Relationship Between Photochemical Quenching and Non-Photochemical Quenching in Six Species of Cyanobacteria Reveals Species Difference in Redox State and Species Commonality in Energy Dissipation

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Although the photosynthetic reaction center is well conserved among different cyanobacterial species, the modes of metabolism, e.g., respiratory, nitrogen and carbon metabolism and their mutual interaction, are quite diverse. To explore such uniformity and diversity among cyanobacteria, here we compare the influence of the light environment on the condition of photosynthetic electron transport through Chl fluorescence measurement of six cyanobacterial species grown under the same photon flux densities and at the same temperature. In the dark or under weak light, up to growth light, a large difference in the plastoquinone (PQ) redox condition was observed among different cyanobacterial species. The observed difference indicates that the degree of interaction between respiratory electron transfer and photosynthetic electron transport differs among different cyanobacterial species. The variation could not be ascribed to the phylogenetic differences but possibly to the light environment of the original habitat. On the other hand, changes in the redox condition of PQ were essentially identical among different species at photon flux densities higher than the growth light. We further analyzed the response to high light by using a typical energy allocation model and found that ‘non-regulated’ thermal dissipation was increased under high-light conditions in all cyanobacterial species tested. We assume that such ‘non-regulated’ thermal dissipation may be an important ‘regulatory’ mechanism in the acclimation of cyanobacterial cells to high-light conditions.

Keywords: Chlorophyll fluorescence measurements • Cyanobacteria • Photochemical quenching • Respiration • State transition • Thermal dissipation.

Abbreviations: NDH, NADPH dehydrogenase; NPQ, non-photochemical quenching; OCP, orange carotenoid protein; PC, phycocyanin; PFD, photon flux density; PQ, plastoquinone.

Introduction

Cyanobacteria are the first life form performing oxygenic photosynthesis and are the evolutionary origin of the chloroplast in plants. Although the photosynthetic machinery of photosynthetic reaction center complexes is almost identical between cyanobacteria and chloroplasts, metabolic interaction of photosynthesis with other cellular processes is fundamentally different. As a photosynthetic prokaryote, cyanobacteria do not have organelles and all the metabolic pathways could directly interact with one another. In particular, photosynthetic electron transport and respiratory electron transport shared several electron transfer components such as plastoquinone (PQ), the Cyt b$_fff$ complex and Cyt c (Aoki and Katoh 1982, Peschek and Schmelter 1982). This direct interaction between photosynthesis and respiration makes cyanobacterial photosynthesis different from photosynthesis of land plants. In the case of land plants, the PQ pool is oxidized in the dark, and gradually reduced along with the elevation in photon flux densities (PFDs). In the case of cyanobacteria, however, the PQ pool is already reduced in the dark due to respiratory electron transfer (Mullineaux and Allen 1986, Schreiber et al. 1995, Campbell and Oquist 1996). Low light illumination oxidizes, not reduces, the PQ pool in the case of cyano bacteria (Campbell and Oquist 1996, Campbell et al. 1998). A further increase in the PFD reduces the PQ pool again.

