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Differences in energy transfer of a cyanobacterium, *Synechococcus* sp. PCC 7002, grown in different cultivation media

Kenta Niki · Shimpei Aikawa · Makio Yokono · Akihiko Kondo · Seiji Akimoto

Abstract Currently, cyanobacteria are regarded as potential biofuel sources. Large-scale cultivation of cyanobacteria in seawater is of particular interest because seawater is a low-cost medium. In the present study, we examined differences in light-harvesting and energy transfer processes in the cyanobacterium *Synechococcus* sp. PCC 7002 grown in different cultivation media, namely modified A medium (the optimal growth medium for *Synechococcus* sp. PCC 7002) and f/2 (a seawater medium). The concentrations of nitrate and phosphate ions were varied in both media. Higher nitrate ion and/or phosphate ion concentrations yielded high relative content of phycobilisome. The cultivation medium influenced the energy transfers within phycobilisome, from phycobilisome to photosystems, within photosystem II, and from photosystem II to photosystem I. We suggest that the medium also affects charge recombination at the photosystem II reaction center and formation of a chlorophyll-containing complex.

K. Niki · S. Akimoto
Graduate School of Science, Kobe University, Kobe 657-8501, Japan

S. Aikawa · A. Kondo
Graduate School of Engineering, Kobe University, Kobe 657-8501, Japan

S. Aikawa · M. Yokono · A. Kondo · S. Akimoto
CREST, JST, Kobe 657-8501, Japan

M. Yokono · S. Akimoto (✉)
Molecular Photoscience Research Center, Kobe University, Kobe 657-8501, Japan
e-mail: akimoto@hawk.kobe-u.ac.jp

Abbreviations
APC	Allophycocyanin
Car	Carotenoid
Chl	Chlorophyll
FDAS	Fluorescence decay-associated spectrum (spectra)
PBS	Phycobilisome
PC	Phycocyanin
PE	Phycoerythrin
PS	Photosystem
TRFS	Time-resolved fluorescence spectrum (spectra)

Introduction

Cyanobacteria are promising agents of biofuel production because they grow mainly in aquatic environments, enabling year-long cultivation without requiring arable land and portable water (Dismukes et al. 2008). Large-scale cultivation of cyanobacteria in seawater is of particular interest because of the low cost of seawater (Mary Leema et al. 2010; Aikawa et al. 2014). Cyanobacteria are the most primordial of the oxygenic photosynthetic organisms and are characterized by their phycobilisomes (PBSs), unique antenna complexes of phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) that absorb visible light energy. The light energy was captured by PBS transfers as excitation energy to chlorophyll (Chl) (Gantt 1981; Mimuro and Kikuchi 2003). It is well known that changes in environmental conditions affect light-harvesting

processes (Riethman et al. 1988; Cousins et al. 2014). In different light environments, cyanobacteria change the quality and quantity of their pigment–protein complexes (Ghosh and Govindjee 1966; Bennett and Bogorad 1973; Grossman et al. 1993; Stowe-Evans and Kehoe 2004; Akimoto et al. 2012), and they change energy transfer processes between pigment and protein complexes (Boardman et al. 1966; Yokono et al. 2008; Akimoto et al. 2013). It is also known that red algae, which contain PBSs for light harvesting, regulate energy transfer involving PBSs under different light conditions (Su et al. 2010). Allakhverdiev et al. (2002) reported that light-induced and salt-induced stresses exert very different effects on the cyanobacterium *Synechocystis* sp. PCC 6803; in particular, strong light induces photodamage to photosystem II (PSII), whereas salt stress inhibits the repair of PSII. The pigment compositions of photosynthetic organisms also strongly depend on the nutrient conditions. For instance, nitrogen, sulfur, or phosphorus deficiency induces PBS degradation in cyanobacterial cells (Allen and Smith 1969; Grossman et al. 1993; Collier et al. 1994; Peter et al. 2010; Su et al. 2010), whereas iron deficiency leads to formation of Chl *a* antenna complexes around PSI trimers (Bibby et al. 2001; Michel and Pistorius 2004). However, although changes in pigment constituents under nutrient stresses have been well documented, the changes in energy transfer induced by nutrients stresses remain incompletely understood.

Among the most useful techniques for investigating energy transfer processes in photosynthetic systems is time-resolved fluorescence spectroscopy. The transition energies of some Chls in PSI and PSII are lower than those of the primary electron donors, so these Chls operate as energy sinks. Chls are named after their fluorescence peak wavelengths, for example, F685 (PSII), F695 (PSII), and F730 (PSI) (Goedheer 1972; Nakayama et al. 1979; Govindjee 2004). The energy transfer between photosynthetic pigments is estimated by monitoring the fluorescence intensities of PE, PC, APC, and Chls over time. In addition, the energy transfer between two PSs can be elucidated from the delayed fluorescence (tens of nanoseconds) at 77 K. The delay at this temperature manifests from charge recombination at the PSII reaction center. Whereas the lifetime of fluorescence from an isolated PSI does not exceed 5 ns (Mimuro et al. 2010), intact cells contain components with long lifetimes in the PSI fluorescence region (Yokono et al. 2011). This behavior indicates energy transfer from PSII to PSI (spillover) in intact cells.

Previously, we reported that energy transfer in the cyanobacterium *Arthrospira platensis* depends on the cultivation medium (Arba et al. 2013). In our previous study, pre-cultivated *A. platensis* cells were cultured in the optimal *A. platensis* growth medium or in seawater medium. In the seawater medium, the relative intensities of low-energy Chl and PBS decreased while that of carotenoid (Car) increased. Time-resolved fluorescence measurements revealed lower efficiencies of PBS–PSs and PSII–PSI energy transfers in the seawater medium than in the optimal medium. It was revealed that light energy not consumed in photosynthesis remained unquenched in the *A. platensis* cells grown in seawater medium. In the present study, we examine energy transfer in the cyanobacterium *Synechococcus* sp. PCC 7002 cells grown in different media by means of time-resolved fluorescence spectroscopy. We also investigate the effects of nitrate and phosphate ions on the energy transfer processes within and between PBS, PSI, and PSII.

