PSII and PSI increased by 14.3% and 2.3%, respectively, at 0 °C, which implies that the back transfer from PSII to APC is also depressed. Furthermore, quantitatively, with excitation at 436 nm and at 0 °C, the increase in PSI fluorescence (2.3%) is far smaller than that in the PSII (14.3%), which could be an additional evidence for the direct energy transfer from C-PC to PSI. In fact, 2.3% is too small to be considered a meaningful increase because of 3% restriction on the relative error for the deconvolution. Whether at RT or 0 °C, the so-called back transfer from PSI to C-PC is never possible because of negligible spectral overlaps of the fluorescence spectrum of PSI with the absorption of C-

Table 2
Percentages of increases (positive) and decreases (negative) in the fluorescence yields at 0 °C relative to those at RT

Component	Ex = 540 nm	Ex = 550 nm	Ex = 560 nm	Ex = 570 nm	Ex = 580 nm	Ex = 436 nm
C-PC (647 nm)	− 23.4	− 19.4	− 8.2	− 15.6	− 26.3	–
APC (660 nm)	+31.2	+25.6	+26.8	+49.2	+38.3	− 7.5
PSII (683 nm)	− 29.1	− 12.4	− 10.4	− 8.7	− 23.8	+14.3
PSI (715 nm)	+11.9	+6.7	+8.7	+9.9	+7.6	+2.3

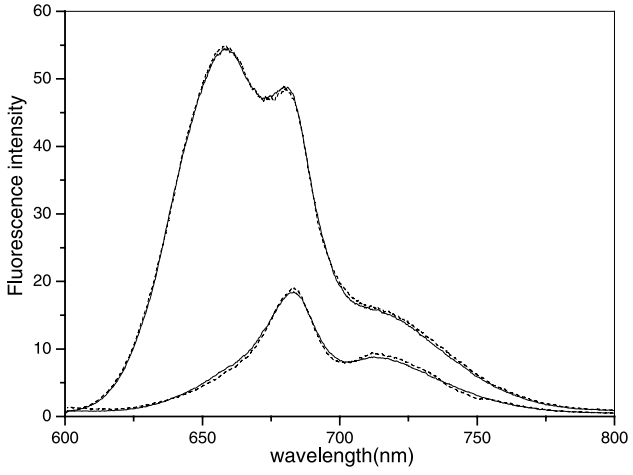

Fig. 5. Fluorescence spectra of the betaine-treated cells excited at 580 nm at RT (solid line) and 0 °C (dashed line), and excited at 436 nm at RT (solid line) and 0 °C (dashed line).

PC. In conclusion, whether excited at 436 or 580 nm, the fluorescence fluctuations for C-PC, APC, PSII and PSI can be uniquely explained by temperature-induced migration of a PBS along the thylakoid membrane. It may be further concluded that the migration makes the APC core move away from the PSII while allowing the C-PC in PBS rods to be more efficiently coupled with PSI.

In fact, if the distribution of excitation energy among PBS, PSII and PSI obeyed the "spillover" model, the fluorescence yield for PSI should have been varied synchronously with that for PSII. On the other hand, if the energy transfer from a PBS to the photosystems took the only way by PBS core (APC), the fluorescence yield for C-PC should have been varied synchronously with that for APC. Therefore, the results provided an evidence for the "mobile PBS" model and the "parallel connection" model but not for the "spillover" model.

3.3. Effect of betaine on connection of PBS with the thylakoid membrane in intact cells

It was found that betaine could fix PBS firmly on the thylakoid membrane [17]; therefore, it can be used to search for the mechanism of the temperature-induced decoupling of a PBS from the photosystems. Fig. 5 shows that the fluorescence spectrum of the betaine-treated cells at 0 °C is almost the same as that at RT, which means that temperature-induced decoupling of PBS from the photosystems will not occur in the betaine-treated cells. These results further confirmed that the temperature-induced decoupling should originate from a position shift of PBS relative to the photosystems. Based on the results above as well as the temperature-induced reversible decoupling and recoupling, it can be suggested that a PBS should translate on the surface of the membrane as an integral entity rather than the detached rod elements [12,16].

4. Conclusion

Based on the results in the current work, several points can be derived. Firstly, an isolated PBS–thylakoid membrane complex remains basically the structural feature of that in an intact cell; therefore, the isolated complex can be used as a model for probing the connection of a PBS with the two photosystems. Secondly, a PBS should be connected with the two photosystems parallelly at physiological condition, that is, it is coupled not only with PSII by the APC but also with PSI by the C-PC. Thirdly, it is the spatial position of a PBS relative to those of the photosystems, rather than the individual structure of PBS, PSI or PSII, that will vary when temperature changes from RT to 0 °C. Furthermore, it can be suggested that the temperature-induced decoupling of a PBS from the reaction centers should originate from a translation of the PBS along the thylakoid membrane, which makes the PBS core partially detached from PSII while making the C-PC in the PBS rods more efficiently coupled with PSI.

