Characteristics of photosynthesis in cyanobacteria probed by chlorophyll fluorescence measurements

クロロフィル蛍光測定により明らかになったシアノバクテリアの光合成の特徴

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Contents

Abbreviations

CCM	carbon concentrating mechanism
DCMU	3-(3, 4-dichlorophenyl)-1, 1-dimethylurea
ETR	electron transport rate
Flv	flavodiiron protein
MV	methyl viologen
NDH-1	type 1 NAD(P)H dehydrogenase
OCP	orange carotenoid protein
OD	optical density
PAR	photosynthetic active radiation
PFD	photon flux density
PSI	Photosystem I
PSII	Photosystem II
SD	standard deviation
WT	the wild-type cells

Abbreviations concerned with chlorophyll fluorescence measurements

Fm	maximum fluorescence determined under oxidized plastoquinone conditions
Fm'	maximum fluorescence determined under reduced plastoquinone conditions
Fo	minimum fluorescence under oxidized plastoquinone pool conditions
Fo'	minimum fluorescence under reduced plastoquinone pool conditions
Fs	fluorescence under steady-state conditions
Fv/Fm	chlorophyll fluorescence parameter representing the maximum quantum yield of Photosystem II calculated as (Fm-Fo)/Fm
NPQ	chlorophyll fluorescence parameter representing the level of non-photochemical quenching calculated as (Fm-Fm')/Fm'
NPQ_{Dark}	NPQ determined with dark acclimated cells
NPQ_{LL}	NPQ determined with low light acclimated cells
$\Phi_{f,D}$	chlorophyll fluorescence parameter representing the energy distribution to non-regulated energy dissipation calculated as Fs/Fm
Φ_{NPQ}	chlorophyll fluorescence parameter representing the energy distribution to regulated energy dissipation calculated as Fs/Fm'-Fs/Fm
Φ_{PSII}	chlorophyll fluorescence parameter representing the effective quantum yield of electron transport in photosystem II (i.e. the energy distribution to photosynthesis) calculated as (Fm'-Fs)/Fm'
qN	chlorophyll fluorescence parameter representing the level of non-photochemical quenching calculated as 1-(Fm'-Fo')/(Fm-Fo)
qP	chlorophyll fluorescence parameter representing the level of photochemical quenching calculated as (Fm'-Fs)/(Fm'-Fo')
rETR	relative rate of electron transport

General Introduction

Photosynthesis is a process converting light energy absorbed by photosynthetic organisms to chemical energy. ATP and NADPH produced by photosynthetic electron transport (**Fig. 1**) are utilized to fix CO_2 to carbohydrate through the reductive pentose phosphate cycle, so called Calvin cycle. Carbohydrate produced by photosynthetic organisms is the basis of the primary production of the ecosystem on earth. Land plants certainly contribute to photosynthesis but nearly half of the total primary production on earth was reported to be produced by oceanic algae (Field et al. 1998). To understand the ecosystem and the global climate change on earth, the precise estimation of algal photosynthesis is quite important. Significance of algal photosynthesis is not limited to the influence to the ecosystem on earth. The ability of algae to convert light energy to chemical energy is expected to be used for supplying the energy resources for future mankind. The mechanism of oil production by micro algae, such as *Botryococcus braunii*, has been extensively studied to replace the limited resources of fossil fuels by algal oil (Chisti 2007; Schenk et al. 2008). Compared with the bioethanol produced from land crops, there are several advantages of the biofuel produced from micro algae. It is possible to harvest algal oil all year round and to utilize sea water as culture medium. On the other hand, there are also problems, such as a high cost or the necessity of CO_2 supply for high efficiency production. Precise estimation of algal photosynthesis is also essential for effective production of biofuel.

There are several ways to evaluate photosynthesis. In the past, photosynthesis has been determined by gas-exchange measurements, such as CO_2- or O_2-exchange. In recent years, chlorophyll fluorescence has been utilized for a non-destructive and easy methods to determine photosynthesis. Already in 1931, it had been reported that a transient kinetics of chlorophyll fluorescence following illumination of dark-acclimated leaves reflected the condition of photosynthesis (Kautsky and Hirsch 1931). Subsequently, chlorophyll fluorescence measurements have been developed as an indicator for photosynthetic electron transport in photosynthetic organisms (Krause and Weis 1991; Govindjee 1995; Campbell et al. 1998). The general principle of chlorophyll fluorescence measurement could be briefly described as follows (the details are described in Introduction of **Chapter III**).

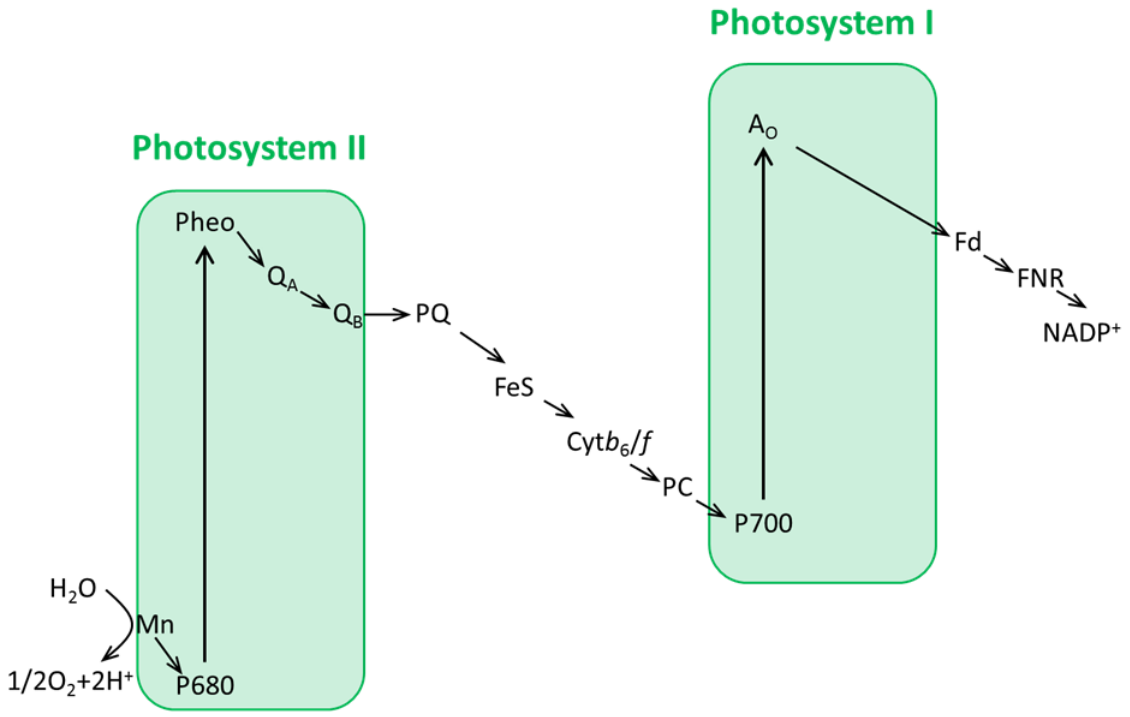

Fig. 1 A scheme of electron transport chain of photosynthesis. **Mn**, manganese cluster (the site of photosynthetic oxygen evolution); **P680**, the reaction center chlorophyll of PSII; **Pheo**, the primary electron acceptor pheophytin of PSII; $\mathbf{Q_A}$, the secondary electron acceptor quinone of PSII; $\mathbf{Q_B}$, the terminal electron acceptor plastoquinone of PSII; PQ, plastoquinone; **FeS**, an iron-sulfur cluster; **Cytb_6/f**, cytochrome b_6/f complex; **PC**, plastocyanin (a type I copper protein which transports electron from cytochrome *f* to P700); **P700**, the reaction center chlorophyll of PSI; $\mathbf{A_0}$, the primary electron acceptor chlorophyll of PSI; **Fd**, ferredoxin (an iron-sulfur protein); **FNR**, ferredoxin:$NADP^+$ oxidoreductase

Not all the light energy absorbed by chlorophyll is utilized for photosynthesis, and the excess energy is either dissipated as heat or emitted as fluorescence (**Fig. 2**). In other words, the total absorbed energy is equal to the sum of the energy utilized for photosynthesis, heat dissipation and fluorescence emission. Since photosynthesis competes with heat dissipation and fluorescence emission, quenching of fluorescence, i.e. the decrease in the yield of fluorescence, is caused by the increase in energy utilization for photosynthesis (photochemical quenching) and/or by the increase in heat dissipation

(non-photochemical quenching). Since most of chlorophyll fluorescence is emitted from chlorophylls of Photosystem II (PSII) at room temperature (Krause and Weis 1984; Krause and Weis 1991), information of PSII efficiency could be obtained from chlorophyll fluorescence if we can distinguish the two causes of the decrease in chlorophyll fluorescence yield, i.e. photochemical quenching and non-photochemical quenching.

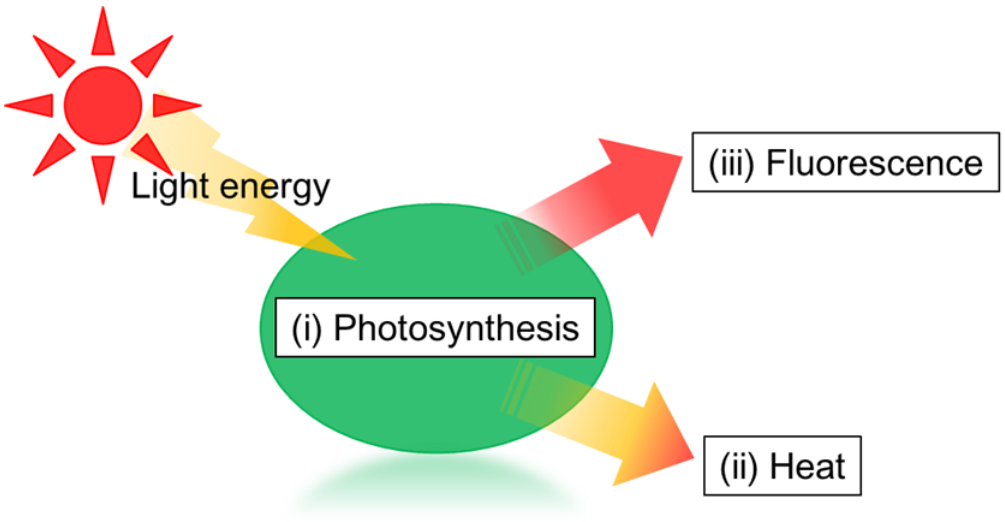

Fig. 2 A scheme of the fate of absorbed light energy by chlorophylls. Absorbed energy is either (i) utilized for photosynthesis, (ii) dissipated as heat, or (iii) emitted as fluorescence.

The problem to distinguish the two quenching mechanisms had been resolved by introducing a saturating pulse. Application of the light pulse, which is strong enough to suppress photochemical quenching through full reduction of Q_A, the electron acceptor of PSII (see **Fig. 1**), but is too short to induce additional non-photochemical quenching, gives only information of non-photochemical quenching developed before the application of light pulse. Once the degree of non-photochemical is determined, the degree of photochemical quenching can be also calculated. This method, so called quenching analysis, has been successfully applied to many photosynthetic organisms in the combination with Pulse Amplitude Modulation (PAM) chlorophyll fluorometer, in which the modulated measuring light was employed to increase sensitivity and to exclude the direct effect of actinic light on fluorescence signal (Schreiber 1986; Schreiber et al. 1986) (**Fig. 3**).

However, the application of PAM analysis to micro algae is sometimes complicated, reflecting the fact that PAM chlorophyll fluorescence measurement has been originally developed for land plants (Schreiber et al. 1995; Campbell et al. 1998). Especially in cyanobacteria that are photosynthetic prokaryotes, problems in PAM chlorophyll fluorescence measurement is more conspicuous (Campbell et al. 1998), since cyanobacteria have no organelle and photosynthesis could be affected by the condition of other metabolic pathways, possibly resulting in the change of chlorophyll fluorescence. In particular, cyanobacterial respiratory electron transport chain shares several components such as plastoquinone (PQ) with photosynthetic electron transport chain (Aoki and Katoh 1982; Peschek and Schmetterer 1982; see the scheme in **Fig. 4**). In the dark where photosynthesis is not conducted and respiration dominates, the PQ pool is reduced by the type 1 NAD(P)H dehydrogenase (NDH-1) complex in the respiratory chain in cyanobacteria, while the pool is oxidized in land plants. Therefore, different methods of PAM chlorophyll fluorescence measurement from those in land plants must be adopted in cyanobacteria to eliminate the influence of respiration on photosynthesis (Campbell and Öquist 1996; Campbell et al. 1998). Another factor complicating PAM chlorophyll fluorescence measurement in cyanobacteria is fluorescence from pigments except for chlorophylls binding to PSII. One of the cyanobacterial pigments disturbing PAM chlorophyll fluorescence measurement is phycobilins, which is a light-harvesting antenna for PSII and highly fluorescent (Campbell et al. 1998; El Bissati and Kirilovsky 2001). In addition to phycobilins, chlorophylls binding to Photosystem I (PSI) might also affect PAM chlorophyll fluorescence signals, since PSI binds up to 90% of the total chlorophylls and the ratio of PSI/PSII is high in cyanobacteria compared to land plants (Campbell et al. 1996; Campbell et al. 1998). Since the yield of fluorescence from phycobilins or PSI chlorophylls does not depend on the condition of PSII and is constant, the condition of photosynthesis evaluated by the change in fluorescence yield upon saturating pulse could be misevaluated (Campbell et al. 1998).

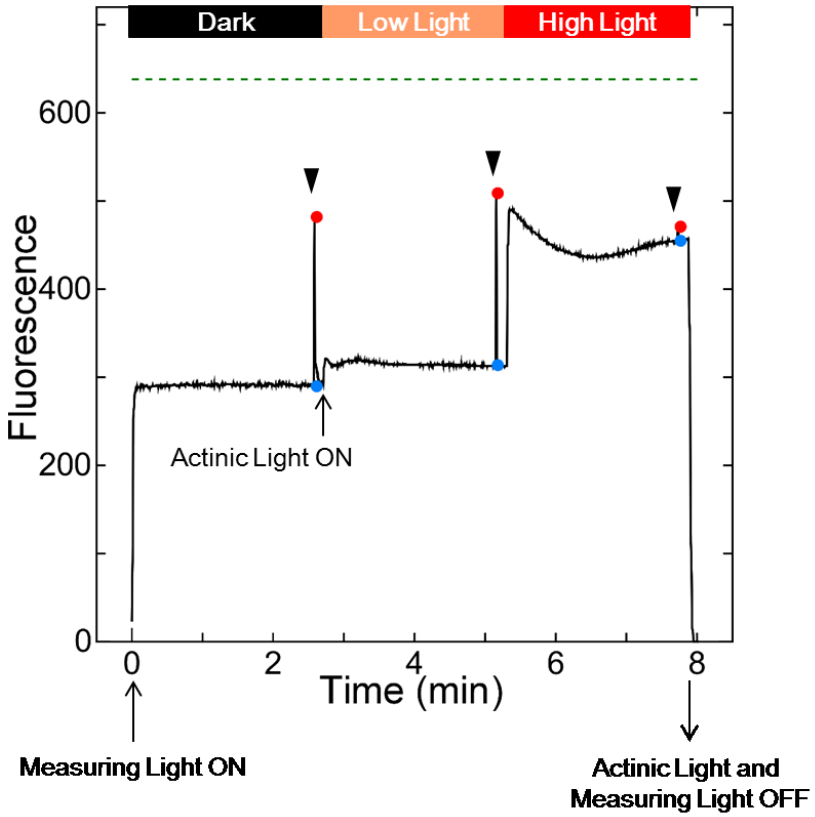

Fig. 3 An example of quenching analysis by PAM chlorophyll fluorometer in the cyanobacterium *Synechocystis* sp. PCC 6803. Fluorescence level is recorded along with the time course (black solid line). Black inverted triangles represent the time of the illumination by saturating pulse. Fluorescence level is increased from Fs level (represented by blue circles) to Fm' level (represented by red circles) followed by the illumination with saturating pulse, upon suppression of photochemical quenching. Green dashed line represents the Fm level determined under illumination in the presence of DCMU.

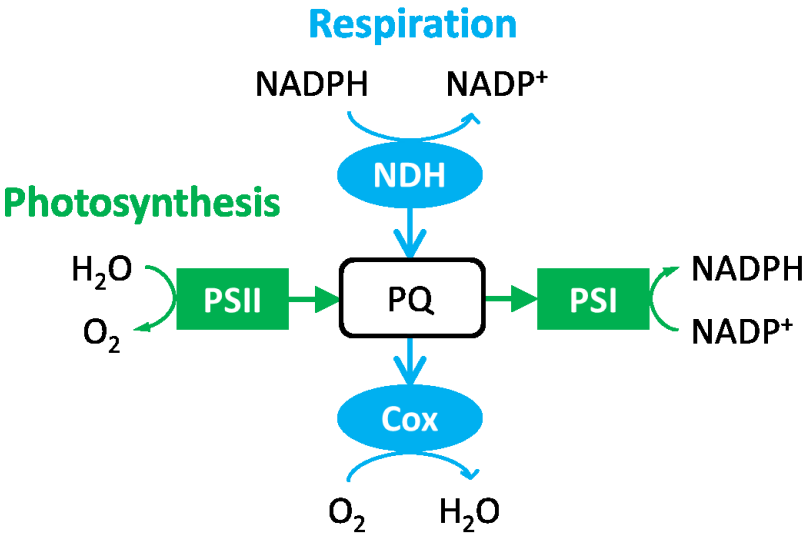

Fig. 4 Scheme of the electron transport chain between photosynthesis and respiration in cyanobacteria. PSII, Photosystem II; PSI, Photosystem I in photosynthetic electron transport chain. NDH, type 1 NAD(P)H dehydrogenase; Cox, cytochrome *c* oxidase in respiratory electron transport chain. Photosynthetic electron transport chain shares several components such as plastoquinone (PQ) with respiratory chain.

In spite of the problems of PAM chlorophyll fluorescence measurement in cyanobacteria as described above, many researchers in the field of algal photosynthesis are using PAM chlorophyll fluorescence technique. Actually, among the papers published in last year in the field of algal photosynthesis (~3,900 papers), approximately 20% papers used or at least refer to PAM fluorescence measurements. Under such circumstances, it is important to establish the method to accurately estimate algal photosynthesis by chlorophyll fluorescence measurements. To achieve the goal, it is essential to understand the influence of not only photosynthesis but other metabolic pathways on chlorophyll fluorescence in algae and cyanobacteria. In this study, I (1) investigated interactions between photosynthesis and other metabolic pathways, (2) examined the influence of the interactions on chlorophyll fluorescence measurement, and (3) developed solutions of problems of the chlorophyll fluorescence measurement in cyanobacteria. Among cellular metabolic pathways in cyanobacteria, I focused on the two, respiration and CO_2 concentrating mechanism (CCM), that are closely involved in photosynthetic

electron transport; respiration shares several components of electron transport chain with photosynthesis and inorganic carbon concentrated in cell by CCM is fixed by utilization with NADPH produced by photosynthetic electron transport. I described the influence of respiration (**Chapter I**) and CCM (**Chapter II**) on photosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803, and finally gave solutions of problems of the chlorophyll fluorescence measurement caused by the interactions among cellular metabolic pathways in cyanobacteria (**Chapter III**).

Chapter I

Influence of Respiration on Photosynthesis

Introduction

There are always interactions among cellular metabolic pathways, although each metabolic pathway such as photosynthesis or respiration is often independently described in textbooks. Sublocalization of the pathways, e.g. photosynthesis in chloroplasts and respiration in mitochondria, does not completely prevent interactions between them. In the case of prokaryotes, the situation is more direct and pronounced, with no organelle for sublocalization of each metabolic pathway. For example, the cyanobacterial photosynthetic electron transport chain chares several components, i.e. plastoquinone (PQ), cytochrome b_6/f complexes and plastocyanin, with the respiratory electron transport chain (Aoki and Katoh 1982; Peschek and Schmetterer 1982; see also **Fig. 4** in **General Introduction**). There should be a similar interaction among other metabolic pathways, albeit in a less direct way compared with the case of respiration and photosynthesis.

The interaction of photosynthesis and respiration in cyanobacteria was originally proposed 60 years ago based on the inhibitory effect of light on the respiratory oxygen uptake (Brown and Webster 1953; Hoch et al. 1963; Jones and Myers 1963). The effect of respiration on the redox state of photosynthetic electron carriers is directly shown by the determination of the absorption kinetics of cytochrome b_6/f (Hirano et al. 1980) or P700 (Nanba and Katoh 1984) in a thermophilic cyanobacterium. The presence of respiratory electron flow in the dark also prevents the full oxidation of PQ (Schreiber et al. 1995; Campbell and Öquist 1996; Sonoike et al. 2001). It was demonstrated that the redox state of the photosynthetic electron transfer chain is modified in the absence of type 1 NAD(P)H dehydrogenase (NDH-1) complexes in the respiratory chain, based on the absorption kinetics of P700 (Mi et al. 1992) or on the fluorescence kinetics of chlorophyll (Mi et al. 1994). Thus, it is no wonder that chlorophyll fluorescence kinetics are affected by the disruption of genes encoding subunits of NDH-1. However, it is noteworthy that the disruptant of *ndhF1* gene showed the most prominent phenotype in the induction

kinetics of chlorophyll fluorescence among the disruptants of genes, including those responsible for photosynthesis, when I searched the database, Fluorome (http:///www.photosynthesis.jp/flurome/).