Materials and methods

Culture conditions

The cyanobacteria *Synechococcus* sp. PCC 7002 was obtained from the Pasteur Culture Collection (Paris, France). Cells were pre-cultured in 500 mL Erlenmeyer flasks containing 250 mL of modified medium A (hereafter referred to as A) (Aikawa et al. 2014) (Supplementary Table S1), agitated at 100 rpm under continuous illumination of 50 µmol photons m^{-2} s^{-1}. Pre-cultivation was continued for 7 days in air at (30 ± 2) °C in an NC350-HC plant chamber (Nippon Medical and Chemical Instruments, Osaka, Japan). Light intensity was measured in the middle of the medium by an LI-250A light meter (LI-COR, Lincoln, Nebraska, USA) equipped with an LI-190SA quantum sensor (LI-COR). To study the differences in energy transfer between the standard and seawater media, pre-cultured cells were inoculated into A (brackish water medium; salinity 2.7 %) or f/2 medium (seawater medium; salinity 4.0 %, hereafter referred to as F) (Guillard and Ryther 1962) (Supplementary Table S2). The dried biomass concentration of the inoculum was 0.01 g dry-cell weight L^{-1}, and the culture conditions were those of the pre-cultivation. Next, we focus on differences between the concentrations of the common major components: nitrogen and phosphorus (Supplementary Tables S1 and S2). To examine the effects of nitrogen and/or phosphorus concentration on the energy transfer processes, the $NaNO_3$ and KH_2PO_4 levels in the media were varied as described below. For the A-based media (A, An, Ap, and Anp), the $NaNO_3$ and KH_2PO_4 levels were, respectively, set to (A) 1.0 g L^{-1} and 50 mg L^{-1}, (An) 75 and 50 mg L^{-1}, (Ap) 1.0 g L^{-1} and 5.0 mg L^{-1}, and (Anp) 75 and 5.0 mg L^{-1}. Note that in the An and Ap media, the $NaNO_3$ level and KH_2PO_4 level, respectively, were reduced to that in F, while Anp was deficient in both $NaNO_3$ and KH_2PO_4. For the F-based media (F, FN, FP, and FNP), the $NaNO_3$ and

KH_2PO_4 levels were, respectively, set to (F) 75 and 5.0 mg L^{-1}, (FN) 1.0 g L^{-1} and 5.0 mg L^{-1}, (FP) 75 and 50 mg L^{-1}, and (FNP) 1.0 g L^{-1} and 50 mg L^{-1}. In the FN and FP media, the $NaNO_3$ level and KH_2PO_4 level, respectively, were increased to that in A, while both of $NaNO_3$ and KH_2PO_4 were enriched in FNP.

Measurements and analysis

Steady-state absorption spectra were measured at room temperature by a spectrometer equipped with an integrating sphere (JASCO V-650/ISV-722). Steady-state fluorescence spectra were measured at 77 K by a spectrofluorometer (JASCO FP-6600/PMU-183). Time-resolved fluorescence spectra (TRFS) were measured at 77 K by a time-correlated single-photon counting system (Akimoto et al. 2012). The excitation source was the second harmonic (400 nm) of a Ti:Sapphire laser, which simultaneously excites all pigments. The time interval of data acquisition was set to 2.4 or 24.4 ps/channel. For the TRFS measurements, a homogenous ice was obtained by adding polyethylene glycol (average molecular weight 3,350, final concentration 15 % (w/v), Sigma-Aldrich, USA) to the sample solutions. All samples were prepared under dimly lit conditions. To precisely understand excitation relaxation in *Synechococcus* sp. PCC 7002 cells, we carried out a global analysis and obtained fluorescence decay-associated spectra (FDAS) as previously reported (Yokono et al. 2008; Akimoto et al. 2012). The fluorescence rise and decay curves at different wavelengths were fitted by sums of exponentials with common time constants. The FDAS of each time constant was obtained from the amplitude versus wavelength plot. In FDAS, positive and negative amplitudes indicate fluorescence decay and rise over time, respectively.

Results

Absorption spectra

Figure 1 shows the absorption spectra of *Synechococcus* sp. PCC 7002 cells grown in different media. All absorption spectra were normalized by the Chl Qy (0,0) absorption band. The relative intensities of the Car and PBS bands (Car/Chl and PBS/Chl, respectively) depend on the cultivation media. Table 1 summarizes the intensities of the Car and PBS band (relative to the Chl Qy (0,0) band) and the peak wavelengths of the Chl Qy (0,0) band. As the Car band we selected the 490-nm band, which is not readily absorbed by PC, APC, or Chl (Mimuro and Kikuchi 2003). The Qy (1,0) absorption band of Chl is around 630 nm. Here, to estimate the intensities of the PBS band in
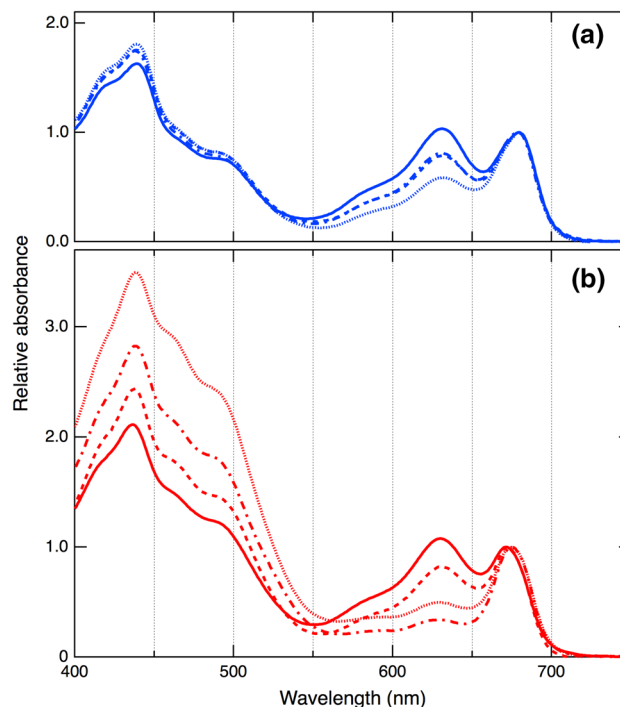