The PQ pool is one of the regulatory sites of photosynthetic electron transfer and energy dissipation. The redox state of the PQ pool or neighboring electron carriers is known to regulate state transition both in land plants and in cyanobacteria. Light energy absorbed by the PSII antenna complex may be directed either to the PSII reaction center complex or to the PSI reaction center complex, depending on the redox state of the PQ pool during the process of state transitions, both in land plants and in cyanobacteria. There are at least two different mechanisms in cyanobacterial state transition. In low-light-acclimated cells of cyanobacteria, the RpaC-dependent state transition is induced to maximize the efficiency of photosynthesis (Emlyn-Jones et al. 1999). On the other hand, the PsAK2-dependent state transition is induced to avoid photoinhibitory damage in high-light-acclimated cells (Fujimori et al. 2005). State transition seems to have a protective role in addition to a regulatory role in land plants too (Bellaﬁore et al. 2005).
The reduced PQ pool brings cyanobacterial cells into State 2 in the PQ pool (Mi et al. 1992, Mi et al. 1994, Ogawa et al. 2013). From respiratory NADPH dehydrogenase (NDH) complexes to the PQ pool is not oxidized in the dark due to electron flow as PQ (Aoki and Katoh 1982, Peschek and Schmetterer 1982). Electron transport in cyanobacteria share several components such as PQ among different cyanobacterial species, the modes of metabolism and their mutual interaction are quite diverse. There are few studies comparing the interaction of photosynthesis and other metabolic pathways or light energy dissipation among different species of cyanobacteria grown under certain conditions. Here we examined the effect of respiration of photosynthetic electron transfer and energy dissipation in six cyanobacterial species using Chl fluorescence measurements. We found that the redox state of the PQ pool in the dark is totally different among different cyanobacterial species. Furthermore, the correlation between the redox state of PQ and the yield of energy dissipation in the dark to the growth light range is different from that in growth light to the high light range. Apparently, some process of energy dissipation was induced only under high-light conditions. The results obtained here demonstrate species differences in the redox state and species commonality in energy dissipation in cyanobacteria.

Since photosynthetic electron transport and respiratory electron transport in cyanobacteria share several components such as PQ (Aoki and Katoh 1982, Peschek and Schmetterer 1982), the PQ pool is not oxidized in the dark due to electron flow from respiratory NADPH dehydrogenase (NDH) complexes to the PQ pool (Mi et al. 1992, Mi et al. 1994, Ogawa et al. 2013). The reduced PQ pool brings cyanobacterial cells into State 2 in the dark, decreasing fluorescence yield. This decrease of fluorescence yield is clearly observed in Anabaena sp. cells grown at 30°C and bubbled with air under continuous illumination at 200 μmol m⁻² s⁻¹ for 24 h. The measurement was initiated by applying a modulated measuring light (ML on) after 15 min dark acclimation of the cells. Shortly after the start of the measurement, a 0.8 s pulse of saturating light was applied to close PSII temporarily for the determination of F₇₀₀. After actinic light was turned on (AL on), the level of fluorescence changed slightly and then settled down to the steady state (Fₛ) during 5 min under each PFD. At the end of each 5 min period, saturating pulses were given to observe the recovery kinetics of F₇₀₀ in the absence of actinic light. Finally, DCMU was added in the presence of actinic light to bring cells fully to State 1 for the determination of F₉₅. The black bar indicates the absence of actinic light. Each number in the white bar indicates the respective PFDs of actinic light (μmol m⁻² s⁻¹).

**Results**

Since photosynthetic electron transport and respiratory electron transport in cyanobacteria share several components such as PQ (Aoki and Katoh 1982, Peschek and Schmetterer 1982), the PQ pool is not oxidized in the dark due to electron flow from respiratory NADPH dehydrogenase (NDH) complexes to the PQ pool (Mi et al. 1992, Mi et al. 1994, Ogawa et al. 2013). The reduced PQ pool brings cyanobacterial cells into State 2 in the dark, decreasing fluorescence yield. This decrease of fluorescence yield is clearly observed in Fig. 1, in which the result of quenching analysis of Anabaena sp. PCC 7120 is shown. The level of maximum fluorescence of the dark-acclimated cells upon a saturating pulse (Fig. 1, closed arrowhead), designated as F₉₅, was low, compared with the F₉₅ value (open arrow) obtained with illumination in the presence of DCMU, which locked the cells into State 1. When the PFD of the actinic light was raised in a stepwise manner (31.5, 167, 562 and 1,190 μmol m⁻² s⁻¹), the maximum fluorescence level (F₉₅, open arrowheads) increased once at around the growth light PFD (200 μmol m⁻² s⁻¹), and decreased again under higher PFD (Fig. 1).