Fluorome is the database of the dark-light induction kinetics of chlorophyll fluorescence, so-called Kautsky induction (an example is presented in **Fig. I-9**). The measurements of chlorophyll fluorescence including the Kautsky curve has been widely used for the analysis of photosynthesis (Govindjee 1995). Previously, a Kautsky induction curve database of cyanobacterial disruptants of 500 genes created by random mutagenesis using transposon was made by Ozaki et al. (2007). Since high-throughput analysis was aimed at in the study, each disruptant used for the Fluorome analysis was not necessarily segregated to guarantee the full inactivation of each gene. The effect of the gene disruption on chlorophyll fluorescence kinetics could be quantitatively analyzed based on the squared deviation from the kinetics of the wild-type cells (Ozaki and Sonoike 2009). Among the 500 disruptants analyzed in the previous work, as well as among 750 disruptants analyzed in the updated database (Fluorome ver. 2.01 updated on October 28, 2011), the most prominent difference was observed in the disruptant of the *ndhF1* gene. NdhF1 is a subunit of NDH-1L complexes serving for respiratory electron transfer (Schluchter et al. 1993; Battchikova and Aro 2007). It was rather unexpected that the disruption of a gene involving respiration showed the most prominent phenotype, considering that many genes involved in photosynthesis were also included in the Fluorome database. It could be assumed that the explicit phenotype of the *ndhF1* disruptant would be due to the specific interaction between respiration and photosynthesis.

In this chapter, I revealed the mechanism by which the genes involved in respiration could affect chlorophyll fluorescence to the greatest degree. Furthermore, the present study showed that the misevaluation of photosynthesis could be caused by modified chlorophyll fluorescence, although chlorophyll fluorescence is widely used for the estimation of the efficiency and activity of photosynthesis. I found that the defect in respiration induced a large modification in chlorophyll fluorescence spectra through the state transition, the mechanism of energy redistribution between two photosystems (**Fig. 5**), as leverage. The estimation of the photosynthetic rate of cyanobacteria by chlorophyll fluorescence measurements requires careful evaluation when the

redox state of the PQ pool is modified by the defect in respiration.

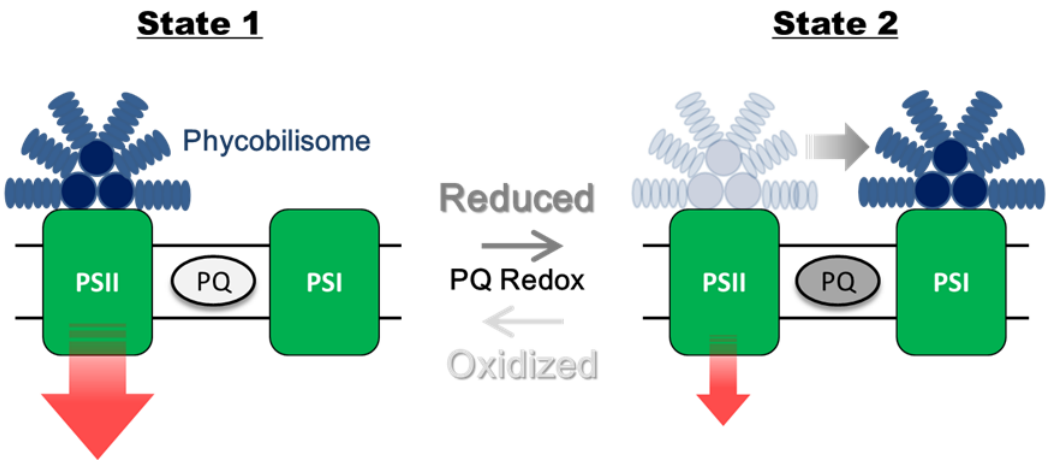

Fig. 5 A scheme of state transition, which is the mechanism regulating distribution of excitation energy between Photosystem II (PSII) and Photosystem I (PSI), in cyanobacteria. Movement of phycobilisome, the light-harvesting antenna, between PSII and PSI is involved in state transition, which is regulated by the redox state of plastoquinone (PQ) in cyanobacteria (Mullineaux and Allen 1986; Mullineaux et al. 1997). When PSI is predominantly excited and the PQ pool is oxidized, phycobilisome binds to PSII and distributes excitation energy to PSII, resulting in high yield of chlorophyll fluorescence, since most of chlorophyll fluorescence is emitted from chlorophylls of PSII at room temperature (Krause and Weis 1984; Krause and Weis 1991). On the other hand, when PSII is more excited and the PQ pool is reduced, phycobilisome migrates to PSI and distributes more energy to PSI, resulting in low yield of chlorophyll fluorescence.

Results

The disruption of *ndhF1* gene in the cells of *Synechocystis* sp. PCC 6803 induced a drastic change in the chlorophyll fluorescence kinetics upon illumination. The relative height of the fluorescence peak at around 0.4 s after the onset of actinic light illumination observed in the *ndhF1* disruptant is far higher than that of any other disruptants (Fluorome: The Cyanobacterial Chlorophyll Fluorescence Database, see also **Fig. I-9** and compare red lines

with black lines) when fluorescence kinetics are searched in Fluorome, the cyanobacterial chlorophyll fluorescence database. The relative change from Fo (initial fluorescence level) to Fp (peak fluorescence level), i.e. (Fp-Fo)/Fp, is presumably supposed to reflect photosynthetic efficiency similarly to the case of Fv/Fm calculated as (Fm-Fo)/Fm. Thus, the large (Fp-Fo)/Fp in the *ndhF1* disruptant might indicate a higher photosynthetic efficiency in the *ndhF1* disruptant than in the wild-type cells, which is rather surprising.

Table I-1 Photosynthetic parameters determined by chlorophyll fluorescence measurements of cells of the wild-type and the *ndhF1* disruptant under the growth light condition (50 µmol m^{-2} s^{-1}).

Condition	Parameter	Wild-type cells	Δ*ndhF1*
Dark, Light/DCMU	Fv/Fm*	0.510 ± 0.033	0.616 ± 0.009
Dark acclimated	Φ_{PSII}	0.391 ± 0.042	0.551 ± 0.013
	Φ_{NPQ}	0.119 ± 0.015	0.066 ± 0.005
	$\Phi_{f,D}$	0.490 ± 0.033	0.384 ± 0.009
Light acclimated (growth light)	Φ_{PSII}	0.406 ± 0.042	0.519 ± 0.009
	Φ_{NPQ}	0.107 ± 0.019	0.061 ± 0.005
	$\Phi_{f,D}$	0.487 ± 0.024	0.420 ± 0.005
	qP	0.879 ± 0.021	0.890 ± 0.006
	Fv'/Fm'	0.402 ± 0.053	0.560 ± 0.013
	qN	0.259 ± 0.031	0.173 ± 0.010

*Fm was determined under illumination in the presence of DCMU.

The photosynthetic parameter, Fv/Fm, was actually higher in the *ndhF1* disruptant than in the wild-type cells. Φ_{PSII}, the effective quantum yield of electron transport, was also higher in the *ndhF1* disruptant under the growth light condition (50 µmol m^{-2} s^{-1}) (**Table I-1**). The increase in quantum yield in the *ndhF1* disruptant was also observed under other light conditions than the growth light condition. With increasing actinic photon flux densities (PFD), Φ_{PSII} decreased both in the wild-type cells and in the *ndhF1* disruptant due to

the closure of Photosystem II (PSII) reaction center (**Fig. I-1**). The *ndhF1* disruptant always showed higher photosynthetic efficiency than the wild-type cells, with a smaller difference under higher actinic light (**Fig. I-1**). In other words, the *ndhF1* disruptant showed higher photosynthesis judging from the fluorescence measurements.

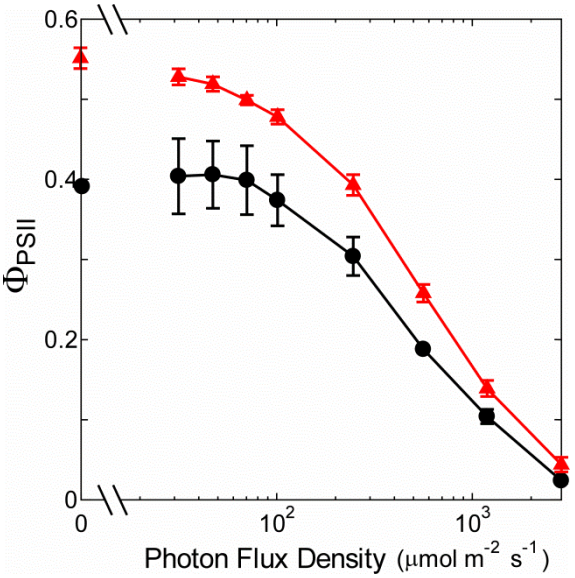

Fig. I-1 Actinic light dependence of Φ_{PSII}, a chlorophyll fluorescence parameter indicating the effective quantum yield of electron transport. Cells were grown at 30°C and bubbled with air under continuous illumination at 50 μmol m^{-2} s^{-1} for 24 h. Black circles, the wild-type cells; red triangles, the *ndhF1* disruptant. Vertical bars indicate the SD (n=3).

The photosynthetic oxygen-evolving rate determined by oxygen electrode, however, indicates that the photosynthetic activity is higher in the wild-type cells than in the *ndhF1* disruptant irrespective of the PFD of the actinic light (**Fig. I-2A**). The rate of oxygen evolution under saturated light conditions in the *ndhF1* disruptant was about half of that in the wild-type cells as well as under light-limiting conditions. The results of the photosynthetic oxygen-evolving rate suggest that the *ndhF1* gene disruption induces a decrease in photosynthetic activity rather than an increase. The relative rate of electron transport (rETR) was estimated from chlorophyll fluorescence measurements and compared with the rate of oxygen evolution (**Fig. I-2B**). The rETR could be calculated as the product of Φ_{PSII} and PFD of the actinic light. Actinic light dependence of oxygen evolution (**Fig. I-2B**, filled circles) and that of rETR (open circles) was similar in the wild-type cells. In the *ndhF1* disruptant, however, the rate of oxygen evolution was considerably lower than the corresponding rETR (**Fig. I-2B**, red triangles). The oxygen-evolving activity is shown on a chlorophyll basis in **Fig. I-2**, but the situation is the same if the

activity is shown on an optical density (OD) basis (**Fig. I-3**). To exclude the possibility of secondary mutation in the original *ndhF1* disruptant, I determined the oxygen evolution of another deletion mutant of *ndhF1* made by Ohkawa et al. (2000). The results are basically the same: lower oxygen evolution than the wild-type cells (**Fig. I-4A**) and a higher rETR than the wild-type cells (**Fig. I-4B**). The apparent increase of the Fv/Fm, Φ_{PSII} or rETR in the *ndhF1* disruptant (**Table I-1**, **Figs. I-1 and 2B**) could not be simply due to the increase in photosynthesis.

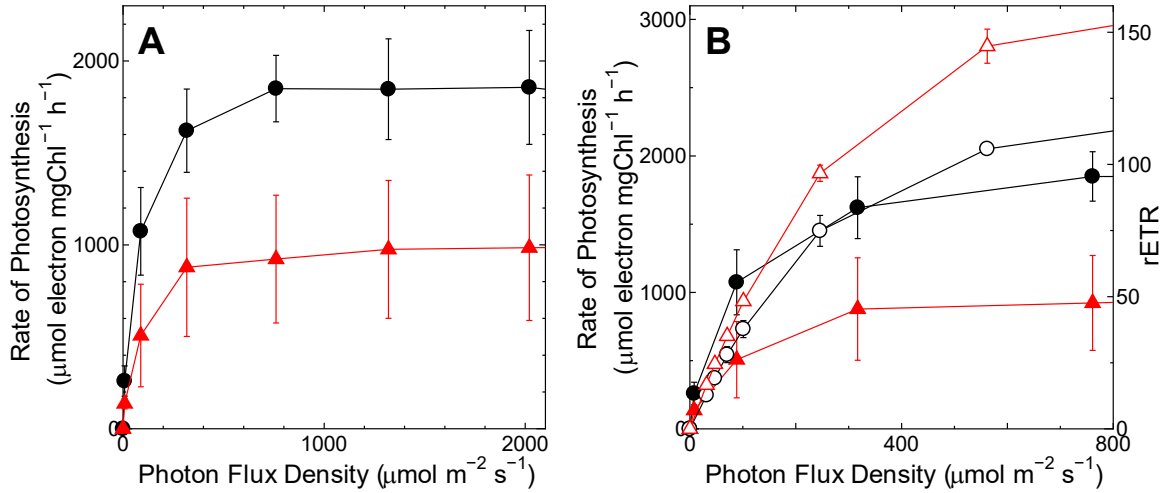

Fig. I-2 Light curve of photosynthesis estimated by oxygen evolution or chlorophyll fluorescence. (**A**) The rate of photosynthesis determined by the oxygen evolution rate with an oxygen electrode. Black circles, the wild-type cells; red triangles, the *ndhF1* disruptant. Vertical bars indicate the SD (n=3). (**B**) Comparison of the rate of photosynthesis determined by an oxygen electrode (filled symbols) and chlorophyll fluorescence (open symbols). The rETR was calculated as rETR=PAR×Φ_{PSII}. Black circles, the wild-type cells; red triangles, the *ndhF1* disruptant. Vertical bars indicate the SD (n=3).

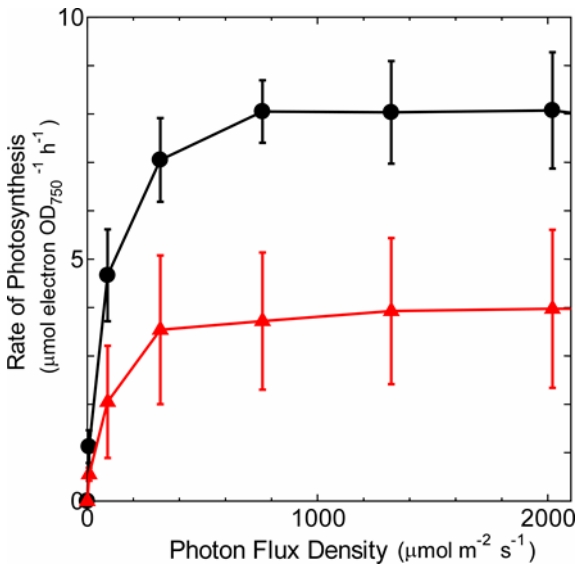

Fig. I-3 The rates of photosynthesis of the wild-type cells (black circles) and the *ndhF1* disruptant (red triangles) calculated on OD basis. Other experimental conditions are the same as **Fig. I-2A**.

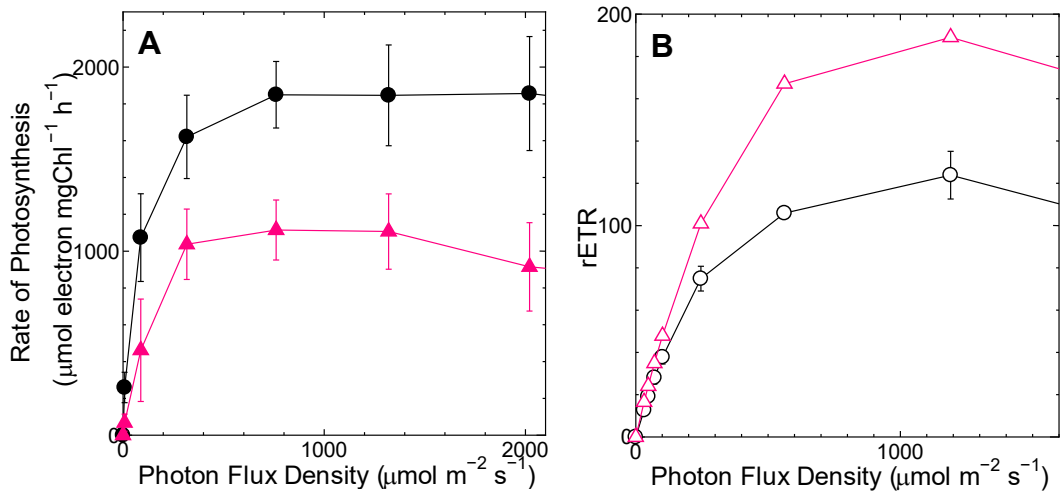

Fig. I-4 The rates of photosynthesis estimated by oxygen evolution (**A**) or by chlorophyll fluorescence (**B**) of the wild-type cells (black circles) of the disruptant of the *ndhF1* gene with erythromycin resistant cartridge (pink triangles). Other experimental conditions are the same as **Fig. I=2**.

To clarify the cause of the apparent increase of Φ_{PSII} in the *ndhF1* disruptant, the energy distribution to photosynthesis (Φ_{PSII}), to regulated energy dissipation (Φ_{NPQ}) or to non-regulated energy distribution ($\Phi_{f,D}$) was examined by chlorophyll fluorescence measurement (**Fig. I-5**). The sum of Φ_{PSII}, Φ_{NPQ} and $\Phi_{f,D}$ is unity, so that it is possible to estimate the energy

distribution between photosynthesis and dissipation as heat of fluorescence (Hendrickson et al. 2004). The apparent increase of Φ_{PSII} in the *ndhF1* disruptant was accompanied by the decrease of Φ_{NPQ} and $\Phi_{f,D}$ (**Fig. I-5B**, **Table I-1**), suggesting that the decrease of the energy dissipation would be the cause of the apparent increase of Φ_{PSII}. Generally speaking, the amount of phycobilisome or Photosystem I (PSI) relative to PSII would affect the level of $\Phi_{f,D}$, not that of Φ_{NPQ}, since the yield of fluorescence from phycobilisome or from PSI is not usually regulated in response to environmental conditions. Although the contribution of these factors is relatively small in the case of land plants, it could not be ignored in the case of cyanobacteria that contain high amounts of phycobiliprotein with a high PSI/PSII ratio (Campbell et al. 1996). As for phycobilisome content, however, there seems to be only a small difference between the wild-type cells and the *ndhF1* disruptant. When absorption spectra of intact cells were determined, the height of the peak at around 620 nm due to phycocyanin absorbance relative to that around 675 nm due to chlorophyll absorbance showed <10% difference between the two strains (**Fig. I-6**).

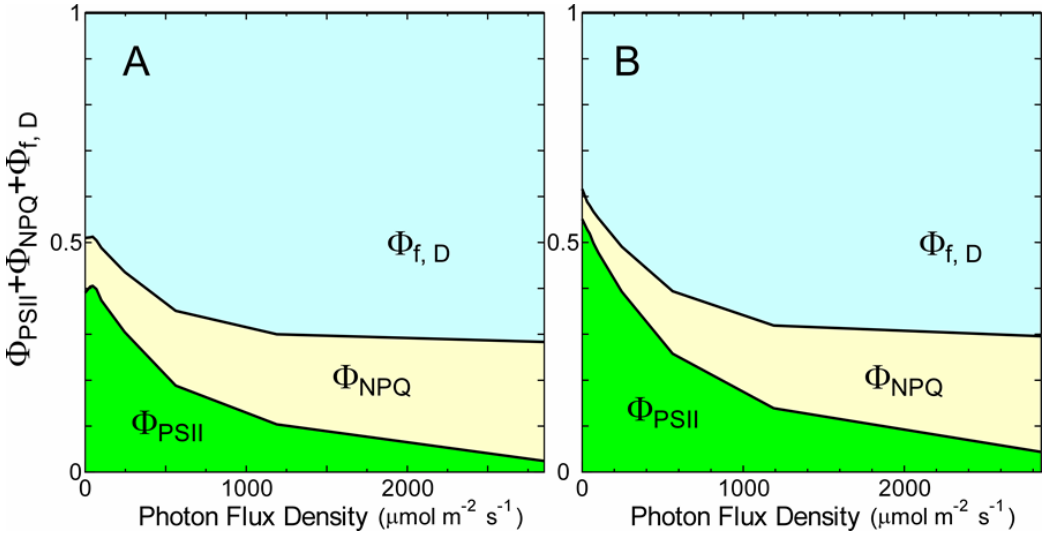

Fig. I-5 The energy distribution to photosynthesis (Φ_{PSII}; green), to regulated energy dissipation (Φ_{NPQ}; pale yellow) or to non-reguated energy dissipation ($\Phi_{f,D}$; blue). (**A**) The wild-type cells. (**B**) The *ndhF1* disruptant.

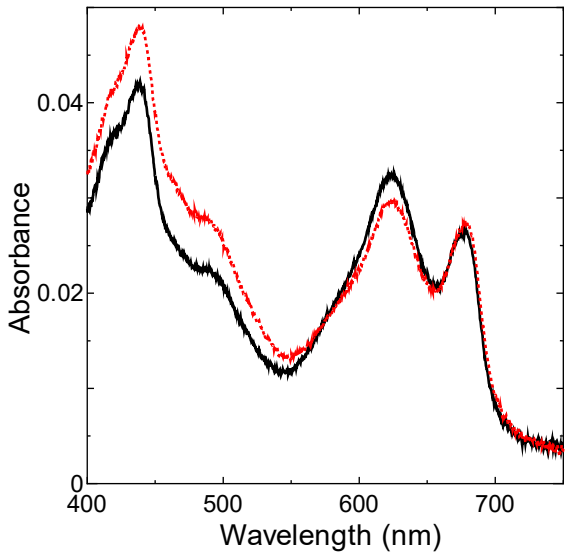

Fig. I-6 Absorption spectra of intact cells adjust to OD_{750}=0.2 in growth medium. Black solid line, the wild-type cells; red broken line, the *ndhF1* disruptant.