Fig. 1 Absorption spectra of *Synechococcus* sp. PCC 7002 cells grown in A-based media (**a**) and F-based media (**b**): A (brackish water medium, *blue solid line*), An (nitrogen-deficient A, *blue-dotted line*), Ap (phosphorous-deficient A, *blue broken line*), Anp (nitrogen and phosphorous-deficient A, *blue dot-dashed line*), FNP (nitrogen and phosphorous-enriched F, *red solid line*), FP (phosphorous-enriched F, *red-dotted line*), FN (nitrogen-enriched F, *red broken line*), and F (seawater medium, *red dot-dashed line*)

Table 1 Intensities of the carotenoid (Car) band ($\sim$490 nm) and the phycobilisome (PBS) band ($\sim$630 nm) relative to the chlorophyll (Chl) Qy (0,0) band in absorption spectra of *Synechococcus* cells grown in different media

	PBS/Chl	Car/Chl	Chl Qy peak (nm)
A	0.88	0.76	680
An	0.44	0.82	680
Ap	0.64	0.82	679
Anp	0.66	0.79	679
FNP	0.93	1.2	671
FP	0.39	2.3	675
FN	0.68	1.4	672
F	0.22	1.8	675

Peak wavelengths of the chlorophyll Qy (0,0) band are also listed

Synechococcus sp. PCC 7002 cells, we assumed that the intensity ratio of the Qy (1,0) band to the Qy (0,0) band is identical in protein and solution. To locate the peak position of the Qy (0,0) band in solution at its peak wavelength in the cells, we red-shifted the absorption spectrum of Chl *a* in solution. By this procedure, the absorbance of the Qy

(1,0) band around 630 nm was estimated as ∼0.15. The net values listed in Table 1 are the PBS/Chl values, obtained by subtracting 0.15 from the apparent relative absorbance around 630 nm (Fig. 1). The relative intensity of PBS was lower in the F medium than in the A medium (0.22 vs. 0.88), while that of Car was higher (1.8 vs. 0.76). Similar behaviors were reported by Arba et al. (2013), who compared the pigment ratios between *A. platensis* grown in seawater and optimal medium.

In the A-based media, deficiencies of nitrogen and/or phosphorus significantly altered the PBS/Chl ratio but scarcely affected the Car/Chl ratio. The PBS/Chl ratio was severely reduced in cells grown in nitrogen-poor, phosphate-sufficient medium (An-grown cells); conversely, the PBS/Chl ratio was insensitive to nitrate ion concentration under phosphorous-deficient conditions (Ap-grown and Anp-grown cells). In the F-based media, both the Car/Chl and PBS/Chl ratios were largely altered by the changing nutrient conditions; nitrogen enrichment increased the PBS/Chl ratio and decreased the Car/Chl ratio, whereas phosphorous enrichment increased the PBS/Chl ratio. By enriching the F medium with both nitrate and phosphorous (FNP-grown cells), the relative PBS content was increased to that of the A-grown cells, although the relative Car content remained larger than in the A-grown cells. The PBS/Chl ratios decreased to 0.44 and 0.39 in the An-grown and FP-grown cells, respectively, whereas the ratios were 0.64 and 0.68 in Ap-grown and FN-grown cells, respectively. These results indicate that the relative amount of PBS was reduced to a greater extent by nitrogen-deficient than by phosphorus-deficient conditions.

The wavelength of the Chl Qy band was more than 5 nm shorter in the F-based media than in the A-based media. Among the A-based media, the wavelengths of the Chl Qy band were essentially the same (679–680 nm; see Table 1). On the other hand, in the F-based media, nitrogen enrichment caused a 3–4-nm blue shift of the Chl Qy band (cf. F and FN or FP and FNP in Table 1), whereas phosphorous enrichment did not affect the peak wavelength (cf. F and FP or FN and FNP in Table 1). These behaviors indicate an increase in the relative content of higher energy Chls in the cells grown in F-based media, and that (an) ion(s) not containing nitrogen or phosphorous affect(s) the quantity of low-energy Chls.

Steady-state fluorescence spectra

Steady-state fluorescence spectra of *Synechococcus* sp. PCC 7002 cells grown in the different media are shown in Fig. 2. The excitation wavelengths were 440 nm (Chl *a* Soret band) and 590 nm (PC band). The fluorescence spectra of the cells grown in the A-based media were little affected by the media composition (blue lines, Fig. 2).

Excitation at 440 nm (solid lines, Fig. 2) induced a large peak around 720 nm in the PSI fluorescence region, with two faint shoulders around 685 and 695 nm in the PSII fluorescence region. Under 590 nm excitation (dotted lines, Fig. 2), additional bands were found in the 630–670-nm region, in which PC and APC peaked around 650 and 665 nm, respectively. The enhanced fluorescence intensity around 750 nm is attributable to the PSII fluorescence tail, whose relative intensity increases under 590 nm excitation (PC excitation). On the other hand, the fluorescence spectra of the cells grown in the F-based media were highly dependent on medium composition (red lines, Fig. 2). The relative intensities of the PSII to PSI fluorescences were larger than those of cells grown in the A-based media; this tendency was increased by nitrogen enrichment (FN-grown and FNP-grown cells). In the spectra excited at 590 nm (dotted lines, Fig. 2), the relative intensity of PC to APC is reduced in cells grown in the nitrogen-deficient media (F and FP), reflecting the lower content of PC in these cells (Fig. 1). In addition, the relative intensities of APC to PSII fluorescences increased in cells grown in nitrogen-deficient media (F and FP), indicating reduced energy transfer efficiency from APC to Chl. The two peaks found in the >700 nm region were assigned to the tails of the PSII and APC fluorescences (Yokono et al. 2008).

Time-resolved fluorescence spectra and florescence decay-associated spectra

Figure 3 depicts the TRFS of *Synechococcus* sp. PCC 7002 cells grown in the different media. The TRFSs of A-grown cells and F-grown cells are clearly different. The spectra of A-grown cells initially features two broad bands around 650 and 700 nm, assigned to the PBS and Chl fluorescences, respectively. After 9.8 ps, the fluorescence was dominated by PSI. On the other hand, in the F-grown cells, the origin of the main fluorescence bands changed over time; the PSII fluorescence maximum immediately after excitation was followed by dominant fluorescences from the red Chls of PSI up to ∼4 ns. In the later time region, the fluorescence was maximized in the APC fluorescence region (669 nm). This indicates that some excitation energy was trapped in APC and did not transfer to Chl.