This change in the levels of F₉₅ reflects the change in the NPQ of Chl fluorescence. ΦNPQ, a parameter which represents non-photochemical quenching by regulated thermal dissipation, was high in the dark in the case of Anabaena sp. but decreased around the growth light PFD and increased again under higher PFD.
under higher PFD, giving a concave dependence on actinic PFD (Fig. 2, filled circles; see also Campbell and Öquist 1996). Very similar tendencies were observed in Nostoc sp. HK-01 (filled triangles) and Synechocystis sp. PCC6803 (open circles). Arthrospira platensis NIES-39 (open triangles) also showed a more or less similar tendency, but Nostoc punctiforme ATCC29133 (red circles) and Acaryochloris marina MBIC11017 (red triangles) showed a rather linear dependence starting at a low NPQ in the dark (i.e. no actinic light). Apparently, the levels of NPQ under high actinic PFD are similar among cyanobacterial species, while those under low PFD are variable depending on the species.

Cyanobacterial NPQ is ascribed either to state transition (Mullineaux and Allen 1990, Campbell and Öquist 1996) or to thermal dissipation by OCP (Kirilovsky 2007). Since red light from a light-emitting diode (LED; peak at 660 nm) was used here for actinic light, it is not necessary to take the involvement of OCP into consideration. To explore the relationship between state transition and NPQ in the dark, the index of State 2 in the dark was estimated from the relative peak height of the Chl fluorescence spectra determined at 77 K. The degree of state transition was estimated as the difference in the ratio of PSI fluorescence and PSII fluorescence determined at 77 K between the dark-acclimated condition and the State 1-induced condition. Mean values of at least three independent measurements and their respective SD are presented. The line in the figure represents the linear fitting result ($R^2 = 0.95$).

We next examined the relationship between the $\Phi_{\text{NPQ}}$ and qP, another parameter representing the redox state of the PQ pool, during the course of increasing actinic light in six cyanobacterial species to examine the resulting change in $\Phi_{\text{NPQ}}$ (Fig. 4). Apparently, the overall relationship was not linear in Anabaena sp. (filled circles), Nostoc sp. HK-01 (filled triangles) and A. platensis NIES-39 (open triangles). From darkness to the growth light condition, the increase of qP (i.e. oxidation of the PQ pool) accompanied the decrease of $\Phi_{\text{NPQ}}$ (i.e. transition to State 1) in these three cyanobacterial species. From growth light to the high-light condition, however, qP decreased again accompanying the increase of the $\Phi_{\text{NPQ}}$ but with a different slope. As a result, the overall relationship showed a V-shape consisting
of two lines with different slopes. In the case of Synechocystis sp. PCC6803, the increase in qP was not apparent (open circles). As for the other two cyanobacterial species, i.e. N. punctiforme ATCC29133 (red circles) and A. marina MBIC11017 (red triangles), the data point obtained in the dark is already near the bottom of the V-shape, and the monotonic increase of $F_{v}/F_{m}$ (angle), the data point obtained in the dark is already near the $A. marina$ ATCC29133 (red circles) and for the other two cyanobacterial species, i.e. $PCC6803$, the increase in qP was not apparent (open circles). As for $Arthrospira platensis$ and $A. platensis NIES-39$, the dashed lines just connect data points to show the course of the increase in actinic PFD.