Photosystem stoichiometry (PSI/PSII ratio) could be determined by low temperature chlorophyll fluorescence spectra with chlorophyll excitation, since the effect of state transition, regulated by mobile phycobilisomes between PSII and PSI (Mullineaux et al. 1997; see **Fig. 5**), is limited under chlorophyll excitation. The height of the PSII fluorescence peak at around 695 nm relative to the PSI peak at around 725 nm was slightly different depending on the condition of the cells, e. g. dark-acclimated or light-acclimated, but the actual PSI/PSII ratio seems to be more or less similar between the wild-type cells (**Fig. I-7A**) and the *ndhF1* disruptant (**Fig. I-7B**).

However, the low temperature chlorophyll fluorescence spectra showed a large difference depending on the condition of the cells upon phycobilisome excitation, under which state transition could be more clearly observed. When the spectra were determined in the cells illuminated in the presence of 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU), which is the inhibitor of electron transfer from Q_A to Q_B (see **Fig. 1** in **General Introduction**) and oxidizes the PQ pool in the light, the relative height of the PSII fluorescence peak to that of the PSI fluorescence peak is high (**Fig. I-8A**, red solid line), suggesting that the cells are in State 1 (refer to **Fig. 5**) due to the oxidation of the PQ pool. Upon dark acclimation of the wild-type cells, the relative PSII fluorescence decreased either in the absence or in the presence of KCN (**Fig. I-8A**, black broken line and black solid line, respectively), suggesting the induction of State 2 through the reduction of the PQ pool by NDH-1 complexes.

The relative PSII fluorescence of light-acclimated cells is in between the two (**Fig. I-8A**, red broken line). The low temperature fluorescence spectra of dark-acclimated cells of the *ndhF1* disruptant in the absence of KCN (**Fig. I-8B**, black broken line) showed a much higher PSII peak than that of the dark-acclimated cells in the presence of KCN (**Fig. I-8B**, black solid line) and was similar to that of the illuminated cells in the presence of DCMU (**Fig. I-8B**, red solid line). Thus, the dark acclimation does not lead to the reduction of the PQ pool in the *ndhF1* disruptant. The results clearly showed that the yield of PSII fluorescence could be affected by the change in the activity of respiration through state transition. Furthermore, the light-acclimated cells of the *ndhF1* disruptant also showed almost similar fluorescence spectra to those of dark-acclimated cells, which contrasts with the case of the wild-type cells. The results suggest that the absence of NDH-1L could affect not only the redox condition of the PQ pool in the dark but also that in the light. The change in the redox condition of the PQ pool must be the cause of the apparent high Φ_{PSII} in the *ndhF1* disruptant **(Fig. I-1**) as well as the large Kautsky induction of the *ndhF1* disruptant. The assumption was supported by the fact that the large induction of chlorophyll fluorescence in the *ndhF1* disruptant (**Fig. I-9**, red lines) was effectively suppressed by the addition of KCN (**Fig. I-9**, yellow lines), while the fluorescence kinetics of the wild-type cells were not affected so much by the addition of KCN (**Fig. I-9**, black and blue lines). Although the apparent higher sensitivity of the *ndhF1* disruptant to KCN could be induced by some change in the terminal oxidase, the target of KCN inhibition, it would be simplest to assume a more direct effect of the NDH-1L function on the PQ pool.

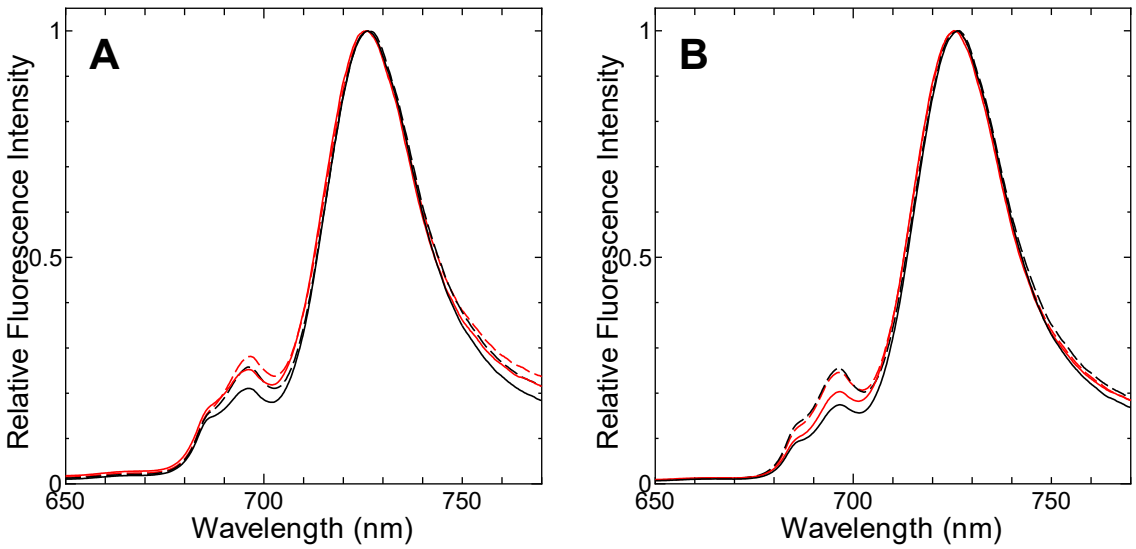

Fig. I-7 77 K chlorophyll fluorescence emission spectra with chlorophyll excitation at 435 nm in the wild-type cells (**A**) and the *ndhF1* disruptant cells (**B**). Black solid line, dark-acclimated cells in the presence of 0.2 mM KCN; black broken line, dark-acclimated cells without any addition; red broken line, growth light-acclimated cells; and red solid line, illuminated cells in the presence of 10 μM DCMU. Each fluorescence spectrum was normalized at the PSI fluorescence peak at around 725 nm. The height of the PSII fluorescence peak at around 695 nm relative to the PSI peak at around 725 nm reflects photosystem stoichiometry (PSI/PSII ratio). The average of spectra of three independent cultures is presented.

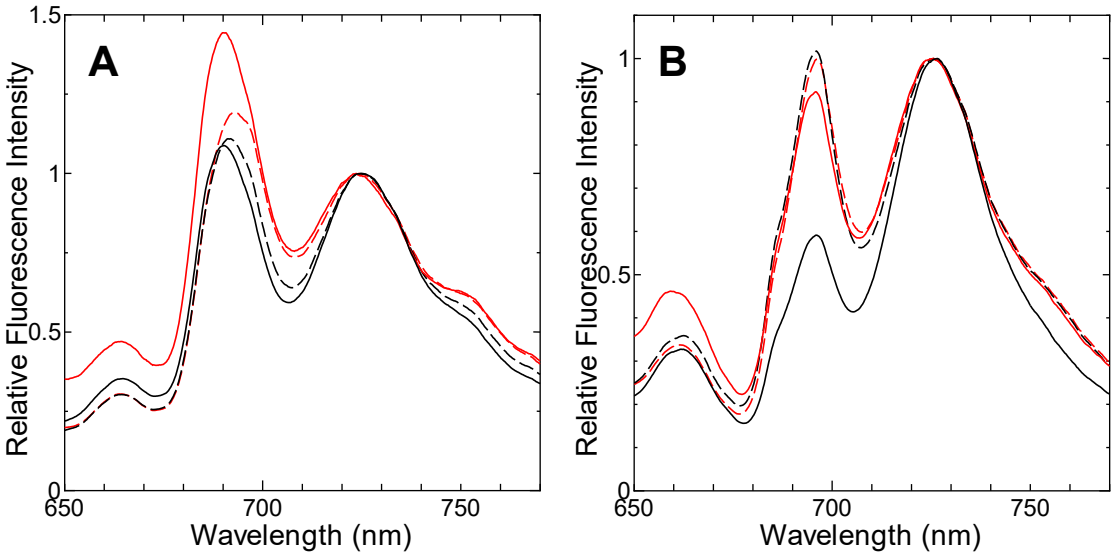

Fig. I-8 77 K Chlorophyll fluorescence emission spectra with phycocyanin excitation at 625 nm in the wild-type cells (**A**) and the *ndhF1* disruptant cells (**B**). Black solid line, dark-acclimated cells in the presence of 0.2 mM KCN; black broken line, dark-acclimated cells without any addition; red broken line, growth light-acclimated cells; and red solid line, illuminated cells in the presence of 10 μM DCMU. Each fluorescence spectrum was normalized at the PSI fluorescence peak at around 725 nm. The height of the PSII fluorescence peak at around 695 nm (F_{PSII}) relative to the PSI peak at around 725 nm (F_{PSI}) reflects energy distribution to PSII that depends on state transition regulated by the redox state of the PQ pool. In other words, high F_{PSII}/F_{PSI} ratio indicates the oxidation of the PQ pool, while low F_{PSII}/F_{PSI} ratio indicates the reduction of the PQ pool. The average of spectra of three independent cultures is presented.

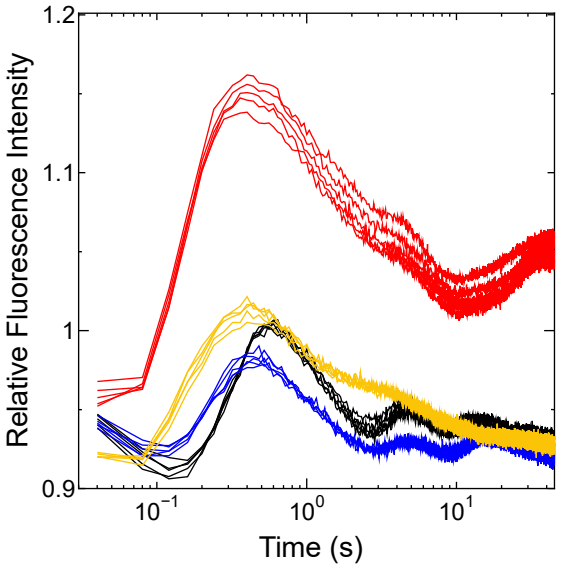

Fig. I-9 Chlorophyll fluorescence kinetics (so called Kautsky curve) of the wild-type cells and the *ndhF1* disruptant in the presence or absence of KCN. Black lines, the wild-type cells; blue lines, the wild-type cells in the presence of KCN; red lines, the *ndhF1* disruptant; yellow lines, the *ndhF1* disruptant in the presence of KCN. Cells were dark acclimated for 15 min prior to the measurement. Fluorescence kinetics of five cyanobacterial patches of each condition are presented as five lines. The fluorescence intensity was normalized at the onset of actinic illumination.

Discussion

Respiration affects chlorophyll fluorescence through state transition

The results presented here clearly show that the defect in respiration could induce a very big change in the induction kinetics of chlorophyll fluorescence through the redox state of the PQ pool. The interaction between photosynthesis and respiration itself is not surprising, since the PQ pool is shared by photosynthesis and respiration in cyanobacteria (Aoki and Katoh 1982; Peschek and Schmetterer 1982). However, the very large effect of disruption of the *ndhF1* gene on chlorophyll fluorescence is unexpected. Actually, the effect of disruption of the *ndhF1* gene on the chlorophyll fluorescence induction kinetics is the largest among the 750 disruptants analyzed in the Fluorome (the cyanobacterial chlorophyll fluorescence database). It is worth noting that the second largest change in the fluorescence

kinetics is induced by the disruption of the *ndhD1* gene, when the similarity of the kinetics in the Fluorome is analyzed by the 'derivative comparison' (Ozaki and Sonoike 2009). Both NdhF1 and NdhD1 were reported to form an NDH-1L complex involved in respiratory electron transfer (Battchikova and Aro 2007; Ohkawa et al. 2000; Battchikova et al. 2011). Although the extent of the change in the fluorescence kinetics in the Fluorome could not be directly compared with different disruptants due to possible differences in segregation, the effect of the disruption of *ndh* genes seems to be rather peculiar. The defects in respiratory electron transfer have an obviously great impact on chlorophyll fluorescence in cyanobacteria, although the respiratory rate is usually about one order smaller than the maximum photosynthesis rate. The chlorophyll fluorescence measurements would also be useful not only for the analysis of photosynthesis but also for that of respiration, at least in the case of cyanobacteria.

The striking change in the chlorophyll fluorescence observed on disruption of the *ndh* genes (Fluorome, see also **Fig. I-9**) seems to be induced by the change in state transition (**Figs. I-7 and 8**). It was shown that the main component of non-photochemical quenching (NPQ) in cyanobacteria was not energy-dependent quenching but state transition (Campbell and Öquist 1996). Under State 2 conditions, the energy flow to PSI, which has a low yield of fluorescence with negligible variable fluorescence, should result in higher NPQ as well as a lower Φ_{PSII}. Since the state transition is regulated by the redox state of the PQ pool (Mullineaux and Allen 1986), respiratory electron transfer should affect the state transition. The inactivation of NDH-1complexes involving electron transfer from NAD(P)H to PQ would result in the oxidation of the PQ pool, leading to the transition to State 1, where the yield of chlorophyll fluorescence is high. Energy allocation to PSII induced in State 1 should cause the increase in Fv, leading to the apparent increase of Fv/Fm as well as that of Φ_{PSII}. Although the *ndhF* disruptant is locked in State 1, the mechanism of the state transition is not perturbed, since the disruptant could be brought in State 2 by the addition of KCN. The mutant M55, which lacks the functional *ndhB* gene vital for the assembly of NDH-1 complexes (Zhang et al. 2004), was also reported to be locked in State 1 even after dark acclimation (Schreiber et al. 1995). Apparently, respiration, though its rate is far lower than that of photosynthesis, could affect chlorophyll fluorescence using the state

transition as leverage.

Validity of the chlorophyll fluorescence parameter

Chlorophyll fluorescence is widely used for the assessment of photosynthesis. The fluorescence parameter, Fv/Fm, is a measure of the maximum quantum yield of PSII (Kitajima and Butler 1975), while the ETR is a measure of the rate of photosynthetic electron transfer (Genty et al. 1989). These parameters were, however, developed mainly for land plants, and caution is necessary for their application to algae and cyanobacteria. For example, fluorescence could not be attained to the maximum level by the application of a saturating pulse to dark acclimated cyanobacterial cells (see **Fig. 3** in **General Introduction**), since dark acclimation induce the reduction of the PQ pool instead of the oxidation of it in cyanobacteria (Schreiber et al. 1995; Campbell and Öquist 1996; Sonoike et al. 2001). The addition of DCMU in the presence of light to oxidize PQ pool is necessary to obtain a true Fm level. Fv/Fm presented in **Table I-1** in this study was determined using such procedure. It was also shown that the development of NPQ is very rapid in algae and cyanobacteria, resulting in the requirement for special care to determine the maximum fluorescence yield (Schreiber et al. 1995).

On the other hand, particular attention has not been paid to the use of ETR and Φ_{PSII} as indicators of the rate and yield of photosynthesis in cyanobacteria. It is evident, however, that the ETR or Φ_{PSII} is not reliable to estimate photosynthesis in cyanobacteria. The *ndhF1* disruptant showed lower oxygen-evolving activity with higher level of Φ_{PSII} or rETR compared with the wild-type cells (**Figs. I-1 and 2**). A part of the discrepancy could be ascribed to the oxygen consumption reaction such as the Mehler reaction in the disruptant. The photosynthetic oxygen evolution concomitant with oxygen consumption should result in the apparent low photosynthetic activity. Although the precise nature of the oxygen consumption reaction is unknown, electron transfer to molecular oxygen at the reducing side of PSI would be a possible candidate. Specific flavoproteins are reported to be involved in the Mehler reaction of *Synechocystis* sp. PCC 6803 (Helman et al. 2003). A heterodimer of the two flavoproteins, Flv1 and Flv3, functions in the "Mehler-like" reaction leading to the suppression of photorespiration under low inorganic carbon conditions (Allahverdiyeva et al. 2013; **Fig. 6**). It would be

worth to examine the expression of these flavoproteins in the *ndhF1* disruptant. Respiration also consumes oxygen, but the rate of respiration is low in the mutant, at least in the dark (30.8±15.7 μmol electron $mgChl^{-1}$ h^{-1}, compared to the value of 111 ± 9 μmol electro $mgChl^{-1}$ h^{-1} in the wild-type cells).

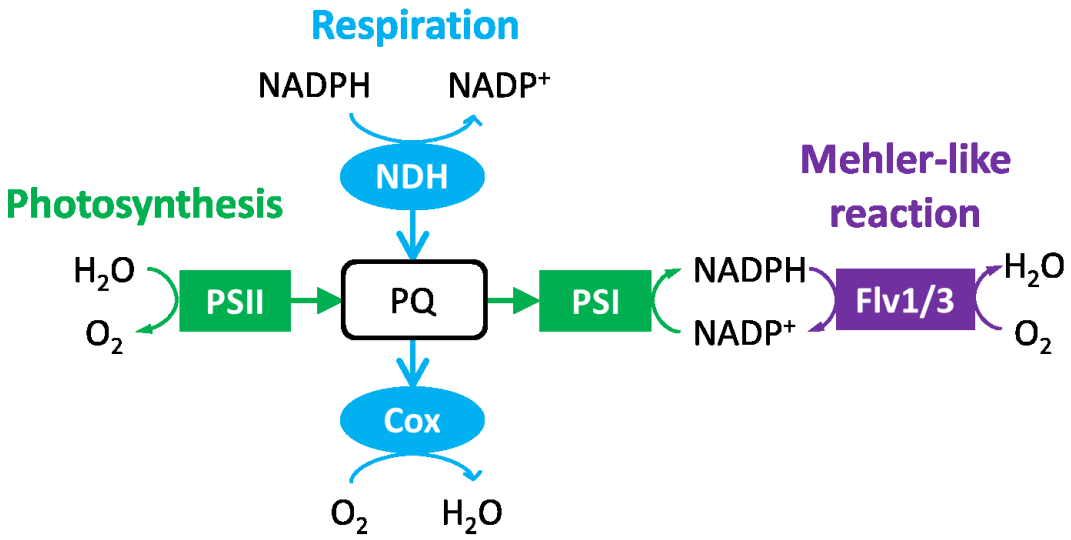

Fig. 6 Scheme of the electron transport chain involved in O_2 evolution or O_2 consumption. Flv1/3, flavodiiron proteins functioning in "Mehler-like" reaction. See **Fig. 4** in **General Introduction** for the other abbreviations.

While enhanced oxygen consumption could explain the cause of the lower oxygen-evolving activity, it could not fully explain the higher rETR determined by chlorophyll fluorescence measurements. Although increased electron flow to oxygen may result in an increase in rETR under light-saturating conditions, the difference in Φ_{PSII} between the wild-type cells and the *ndhF1* disruptant is more evident in the light-limiting condition (**Fig. I-2**), suggesting that some other factor is involved. The increased rETR in the *ndhF1* disruptant could be ascribed in part to the decreased level of NPQ in the *ndhF1* disruptant, as discussed above. The PQ pool is more oxidized in the *ndhF1* disruptant than in the wild-type cells under the growth light as well as in the dark (**Fig. I-8B**), and that could be the cause of the increased rETR in the *ndhF1* disruptant. A similar discrepancy between oxygen-evolving activity and the chlorophyll fluorescence measurements was reported for a desiccation-tolerant

cyanobacterium under stress conditions (Ohad et al. 2010), although the mechanism would be totally different from the case of the *ndhF1* disruptant. The main component of NPQ was shown to be state transition in cyanobacteria (Campbell and Öquist 1996), so that one must be cautious in using the chlorophyll fluorescence parameter to estimate photosynthesis in mutants defective in state transition, such as the disruptants of *ndh* genes examined in this work. In the case of respiratory mutants, the addition of KCN or illumination with blue light, which predominantly excites PSI in cyanobacteria (discussed in **Chapter III**), would be one of the ways to solve the problem, as shown in **Figs. I-8 and 9**.

Materials and Methods

Strains and growth conditions

The *ndhF1* disruptant, which was originally used for the collection of the fluorescence kinetics in Fluorome, had been constructed by transposon mutagenesis of *Synechocystis* sp. PCC 6803 (Ozaki et al. 2007). The transposon, which confers chloramphenicol resistance on cyanobacteria, was inserted at position 2,857,968 in the genome according to the numbering in Cyanobase (http://genome.microbedb.jp/cyanobase/), which is 91 nucleotides downstream of the initial ATG of the coding region of the *ndhF1* gene. To exclude the possibility of a second mutation in the disruptant, another *ndhF1* disruptant (No. 5-20), a kind gift from professor Ogawa, was used. To construct this disruptant, an erythromycin resistance cassette was inserted into the StuI site (530 bases downstream of the initial ATG) of the *ndhF1* gene. The wild-type cells and the disruptant were grown at 30°C in BG11 medium (Allen 1968), buffered with 20 mM TES-KOH (pH 8.0) and bubbled with air under continuous illumination of 50 µmol m^{-2} s^{-1} for 24 h. Chloramphenicol at 25 µg ml^{-1} or erythromycin at 25 µg ml^{-1} was added to the culture medium for the growth of disruptants.