In the global analysis, the TRFS differences between the cells grown in different media are represented by combinations of differences in the FDAS time constants and the spectral shapes of FDAS. The FDAS of *Synechococcus* sp. PCC 7002 cells grown in different media are shown in Fig. 4. In the A-grown cells, energy was transferred from PC to APC with a time constant of 15 ps. Energy transfer from PBS to PSII was completed in 35 ps, and the PSI red Chls sequentially received excitation energy with time constants of 15 and 35 ps. In addition, delayed

Fig. 2 Steady-state fluorescence spectra of *Synechococcus* sp. PCC 7002 cells grown in different media. Each spectrum is normalized by its maximum intensity. Excitation wavelength is 440 nm (*solid line*) or 590 nm (*dotted line*)
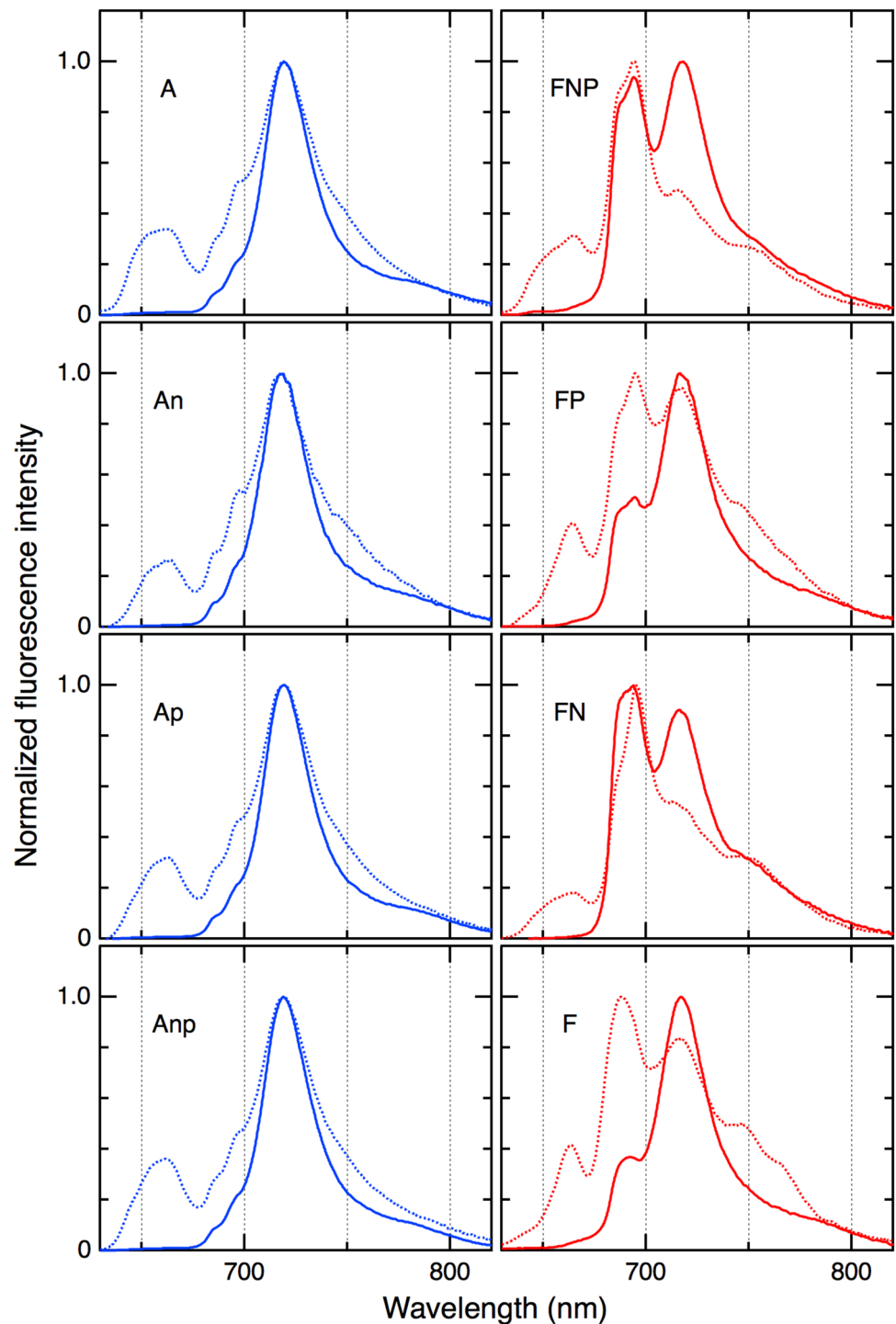
fluorescence (lifetime exceeding 10 ns) was recognized in the PSII and PSI fluorescence regions. This delayed fluorescence originates from charge recombination at the PSII reaction center (Mimuro et al. 2007). Therefore, existence of the PSI fluorescence in the final FDAS ($\geq$10 ns) indicates energy transfer from PSII to PSI in the A-grown cells. On the other hand, the second phase of the energy transfer to PSI was slower in the F-grown cells, occurring with a time constant of 80 ps. The 6th FDAS (at 6.6 ns) was dominated by APC fluorescence, indicating that energy transfer from APC to PSII was suppressed in the F-grown cells. In contrast to the excitation relaxation in the A-grown cells, no delayed fluorescence was observed in these cells.
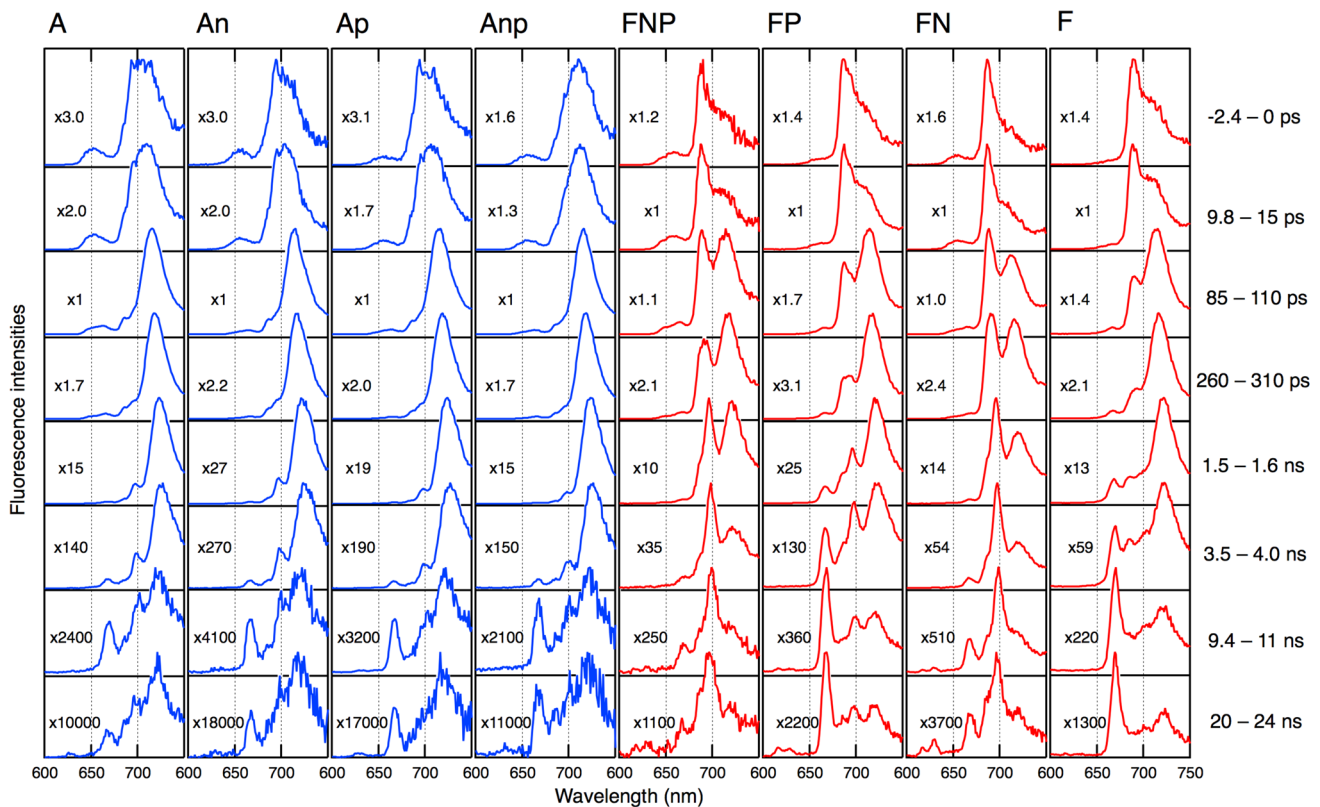
Fig. 3 Time-resolved fluorescence spectra of *Synechococcus* sp. PCC 7002 cells grown in different media. Excitation wavelength is 400 nm