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References

[1] Y. Fujita, A. Murakami, K. Aizawa, K. Ohki, Short-term and long-term adaptation of the photosynthetic apparatus: homeostatic properties of thylakoids, in: D.A. Bryant (Ed.), The Molecular Biology of Cyanobacteria, Kluwer Academic Publishing, Dordrecht, 1994, pp. 677–692.
[2] A. Melis, Dynamics of photosynthetic membrane composition and function, Biochim. Biophys. Acta 1058 (1991) 87–106.
[3] C.P. Rijgersberg, R.V. Grondelle, J. Amesz, Energy transfer and bacteriochlorophyll fluorescence in purple bacteria at low temperature, Biochim. Biophys. Acta 592 (1980) 53–64.
[4] D. Bald, J. Kruip, M. Rögner, Supramolecular architecture of cyanobacterial thylakoid membranes: how is the phycobilisome connected with the photosystems? Photosynth. Res. 49 (1996) 103–118.
[5] B.M. Chereskin, J. Clement-Metral, E. Gantt, Characterization of a purified PSII-phycobilisome particle preparation from *Porphyridium cruentum*, Plant Physiol. 77 (1985) 626–629.
[6] C.W. Mullineaux, Excitation energy transfers from phycobilisomes to photosystem I in a cyanobacterium, Biochim. Biophys. Acta 1100 (1992) 285–292.
[7] A.N. Glazer, Y.M. Gindt, C.F. Chan, K. Sauer, Selective disruption of energy flow from phycobilisomes to photosystem I, Photosynth. Res. 40 (1994) 167–173.
[8] E. Bittersmann, W. Vermaas, Fluorescence lifetime studies of cyanobacterial photosystem II mutants, Biochim. Biophys. Acta 1098 (1991) 105–116.
[9] W.C. Mullineaux, Excitation energy transfer from phycobilisomes to photosystem I in a cyanobacterial mutant lacking photosystem II, Biochim. Biophys. Acta 1184 (1994) 71–77.
[10] X. Su, P.G. Fraenkel, L. Bogorad, Excitation energy transfer from phycocyanins to chlorophyll in an apcA-defective mutant of *Synechocystis* sp. PCC 68023, J. Biol. Chem. 267 (1992) 22944–22950.
[11] A.C. Ley, W.L. Butler, Energy distribution in the photochemical apparatus of *Porphyridium cruentum* in state 1 and state 2, Biochim. Biophys. Acta 592 (1980) 349–363.
[12] C.W. Mullineaux, M.J. Tobin, G.R. Jones, Mobility of photosynthetic complexes in the thylakoid membranes, Nature 390 (1997) 421–424.
[13] M. Sacina, M.J. Tobin, C.W. Mullineaux, Diffusion of phycobilisomes on the thylakoid membrane of the cyanobacterium *Synechococcus* 7942. Effect of phycobilisome size, temperature, and membrane lipid composition, J. Biol. Chem. 276 (2001) 46830–46834.
[14] M.G. Rakhimberdieva, V.A. Boichenko, N.V. Karapetyan, I.N. Stadnichuk, Interaction of phycobilisomes with photosystem II dimers and photosystem I monomers and trimers in the cyanobacterium *Spirulina platensis*, Biochemistry 40 (2001) 15780–15788.
[15] U. Schreiber, C.P. Rijgersberg, J. Amesz, Temperature-dependent reversible change in phycobilisome thylakoid membrane attachment in *Anacystis nidulans*, FEBS Lett. 104 (1979) 327–331.
[16] D. Foguel, R.M. Chaloub, J.L. Silva, A.R. Crofts, G. Weber, Pressure and low temperature effects on the fluorescence emission spectra and lifetimes of the photosynthetic components of cyanobacteria, Biophys. J. 63 (1992) 1613–1622.
[17] Y. Li, J.P. Zhang, J. Xie, J.Q. Zhao, L.J. Jiang, Temperature-induced

decoupling of phycobilisomes from reaction centers, Biochim. Biophys. Acta 1504 (2001) 229–234.
[18] V.V. Shubin, S.D.S. Murthy, N.V. Karapetyan, P. Mohanty, Origin of the 77 K variable fluorescence at 758 nm in the cyanobacterium *Spirulina platensis*, Biochim. Biophys. Acta 1060 (1991) 28–36.
[19] D. Bruce, S. Brimble, D.A. Bryant, State transitions in a cyanobacterium *Synechococcus* sp. PCC7002, Biochim. Biophys. Acta 974 (1989) 66–73.
[20] J. Biggins, D. Bruce, Regulation of excitation energy transfer in organisms containing phycobilins, Photosynth. Res. 1089 (1989) 1–34.