Chlorophyll fluorescence measurement

Chlorophyll fluorescence was measured with a pulse-amplitude fluorometer (WATER-PAM, Walz). The OD of the cell suspension at 750 nm was adjusted to 0.2 and dark acclimated for 10 min prior to measurements. Minimal

fluorescence (Fo), fluorescence under steady-state conditions (Fs), the maximum fluorescence of light-acclimated cells (Fm') and the maximum fluorescence determined in the presence of DCMU (Fm) were used for calculation of parameters; qP=(Fm'-Fs)/(Fm'-Fo'), qN=1-(Fm'-Fo')/(Fm-Fo) (van Kooten and Snel 1990), Φ_{PSII}=(Fm'-Fs)/Fm' (Genty et al. 1989; Bilger et al. 1995), Φ_{NPQ}=Fs/Fm'-Fs/Fm and $\Phi_{f,\ D}$=Fs/Fm (Hendrickson et al. 2004). Fo' was calculated as Fo' =Fo/(Fv/Fm+Fo/Fm') (Oxborough and Baker 1997). A 0.8 s flash of saturating light was given to determine Fm' after illumination by actinic light for 2 min 30 s. Fm was measured in the presence of 20 μM DCMU with illumination by actinic light and saturating light. The rETR was calculated as rETR=Φ_{PSII}×PAR (photosynthetic active radiation). Although it is possible to calculate the absolute value of the ETR with several assumptions, such assumptions used for the measurements of land plants could not be applied to cyanobacteria. Thus, only the rETR was presented in this study. PAR was determined in a cuvette filled with water by a spherical micro-sensor (US-SQS/L, Walz) with a light meter (LI-250, LI-COR Biosciences).

Chlorophyll induction kinetics (Kautsky kinetics) were determined by a fluorescence CCD camera (FluorCam 700MF, Photon System Instruments) as described previously (Ozaki et al. 2007). To examine the effect of KCN, 10 μl of 100 mM KCN solution was dropped on a cyanobacterial patch grown on an agar plate just before dark acclimation.

Assay of oxygen-evolving activity

Oxygen evolution of intact cells was measured in the presence of 1 mM $NaHCO_3$ with an oxygen electrode (Oxygraph, Hansatech Instruments) under illumination from a light source (PICL-NRX, Nippon P. I.) at 25°C. The OD of the cell suspension at 750 nm was adjusted to 0.2 in growth medium. PAR was determined as above.

Absorption spectra

Absorption spectra were measured with a spectrophotometer (V-650, JASCO) equipped with an integrating sphere (ISV-722, JASCO). The absorption of the cell suspension (OD_{750}=0.2) was determined in a cuvette with light path length of 5 mm.

77 K fluorescence emission spectra

77 K fluorescence emission spectra were measured with a fluorescence spectrometer (FP-8500, JASCO) with a low temperature attachment (PU-830, JASCO). Cell suspension were adjusted to a concentration of 10 μg chlorophyll ml^{-1}. Prior to the measurements, the cells were either dark acclimated without any addition, dark acclimated in the presence of 0.2 mM KCN, illuminated at 50 μmol m^{-2} s^{-1} without any addition or illuminated at 550 μmol m^{-2} s^{-1} in the presence of 10 μM DCMU, for 10 min. The samples were excited by the light at 435 nm for chlorophyll excitation or 625 nm for phycocyanin excitation with an excitation slit width of 10 nm. The fluorescence spectra were recorded with a fluorescence slit width of 2.5 nm and a resolution of 0.2 nm. The spectra were recorded five times for one sample, and the averages of the data of three independent cultures were presented. The spectra were corrected for the sensitivity of the photomultiplier and the spectrum of the light source using a secondary standard light source (ESC-842, JASCO). Each fluorescence spectrum was normalized at the PSI fluorescence peak.

Contents in Chapter I had been published in 2013 as a paper titled "Disruption of the *ndhF1* gene affects chlorophyll fluorescence through state transition in the cyanobacterium *Synechocystis* sp. PCC 6803" in Plant & Cell Physiology 54: 1164-1171 (doi: 10.1093/pcp/pct068) by Oxford University Press.

Chapter I

Chapter II

Influence of CO_2 Concentrating Mechanism on Photosynthesis

Introduction

Yield of chlorophyll fluorescence of photosynthetic organisms rapidly changes as photosynthesis is affected by environmental factors or by cellular metabolism (Govindjee 1995). Such changes of fluorescence yield could be used to analyze photosynthetic electron transport (Krause and Weis 1991). In cyanobacteria, respiration and photosynthesis share several components of electron transport chain such as plastoquinone (PQ) and cytochrome b_6/f complex (Aoki and Katoh 1982; Peschek and Schmetterer 1982, see also **Fig. 4** in **General Introduction**) so that not only photosynthesis but also respiration could directly affect chlorophyll fluorescence. In fact, it was demonstrated that the type 1 NAD(P)H dehydrogenase (NDH-1) complex serving for cyanobacterial respiratory electron transport donates electron to the PQ pool and affects chlorophyll fluorescence (Mi et al. 1992).

In cyanobacteria, most of the *ndh* genes encoding the subunits of the NDH-1 complex exist as a single copy in the genome, but six *ndhD* genes (*ndhD1*-*ndhD6*) and three *ndhF* genes (*ndhF1*, *ndhF3* and *ndhF4*) are found in *Synechocystis* sp. PCC 6803 (Kaneko et al. 1996; Battchikova et al. 2011; Cyanobase, http://www.kazusa.or.jp/cyano/). Cyanobacteria have functionally distinct NDH-1 complexes with different set of NdhD and NdhF subunits. This diversity in Ndh subunits and multiplicity of functions of NDH-1 complexes have been revealed by investigating the phenotype of *ndh* mutants. NDH-1L complex and NDH-1L' complex which contain NdhF1/D1 and NdhF1/D2 respectively (Zhang et al. 2004; Battchikova et al. 2011) are essential for the cyclic electron transport around Photosystem I (PSI) as well as the respiratory electron transport, since the *ndhD1*/*D2* double disruptant lacking the NDH-1L complexes exhibits low rate of oxygen uptake in the dark and the high level of oxidized PSI reaction center ($P700^+$) under weak far red light (Ohkawa et al. 2000a). In addition to respiratory and PSI cyclic electron transport, cyanobacterial NDH-1 complexes are involved in carbon concentrating

mechanism (CCM) (Ogawa 1991). NDH-1MS and NDH-1MS' complexes are considered to be essential for CCM, based on the results that double disruptant of NdhD3/D4 subunits showed decrease in CO_2 uptake (Ohkawa et al. 2000a; Shibata et al. 2001; Maeda et al. 2002). NDH-1MS or NDH-1MS' complex consists of NDH-1M subcomplex and NDH-1S or NDH-1S' subcomplex (Herranen et al. 2004). The subunits composing NDH-1M subcomplex are common to those composing NDH-1L complex except for NdhD1 and NdhF1 in the NDH-1L complex (Battchikova et al. 2005). NDH-1S subcomplex or NDH-1S' subcomplex has NdhD3 and NdhF3 or NdhD4 and NdhF4, respectively.

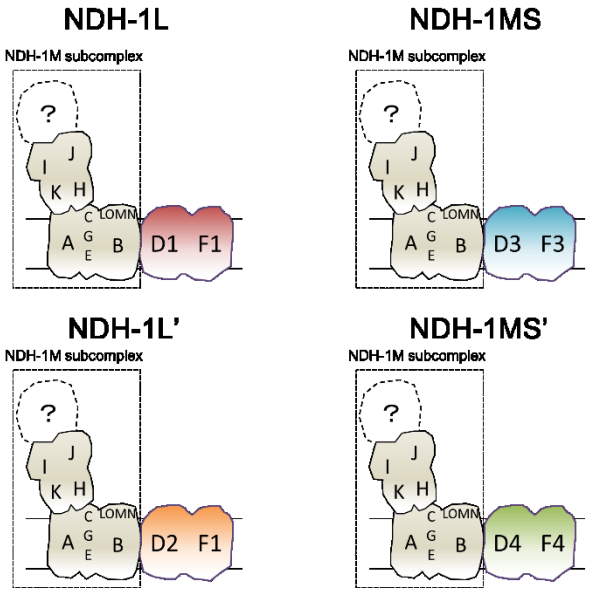

Fig. 7 A scheme of cyanobacterial NDH-1 complex referring to Battchikova et al. (2011). NDH-1M subcomplex is common to all 4 functionally and structurally distinct NDH-1 complexes. NdhD and NdhF subunits are different among the 4 complexes; NDH-1L complex has NdhD1/F1 subunits, NDH-1L' has NdhD2/F1, NDH-1MS has NdhD3/F3 and NDH-1MS' has NdhD4/F4, respectively.

Since P700 is oxidized under weak far red light in the *ndhD1*/*D2* disruptant (Ohkawa et al. 2000a) and that the *ndhF1* disruptant is locked in State 1 in the dark (Ogawa et al. 2013; **Chapter I**), the *ndhD1* and *ndhF1* genes which compose the NDH-1L complex must have great influence on photosynthetic electron transport along with the respiratory electron transport. On the other hand, disruptions of *ndhD3*/*D4* genes hardly affect PSI cyclic electron transport (Ohkawa et al. 2000a) in spite of its presumed involvement in CO_2 uptake (Ogawa 1985; Li and Canvin 1998; Maeda et al. 2002). Apparently, the essential role of NdhD3/D4 subunits in CCM could not be ascribed solely to the function in PSI cyclic electron transport. It is suggested that the CupA/CupB protein in the NDH-1S/S' subcomplex converts CO_2 to HCO_3^- while

the NDH-1M subcomplex is involved in PSI cyclic electron transport (Price et al. 2002; Zhang et al. 2004). However, it is not clear whether the NdhF3/F4 and NdhD3/D4 subunits could affect the photosynthetic electron transport.

In this chapter, I monitored a fluorescence parameter, NPQ, which represents the state transition that is triggered by the reduction of PQ pool in cyanobacteria, to investigate the effect of the defect in either respiration or CCM on photosynthetic electron transfer. It was revealed that NPQ measured both in the dark and light was lower in the *ndhD1*/*D2* and *ndhF1* disruptants compared with the wild-type cells due to insufficient electron supply to the PQ pool in respiratory electron transport. Moreover, I found that NPQ in the *ndhD3*/*D4* and *ndhF3*/*F4* disruptants under low light condition was lower than that in the wild-type cells with apparent increase in PSI content. The result indicates that the disruption of NdhD and NdhF subunits essential for CCM affects the redox state of the PQ pool in the light through the change in photosystem stoichiometry (i.e. PSI/PSII ratio), while disruption of genes essential for respiration oxidizes the PQ pool both in the dark and under the low light condition. Measuring NPQ, reflecting the redox state of the PQ pool, in the dark and in the light is a simple method to evaluate the condition of both respiration and photosynthesis.

Results

I have shown in **Chapter I** that chlorophyll fluorescence of cyanobacteria was quenched upon reduction of PQ pool due to the respiratory electron transfer in the dark, which could be diminished by the disruptant of the *ndhF1* gene coding a subunit of NDH-1 complex serving for respiration (Ogawa et al. 2013). The disruption of the *ndhF1* gene of the cyanobacterium *Synechocystis* sp. PCC 6803 affects not only the quenching of chlorophyll fluorescence in the dark, but also that in the low light (**Fig. II-1**, red triangles compared to black circles). In this study, since the red actinic light (LEDs peaking at 660 nm) was used for pulse-amplitude chlorophyll fluorescence measurement, it is assumed that the quenching of the chlorophyll fluorescence was primarily induced by state transition but not by the action of orange carotenoid protein (OCP), which induces fluorescence quenching upon illumination by high intensities of blue light (Kirilovsky 2007). The level of

fluorescence quenching estimated as NPQ=(Fm-Fm')/Fm' was high in the dark and decreased to lower levels under low light condition, but then increased again under higher photon flux density (PFD), giving concave dependency (**Fig. II-1**, black circles for the wild-type cells). This result indicates that PQ pool is reduced in the dark and under high light while oxidized under low light condition. Similar results have been reported for qN (Campbell and Öquist 1996; Sonoike et al. 2001), which is another parameter representing chlorophyll quenching calculated as 1-(Fm'-Fo')/(Fm-Fo), also reflecting state transition (Campbell and Öquist 1996). The disruption of the *ndhF1* gene significantly decreased the NPQ level in the dark (**Fig. II-1**, red triangles) as reported earlier (Ogawa et al. 2013), and the difference between the values in the wild-type cells and those in the *ndhF1* disruptant persisted even in the light, although the difference became similar with higher actinic light PFD. Apparently, the defect in respiration could affect photosynthesis in the light as well as in the dark as shown in **Chapter I**.

To explore the functional divergence of the NDH subunits, I also examined the actinic PFD dependency of NPQ in the disruptant of *ndhF3*/*F4* genes, which is reported to have defect in CO_2 uptake (Ohkawa et al. 2000a; Shibata et al. 2001; Maeda et al. 2002). The *ndhF3*/*F4* disruptant showed totally different dependency from that of the disruptant of *ndhF1* gene: NPQ in the dark (NPQ_{Dark}) of the *ndhF3*/*F4* disruptant was similar to that of the wild-type cells while NPQ under low light (NPQ_{LL}) was lower than that of the wild-type cells, showing steep decrease from NPQ_{Dark} to NPQ_{LL} **(Fig. II-1**, green diamonds). Since main component of the NPQ in cyanobacteria is state transition (Campbell and Öquist 1996) and the state transition is regulated by the redox state of PQ pool (Mullineaux and Allen 1986), the levels of NPQ could be used to roughly estimate the relative redox state of the PQ pool. The results obtained in **Fig. II-1** suggest that, in the disruptant of *ndhF3*/*F4* genes, the redox state of PQ pool is similar to that of the wild-type cells in the dark and more oxidized under low light than in the wild-type cells.

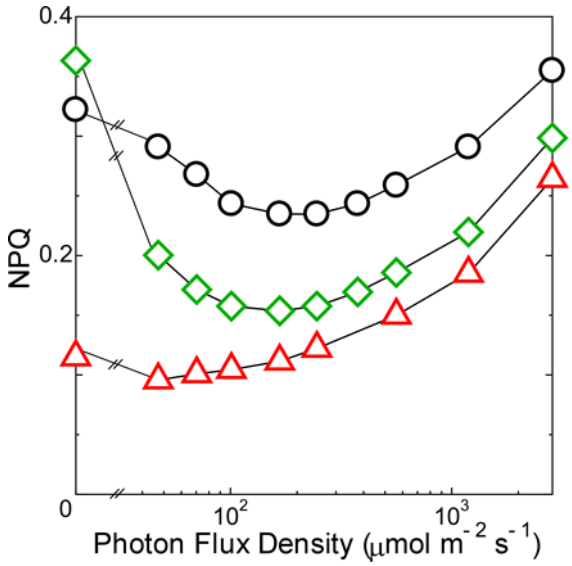

Fig. II-1 The response of NPQ, which reflects the relative redox state of the PQ pool, to the actinic photon flux densities of the cells grown at pH 8. NPQ in the dark (at 0 μmol m^{-2} s^{-1}) was determined by the second saturating pulse in the dark as described in Materials and Methods. Black circles, the wild-type cells; red triangles, the *ndhF1* disruptant; green diamonds, the *ndhF3*/*F4* disruptant. High NPQ indicates reduced PQ pool, while low NPQ indicates oxidized PQ pool.

The levels of NPQ of the wild-type cells, the *ndhF1* disruptant, *ndhD1*/*D2* disruptant, *ndhF3*/*F4* disruptant and *ndhD3*/*D4* disruptant in the dark as well as under low light were compared in **Fig. II-2**. The levels of NPQ_{Dark} of the *ndhF1* and *ndhD1*/*D2* disruptants were lower than that of the wild-type cells, while those of the *ndhF3*/*F4* and *ndhD3*/*D4* disruptants were similar to that of the wild-type cells (**Fig. II-2**, black bars). The NdhF1 subunit, together with the NdhD1 or NdhD2 subunit, composes the NDH-1L or NDH-1L' complex (**Fig. 7**) participating in respiratory and PSI cyclic electron transport (Zhang et al. 2004; Battchikova et al. 2011). Thus, it is reasonable to assume that the lowered NPQ_{Dark} in the *ndhF1* and *ndhD1*/*D2* disruptants reflects the oxidation of the PQ pool due to the insufficient electron supply from NDH-1 complexes to PQ pool in respiratory electron transport. It has been also shown in **Chapter I** that the PQ pool of the *ndhF1* disruptant is oxidized in the dark, based on the chlorophyll fluorescence spectra determined at 77 K (Ogawa et al. 2013). Contrary to the case of the *ndhF1* and *ndhD1*/*D2* disruptants, the NdhF3/D3 or NdhF4/D4 subunits, which compose the NDH-1MS or NDH-1MS' complex respectively (**Fig. 7**) and are reported to be essential for CO_2 uptake (Herranen et al. 2004; Battchikova et al. 2011), apparently do not contribute to the reduction of PQ pool in the dark judging from the similar NPQ_{Dark} to the

wild-type cells observed in the *ndhF3*/*F4* and *ndhD3*/*D4* disruptants.

On the other hand, NPQ_{LL} of all the *ndh* mutants examined were lower than that of the wild-type cells (**Fig. II-2**, white bars). In other words, larger decrease of NPQ upon illumination (from NPQ_{Dark} to NPQ_{LL}) was observed only in the *ndhF3*/*F4* and *ndhD3*/*D4* disruptants, and not in the *ndhF1* and *ndhD1*/*D2* disruptants. Considering the reported role of NDhF3/F4/D3/D4 subunits, the change in NPQ upon the transition from dark to light could be ascribed to the limitation in CO_2.

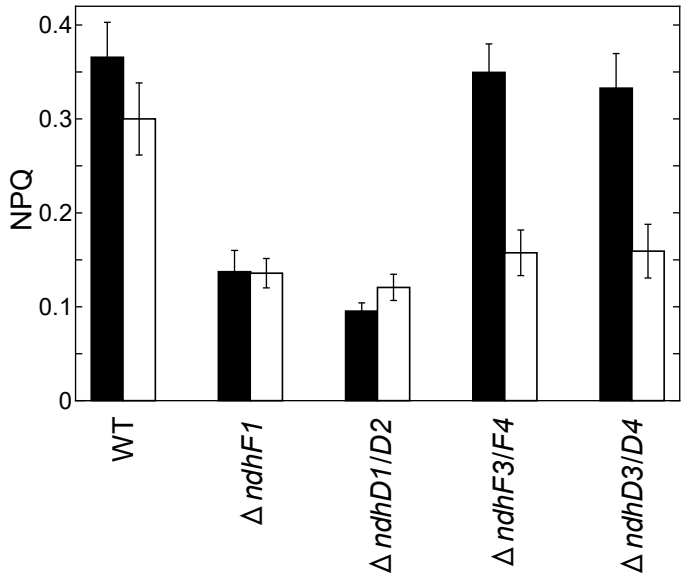

Fig. II-2 NPQ, which reflects the relative redox state of the PQ pool, determined in the wild-type cells (WT) and *ndh* mutants grown at pH 8 determined in the dark (black bars) or under the low light (100 μmol m^{-2} s^{-1}, white bars). Results are averages ±SD of measurements of three independent cultures.

If the NPQ_{Dark} reflects respiratory electron transfer to PQ, it should be increased by the addition of KCN, an inhibitor of terminal oxidase that mediates respiratory removal of electrons from the PQ pool. In fact, NPQ_{Dark} of the *ndhF1* disruptant was increased by the addition of KCN (**Fig. II-3A**). The effect of KCN on the *ndhF3*/*F4* disruptant was much smaller. As for CO_2 limitation, it would be relieved under alkaline pH condition, since inorganic carbon should be supplied in the form of bicarbonate at high pH. Thus, if the decrease of NPQ upon light illumination (i.e., the difference between NPQ_{Dark} and NPQ_{LL}; in other words, the difference of redox state of the PQ pool between dark and low light) reflects CO_2 limitation, it should be affected by the changes in the growth pH. When the cells were grown at pH 8, the relative difference between NPQ_{Dark} and NPQ_{LL} calculated as $(NPQ_{Dark}-NPQ_{LL})/NPQ_{LL}$ was small

in the wild-type cells as well as in the *ndhF1* disruptant while it was large in the *ndhF3*/*F4* disruptant (**Fig. II-3B**, gray bars), reflecting the actinic light dependency of NPQ shown in **Fig. II-1**. Upon the shift of growth pH to 9, however, the relative difference between NPQ_{Dark} and NPQ_{LL} of the *ndhF3*/*F4* disruptant became also small (**Fig. II-3B**, dotted bars). These results support the assumption that the lowered NPQ_{Dark} in the *ndhF1* disruptant represents the limitation in respiration while the large difference between NPQ_{Dark} and NPQ_{LL} in the *ndhF3*/*F4* disruptant is ascribed to the limitation in CO_2 as inorganic carbon source.