The time courses of the TRFS for cells grown in A-based media were similar (blue lines, Fig. 3), except in the later post-excitation time period (20–24 ns), when the relative intensities of the APC fluorescence increased under deficient nitrate ion and/or phosphate ion conditions (An, Ap, and Anp). When both ions were deficient (Anp), the increase in the relative intensity of the APC fluorescence was apparent at an earlier period (9.4–11 ns). In contrast, the TRFS of cells grown in the F-based media were clearly different (red lines, Fig. 3). Especially, in nitrogen-enriched media (FN, FNP), the relative intensities of the PSI to PSII fluorescences increased more slowly than in the nitrogen-deficient media (F, FP). The FN-grown and FNP-grown cells also exhibited PBS fluorescence from the initial time period. After 1.5 ns, the contribution of the APC fluorescence was reduced under high nitrate ion conditions (FN, FNP). Delayed fluorescence appeared in the PSII fluorescence region in the FN-grown, FP-grown, and FNP-grown cells, although its contribution was small in the FP-grown cells.

During the early stages, the PBS fluorescence region of the A-grown cells was relatively unaffected by media composition (blue lines, Fig. 4). This indicates that the energy transfer processes in PBS are not largely affected by nitrate ion or phosphate ion concentrations in the

A-based media. In the 6th FDAS (4.4–6.6 ns), the fluorescence peak of lower energy Chl shifted to a shorter wavelength ($\sim$716 nm) in the An-grown and Anp-grown cells. It appears that the reddest Chl (F735, which emits at 735 nm) is not easily formed under nitrogen-deficient conditions. All of the A-based media demonstrated delayed fluorescences in both the PSII and PSI fluorescence regions. This suggests that energy is transferred from PSII to PSI in the A medium even under nitrogen-deficient and/or phosphorous-deficient condition(s). In the 1st and 2nd FDAS (15–25 and 50–80 ps, respectively) of the FN-grown, FP-grown, and FNP-grown cells, clear peaks appear in the PBS fluorescence region, which are absent in the spectra of F-grown cells (red lines, Fig. 4). This indicates that additional nitrate ion and/or phosphate ion triggers some energy transfer processes in PBS. The APC fluorescence is diminished in the 6th FDAS of FN-grown and FNP-grown cells (at 5.4 and 5.5 ns, respectively). Therefore, we have confirmed that adding nitrate ion to the F medium increases the efficiency of energy transfer from APC to Chl. In the FN-grown, FP-grown, and FNP-grown cells, delayed fluorescence was mainly observed in the PSII fluorescence region ($\geq$10 ns). Therefore, energy may not be preferentially transferred from PSII to PSI in these cells.
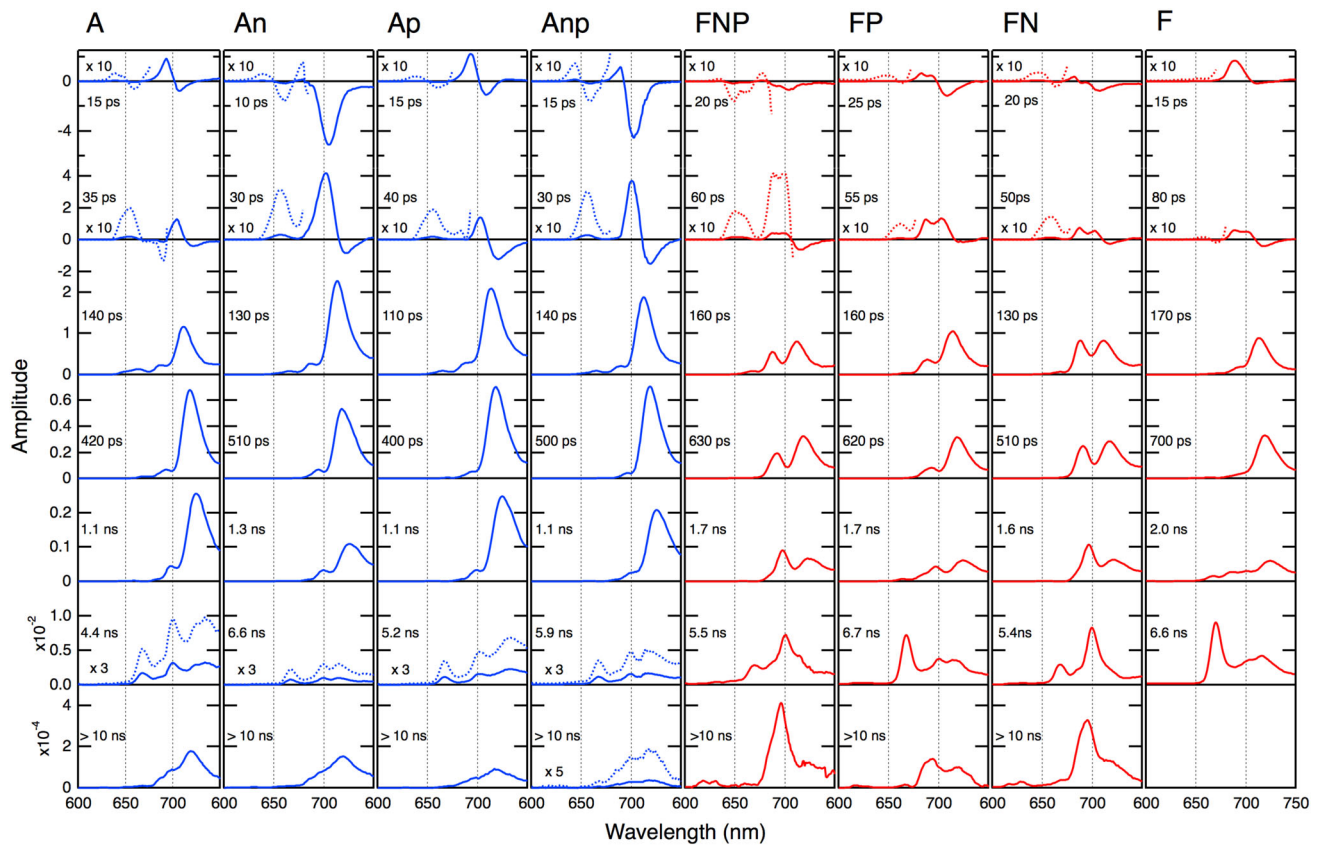
Fig. 4 Fluorescence decay-associated spectra of *Synechococcus* sp. PCC 7002 cells grown in different media, obtained by global analysis of the time-resolved fluorescence spectra (Fig. 3). *Dotted lines* are magnified spectra; the magnification factor is shown in each window

Discussion

Changes in energy transfer from phycobilisome