Finally, the cause of the difference in $F_{v}/F_{m}$ among cyanobacterial species was examined. $F_{v}/F_{m}$ values showed some variation dependent on the cyanobacterial species ($Table 1$). Although the value ($F_{m}/F_{o}$) for $A. marina$ MBIC11017 was greatly affected by the degree of state transition, $F_{v}/F_{m}$ values determined in the presence of DCMU were not affected by the state transition ($Table 1$), since the cells were locked in State 1 in the presence of DCMU. $F_{v}/F_{m}$ is well known to be a parameter representing the maximum quantum yield of PSII but was reported to be affected by the cellular content of phycobiline (Campbell et al. 1998). We determined the absorbance spectra of intact cells ($Fig. 6$) to calculate the phycocyanin (PC)/Chl ratio for the six cyanobacterial species. When the correlation between $F_{v}/F_{m}$ and the PC/Chl ratio was plotted, a negative correlation was observed ($Fig. 7$), suggesting the variation of the PC content as the cause of the difference in $F_{v}/F_{m}$. In other words, although the six cyanobacteria were grown at the same temperature and the same PFD, the pigment composition ratio was different among the species and the variation in the pigment composition among the species caused the species difference of $F_{v}/F_{m}$.

**Discussion**

Cyanobacterial diversity observed in the PQ reduction in the dark

It is well known that the PQ pool is mostly reduced in dark-acclimated cells of cyanobacteria (Campbell et al. 1998), which is quite different from the case of land plants. Apparently, such a dark reduction of the PQ pool is not observed in all cyanobacterial species. Among the six cyanobacterial species examined in this study, the PQ pool was reduced to different extents in four species but it is oxidized in the dark-acclimated cells of $N. punctiforme$ ATCC29133 and A. marina MBIC11017. All the cells are grown at the same temperature and under the same PFD, so the difference among species could not be ascribed to the difference in growth conditions, but to the intrinsic nature of the cyanobacterial species.
Since the two *Nostoc* species, *Nostoc punctiforme* ATCC29133 and *Nostoc* sp. HK-01, showed totally different redox conditions of their PQ pool in the dark, the diversity could not be explained solely by phylogenetic factors. *Nostoc punctiforme* ATCC29133 was first isolated as a symbiont from a root of a gymnosperm cycad in Australia (Rippka and Herdman 1992). *A. marina* MBIC11017, another cyanobacterium with an oxidized PQ pool in the dark, was also originally isolated from inside of an ascidian in Palau (Miyashita et al. 1996). The light environments of the original habitats of these cyanobacteria should be rather weak, and adaptation to such environments may be the evolutionary cause of the PQ pool characteristics. The life style as a symbiont may be also considered as the cause of PQ oxidation in the dark, but it is difficult to assume such a concrete connection between them.

As for the actual mechanism of the variation in the redox condition of the PQ pool, there are three possibilities: first, the redox state of the cytosol may affect the condition of the PQ pool. For example, Mi et al. (1994) showed that dark-starved cells of *Synechocystis* sp. PCC6803 had an oxidized PQ pool that might have resulted from starvation of the respiratory substrates in the cytosol. In our experiments, however, the cells were grown photoautotrophically under continuous light so that the concentration of respiratory substrates should not be particularly different among species. Secondly, the difference in the activities of the NDH complex upstream of the PQ pool may result in the variation in the PQ redox. Since the inactivation of the NDH complex resulted in the oxidation of the PQ pool (Ogawa et al. 2013), the difference in the activity of the NDH complex would be a plausible candidate for the cause of the species difference of PQ redox. Thirdly, the difference in Cyt c oxidase (COX) activity in the dark may contribute to the variation in PQ redox. It was reported that *Synechocystis* sp. PCC6803 could grow heterotrophically in the dark when a mutation was introduced in the Cyt cM gene (Hiraide et al. 2014). It was implied that Cyt cM would act as a negative regulator for COX activity in cyanobacteria lacking the ability to grow heterotrophically in the dark. In cyanobacteria with the ability to grow heterotrophically in the dark, e.g. *N. punctiforme*, COX activity would be kept relatively high in the dark, resulting in the oxidation of the PQ pool in the dark. The actual mechanism, however, should be tested in the near future.

### Regulatory energy dissipation is not limited to NPQ in cyanobacteria

As discussed above, the PQ pool is in a reduced condition which could be monitored as a low qP level in the dark-acclimated cyanobacterial cells, and the PQ reduction induced the State 2 condition which could be monitored as high $\Phi_{NPQ}$. Upon the increase of actinic illumination, the PQ pool is gradually oxidized (i.e. qP is gradually increased), inducing transition to State 2.