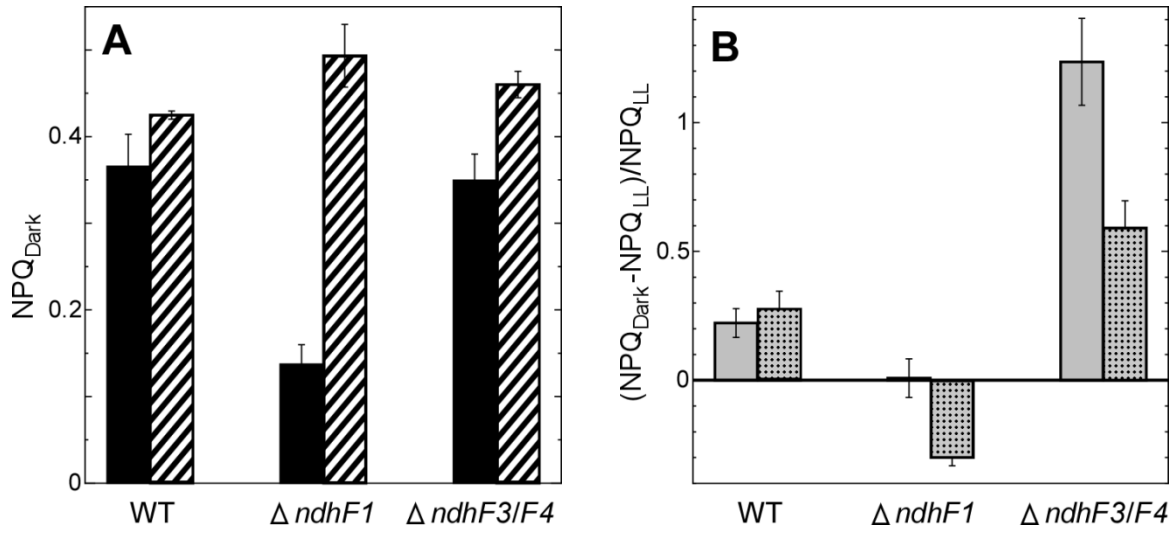

Fig. II-3 (**A**) Effects of KCN on NPQ_{Dark} in the wild-type cells, the *ndhF1* disruptant or the *ndhF3*/*F4* disruptant. Black bars, no addition; hatched bars, in the presence of 0.1 mM KCN. (**B**) Effects of growth pH on the relative difference between NPQ_{Dark} and NPQ_{LL} calculated as $(NPQ_{Dark}-NPQ_{LL})/NPQ_{LL}$ in the wild-type cells, the *ndhF1* disruptant or the *ndhF3*/*F4* disruptant. Gray bars, grown at pH 8; dotted bars, grown at pH 9. Results are averages ±SD of measurements of three independent cultures.

As a cause of the decrease of NPQ in the *ndhF3*/*F4* and *ndhD3*/*D4* disruptants upon dark to light transition, it is natural to assume that photosynthetic machinery is somehow affected in these disruptants. Decrease in Photosystem II (PSII) activity or increase in PSI activity would be the simplest assumption as a cause of oxidation of the PQ pool in the light relative to in the

dark. Thus, I investigated photosystem stoichiometry (PSI/PSII ratio) in the wild-type cells and the *ndh* mutants by 77 K fluorescence emission spectra with chlorophyll excitation (**Fig. II-4A**). The peak height around 725 nm fluorescence emitted from PSI relative to that around 695 nm fluorescence emitted from PSII was higher in the *ndhF3*/*F4* disruptant (**Fig. II-4A**, green dotted line) than that in the wild-type cells (black solid line) or in the *ndhD1*/*D2* disruptant (yellow broken line). The ratio of the heights of two fluorescence peaks, F_{PSI}/F_{PSII}, could be used as an indicator of PSI/PSII ratio and the relationship between F_{PSI}/F_{PSII} and chlorophyll content per cell is plotted in **Fig. II-4B**. F_{PSI}/F_{PSII} ratio is higher both in the *ndhF3*/*F4* disruptant (**Fig. II-4B**, open green diamond) and in the *ndhD3*/*D4* disruptant (open blue square) compared with the wild-type cells (open black circle). This increase of the F_{PSI}/F_{PSII} ratio is accompanying the increase in the chlorophyll content per cell, suggesting that the increase of the PSI content, not the decrease of the PSII content is the cause of the increase in the F_{PSI}/F_{PSII} ratio, since PSI generally binds up to 90% of the total chlorophyll in cyanobacteria. Furthermore, in the *ndhF3*/*F4* disruptant, both the chlorophyll content and the PSI/PSII ratio were decreased by the elevation of growth pH to 9 (closed green diamond), suggesting that PSI content is regulated by the amount of available inorganic carbon source. The *ndhF1* disruptant grown at pH 9 showed lower F_{PSI}/F_{PSII} ratio (closed red triangle) compared with other disruptants with smaller chlorophyll content. This may be due to the increase in PSII content but the mechanism of this change is not clear.

In any event, the above results suggest that the decrease of NPQ upon dark to light transition observed in the *ndhF3*/*F4* and *ndhD3*/*D4* disruptants is caused by the increased content of PSI in the disruptants. To verify the relationship between PSI/PSII ratio and relative NPQ change upon dark to light transition, $(NPQ_{Dark}-NPQ_{LL})/NPQ_{LL}$ is plotted against F_{PSI}/F_{PSII} ratio (**Fig. II-5**). Positive correlation (R^2=0.769) was observed confirming the relationship.

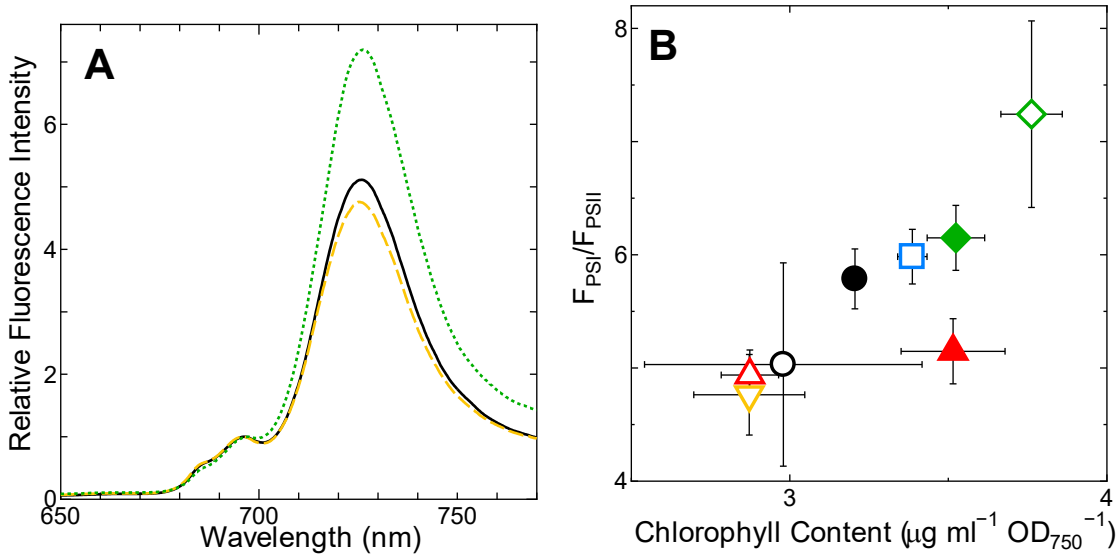

Fig. II-4 (**A**) 77 K chlorophyll fluorescence emission spectra with chlorophyll excitation at 435 nm. Prior to measurements, the cells were illuminated at 550 μmol m^{-2} s^{-1} for 10 min in the presence of 10 μM DCMU. Each fluorescence spectrum was normalized at the PSII fluorescence peak at around 695 nm. The averaged spectra of at least three independent cultures are presented. Black solid line, the wild-type cells; yellow broken line, the *ndhD1/D2* disruptant; green dotted line, the *ndhF3/F4* disruptant. (**B**) Fluorescence peak ratio (F_{PSI}/F_{PSII}), which is an indicator of PSI/PSII ratio, plotted against chlorophyll content per a cell, which is an indicator of PSI content. F_{PSI}/F_{PSII} was calculated from 77 K chlorophyll fluorescence emission spectra with chlorophyll excitation at 435 nm. Black circles, the wild-type cells; red triangles, the *ndhF1* disruptant; yellow inverted triangle, the *ndhD1/D2* disruptant; green diamonds, the *ndhF3/F4* disruptant; blue square, the *ndhD3/D4* disruptant. Open symbols represent cells grown at pH 8, while closed symbols represent cells grown at pH 9. Results are averages ±SD of measurements of at least three independent cultures.

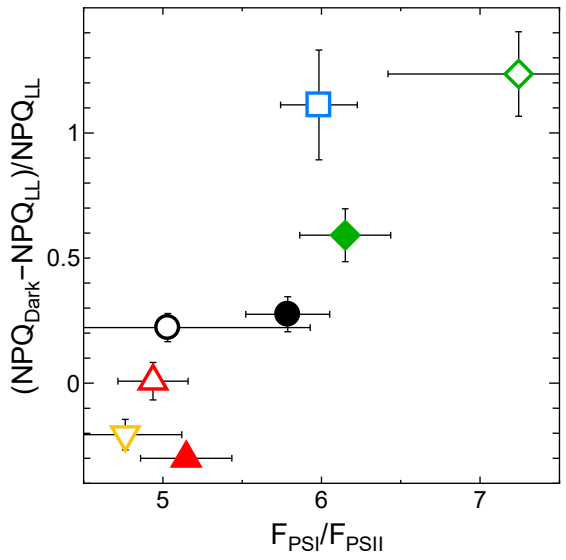

Fig. II-5 Relative NPQ difference between dark and low light calculated as $(NPQ_{Dark}-NPQ_{LL})/NPQ_{LL}$ plotted against PSI/PSII ratio. Black circles, the wild-type cells; red triangles, the *ndhF1* disruptant; yellow inverted triangle, the *ndhD1/D2* disruptant; green diamonds, the *ndhF3/F4* disruptant; blue square, the *ndhD3/D4* disruptant. Open symbols represent cells grown at pH 8, while closed symbols represent cells grown at pH 9. Results are averages ±SD of measurements of at least three independent cultures.

Discussion

Evaluation of photosynthetic and respiratory condition by chlorophyll fluorescence

In this study, effects of gene disruption and growth condition on the photosynthetic and respiratory electron transfer was evaluated by the two different parameters of chlorophyll fluorescence, NPQ_{Dark} and NPQ_{LL}. NPQ_{Dark}, the level of NPQ of dark acclimated cells, primarily reflects the condition of state transition in the dark that is regulated by the redox state of PQ pool. The assumption is supported by the decrease of NPQ_{Dark} upon PQ oxidation by the disruption of the *ndhF1* gene or *ndhD1/D2* genes (**Fig. II-2**), as well as by the increase of NPQ_{Dark} upon PQ reduction by the addition of KCN in the *ndhF1* disruptant (**Fig. II-3A**). The effect of the defect in respiration on state transition was originally reported in the M55 mutant deficient in *ndhB* gene. This mutant, lacking both NDH-1L and NDH-1M complexes (Zhang et al. 2004), is locked in State 1 (**Fig. 5**) in the dark as well as under illumination by blue

light that selectively excites PSI (Schreiber et al. 1995). Effect of respiratory change on the redox state of PQ pool was directly revealed by the measurements of chlorophyll fluorescence spectra at liquid nitrogen temperature in **Chapter I** (Ogawa et al. 2013). Defect in NDH-1 complex was also reported to affect the redox state of Q_A and P700 (Mi et al. 1994). In this context, it is worth to mention that the *cta* disruptants having defects in terminal oxidase showed distinctive fluorescence induction kinetics (Ozaki et al. 2007). Instead of usual increase of chlorophyll fluorescence upon light illumination, these *cta* mutants showed temporal decrease of fluorescence within 100 ms upon weak actinic illumination. The decrease of fluorescence can be induced either by the increase of non-photochemical quenching or photochemical quenching. Increase of non-photochemical quenching may not be the case, since it was shown to decrease upon light illumination as observed in **Figs. II-1 and 2**. Thus, the temporal decrease of fluorescence observed in the *cta* disruptants must be due to increase of photochemical quenching induced by the oxidation of PQ pool by PSI. Apparently, the fluorescence kinetics was affected not only by the changes in the upstream respiratory components but also by the changes in the downstream respiratory components. Furthermore, the results obtained here, together with the Kautsky fluorescence induction of *cta* mutants (Ozaki et al. 2007), may provide the tool to analyze the condition of terminal oxidase.

Contrary to the effects of the disruption of the *ndhF1* gene or the *ndhD1*/*D2* genes, NPQ_{Dark} is not much affected by the disruption of *ndhF3*/*F4* or *ndhD3*/*D4* gene. Instead, extent of the decrease from NPQ_{Dark} to NPQ_{LL} was largely affected (**Fig. II-2**). These genes encode subunits that compose NDH-1 complexes essential for CCM (Zhang et al. 2004; Herranen et al. 2004), based on the phenotype that the disruptants of these genes could not take up CO_2 (Ohkawa et al. 2000a; Shibata et al. 2001; Maeda et al. 2002). Since CO_2 limitation should decrease the demand of ATP and reducing equivalent consumed in Calvin cycle, defects in CO_2 uptake might lead to the increased proton gradient across thylakoid membranes and NPQ. However, this is not the case, since NPQ under light condition decreased, not increased, by the disruption of *ndhF3*/*F4* or *ndhD3*/*D4* genes (**Fig. II-2**).

Alternatively, other regulating mechanisms could be involved. Since PSI/PSII ratio was reported to increase under low CO_2 condition (Manodori and Melis 1984; Murakami et al. 1997), it is reasonable to assume that PSI content

is also increased in the *ndhF3*/*F4* and *ndhD3*/*D4* disruptants, and this actually is the case (**Fig. II-4B**). The physiological relevance of the increased PSI has been explained as the following. Energy requirement for transporting HCO_3^- into cells under low CO_2 stress condition may elevate the cyclic electron transport to the linear one, and as a result the electron transport chain between PSII and PSI may be reduced, and this, in turn, induces the increase of PSI content (Murakami and Fujita 1993; Murakami et al. 1997). This model is consistent with the report that CO_2 uptake is also supported by PSI electron transport (Ogawa et al. 1985; Li and Canvin 1998; Maeda et al. 2002) and that the rate of cyclic electron transport is higher under low CO_2 condition (Deng et al. 2003; Zhang et al. 2004). Causal relationship could be reverse, however, and the increase in PSI due to energy requirement for CCM may be the first event, and this, in turn, increase the activity of cyclic electron transport.

At all events, the results presented here show that the defects in CCM in the *ndhF3*/*F4* and *ndhD3*/*D4* disruptants affect chlorophyll fluorescence through the change in PSI stoichiometry. It should be also noted that the difference between NPQ_{Dark} and NPQ_{LL} became larger upon disruption of the *pmgA* gene (Sonoike et al. 2001), which results in the insufficient suppression of PSI content under high light condition (Hihara et al. 1998). Another photosystem stoichiometry mutant, the disruptant of *sll1961* gene, was shown to share many characteristics of chlorophyll fluorescence kinetics with the *pmgA* disruptant (Fujimori et al. 2005). Although the actual mechanism of the *pmgA* gene or the *sll1961* gene function is still not known, PSI content is higher in these mutants compared with the wild-type cells (Hihara et al. 1998; Fujimori et al. 2005). Thus, the difference between NPQ_{Dark} and NPQ_{LL} seems to be useful for the detection of the change in PSI content as well as any physiological alteration that is accompanying the change in photosystem stoichiometry, such as changes in CO_2 availability.

The defects in NDH-1L and NDH-1L' complexes by the disruption of *ndhF1* and *ndhD1*/*D2* genes decrease both NPQ_{Dark} and NPQ_{LL} due to the PQ oxidation in the dark and in the light by the decreased activity of respiratory electron transport (**Fig. II-2**). Furthermore, $(NPQ_{Dark}-NPQ_{LL})/NPQ_{LL}$ is small in the *ndhF1* and *ndhD1*/*D2* disruptants (**Figs. II-2 and 5**), suggesting the small difference of the redox state of the PQ pool between in the dark and in the light. On the other hand, the defect in NDH-1MS and NDH-1MS' complexes due

to the disruption of the *ndhF3*/*F4* and *ndhD3*/*D4* genes results in the large difference between NPQ_{Dark} and NPQ_{LL} (**Figs. II-2 and 5**), suggesting that the PQ pool is oxidized only in the light. In this way, it would be possible to judge which type of the NDH-1 complex is involved in a phenomenon by simply measuring the two parameters, NPQ_{Dark} and NPQ_{LL}.

It must be noted, however, that this type of evaluation is not valid when several different cellular metabolic pathways are simultaneously altered. It was pointed out that PFD that gives minimum qN in the actinic light-dependent curves of qN was proportional to growth PFD (Campbell and Öquist 1996) and this seems to be due to the increased non-photochemical quenching under State 2 in the dark at least in part (Sonoike et al. 2001). Apparently, the growth light condition significantly affects both photosynthesis and respiration, leading to the altered redox condition of PQ and chlorophyll fluorescence. Increased NPQ_{Dark} of the cells grown under high light condition could be explained by the relative increase of respiratory activity due to the suppression of photosynthesis under high light. The difference between NPQ_{Dark} and NPQ_{LL} is larger in the cells grown under high light condition than in the cells grown under moderate light condition (Sonoike et al. 2001). This is rather hard to explain, since PSI/PSII ration must be lower in the cells grown under high light condition (Hihara and Sonoike 2001). High light response of cyanobacteria is quite complicated phenomenon involving many acclimatory processes (Muramatsu and Hihara 2012) and dissection of such complicated processes are apparently not possible by the simple comparison of the two chlorophyll fluorescence parameters.

Growth pH affects light-response of NPQ through the change in photosystem stoichiometry

Increase of the PSI content induced by the disruption of *ndhF3*/*F4* genes is less obvious when cells were grown at alkaline pH at 9 (**Fig. II-4B**, green diamonds). Apparently, the increased supply of the inorganic carbon as a form of HCO_3^- must relieve the cells from insufficient CO_2 availability caused by the gene disruption. On the other hand, elevation in growth pH did not much affect the PSI content in the wild-type cells (**Fig. II-4B**). Since the wild-type cells could utilize CO_2 as inorganic carbon source for photosynthesis in contrast to the case of the *ndhF3*/*F4* disruptant, it is natural to assume that increased

supply of HCO_3^- due to elevation in pH did not have great influence on the availability of inorganic carbon in the case of the wild-type cells. These results suggest that the availability of inorganic carbon, not pH or concentration of CO_2 itself, is the factor that regulates stoichiometry of PSI.

The elevation in growth pH did not affect a light-response of NPQ in the wild-type cells (**Fig. II-3B**). This is apparently consistent with the report that CO_2 supply does not affect the typical pattern of qN versus light where qN decreases to a minimum around growth light condition (Campbell and Öquist 1996). In the case of CCM mutants, however, situation seems to be different. Light response of NPQ is altered by the disruption of *ndhF3*/*F4* and *ndhD3*/*D4* genes, and the change was further modified by the shift of pH from 8 to 9 (**Figs. II-2 and 3B**). Apparently, the amount of available inorganic carbon affects the light response curve of NPQ only when cells cannot utilize CO_2 by the defects in CO_2 uptake. *Synechocystis* sp. PCC 6803 possesses five systems for inorganic carbon acquisition (Price et al. 2008); two CO_2 uptake systems (Shibata et al. 2001) and three HCO_3^- transporters (Omata et al. 1999; Shibata et al. 2002; Price et al. 2004). Use of mutants defective in these systems for inorganic carbon uptake, together with the different growth condition of pH or CO_2 concentration, would enable us to analyze the effects of inorganic carbon uptake on cyanobacterial photosynthesis, and for that purpose, determination of NPQ_{Dark} and NPQ_{LL} would be a rapid method to monitor the effects on photosynthesis non-destructively.

Materials and Methods

Strains and growth conditions

The wild-type cells and the *ndh* gene disruptants of *Synechocystis* sp. PCC 6803 were grown at 30°C in BG11 medium, buffered with 20 mM TES-KOH (pH 8.0) or 20 mM CHES-KOH (pH 9.0) and bubbled with air for 24 h under continuous illumination using fluorescent lumps from two sides. PFD of the growth light was determined with a light meter (LI-250, LI-COR Biosciences). Growth light PFD was 50 µmol m^{-2} s^{-1} when determined by a flat sensor (QUANTUM, LI-COR Biosciences) facing to one side, which corresponds to 120 µmol m^{-2} s^{-1} determined by a spherical micro-sensor (US-SQS/L, Walz) to

detect the light from all directions. The *ndhF1* disruptant was constructed by transposon mutagenesis and conferred resistance to chloramphenicol (Ozaki et al. 2007). Other *ndh* gene disruptants, a kind gift from Professor Teruo Ogawa, were constructed by inserting cassettes that confer resistance to several different antibiotics (Ohkawa et al. 2000b). The constructs used to generate single mutants were also used to generate double mutants (Ohkawa et al. 2000a; Bernát et al. 2011). The *ndhD1* and *ndhD2* genes double disruptant (the *ndhD1*/*D2* disruptant), the *ndhD3* and *ndhD4* genes double disruptant (the *ndhD3*/*D4* disruptant) and the *ndhF3* and *ndhF4* genes double disruptant (the *ndhF3*/*F4* disruptant) were resistant to kanamycin/chloramphenicol, kanamycin/spectinomycin and kanamycin/hygromycin, respectively, and were maintained in the presence of appropriate antibiotics. Concentration of the antibiotics in the culture medium is as follows: Chloramphenicol at 25 μg ml^{-1}, kanamycin at 20 μg ml^{-1}, spectinomycin at 20 μg ml^{-1} or hygromycin at 20 μg ml^{-1}. The antibiotics were added to the pre-culture medium but not to the culture medium used for the measurements of chlorophyll fluorescence.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured as described in Ogawa et al. 2013 using a pulse-amplitude chlorophyll fluorometer (WATER-PAM, Walz). The peak wavelength of the measuring light LEDs is 650 nm and that of the actinic light LEDs is 660 nm. The maximum fluorescence of light-acclimated cells (Fm') and the maximum fluorescence determined in the presence of DCMU (Fm) were used for calculation of NPQ as (Fm-Fm')/Fm' (Bilger and Björkman 1990). A 0.8 s pulse of saturating light from 660 nm LEDs was given to determine Fm' after illumination by actinic light for 150 s. PFD of the actinic light was determined in a cuvette filled with water using a spherical micro-sensor (US-SQS/L, Walz) with a light meter (LI-250, LI-COR Biosciences). Fm was measured in the presence of 20 μM DCMU with illumination by the actinic light and the saturating light. The response of NPQ to the actinic PFD (**Fig. II-1**) was measured with changing in actinic light intensity continuously from dark to high light. NPQ in the dark was determined by the second saturating pulse applied after 150 s following the first pulse in the dark, in order to make the interval of the saturating pulse constant between the measurements in the dark and in the light.