Clear changes in the relative absorbance of PC (Fig. 1) reveal that the relative PBS contents depend on the nitrate and phosphate ion concentrations. The nitrogen-deficient condition induced a larger decrease in the PBS/Chl ratio than the phosphorus-deficient condition did (Table 1). This result suggests deficiencies of phosphorus and nitrogen in Anp and F, respectively, although the concentrations of nitrogen and phosphorus nutrients are the same in Anp and F. This difference might be caused by differences in concentrations of ions that do not contain nitrogen or phosphorous. Nitrogen and/or phosphorus deficiencies in the A-based media reduced the relative intensities of PBS absorption, as reported for other cyanobacteria: *Anacystis nidulans* (*Synechococcus* sp. PCC 6301) under nitrogen-deficient conditions (Allen and Smith 1969; Grossman et al. 1993), *Synechococcus* sp. PCC 7942 under nitrogen-, sulfur-, or phosphorus-deficient conditions (Collier et al. 1994), and *Spirulina platensis* (*A. platensis*) under nitrate-

deficient conditions (Peter et al. 2010). However, nitrogen and/or phosphorous deficiency little affected the FDAS in the PBS fluorescence region of the *Synechococcus* sp. PCC 7002 cells (blue line, Fig. 4). Nutrient deficiencies in the A-based media caused a reduction in the relative PBS content without changing the energy transfer in PBS. On the other hand, the relative PBS content was increased by adding nitrate ion and/or phosphate ion to the F medium (Fig. 1; Table 1). However, the relative fluorescence intensity from phycobiliprotein, which is not involved in energy transfer processes, increased in the later time region of the TRFS (20–24 ns) and in the FDAS with the longest lifetime ($\geq$10 ns) (Figs. 3 and 4). Therefore, the increased PBS content induced by the nitrogen and/or phosphorous enrichment likely does not improve the light harvesting by PC in the F-based medium. Phycobiliproteins not involved in energy transfer were also identified in *A. platensis* cells grown in seawater medium (Arba et al. 2013).

For cells grown in the F medium, large APC fluorescence appeared in the later time region of the TRFS (after 3.5 ns) and in the 6.6-ns FDAS (Figs. 3 and 4), indicating suppression of the energy transfer from APC to Chl. The

APC fluorescence was unaffected by phosphorus enrichment (FP-grown cells) but was reduced by nitrogen enrichment, along with increased PSII fluorescence (see 5.4 ns FDAS of the FN-grown cells and 5.5 ns FDAS of the FNP-grown cells). In contrast, the PSI fluorescence was unaltered by enrichment of either nutrient. In the 6th FDAS of the cells grown in A-based media (Fig. 4), the APC and PSII fluorescences were accompanied by PSI fluorescence. Therefore, it appears that nitrogen enrichment selectively improves the energy transfer route from APC to PSII. In a previous report, no energy transfer from PSII to PSI was detected in a supercomplex of PBS, PSI, and PSII (known as the PSI–PBS–PSII supercomplex) from *Synechocystis* sp. PCC 6803 (Liu et al. 2013). If also true in *Synechococcus* sp. PCC 7002, PSII cannot share the energy accepted from PBS with PSI. In the FN-grown and FNP-grown cells, light harvesting by PBS alone might disrupt the excitation energy balance between PSI and PSII.