### Table 1 Photosynthetic parameters and the PC/Chl ratio of the six cyanobacterial species

| Species              | $F_v/F_m$ | $(F_m' - F_o)/F_m'\text{ dark}$ | PC/Chl |
|----------------------|----------|---------------------------------|--------|
| *Acaryochloris marina* | 0.669 ± 0.029 | 0.617 ± 0.035                  | –      |
| *Arthrospira platensis* | 0.642 ± 0.017 | 0.503 ± 0.019                  | 4.33 ± 0.04 |
| *Nostoc* sp.         | 0.599 ± 0.010 | 0.369 ± 0.054                  | 4.80 ± 0.27 |
| *Nostoc punctiforme* | 0.591 ± 0.030 | 0.510 ± 0.039                  | 5.76 ± 0.12 |
| *Anabaena* sp.       | 0.575 ± 0.009 | 0.379 ± 0.023                  | 6.72 ± 0.46 |
| *Synechocystis* sp.  | 0.561 ± 0.022 | 0.389 ± 0.035                  | 8.03 ± 0.64 |

Values represent the average ± SD with at least three independent cultures.

**Fig. 6** Absorption spectra of the intact cells of six cyanobacterial species in the growth medium. Each absorption spectrum was normalized at the peak height of the respective highest peak. Averages of spectra with three independent cultures are presented.

**Fig. 7** The relationship between $F_v/F_m$ and the phycocyanin content. Mean values of at least three independent measurements and their respective SD are presented.
1 that could be monitored as the decrease in $\Phi_{NPQ}$. Until the actinic light reached the growth light level, the relationship between $qP$ and $\Phi_{NPQ}$ is linear, suggesting that this process is a simple state transition to State 1 induced by the oxidation of the PQ pool. No other energy dissipation process was induced under the growth condition, since $\Phi_{I,D}$ was constant during this process. Although the initial levels of the PQ redox state in the dark varied among cyanobacterial species, the $qP$–$\Phi_{NPQ}$ relationships and $\Phi_{PSII}$–$\Phi_{NPQ}$ relationships were more or less similar, with the same slope between the dark condition and growth light condition. In this sense, *Synechocystis* sp. PCC 6803 is somewhat peculiar, showing a decrease in $\Phi_{NPQ}$ in the dark to growth light transition without apparent PQ oxidation. A similar change was observed by Campbell et al. (1998), but the mechanism of this change is unknown. Apparently, *Synechocystis* sp. PCC 6803 is not a typical cyanobacterium from the view of the relationship between PQ redox and state transition.

The reverse change was observed in the range from the growth light to high light, i.e. the level of $qP$ decreased while the level of $\Phi_{NPQ}$ increased again. Although the change in this range could be partly attributed to the reverse transition to State 2 induced by the reduction of the PQ pool, the slope of the $qP$–$\Phi_{NPQ}$ relationships (or $\Phi_{PSII}$–$\Phi_{NPQ}$ relationships) was different from that in the range from the dark condition to the growth light condition. The relationship between $\Phi_{PSII}$ and $\Phi_{NPQ}$ indicates that $\Phi_{I,D}$ was increased by high light together with the state transition induced by the reduction of the PQ pool. In many land plant species, the level of $\Phi_{I,D}$ was reported to be relatively constant with a wide range of actinic PFD, and thus $\Phi_{I,D}$ was regarded as ‘non-regulatory’ energy dissipation as fluorescence or as heat (Hendrickson et al. 2004, Zhang et al. 2011, Park 2013). In cyanobacteria, however, an increase of $\Phi_{I,D}$ was observed in all the species tested here, and the rates of the increase of $\Phi_{I,D}$ (i.e. the slopes of the data lines in the $\Phi_{PSII}$–$\Phi_{NPQ}$ relationships) were similar among species. Hendrickson et al. (2004) showed that $\Phi_{I,D}$ in *Vitis vinifera* increased along with the increase of actinic light to 500 μmol m$^{-2}$ s$^{-1}$ at 25°C, but not at 10°C. Since $\Phi_{I,D}$ decreased, not increased, in the higher actinic light range (750–2,000 μmol m$^{-2}$ s$^{-1}$) in their experiments, the mechanism of $\Phi_{I,D}$ regulation in land plants would be different from the one in the cyanobacteria observed here. Judging from the full recovery of $F_{m}'$ in the dark within 5–10 min (Fig. 1), significant photoinhibition was not induced by high light, which could be the cause of the apparent increase in $\Phi_{I,D}$. Furthermore, no persistent photoinhibition under the growth condition was induced, since the change in $F_{s}/F_{m}$ was mostly explained by the change in PC contents. Thus, photoinhibition is not the cause of the increase in $\Phi_{I,D}$.