Fluorescence emission spectra determined at 77 K

The ratio of PSI/PSII was estimated by 77 K fluorescence emission spectra measured as described in Ogawa et al. 2013 using a fluorescence spectrometer (FP-8500, JASCO) with a low temperature attachment (PU-830, JASCO). The cells were illuminated by white light from a light source (Cold Spot, PICL-NRX, Nippon P. I.) in the presence of 10 μM DCMU for 10 min prior to the measurements. PFD of the illumination was determined as 550 μmol m^{-2} s^{-1} by a flat sensor (QUANTUM, LI-COR Biosciences) with a light meter (LI-250, LI-COR Biosciences). The samples were excited by the light at 435 nm for chlorophyll excitation with an excitation slit width of 10 nm. The ratio of PSI/PSII was evaluated by the ratio of PSI fluorescence peak (F_{PSI}) and PSII fluorescence peak (F_{PSII}).

Estimation of the chlorophyll content per cell

Chlorophyll content per cell was estimated by chlorophyll concentration (μg ml^{-1}) per OD of the cell suspension at 750 nm. Chlorophyll was extracted into methanol and chlorophyll concentration (μg ml^{-1}) was determined as described in Grimme and Boardman 1972. The OD of the cell suspension at 750 nm and the absorbance of the chlorophyll solution at 665 nm were measured with a spectrophotometer (V-650, JASCO).

Contents in Chapter II had been published in 2015 as a paper titled “Dissection of respiration and photosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 by the analysis of chlorophyll fluorescence” in Journal of Photochemistry and Photobiology B: Biology 144: 61-67 (doi: 10.1016/j.photobiol.2015.02.005) by Elsevier.

Chapter III

Problems and Its Solutions of Chlorophyll Fluorescence Measurements in Cyanobacteria

Introduction

Chlorophyll fluorescence has been widely used to assess the condition of photosynthesis mainly due to the non-destructive nature of the measurements (Krause and Weis 1991; Govindjee 1995; Campbell et al. 1998). Since the yield of fluorescence from Photosystem II (PSII) is much higher than that from Photosystem I (PSI) at room temperature, total fluorescence mainly reflects the condition of PSII. Chlorophyll fluorescence is high when the PSII electron acceptor, Q_A (**Fig. 1** in **General Introduction**), is reduced, while it is low when Q_A is oxidized. In other words, under the latter condition where the PSII reaction center is open, energy could be used for photosynthesis and the yield of fluorescence is low (see **Fig. 2** in **General Introduction**). Since utilization of absorbed energy for photosynthesis could compete with heat dissipation or fluorescence emission, the change in the yield of chlorophyll fluorescence reflects that of photosynthesis or of heat dissipation. Thus, the decrease in the yield of fluorescence (i.e. fluorescence quenching) could be induced either by photochemical quenching due to energy utilization for photosynthesis or by non-photochemical quenching due to energy dissipation as heat. These two types of quenching can be distinguished by applying short saturating pulses, which are too short to induce non-photochemical quenching but are sufficient to reduce Q_A fully and suppress photochemical quenching (Schreiber et al. 1986; Krause and Weis 1991). In the absence of non-photochemical quenching (e. g. as in dark-acclimation leaves of land plants), the fluorescence yield is at its minimum (Fo) while the application of a saturating pulse induces the maximum level of fluorescence (Fm), providing information on the maximum photosynthetic efficiency of PSII as Fv/Fm calculated as (Fm-Fo)/Fm (Kitajima and Butler 1975). Healthy plant leaves always give Fv/Fm values of 0.8-0.85.

In the case of the cyanobacterium *Synechocystis* sp. PCC 6803, however, the situation is quite different from that of land plants. Simple application of a

saturating pulse to dark-acclimated cyanobacterial cells gives an apparent Fv/Fm value of 0.4. In cyanobacteria, the respiratory electron transport chain shares several components such as plastoquinone (PQ) with the photosynthetic electron transport chain (Aoki and Katoh 1982; Peschek and Schmetterer 1982; **Fig. 4** in **General Introduction**), and the PQ pool is reduced even in the dark-acclimated cells. The redox state of the PQ pool affects chlorophyll fluorescence quenching in cyanobacteria, since the main component of cyanobacterial non-photochemical quenching is not energy-dependent quenching but the state transition (Campbell and Öquist 1996), which is regulated by the redox state of the PQ pool (Mullineaux and Allen 1986). Thus, State 2 is induced by the reduction of the PQ pool in dark-acclimated cyanobacterial cells, and the maximum fluorescence level cannot be obtained by dark acclimation, in contrast to the case of land plants. In the case of cyanobacteria, Fm should be determined under illumination in the presence of DCMU, which inhibits electron transfer from Q_A to Q_B (see **Fig. 1** in **General Introduction**), thus oxidizing the PQ pool to eliminate the reducing pressure from respiratory electron transport (Campbell and Öquist 1996), resulting in State 1. On the other hand, the fluorescence level in the dark has often been regarded as Fo in cyanobacteria as well as in land plants, although photochemical quenching must be partly suppressed and State 2 partly induced in the dark due to the reduction of the PQ pool. In fact, under background illumination of weak blue light, which preferentially excites PSI, the yield of minimum fluorescence was higher than in the dark, and application of a saturating pulse also gave a higher yield of maximum fluorescence under background weak blue light than in the dark (Schreiber et al. 1995; El Bissati et al. 2000; see also **Fig. III-1**), suggesting suppression of State 2 by background weak blue light. The result shows that background weak blue light might be necessary to obtain the 'true' Fo in cyanobacteria.

Evaluation of photosynthetic conditions by chlorophyll fluorescence measurement becomes further complicated due to the influence of respiration in cyanobacteria. I have reported that the photosynthetic electron transport rate evaluated by chlorophyll fluorescence measurement is underestimated due to reduction of the PQ pool by respiration in *Synechocystis* sp. PCC 6803 (Ogawa et al. 2013; see also **Figs. I-1 and 2** in **Chapter I**). This indicates that the influence of respiration on photosynthesis would disturb the precise estimation

of photosynthesis by chlorophyll fluorescence measurements in cyanobacteria. Furthermore, the presence of a large amount of phycobilins, which are highly fluorescent even under functional conditions, also leads to the misevaluation of photosynthesis. The influence of fluorescence from phycobilins on the chlorophyll fluorescence measurement would not be negligible in cyanobacteria. It has been reported that the minimum chlorophyll fluorescence level in the dark is dependent on the phycocyanin/chlorophyll ratio, and in an extreme case, the minimum fluorescence is very low in a mutant of *Synechococcus* sp. PCC 7942 lacking phycocyanin (Campbell et al. 1998). Similar low chlorophyll fluorescence in a phycobilisome-less mutant was also reported in *Synechocystis* sp. PCC 6803 (El Bissati and Kirilovsky 2001). As a result, the values of the photosynthetic parameters estimated from chlorophyll fluorescence yield changed according to the change in phycocyanin content (Campbell et al. 1998; El Bissati and Kirilovsky 2001). Furthermore, fluorescence from PSI chlorophyll might also affect the fluorescence signals in cyanobacteria, since PSI generally binds up to 90% of the total chlorophyll and the contribution of PSI to the total fluorescence signals is much higher in cyanobacteria than in land plants (Campbell et al. 1998).

A similar problem is also observed in land plants, albeit to a lesser extent. At wavelength longer than 700 nm, PSI significantly contributes to the level of fluorescence even in land plants (Genty et al. 1990; Pfündel et al. 2013). It was reported that the influence of non-photochemical quenching on the minimum fluorescence emission at 730 nm is smaller than that at 690 nm in *Hordeum vulgare* due to a higher contribution of PSI fluorescence in the longer wavelength region (Genty et al. 1990). To correct the influence of PSI fluorescence, Pfündel et al. (2013) compared the determined quenched minimum fluorescence (Fo') level and the estimated Fo' level calculated by the equation developed by Oxford and Baker (1997). By assuming that the difference between the two values came from the PSI fluorescence level, they estimated the contribution of PSI fluorescence, which turned out to be about 25% of total fluorescence in C_3 plants (Pfündel et al. 2013). Theoretically, this method could also be applied for the estimation of the contribution of the phycobilins and PSI fluorescence in cyanobacteria. In non-diazotrophic cyanobacteria, which cannot utilize molecular dinitrogen, the degradation of phycobilisome is induced under nitrogen-deficient conditions, a phenomenon

that is called bleaching or chlorosis because of the yellow color of the cell culture (Allen and Smith 1969; Grossman et al. 1993). Although the phycobilisome contents were reported to change dynamically upon nitrogen starvation in cyanobacteria, it was virtually impossible to estimate the efficiency pf PSII photochemistry under nitrogen starvation by chlorophyll fluorescence measurements. This problem might be solved by the estimation of the basal fluorescence level from phycobilisomes and PSI.

In this chapter, I tried to exclude the factors which disturb the chlorophyll fluorescence measurements. Background illumination with weak blue light during the measurements relieved the influence of respiratory electron chain on chlorophyll fluorescence. Furthermore, I estimated the contribution of the basal fluorescence by applying the method of Pfündel et al. (2013) to the cyanobacterium *Synechocystis* sp. PCC 6803 grown in the presence of various concentrations of nitrogen. When the levels of Fo under weak blue light and Fm under illumination in the presence of DCMU were corrected for the basal fluorescence in the calculation of the chlorophyll fluorescence parameters, the 'true' maximum quantum yield of PSII was around 0.8 under nitrogen-sufficient conditions, which was similar to the value observed in land plants. The results indicate that subtraction of basal fluorescence from phycobilins or PSI is essential for the precise evaluation of the photosynthetic condition of cyanobacterial cells.

Results

The levels of chlorophyll fluorescence were compared in the cells of the cyanobacterium *Synechocystis* sp. PCC 6803 dark-acclimated for 10 min and those under weak background illumination with blue light (**Fig. III-1** and **Table III-1**). The minimum fluorescence and maximum fluorescence upon a saturating pulse were higher in the cells under weak blue light than those in the dark, suggesting that the chlorophyll fluorescence was already quenched in the dark (Schreiber et al. 1995; El Bissati and Kirilovsky 2001). The cause of this quenching in the dark was ascribed to the reduction of the PQ pool by respiratory NAD(P)H dehydrogenase (NDH) complexes (Mi et al. 1992; Mi et al. 1994). Application of weak blue light preferentially excites PSI, oxidizes the PQ pool and eliminates the quenching of chlorophyll fluorescence, since PSI

generally binds up to 90% of the total chlorophyll in cyanobacteria. On the other hand, strong blue light at 740 μmol m^{-2} s^{-1} induced non-photochemical quenching due to the photoprotective system regulated by the orange carotenoid protein (OCP) (Wilson et al. 2006; Kirilovsky 2007). Since NPQ, a chlorophyll fluorescence parameter reflecting non-photochemical quenching, showed a minimum value under approximately 60-70 μmol m^{-2} s^{-1} (**Fig. III-2**), I used blue light at 70 μmol m^{-2} s^{-1} to oxidize the PQ pool without significant fluorescence quenching by OCP. A similar weak blue light at 80 μmol m^{-2} s^{-1} was also used for the induction of State 1 in the absence of chlorophyll fluorescence quenching due to OCP (Wilson et al. 2006; Kirilovsky 2007). When cells were illuminated with background weak blue light (70 μmol m^{-2} s^{-1}) during the measurement, difference in Φ_{PSII}, a parameter representing effective quantum yield of electron transfer through PSII, between the wild-type cells and the *ndhF1* disruptant (see **Fig. I-1** in **Chapter I**) was smaller than that in the absence of background blue light (**Fig. III-3**). This suggests that background illumination with weak blue light relieves influence of respiratory chain on photosynthesis and that it is useful for eliminating the influence of respiration on chlorophyll fluorescence measurements. The observed effect of weak blue light was, however, much larger at the maximum fluorescence level (14-18% change) than at the minimum fluorescence level (3.0-3.7% change (**Table III-1**). I have assumed that this discrepancy could be partly ascribed to the 'basal fluorescence' from phycobilins or PSI chlorophylls. In the presence of substantial basal fluorescence, quenching of fluorescence should be underestimated more in terms of the minimum fluorescence than the maximum fluorescence, since the relative contribution of the basal fluorescence is smaller in the fluorescence at the maximum level.

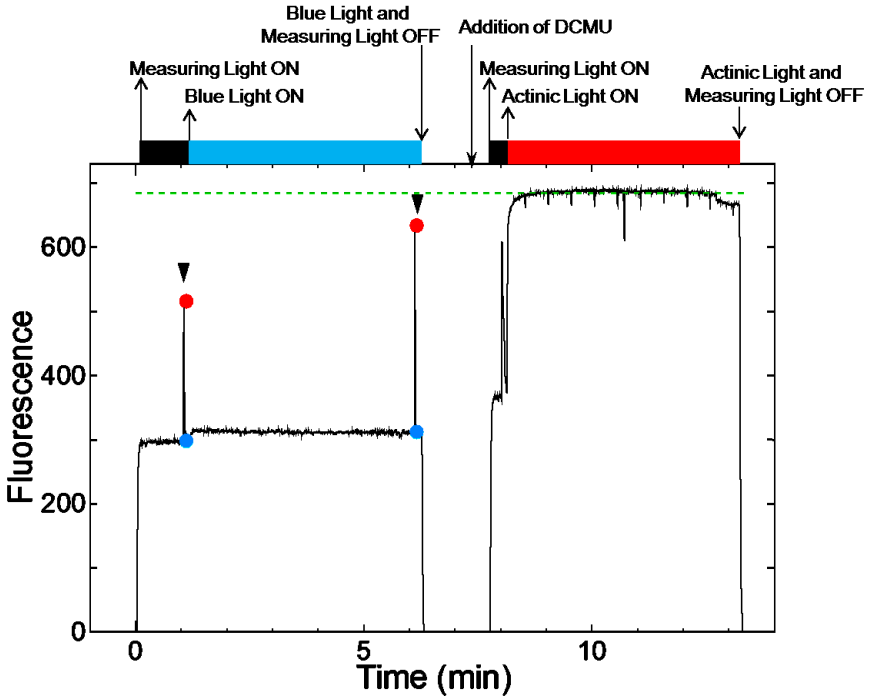

Fig. III-1 A typical example of the measurements of fluorescence levels presented in **Table III-1**. Fluorescence level was recorded along with the time course (black solid line) by using a PAM fluorometer. Black inverted triangles represent the time of the illumination with saturating pulse to determine maximum fluorescence level (represented by red circles). Minimum fluorescence level was determined just before the application of the saturating pulses (blue circles). Green dashed line indicates the fluorescence level determined under illumination in the presence of DCMU. Bars on the top represent the light regime during the measurement; black, blue or red bar represents dark, illumination with weak blue light or illumination with actinic light, respectively.

Table III-1 Minimum or maximum fluorescence levels normalized by chlorophyll concentration.

Condition	Minimum fluorescence level	Maximum fluorescence level
Dark acclimated	91.2 ± 6.1	159 ± 5
Under weak blue light	94.2 ± 6.5	184 ± 8
Light/DCMU	-	194 ± 9
Calculated Fo'*	84.9 ± 4.9	-

*Calculated according to Equation 1 (see also Oxborough & Baker 1997).

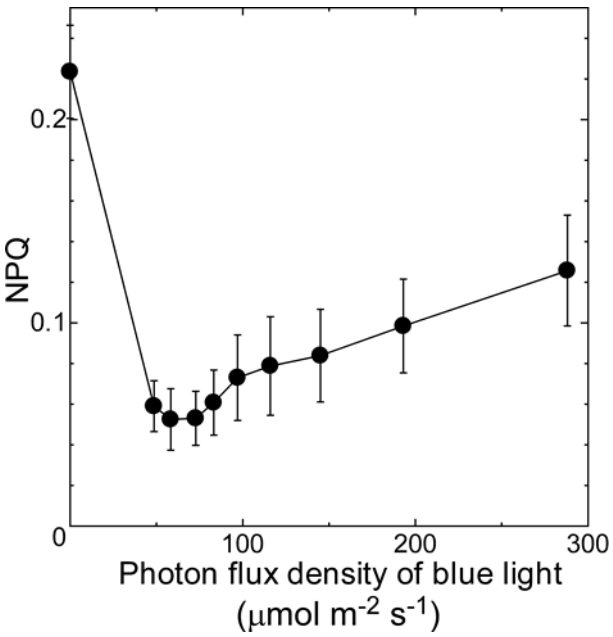

Fig. III-2 The response of NPQ, a chlorophyll fluorescence parameter calculated as (Fm-Fm')/Fm, to the photon flux densities of blue light. Cells were grown under nitrogen-sufficient conditions for 24 h. Averages ±SD for three independent cultures are presented.

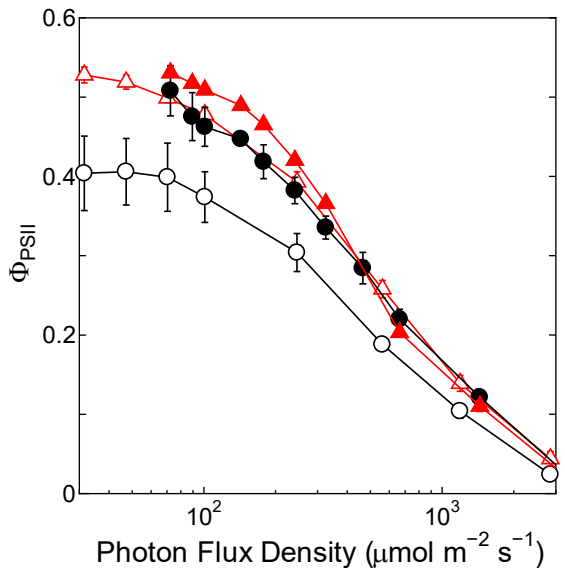

Fig. III-3 The response of Φ_{PSII} to the photon flux densities of actinic light in the wild-type cells (black circles) and the *ndhF1* disruptant (red triangles). Cells were dark acclimated for 10 min prior to the measurements (open symbols), or illuminated with background weak blue light (70 μmol m^{-2} s^{-1}) prior to the measurements as well as during the measurements (closed symbols). Averages ±SD for three independent cultures are presented. Data of open symbols are taken from Ogawa et al. 2013 (see also **Fig. I-1** in **Chapter I**).

I tested this hypothesis using the equation to estimate the Fo' value from Fo, Fm and Fm' values developed by Oxborough and Baker (1997).

$$\mathrm{Fo} = \frac{\mathrm{Fo}}{\mathrm{Fv/Fm} + \mathrm{Fo/Fm'}} \quad (1)$$

This equation is based on the assumption that Fo or Fm is quenched to the level of Fo' or Fm', respectively, by the same increase in the rate of thermal decay. Application of this equation to cyanobacteria is not so simple, since the fluorescence level of cyanobacterial cells was already quenched in the dark, as mentioned above. Thus, the fluorescence level of the dark-acclimated cells could not be used as the Fo value. Here I regarded the minimum fluorescence level under weak blue light as Fo, and that in the dark as quenched Fo'. Similarly, I regarded the maximum fluorescence level obtained in the presence of DCMU under actinic light as Fm, and that in the dark as quenched Fm'. The theoretical Fo' level calculated from the Fo value under weak blue light, the Fm value under illumination in the presence of DCMU and the Fm' value in the dark appeared to be smaller than the observed Fo' determined in the dark (**Table III-1**). Pfündel et al. (2013) explained the difference between measured Fo' and calculated Fo' in *Arabidopsis thaliana* and *Zea mays* by the presence of PSI fluorescence. They introduced the term of PSI fluorescence into Equation 1 and estimated the contribution from PSI fluorescence. Following the method of Pfündel et al. (2013), I assumed that this difference is caused by the basal fluorescence from phycobilins and/or PSI chlorophyll. On such an assumption, the magnitude of the basal fluorescence (defined as '*f*' herein) could be estimated by solving the equation below:

$$\mathrm{Fo'} - f = \frac{\mathrm{Fo} - f}{\mathrm{Fv}/(\mathrm{Fm} - f) + (\mathrm{Fo} - f)/(\mathrm{Fm'} - f)} \quad (2)$$

It must be noted that all the variables except for f can be directly measured and that this equation can be solved for f by an analytical method as in the following (see also Pfündel et al. 2013).

$$f = \frac{\mathrm{Fo'Fm} - \mathrm{FoFm'} - \sqrt{(Fm - Fo)(Fm' - Fo')(Fo - Fo')(Fm - Fm')}}{(Fm - Fm') - (Fo - Fo')} \quad (3)$$

To verify this hypothesis, the cellular phycobilin content was modified by changing the nitrogen availability in the growth culture of the cyanobacterium. The phycocyanin content per cell declined along with the

decrease in the concentration of nitrogen in the growth medium, while the chlorophyll content was less affected (**Fig. III-4A**) as reported earlier (Allen and Smith 1969; Grossman et al. 1993; Salmon et al. 2013). Since the phycocyanin/chlorophyll ratio decreased with decreasing nitrogen concentration (**Fig. III-4B**), the basal fluorescence must also decrease. It seems that this was the case, and the decrease of the basal fluorescence was clearly observed under low phycocyanin/chlorophyll conditions, i.e. nitrogen-deficient conditions (**Fig. III-5**), indicating the validity of Equation 2 in cyanobacteria as well as in land plants. It must be noted that photosystem stoichiometry, i.e. the PSI/PSII ratio, was more or less constant regardless of the nitrogen concentration in the growth medium (**Fig. III-6**), in accordance with earlier reports on the photosystem stoichiometry in the absence of nitrogen (Li and Sherman 2002; Sato et al. 2008). Thus, the contribution of the PSI fluorescence to the basal fluorescence should be rather limited. Furthermore, the basal fluorescence was almost negligible in the cells with the lowest amount of phycocyanin (**Fig. III-5**). It seems that most of the basal fluorescence in cyanobacteria could be attributed to the fluorescence from phycobilins under the experimental conditions in this study.