Changes in energy transfer within and between photosystems

In the 5th FDAS of all cells except the F-grown cells (FDAS with a time constant of 1.1–2.0 ns), the amplitude of the 695-nm band (CP47) was much larger than that of the 685-nm band (CP43); in the F-grown cells, both amplitudes were comparable (Fig. 4). When two PSII monomer units combine, the Chl exhibiting the 685-nm band transfers energy to the Chl exhibiting the 695-nm band. Therefore, it is suggested that interaction is minimal between the PSII monomers of cells grown in the F medium but can be promoted by nitrogen or phosphorus addition. The F-grown cells alone emitted no delayed fluorescence, indicating a change in charge recombination at the PSII reaction center. At present, we cannot easily determine the effects of nitrogen and phosphorus by correlating the energy and electron transfers; however, we can infer that some pigment–pigment and complex–complex interactions have been altered in the PSII of F-grown cells.

The cells grown in all other media exhibited delayed fluorescence (Figs. 3 and 4). In the delayed fluorescence spectra, the relative intensity of the PSI to PSII fluorescences (PSI/PSII) was roughly classifiable into three categories: (i) PSI/PSII > 1 (cells grown in A-based media), (ii) PSI/PSII ~ 1 (FP-grown cells), and (iii) small PSI/PSII (FN-grown and FNP-grown cells). The PSI/PSII value in the delayed fluorescence reflects the relative amount of PSII that transfers energy to PSI (the PSI–PSII supercomplex); the larger the PSI/PSII, the higher the ratio of PSI–PSII supercomplex to isolated PSII (Yokono et al. 2011). Since energy is transferred from PSII to PSI within 100 ps (Yokono et al. 2011), the relative amount of PSII tied up in

the PSI–PSII supercomplex should be determinable from the PSI/PSII ratio in the 3rd and 4th FDAS, although these ratios are inflated by PSI fluorescence emitted after the direct excitation of PSI. In the 3rd and 4th FDAS (Fig. 4), the PSI fluoresced at much higher amplitude than the PSII fluorescence in group (i), but the amplitudes were comparable in group (iii). The PSI/PSII ratio in group (ii) was intermediate between those of groups (i) and (iii). Assuming that the above analysis is applicable to the F-grown cells, we can estimate the relative content of the PSI–PSII supercomplex in the F-grown cells. Examining the 3rd and 4th FDAS of the F-grown cells (Fig. 4), we find that the amplitude is much higher in the PSI fluorescence than in the PSII fluorescence, consistent with group (i). While energy might be actively transferred from PSII to PSI in the F-grown cells, this cannot be confirmed from the delayed fluorescence.

Origin of the high-energy chlorophylls

The Chl Qy band appears at shorter wavelengths in the F-grown cells than in the A-grown cells (Fig. 1; Table 1). This blue shift of the Chl Qy band is explained by two possible mechanisms, replacement of Chls with higher energy Chls in the pigment–protein complexes that also exist in the A-grown cells or formation of additional pigment–protein complexes containing higher energy Chls, which are absent in the A-based media. A sequential energy transfer to PSI red Chl appears in the 1st and 2nd FDAS (Fig. 4). For example, positive–negative peak pairs locate around 693 and 707 nm in the 1st FDAS and around 704 nm and 722 nm in the 2nd FDAS of the A-grown cells. This sequential energy transfer was recognized in other cells but was distinctly different between cells grown in A-based and F-based media. Only cells grown in the F-based media exhibited two positive peaks in the 2nd FDAS; the peak at shorter wavelength was absent in the FDAS of cells grown in A-based media. This indicates that cells grown in the F-based media transfer energy to PSI red Chl via an alternative pathway involving a higher energy Chl. However, if this higher energy Chl forms part of the PSI complex, its fluorescence should appear in the 1st FDAS and disappear in the 2nd FDAS (Mimuro et al. 2010). A more likely explanation is that cells grown in F-based media form additional pigment–protein complexes containing higher energy Chls, which transfer energy to PSI. The formation of the additional pigment–protein complexes around PSI might relate to the suppression of PSII–PSI energy transfer in the FN-grown, FP-grown, and FNP-grown cells. In this context, enriching the F medium with nitrogen and/or phosphorus might enhance formation of the additional pigment–protein complex. This idea is supported by the

shortened wavelength of the Chl Qy peak in FN-grown and FNP-grown cells (Table 1), indicating that more of the additional pigment–protein complexes exist in these cells than in the other cell groups. This hypothesis also fits the reduced PSI/PSII ratio in the delayed fluorescence of FN-grown and FNP-grown cells (Fig. 4).

Under iron-deficient conditions, antenna complexes containing Chl *a*, CP43' are formed around the PSI trimers of cyanobacterial cells (Bibby et al. 2001; Michel and Pistorius 2004). No additional pigment–protein complexes formed in the An-grown, Ap-grown, and Anp-grown cells, indicating that formation of complexes containing higher energy Chls is not triggered by nitrogen or phosphorus deficiency per se. To elucidate how and why additional pigment–protein complexes form in the F-based media, we should examine the effects of ions not containing nitrogen or phosphorous.

Summary

We examined the effects of nitrogen and phosphorus on the light-harvesting and energy transfer processes of *Synechococcus* sp. PCC 7002 cells grown in optimal medium (A) and a seawater medium (F). The energy transfer process within PSII and charge recombination at the PSII reaction center were suppressed in the F-grown cells. The relative PBS content of cells was increased by higher concentrations of nitrate and/or phosphate ions. Although reducing the relative quantity of PBS did not significantly affect the PBS energy transfer in the A-grown cells, increasing the relative quantity of PBS did not necessarily improve PBS light harvesting in the F-grown cells. The energy transfer from APC to Chl was improved only by improving the energy transfer from APC to PSII. In addition, cells grown in F-based media produced considerable amounts of pycobiliprotein, which is not involved in the energy transfer chain. The PSII–PSI energy transfer was retained in the A-based media, regardless of nitrate and phosphate ion concentration. On the other hand, additional pigment–protein complexes containing Chl were formed in the F-based media, especially when supplemented with nitrogen. These complexes suppress the energy transfer from PSII to PSI. Therefore, we suggest that in cells grown in F medium enriched with nitrogen and/or phosphorus, PBS acts as a light-harvesting complex for PSII alone, while the new Chl-containing complex plays an antenna role for PSI. The effects of ions not containing nitrogen or phosphorous were also discussed.