It was reported that a passive increase of $\Phi_{I,D}$ under high light was observed in the M55 mutant of NDH-1 complexes, which is incapable of state transition (Schreiber et al. 1995). Similarly, an increase in $\Phi_{I,D}$ was observed in the PsbS mutant of rice plants, which is deficient in some mechanism of NPQ (Ishida et al. 2011, Ikeuchi et al. 2014). These results may suggest that the increase in $\Phi_{I,D}$ is induced in the absence of physiological energy dissipation. Since red actinic light was used in our study, OCP was not induced in our experimental conditions. Furthermore, the increase of $\Phi_{I,D}$ was reported under strong blue light in the OCP deletion mutant of *Synechocystis* sp. PCC6803, resulting in the decrease of $\Phi_{NPQ}$ but not in the change of $\Phi_{PSII}$ (Kusama et al. 2015). In our preliminary result, however, the V-shape relationship between $\Phi_{PSII}$ and $\Phi_{NPQ}$ was observed even when blue light was employed as actinic light. Unfortunately, blue light also acts as PSI light, resulting in oxidation of the PQ pool that complicated the interpretation of the results. The involvement of the OCP in the change of $\Phi_{I,D}$ should be further explored in the near future. It should also be noted that we employed rather a high PFD (200 μmol m$^{-2}$ s$^{-1}$) for the growth light. It was reported that there are at least two types of state transition in cyanobacteria. Psak2-dependent state transition was only induced in high-light-acclimated cells (Fujimori et al. 2005), while RpaC-dependent state transition is functional under low-light conditions (Emlyn-Jones et al. 1999). The lack of the RpaC-dependent state transition in high-light-acclimated cells may also bring about the increase of $\Phi_{I,D}$ in the absence of physiological energy dissipation.

In any event, the mechanism of the induction of $\Phi_{I,D}$ could not be ascribed solely to a passive increase, since the increase in $\Phi_{I,D}$ was observed in the actinic light range where the induction of NPQ was not saturated. The increase of $\Phi_{I,D}$ should lead to smaller energy allocation to photosynthetic electron transfer. Similarly to the case of NPQ, the induction of $\Phi_{I,D}$ that has been defined as ‘non-regulatory’ in the past may also contribute to the protection of the photosynthetic machinery from photoinhibition. Alternatively, the increase in $\Phi_{I,D}$ might protect PSI from photoinhibition that could be caused by the excessive electron flow from PSII. The observed increase in $\Phi_{I,D}$ must be some regulatory mechanism or at least some acclimatory response generally observed in cyanobacteria.