Presumably reflecting the decrease of fluorescence from phycobilins, the Fo under weak blue light and Fm under illumination in the presence of DCMU also significantly decreased along with the decrease of the phycocyanin/chlorophyll ratio, due to a high (>60%) contribution of fluorescence from phycobilins (**Fig. III-7**, filled symbols). However, when 'true' Fo and Fm values were calculated by subtracting basal fluorescence as Fo-*f* and Fm-*f*, the effect of the phycocyanin/chlorophyll ratio was much smaller than the apparent Fo and Fm especially in the case of Fo-*f* (**Fig. III-7**, open symbols). The correction using Equation 2 therefore appears to be necessary to estimate the 'true' Fo values for cells with different contents of phycocyanin.

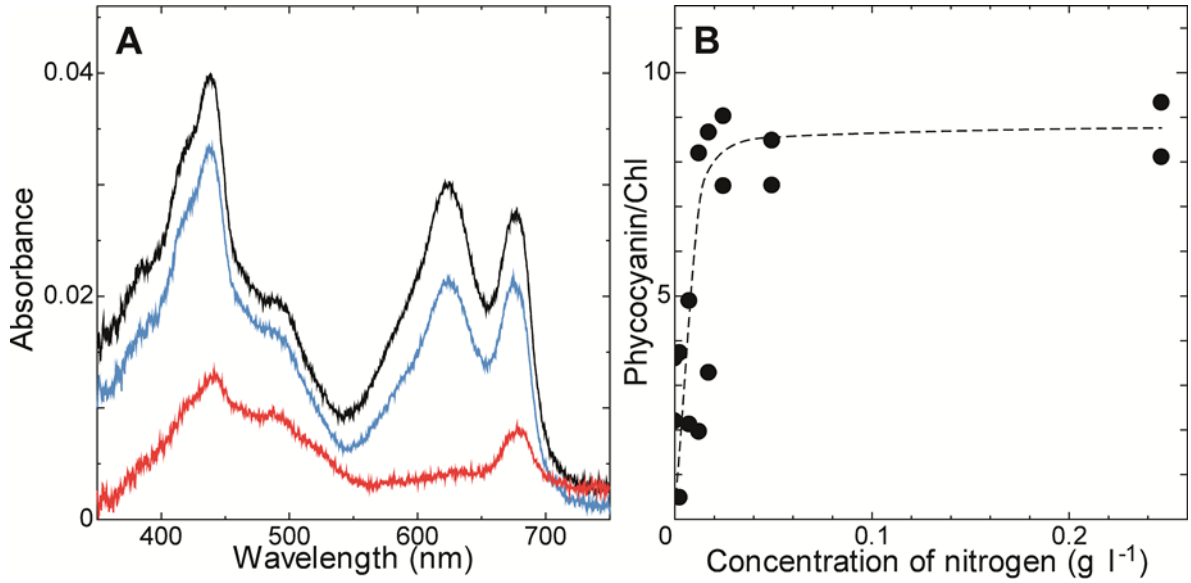

Fig. III-4 Change in the content of photosynthetic pigments due to nitrogen deficiency. (**A**) Absorption spectra of cells grown with different nitrogen concentrations for 48 h. The optical densities of the cell cultures at 750 nm were adjusted to 0.2 for the measurements. A black line, blue line or red line represents cells grown in growth medium containing 0.247, 0.0247 or 0.00 g l^{-1}, respectively. (**B**) The phycocyanin/chlorophyll ratio of cells grown with different nitrogen concentrations for 24 or 48 h.

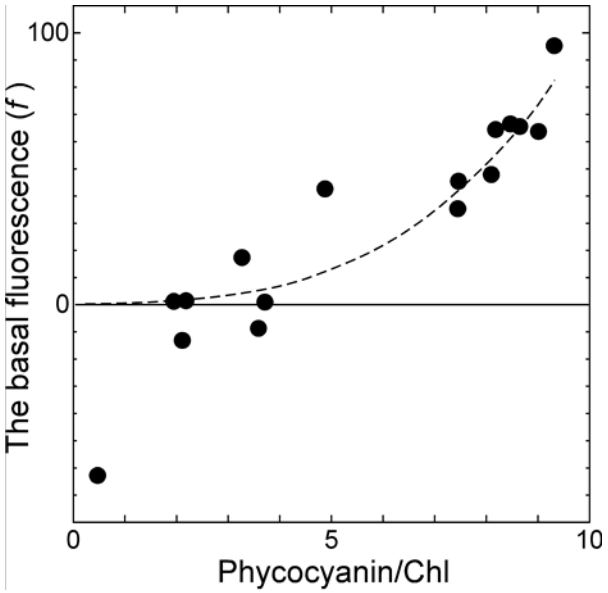

Fig. III-5 The relationship between the change in the basal fluorescence *f*, calculated from Equation 2, and the phycocyanin/chlorophyll ratio of the cells grown with different nitrogen concentrations (R^2=0.867).

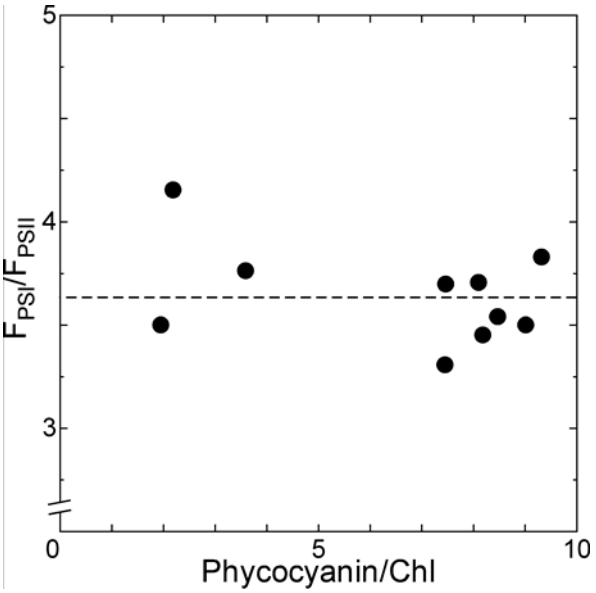

Fig. III-6 The relationship between the ratio of the peak height of PSI fluorescence and PSII fluorescence (F_{PSI}/F_{PSII}), an index for the PSI/PSII ratio, and the ratio of phycocyanin and chlorophyll of the cells grown with different nitrogen availability. F_{PSI}/F_{PSII} was calculated from the chlorophyll fluorescence emission spectra of cells determined at 77 K. Cells were illuminated by white light in the presence of DCMU for 10 min prior to the measurements to lock the cells in State 1. Light at 435 nm was used for the measurements to excite chlorophyll preferentially.

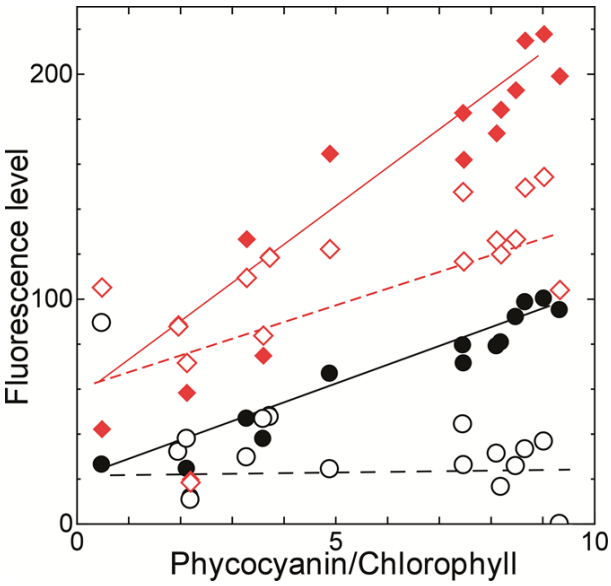

Fig. III-7 The relationship between the fluorescence levels and the phycocyanin/chlorophyll ratio of the cells grown with different nitrogen concentrations. Filled symbols, the observed fluorescence levels; open symbols, fluorescence levels calculated by subtracting the basal fluorescence *f*. Black circles, observed Fo under weak blue light or calculated Fo-*f*; red diamonds, observed Fm under illumination in the presence of DCMU of calculated Fm-*f*.

As regards the values of conventional Fv/Fm calculated as apparent (Fm-Fo)/Fm, they did not show a clear tendency in the course of the decrease in the phycocyanin/chlorophyll ratio during nitrogen starvation (**Fig. III-8A**). On the other hand, the rate of photosynthesis estimated on an O_2 evolution per chlorophyll basis in the presence of bicarbonate as an electron acceptor was reported to decrease to 50% upon nitrogen starvation (e.g. Li and Sherman 2002). This discrepancy could also be ascribed to the presence of basal fluorescence. Cells under nitrogen-sufficient conditions contains a large amount of phycocyanin, and the calculation of Fv/Fm would be underestimated due to the influence of basal fluorescence on Fm but not on Fv [please note that both Fm-Fo and (Fm-*f*)-(Fo-*f*) are the same Fv]. On the other hand, Fv/Fm would not be affected very much by basal fluorescence in the case of cells that were grown under nitrogen-deficient conditions with minimum phycocyanin. To eliminate such interference from the basal fluorescence, Fv/(Fm-*f*) was plotted against the phycocyanin/chlorophyll ratio, instead of apparent Fv/Fm. The value of Fv/(Fm-*f*) in cells grown under nitrogen-sufficient conditions was around 0.8 (**Fig. III-8B**), and comparable with the values observed in land plants (Krause and Weis 1991; Campbell et al. 1998). It was also revealed that Fv/(Fm-*f*) declined with a decrease in the phycocyanin/chlorophyll ratio under nitrogen-deficient conditions (**Fig. III-8B**). This result indicates that Fv/(Fm-*f*) is much more appropriate for the measure of the actual quantum yield of PSII than the conventional Fv/Fm.

To explore the cause of the reduced quantum yield of PSII, the reoxidation kinetics of Q_A^- were compared between cells grown under nitrogen-sufficient condition and nitrogen-deficient conditions (**Fig. III-9**). The rate of the initial decrease of chlorophyll fluorescence, which represents the rate of electron transfer from Q_A to Q_B (Krause and Weis 1991), was not much affected by the nitrogen starvation, while the relative amplitude of the fast decay component decreased by 23% in cells grown under nitrogen-deficient conditions (**Fig. III-9**, open symbols; **Table III-2**). In the presence of DCMU, an inhibitor of electron transport from Q_A to Q_B, the slow component due to recombination between Q_A^- and the donor side of PSII appeared (Vass et al. 1999). There was no obvious difference in these slow fluorescence decay kinetics between cells grown under nitrogen-sufficient and nitrogen-deficient conditions (**Fig. III-9**, filled symbols; **Table III-2**). These results indicate

that nitrogen starvation partly affects the photochemistry of PSII but the large decrease of quantum yield of PSII, Fv/(Fm-*f*) (**Fig. III-8B**), could not be explained by the change observed here.

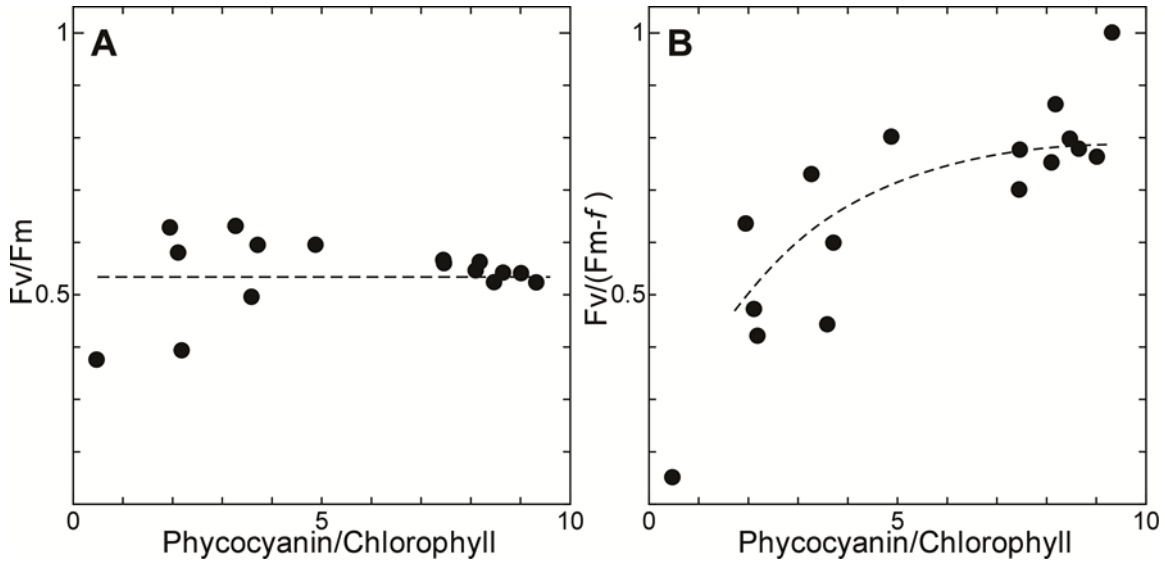

Fig. III-8 The relationship between maximum quantum yield of PSII and the phycocyanin/chlorophyll ratio of the cells grown in the culture medium with different nitrogen concentrations. (**A**) Conventional Fv/Fm. (**B**) Calculated Fv/(Fm-*f*) subtracting the basal fluorescence level. Fo was measured under illumination by weak blue light, and Fm was measured under illumination in the presence of DCMU to eliminate the influence of respiration.

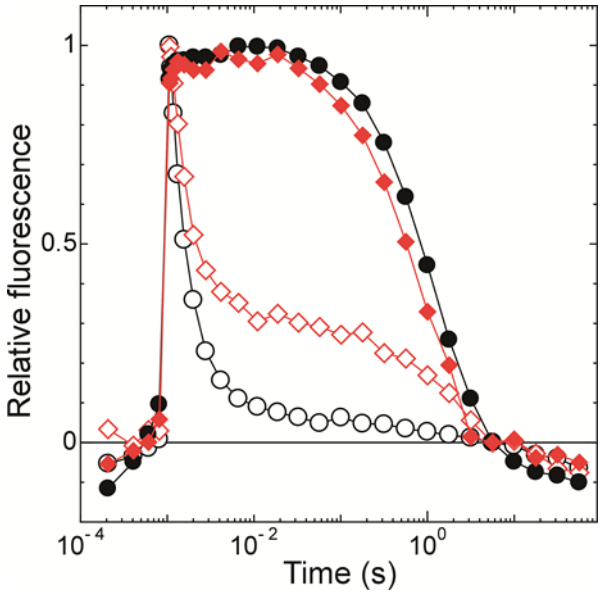

Fig. III-9 Reoxidation kinetics of Q_A^- in the absence (open symbols) or presence of DCMU (filled symbols) of the cells grown under nitrogen-sufficient conditions (black circles) or nitrogen-deficient conditions (red diamonds) for 24 h. The level of the data point at 5 s and the level of the peak were regarded as 0 and 1, respectively, for normalization of the fluorescence signals. Results are averages of measurements of three independent cultures.

Table III-2 Lifetime and amplitude of each component of the fluorescence decay kinetics shown in **Fig. III-9**.

Growth	Condition	Component	Lifetime	Relative amplitude (%)
+N	No addition	Fast	0.53 ± 0.06 (ms)	78.0 ± 2.3
		Middle	6.98 ± 0.91 (ms)	9.63 ± 0.77
		Slow	1.47 ± 1.42 (s)	5.93 ± 1.10
	+DCMU	Middle	256 ± 51 (ms)	16.4 ± 3.4
		Slow	1.08 ± 0.06 (s)	83.0 ± 2.8
-N	No addition	Fast	0.52 ± 0.12 (ms)	60.6 ± 7.5
		Middle	33.2 ± 23.7 (ms)	8.83 ± 4.06
		Slow	1.37 ± 0.39 (s)	29.0 ± 5.4
	+DCMU	Middle	160 ± 53 (ms)	20.9 ± 8.4
		Slow	0.91 ± 0.21 (s)	76.7 ± 9.4

Discussion

Background weak blue light illumination as a procedure to determine Fo and Fm in cyanobacteria

Fo and Fm are the two parameters most easily determined in the dark in land plants, making the parameter Fv/Fm=(Fm-Fo)/Fm useful for the quick assessment of the photosynthetic condition. Fo can be measured as the level of fluorescence of the leaves that have been dark acclimated, and application of a saturating pulse under such conditions would give the Fm value, since the PQ pool could be fully oxidized after the appropriate dark acclimation. The situation for cyanobacteria is totally different because of the presence of respiratory electron transport reducing the PQ pool in the dark (Hirano et al. 1980; Schreiber et al. 1995; Campbell and Öquist 1996). Dark acclimation of cyanobacterial cells does not oxidize the PQ pool but rather reduces it. Light illumination in the presence of DCMU is necessary to obtain the Fm value in cyanobacteria (Campbell and Öquist 1996; Campbell et al. 1998; Sonoike et al. 2001). It was reported that blue light illumination at low intensities, which preferentially excites PSI, gave an Fm value similar to the values obtained in the presence of DCMU (El Bissati et al. 2000; El Bissati and Kirilovsky 2001), and could be also used to obtain the Fm value. In this study, I examined the difference in Fm values measured under blue light illumination or in the presence of DCMU. The maximum fluorescence level under illumination of weak blue light was slightly but significantly lower (4.4-6.8%, **Table III-1**) than that under illumination in the presence of DCMU. The result suggests that the weak blue light in the conditions in this study predominantly excites PSI but slightly excites PSII as well. I use the value obtained in the presence of DCMU as Fm hereafter.

In some earlier studies, fluorescence parameters such as Fv/Fm were calculated based on the 'precise' Fm value that was determined in the presence of DCMU, as described above (Campbell and Öquist 1996; Campbell et al. 1998; El Bissati et al. 2000). Even in those studies, however, the fluorescence level determined in the dark-acclimated cells was used as Fo. This would not be correct, at least in theory, since the Fo level should be quenched to the Fo' level when the PQ pool is reduced. Actually, the increase in the fluorescence level

was observed upon weak blue light illumination in the dark-acclimated cyanobacterial cells (Schreiber et al. 1995; El Bissati et al. 2000; El Bissati and Kirilovsky 2001; Wilson et al. 2006; Jallet et al. 2012). I also observed a 3% increase of the fluorescence level upon weak blue light illumination (**Table III-1**), suggesting the quenching of Fo to Fo' in the dark. This level of Fo under weak blue light may not be accurate due to the possible excitation of PSII as observed in the case of Fm. Unfortunately, the use of DCMU would not be a solution to the problem, since Q_A is reduced in the presence of DCMU, resulting in decreased photochemical quenching while Fo requires maximum photochemical quenching by definition. Here, I assumed that the Fo level under weak blue light could be used as a proxy of the 'true' Fo. Two Fo' levels can be calculated by Equation 1 from the two corresponding Fm' values, one under weak blue light and the other in the presence of DCMU. The difference in the two Fo' levels is only 0.65-0.84%, which is much smaller than the original difference of Fm' (4.4-6.8%). Thus, the Fo level under weak blue light could be used as the 'true' Fo with an error of <1%. I use the value obtained under weak blue light as Fo hereafter.

Although I used only one cyanobacterial species, *Synechocystis* sp. PCC 6803, in this study, it is known that the degree of state transition and redox state of the PQ pool in the dark varies among different cyanobacterial species, presumably due to a difference in the activity of respiratory electron transfer. Even if the cells are grown at the same temperature under the same photon flux density, some cyanobacteria show a highly reduced PQ pool and are in State 2 in the dark while others show an almost oxidized PQ pool and are in State 1 in the dark, resulting in different levels of state transition. However, a comparative study using six cyanobacterial species showed that the relationship between the redox state of the PQ pool and state transition was actually identical under weak light in the range between dark and growth light, irrespective of the PQ redox condition in the dark (Misumi et al. 2016). I presume that background weak blue light could be commonly used for the estimation of the Fo level in most of the cyanobacterial species, although the quenched level of Fo' could only be determined in the dark-acclimated samples in limited cyanobacterial species.