References

Aikawa S, Nishida A, Ho S-H, Chang J-S, Hasunuma T, Kondo A (2014) Glycogen production for biofuels by the euryhaline cyanobacteria *Synechococcus* sp. strain PCC 7002 from an oceanic environment. Biotechnol Biofuels 7:88

Akimoto S, Yokono M, Hamada F, Teshigahara A, Aikawa S, Kondo A (2012) Adaptation of light-harvesting systems of *Arthrospira platensis* to light conditions, probed by time-resolved fluorescence spectroscopy. Biochim Biophys Acta 1817:1483–1489

Akimoto S, Yokono M, Aikawa S, Kondo A (2013) Modification of energy-transfer processes in the cyanobacterium, *Arthrospira platensis*, to adapt to light conditions, probed by time-resolved fluorescence spectroscopy. Photosynth Res 117:235–243

Allakhverdiev SI, Nishiyama Y, Miyairi S, Yamamoto H, Inagaki N, Kanesaki Y, Murata N (2002) Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of *psbA*Genes in *Synechocystis*. Plant Physiol 130:1443–1453

Allen MM, Smith AJ (1969) Nitrogen chlorosis in blue-green algae. Arch Mikrobiol 69:114–120

Arba M, Aikawa S, Niki K, Yokono M, Kondo A, Akimoto S (2013) Differences in excitation energy transfer of *Arthrospira platensis* cells grown in seawater medium and freshwater medium, probed by time-resolved fluorescence spectroscopy. Chem Phys Lett 588:231–236

Bennett A, Bogorad L (1973) Complementary chromatic adaptation in a filamentous blue–green alga. J Cell Biol 58:419–435

Bibby TS, Nield J, Barber J (2001) Iron deficiency induces the formation of an antenna ring around trimeric photosystem I in cyanobacteria. Nature 412:743–745

Boardman NK, Thome SW, Anderson JM (1966) Fluorescence properties of particles obtained by digitonin fragmentation of spinach chloroplasts. Proc Natl Acad Sci USA 56:586–593

Collier JL, Herbert SK, Fork DC, Grossman AR (1994) Changes in the cyanobacterial photosynthetic apparatus during acclimation to macronutrient deprivation. Photosynth Res 42:173–183

Cousins AB, Johnson M, Leakey ADB (2014) Photosynthesis and the environment. Photosynth Res 119:1–2

Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC (2008) Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr Opin Biotechnol 19:235–240

Gantt E (1981) Phycobilisomes. Ann Rev Plant Physiol 32:327–347

Ghosh AK, Govindjee (1966) Transfer of the excitation energy in *Anacystis nidulans* grown to obtain different pigment ratios. Biophys J 6:611–619

Goedheer JC (1972) Fluorescence in relation to photosynthesis. Ann Rev Plant Physiol 23:87–112

Govindjee (2004) Chlorophyll *a* fluorescence: a bit of basics and history. In: Papageorgiou GC, Govindjee (eds) Chlorophyll *a* Fluorescence: a signature of photosynthesis. Kluwer Academic Publishers, Dordrecht, pp 1–42

Grossman AR, Schaefer MR, Chiang GG, Collier JL (1993) The phycobilisome, a light-harvesting complex responsive to environmental conditions. Microbial Rev 57:725–749

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Liu H, Zhang H, Niedzwiedzki DM, Prado P, He G, Gross ML, Blankenship RE (2013) Phycobilisomes supply excitations to both photosystems in a megacomplex in cyanobacteria. Science 342:1104–1107

Mary Leema JT, Kirubagaran R, Vinithkumar NV, Dheenan PS, Karthikayulu S (2010) High value pigment production from *Arthrospira* (*Spirulina*) *platensis* cultured in seawater. Bioresour Technol 101:9221–9227

Michel K-P, Pistorius EK (2004) Adaptation of the photosynthetic electron transport chain in cyanobacteria to iron deficiency: the function of IdiA and IsiA. Physiol Plant 120:36–50

Mimuro M, Kikuchi H (2003) Antenna systems and energy transfer in Cyanophyta and Rhodophyta. In: Green BR, Parson WW (eds) Light-harvesting antennas in photosynthesis. Kluwer Academic Publishers, Dordrecht, pp 281–306

Mimuro M, Akimoto S, Tomo T, Yokono M, Miyashita H, Tsuchiya T (2007) Delayed fluorescence observed in the nanosecond time region at 77 K originates directly from the photosystem II reaction center. Biochim Biophys Acta 1767:327–334

Mimuro M, Yokono M, Akimoto S (2010) Variations in photosystem I properties in the primordial cyanobacterium *Gloeobacter violaceus* PCC 7421. Photochem Photobiol 86:62–69

Nakayama K, Yamaoka T, Katoh S (1979) Chromatographic separation of photosystems I and II from the thylakoid membrane isolated from a thermophilic blue-green alga. Plant Cell Physiol 20:1565–1576

Peter P, Sarma AP, Azeen ul Hasan M, Murthy SDS (2010) Studies on the impact of nitrogen starvation on the photosynthetic pigments through spectral properties of the cyanobacterium, *Spirulina platensis*: identification of target phycobiliprotein under nitrogen chlorosis. Bot Res Int 3:30–34

Riethman H, Bullerjahn G, Reddy KJ, Sherman LA (1988) Pegulation of cyanobacterial pigment-protein composition and organization by environmental factors. Photosynth Res 18:133–161

Stowe-Evans EL, Kehoe DM (2004) Signal transduction during light-quality acclimation in cyanobacteria: a model system for understanding phytochrome-response pathways in prokaryotes. Photochem Photobiol Sci 3:495–502

Su H-N, Xie B-B, Zhang X-Y, Zhou B-C, Zhang Y-Z (2010) The suptramolecular architecture, function, and regulation of thylakoid membranes in red algae: an overview. Photosynth Res 106:73–87

Yokono M, Akimoto S, Koyama K, Tsuchiya T, Mimuro M (2008) Energy transfer processes in *Gloeobacter violaceus* PCC 7421 that possesses phycobilisomes with a unique morphology. Biochim Biophys Acta 1777:55–65

Yokono M, Murakami A, Akimoto S (2011) Excitation energy transfer between photosystem II and photosystem I in red algae: larger amounts of phycobilisome enhance spillover. Biochim Biophys Acta 1807:847–853