### Materials and Methods

#### Strains and growth conditions

*Synechocystis* sp. PCC6803, *Anabaena* sp. PCC7120, *N. punctiforme* ATCC29133, *Nostoc* sp. HK-01, *A. platensis* NIES-39 and *A. marina* MBIC11017 were grown at 30°C and bubbled with air under continuous illumination at 200 μmol m$^{-2}$ s$^{-1}$. *Acaryochloris marina* was grown in IMK medium in sea water (Nihon Pharmaceutical Co., Ltd.), *A. platensis* in SOT medium (Ogawa and Terui 1970) and the other species in BG-11 medium (Allen 1968). Cells of all the strains, except for *Nostoc* sp. HK-01, were sampled in the logarithmic phase. In the case of *Nostoc* sp. HK-01, cells were in a more or less aggregated form so that we could not judge whether this species was in the logarithmic phase, at least not simply from the measurements of optical density of the cells at 750 nm. For optical measurements, we suspended the cells of *Nostoc* sp. HK-01 to make as uniform a suspension as possible.

#### Fluorometer measurements

Chl fluorescence was measured with a pulse-amplitude fluorometer (WATER-PAM Waltz) as described earlier (Ogawa et al. 2013). Cells in 2 ml of liquid culture were dark-adapted for 15 min and the minimum fluorescence level $(F_{0})$ was determined with a measuring light (peak at 650 nm). A 0.8 s flash of saturating light was given to determine $F_{m}'$. Subsequently, red actinic light (peak at 660 nm) of different PFDs (31.5, 167, 562 and 1,190 μmol m$^{-2}$ s$^{-1}$) was applied in a stepwise manner every 5 s to monitor fluorescence under the steady-state condition $(F_{s})$. At the end of each step,
saturating light was applied to monitor the maximum fluorescence of the light-acclimated cells (Fm'). Then, actinic light was turned off and cells were relieved for 20 min from the effect of actinic light. Finally, cells were illuminated by actinic light for 4 min in the presence of 10 μM DCMU, and saturating light was applied to monitor the level of maximum fluorescence (Fm). The cell suspension was stirred at all times during the experiment. Fluorescence parameters were calculated as follows; qP = (Fm' – Fm)(Fm' – Fo') (Van Kooten and Snel 1990), ΦPSII = (Fm' – Fo')/Fm' (Bilger et al. 1995, Genty et al. 1989), ΦNPQ = Fm' – Fo = Fm' – Fo' and ΦPSI = Fo/Fm' (Hendrickson et al. 2004). Fm' was calculated as Fm' = F0m'(Fm/F0m') + (Fo/F0m'). Absorbance spectra Absorbance spectra were determined with a spectrophotometer (V-650; JASCO) equipped with an integrating sphere (ISV-722; JASCO) as described previously (Ogawa et al. 2013) at room temperature. The absorbance of the cell suspension was determined in a cuvette with a light path length of 5 mm. Each absorbance spectrum was normalized at the maximum absorbance in the respective spectrum. PC and Chl content were calculated as [PC] = 138.5 × A680 – 35 A649 and [Chl] = 14.97 × A680 – 0.615 × A665 according to Arnon et al. (1974).

77 K fluorescence emission spectra

77 K fluorescence emission spectra were determined with a fluorescence spectrometer (FP-8500; JASCO) with a low temperature attachment (PLU-830; JASCO) as described earlier (Ogawa et al. 2013). Cell suspensions of cyanobacterial strains were adjusted to a concentration of 2 × 10^8 cells ml^-1. Growth medium. The Chl concentration was determined by extraction with 100% methanol according to Grimme and Boardman (1972). Prior to the measurements, the cells were either dark-acclimated for 15 min or illuminated by using a light source (PICNRLX; NIPPON P-I) in the presence of 10 μM DCMU for 4 min. The samples were excited by 625 nm light (excitation slit width at 10 mm) for PC excitation. The fluorescence spectra were recorded with a fluorescence slit width of 2.5 nm and resolution of 0.2 nm. The spectra were averaged for three independent cultures and corrected for the sensitivity of the photomultiplier and the spectrum of light source using a secondary standard light source (ESC-842; JASCO).

Disclosures

The authors have no conflicts of interest to declare.

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