The method to estimate 'true' Fo and Fm levels in cyanobacteria

As mentioned above, the observed difference between the Fo' level in the dark and the Fo level under weak blue light (3%) is far smaller than the difference between the Fm' level in the dark and the Fm level observed under weak blue light (16%). This controversy prompted me to assume a disturbance of the chlorophyll fluorescence measurements by the large basal fluorescence due to phycobilins in cyanobacteria. In this study, I estimated the contribution of basal fluorescence using Equation 2, which introduces basal fluorescence *f* into Equation 1 as in Pfündel et al. (2013). Upon application of Equation 2 to cyanobacteria, I used the dark-acclimated level of fluorescence Fo' or Fm', not Fo or Fm, for the reasons described above. The estimated basal fluorescence *f* showed a strong correlation with the phycocyanin/chlorophyll ratio (R^2=0.867, **Fig. III-5**) indicating that the basal fluorescence is useful as an index for estimating the level of fluorescence from phycobilins. The contribution of the estimated basal fluorescence *f* to the Fo level was more than 60% in the cells grown under nitrogen-sufficient conditions (see **Fig. III-7**), while the estimated contribution of PSI fluorescence to the Fo level in *A. thaliana* or in *Z. mays* was only 24% or 50%, respectively (Pfündel et al. 2013). So the effect of the basal fluorescence on the calculation of the chlorophyll fluorescence parameter should be much larger in cyanobacteria than in land plants. Indeed, the difference between the 'apparent' Fv/Fm and 'true' Fv/Fm is 4% or 14% in *A. thaliana* or *Z. mays*, respectively (Pfündel et al. 2013), while it was up to 50% in *Synechocystis* 6803 cells containing large amount of phycocyanin (**Fig. III-8A, B**). It must be noted that the 'true' value of Fv/Fm corrected for the basal fluorescence, i.e. Fv/(Fm-*f*), was around 0.8 under nitrogen-sufficient conditions (**Fig. III-8B**), which is comparable with the value of Fv/Fm in land plants. The result indicates that the main cause of the low Fv/Fm value observed in cyanobacteria is the basal fluorescence from the phycobilins. In addition to the phycobilins, PSI fluorescence also potentially contributes to the basal fluorescence in cyanobacteria in spite of its low yield at room temperature (Krause and Weis 1984; Krause and Weis 1991), since the PSI/PSII ratio is much higher in cyanobacteria than in land plants and >90% of chlorophyll molecules are bound on PSI (Campbell et al. 1998). However, the PSI/PSII ratio was not affected much under nitrogen-deficient conditions (**Fig. III-6**). The

contribution of PSI fluorescence to basal fluorescence *f* seems to be negligible in cyanobacteria, presumably due to the low yield of chlorophyll fluorescence.

In any event, the assumption of basal fluorescence in combination with weak blue light excitation employed in this study enables us to calculate 'true' Fo and Fm values, provided that the background weak blue light illumination and the addition of DCMU is possible. The latter requirement may be dispensable, since Fm values could also be estimated under weak blue light illumination if we allow a 5% error level as described above. It has not been possible to compare the absolute values of Fv/Fm between land plants and cyanobacteria, with the only exception of phycobiline-less cyanobacteria mutants (El Bissati and Kirilovsky 2001), but my method makes it possible to estimate the absolute values of Fv/Fm in cyanobacteria containing different quantities of phycocyanin.

Nitrogen deficiency lowers the maximum quantum yield of PSII

The effect of nitrogen deficiency on photosynthetic electron transport is somewhat controversial. There are reports that the rate of photosynthetic oxygen evolution is decreased by nitrogen deficiency (Collier et al. 1994; Görl et al. 1998; Li and Sherman 2002; Krasikov et al. 2012). For example, it was reported that the rate of O_2 evolution (in the presence of bicarbonate as an ultimate electron acceptor) decreased to about 50% when cells were grown in nitrogen-depleted medium (Li and Sherman 2002). It was suggested that the decrease in the photosynthetic oxygen evolution is primarily caused by the decline in CO_2 fixation and photosynthetic electron transfer retains its functionality, based on the results showing that cells under nitrogen-sufficient conditions and those under nitrogen-deficient conditions showed no difference if methyl viologen (MV), an artificial electron acceptor from PSI, was used to monitor the rate of photosynthetic electron transfer (Krasikov et al. 2012).

On the other hand, there are several works reporting a decrease in Fv/Fm under nitrogen-deficient conditions (Sauer et al. 2001; Salomon et al. 2013). Since these values were determined in the dark where the PQ pool is reduced due to respiration, the reported decrease in the 'apparent' Fv/Fm do not necessarily indicate the decrease in the maximum quantum yield of PSII under nitrogen-deficient conditions. To my knowledge, my study is the first attempt to determine the 'true' Fv/Fm value in cells grown under nitrogen-deficient

conditions. The results presented here clearly indicate that nitrogen deficiency lowers the 'true' Fv/Fm to half of the original value (**Fig. III-8B**). The result agrees well with the 50% decrease observed in O_2 evolution upon nitrogen deficiency (Li and Sherman 2002), although the effect of nitrogen deficiency would not be so simple as described above (Krasikov et al. 2012). It must be noted that a significant change in the 'apparent' Fv/Fm was not observed under nitrogen starvation (**Fig. III-8A**), suggesting that the 'apparent' Fv/Fm was underestimated under nitrogen-sufficient conditions due to the basal fluorescence from phycocyanin. As mentioned in the previous section, due to a large contribution of the basal fluorescence to fluorescence signals in cyanobacteria, subtraction of the basal fluorescence is essential to estimate the change in the maximum quantum yield of PSII in the presence of a large amount of phycobilins by chlorophyll fluorescence measurements. For the estimation of PSII efficiency of the cells with different phycobilisome contents by chlorophyll fluorescence measurements, it is necessary to use Fv/(Fm-*f*) instead of the conventional Fv/Fm.

In contrast to the case of nitrogen deficiency, loss of phycobiliproteins by deletion of genes encoding allophycocyanin subunits increases the value of the 'apparent' Fv/Fm measured under weak blue light illumination (Ajlani and Vernotte 1998; El Bissati and Kirilovsky 2001). Apparently, the PSII reaction center is intact in this case, and the change in the 'apparent' Fv/Fm value measured under weak blue light simply reflected that the change in basal fluorescence is due to the decrease in the phycobilisome content. Thus, the cause of the decrease in the 'true' Fv/Fm in cells grown under nitrogen-deficient conditions (**Fig. III-8B**) should be ascribed to the impairment of PSII reaction centers but not to the degradation of phycobilins. Since fluorescence decay kinetics are not greatly affected by the nitrogen deficiency in both the absence and the presence of DCMU (**Fig. III-9**; **Table III-2**), the main cause of the decrease in PSII quantum yield is not clear.

Materials and Methods

Strains and growth conditions

The wild-type cells of *Synechocystis* sp. PCC 6803 were grown at 30°C

in BG11 medium, buffered with 20 mM TES-KOH (pH 8.0) and bubbled with air for 24 or 48 h under continuous illumination using fluorescent lamps from two sides. The photon flux density of the growth light was 120 μmol m^{-2} s^{-1} when measured by a spherical micro-sensor (US-SQS/L, Walz) with a light meter (LI-250, LI-COR Biosciences) inside the growth bottles. To examine the effect of nitrogen-deficient conditions, cells were grown with BG11 medium with reduced $NaNO_3$ contents (0.0494, 0.0247, 0.0173, 0.0124, 0.00741, 0.00247 or 0.00 g nitrogen l^{-1}; the original BG11 medium contains 0.247 g nitrogen l^{-1}). For cultures under nitrogen-deficient conditions, cells were spun down with a centrifuge (MX-305, TOMY) at 15,000 r.p.m. for 6 min and then suspended in BG11 medium without $NaNO_3$. This procedure was repeated once more, and then cells were transferred to BG11 medium with the respective $NaNO_3$ content.

Pulse amplitude chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using a pulse amplitude modulation fluorometer (WATER-PAM, Walz). Cell cultures with an optical density of 0.2 at 750 nm were used for the measurements. The optical density of the cell cultures was determined by a spectrophotometer (V-650, JASCO). Cells were dark-acclimated for 10 min prior to the measurement. After illumination by red measuring light for 1 min, the fluorescence level was determined and this level was regarded as Fo' in the dark (please note that it is not Fo), since State 2 was induced in the dark in cyanobacteria. A 0.8 s saturating light pulse from 660 nm light-emitting diodes (LEDs) was given to determine the maximum fluorescence level under this condition, which was correspondingly regarded as Fm' in the dark (not Fm). After Fo' and Fm' were determined, Fo, the minimum fluorescence level under conditions where photochemical quenching was fully induced and State 2 was suppressed, was determined under illumination (70 μmol m^{-2} s^{-1}) with blue light LEDs peaking at 460 nm for 5 min. Fm, the maximum fluorescence level under conditions where photochemical quenching and State 2 were suppressed, was measured under illumination with actinic light LEDs peaking at 660 nm in the presence of 20 μM DCMU as described in Ogawa et al. (2013). The photon flux density of the blue light was determined in a cuvette filled with water using a spherical micro-sensor (US-SQS/L, Walz) with a light meter (LI-250, LI-COR Biosciences). For calculation of NPQ as (Fm-Fm')/Fm' (Bilger and Björkman 1990), the maximum fluorescence level in

the dark or under illumination of blue light was used for Fm', and that measured under illumination in the presence of DCMU was used for Fm.

Reoxidation kinetics of Q_A^-

Reoxidation kinetics of Q_A^- were measured by using a double-modulated chlorophyll fluorometer (FL200/PS, Photon System Instruments) with no addition or in the presence of 10 μM DCMU. The chlorophyll concentration of the cell suspension was adjusted to 2 μg ml^{-1}. Prior to the measurements, cells were dark-acclimated for 5 min. The cells were illuminated with a 30 μs single-turnover flash for the reduction of Q_A, and subsequent reoxidation kinetics were monitored for 60 s. The measurement of each independent culture was repeated four times.

Absorbance spectra

Absorbance spectra of the intact cells were obtained with a spectrophotometer (V-650, JASCO) equipped with an integrating sphere (ISV-722, JASCO) as described in Ogawa et al. (2013). Concentrations of phycocyanin and chlorophyll estimated from the absorbance spectra (Arnon et al. 1974) were used to evaluate the phycocyanin/chlorophyll ratio.

Estimation of the chlorophyll content per cell

Chlorophyll in cells was extracted into methanol, and the chlorophyll concentration (μg ml^{-1}) was determined as described in Grimme and Boardman (1972). The chlorophyll concentration divided by the optical density of the cell suspension at 750 nm was used as the measure of the chlorophyll contents per cell (Ogawa and Sonoike 2015).

Chlorophyll emission spectra determined at 77 K

The PSI/PSII ratio was estimated from chlorophyll fluorescence emission spectra determined at 77 K as described in Ogawa et al. (2013) and Ogawa and Sonoike (2015) using a fluorescence spectrometer (FP-8500, JASCO) with a low temperature attachment (PU-830, JASCO). Cells were illuminated by white light (50 μmol m^{-2} s^{-1}) from a light source (Cold Spot, PICL-NRX, Nippon P. I.) in the presence of 10 μM DCMU for 10 min prior to the measurement to block the cells in State 1. The samples were excited by light

at 435 nm with an excitation slit width of 10 nm. The PSI/PSII ratio was evaluated by dividing the height of the PSI fluorescence peak (F_{PSI}) by that of the PSII fluorescence peak (F_{PSII}).

Contents in Chapter III had been published in 2016 as a paper titled “Effects of bleaching by nitrogen deficiency on the quantum yield of photosystem II in *Synechocystis* sp. PCC 6803 revealed by chlorophyll fluorescence measurements” in Plant & Cell Physiology 57: 558-567 (doi: 10.1093/pcp/pcw010) by Oxford University Press.

General Discussion

Characteristics of photosynthesis is largely modified by the interaction among cellular metabolic pathways in cyanobacteria

Cyanobacteria are supposed to be the evolutional origin of chloroplasts (Giovannoni et al .1988), and photosynthetic machinery is well conserved between cyanobacteria and land plants (Renger and Renger 2008). As prokaryotes, however, cyanobacteria have no organelle, and photosynthesis coexists with other metabolic pathways within a cell. Thus, all the cyanobacterial cellular metabolic pathways must have some kind of interaction with photosynthesis. In other word, photosynthesis in cyanobacteria could be largely modified by the condition of other metabolic pathways, which is quite different from the case in land plants.

I examined the influence of the defect in respiration (**Chapter I**; Ogawa et al. 2013) or that in CO_2 concentrating mechanism (**Chapter II**; Ogawa and Sonoike 2015) on photosynthetic condition in the cyanobacterium *Synechocystis* sp. PCC 6803 using chlorophyll fluorescence and other spectroscopic measurements as well as electrochemical measurements. I demonstrated that respiration affected photosynthesis through the redox state of the plastoquinone (PQ) pool, resulting in the change of energy distribution between Photosystem II (PSII) and Photosystem I (PSI) (**Chapter I**). On the other hand, CO_2 concentrating mechanism affected the PSI stoichiometry through the content of available inorganic carbon source for the cells presumably accompanying the change in gene expression (**Chapter II**). These results indicate that the mechanism, by which photosynthesis is affected by other metabolic pathways, is totally different between the case of respiration and that of CO_2 concentrating mechanism: respiration affects the redox state of the PQ pool through the change of short-term response, while CO_2 concentrating mechanism affects the photosystem stoichiometry through the change of long-term acclimation.

The difference in the mode of interaction could explain the difference in the influence on chlorophyll fluorescence measurement between respiration and CO_2 concentrating mechanism: respiration disturbs chlorophyll fluorescence

measurement while CO_2 concentrating mechanism does not. Since the change in condition of respiration is reflected in the short-term response mechanism of photosynthesis, the interaction between respiration and chlorophyll fluorescence could be dynamically changed along with the change in measuring conditions such as photon flux density of actinic light (Campbell and Öquist 1996). On the other hand, the change in the condition of CO_2 concentrating mechanism, which is involved in the long-term acclimation accompanying the change in gene expression, would not much affect the chlorophyll fluorescence measurement.

I also found that not only disruptions of genes involved in metabolic pathways but also those of coding aminoacyl-tRNA synthase involved in the translation could affect photosynthesis through the change in the stromal redox status (i.e. the ratio of $NADPH/NADP^+$), resulting in the change of the appearance time of the first peak in the dark-light induction curve of chlorophyll fluorescence. The results presented here indicate that photosynthesis in cyanobacteria would be performed in accordance not only with other metabolic pathways but also with virtually every biological reaction.

Problems in the application of chlorophyll fluorescence measurements to cyanobacteria

Chlorophyll fluorescence reflects the condition of photosynthesis and can be quite easily determined, so that it has been widely used to evaluate the photosynthesis in the fields of plant physiology, ecology and microbiology (Krause and Weis 1991; Govindjee 1995). However, the situation in cyanobacteria is sometimes more complicated than in land plants due to the influence of the interaction among cellular metabolic pathways (Campbell et al. 1998). Other than the influence of respiration discussed above, fluorescence from phycobilisome or PSI would cause the misevaluation of photosynthesis by chlorophyll fluorescence measurement (Campbell et al. 1998). In this study, I developed the methods to precisely evaluate photosynthesis without disturbances from respiration or from phycobilin fluorescence, and revealed that the value of Fv/Fm, a most frequently used fluorescence parameter representing photochemical efficiency of PSII, was similar between cyanobacteria and land plants. Thus, the longstanding discrepancy between apparent large difference in Fv/Fm and quite conserved PSII reaction centers

has been finally dispelled by the careful examination of the procedure of the chlorophyll fluorescence measurements.

The effect of other metabolic pathways than photosynthesis on chlorophyll fluorescence measurement, on the other hand, can be used to evaluate the condition of such metabolic pathways. For example, disruption of many genes, which are not directly involved in photosynthesis, resulted in the changes in dark-light induction curve of chlorophyll fluorescence in the cyanobacterium *Synechocystis* sp. PCC 6803 (Ozaki et al. 2007). I also showed that the condition of respiration or CO_2 concentrating mechanism could be estimated solely by the measurements of NPQ, the chlorophyll fluorescence parameter reflecting the redox state of PQ pool in cyanobacteria (Campbell and Öquist 1996; Ogawa and Sonoike 2015; **Fig. II-2** in **Chapter II**). Needless to say, it is not easy to evaluate the condition of metabolism only by chlorophyll fluorescence, since the interactions among metabolic pathways in a cyanobacterial cell is complicated. Chlorophyll fluorescence measurement is just one of the methods for evaluating the condition of metabolism. For example, let's consider the effect of the changes in aminoacyl-tRNA synthase on photosynthesis. As mentioned above, I found that disruption of genes encoding aminoacyl-tRNA synthase affected photosynthetic electron transport chain possibly through the change in the stromal redox state. Although this peculiar discovery was based on the detailed examination of the chlorophyll fluorescence, other experimental technique was necessary to reveal the mechanism of the influence of translation on photosynthesis. Chlorophyll fluorescence measurement is a non-destructive and easy method, and it is suitable for the first step of analysis. I believe that my results obtained here certainly contribute to such application of chlorophyll fluorescence measurements for the analysis of interaction among cellular metabolic pathways in cyanobacteria.

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References

Ajlani G and Vernotte C (1998) Construction and characterization of a phycobiliprotein-less mutant of *Synechocystis* sp. PCC 6803. *Plant Mol. Biol.* 37: 577-580

Allahverdiyeva Y, Ermakova M, Eisenhut M, Zhang P, Richaud P, Hagemann M, Cournac L and Aro EM (2011) Interplay between flavodiiron proteins and photorespiration in *Synechocystis* sp. PCC 6803. *J. Biol. Chem.* 286: 24007-24014

Allahverdiyeva Y, Mustila H, Ermakova M, Bersanini L, Richaud P, Ajlani G, Battchikova N, Cournac L and Aro EM (2013) Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. *Proc. Natl Acad. Sci. USA* 110: 4111-4116

Allen MM (1968) Simple conditions for growth of unicellular bluegreen algae on plates. *J. Phycol.* 4: 1-3

Allen MM and Smith AJ (1969) Nitrogen chlorosis in blue-green algae. *Arch. Microbiol.* 69: 114-120

Aoki M and Katoh S (1982) Oxidation and reduction of plastoquinone by photosynthetic and respiratory electron transport in a cyanobacterium *Synechococcus* sp. *Biochem. Biophys. Acta* 682: 307-314

Arnon DI, McSwain BD, Tsujimoto HY and Wada K (1974) Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll a and removal of phycocyanin. *Biochim. Biophys. Acta* 357: 231-245

Battchikova N, Zhang P, Rudd S, Ogawa T and Aro EM (2005) Identification of NdhL and Ssl1690 (NdhO) in NDH-1L and NDH-1M complexes of *Synechocystis* sp. PCC 6803. J. Biol. Chem. 280: 2587-2595

References

Battchikova N and Aro EM (2007) Cyanobacterial NDH-1 complexes: multiplicity in function and subunit composition. *Physiol. Plant.* 131: 22-32

Battchikova N, Eisenhut M and Aro EM (2011) Cyanobacterial NDH-1 complexes: novel insights and remaining puzzles. *Biochim. Biophys. Acta* 1807: 935-944

Bernát G, Appel J, Ogawa T and Rögner M (2011) Distinct roles of multiple NDH-1 complexes in the cyanobacterial electron transport network as revealed by kinetic analysis of $P700^+$ reduction in various *ndh*-deficient mutants of *Synechocystis* sp. strain PCC6803. *J. Bacteriol.* 193: 292-295

Bilger W and Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.* 25: 173-185

Bilger W, Schreiber U and Bock M (1995) Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. *Oecologia* 102: 425-432

Brown AH and Webster GC (1953) The influences of light on the rate of respiration of the blue-green alga *Anabaena*. *Amer. J. Bot.* 40: 753-758

Campbell D and Öquist G (1996) Predicting light acclimation in cyanobacteria from nonphotochemical quenching of photosystem II fluorescence which reflects state transition in these organisms. *Plant Physiol.* 111: 1293-1298

Campbell D, Bruce D, Carpenter C, Gustafsson P and Öquist G (1996) Two forms of the Photosystem II D1 protein alter energy dissipation and state transition in the cyanobacterium *Synechococcus* sp. PCC 7942. *Photosynth. Res.* 47: 131-144

Campbell D, Hurry V, Clarke AK, Gustafsson P and Öquist G (1998) Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and

acclimation. *Microbiol. Mol. Biol. Rev.* 62: 667-683

Chisti Y (2007) Biodiesel from microalgae. *Biotechnol. Adv.* 25: 294-306

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

Deng Y, Ye J and Mi H (2003) Effects of low CO_2 on NAD(P)H dehydrogenase, a mediator of cyclic electron transport around photosystem I in the cyanobacterium *Synechocystis* PCC 6803. *Plant Cell Physiol.* 44: 534-540

El Bissati K, Delphin E, Murata N, Etienne AL and Kirilovsky D (2000) Photosystem II fluorescence quenching in the cyanobacterium *Synechocystis* PCC 6803: involvement of two different mechanisms. *Biochim. Biophys. Acta* 1457: 229-242

El Bissati K and Kirilovsky D (2001) Regulation of *psbA* and *psaE* expression by light quality in *Synechocystis* species PCC 6803. A redox control mechanism. *Plant Physiol.* 125: 1988-2000

Field CB, Behrenfeld MJ, Randerson JT and Falkowski P (1998) Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281: 237-240

Fujimori T, Higuchi M, Sato H, Aiba H, Muramatsu M, Hihara Y and Sonoike K (2005) The mutant of *sll1961*, which encodes a putative transcriptional regulator, has a defect in regulation of photosystem stoichiometry in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiol. 139: 408-416

Genty B, Briantais JM and Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990: 87-92

Genty B, Wonders J and Baker NR (1990) Non-photochemical quenching of Fo

in leaves is emission wavelength dependent: consequences for quenching analysis and its interpretation. *Photosynth. Res.* 26: 133-139

Giovannoni SJ, Turner S, Olsen GJ, Barns S, Jane DJ and Pace NR (1988) Evolutionary relationships among cyanobacteria and green chloroplasts. *J. Bacterial.* 170: 3584-3592

Govindjee (1995) Sixty-three years since Kautsky: chlorophyll *a* fluorescence. *Aust. J. Plant Physiol.* 22: 131-160

Görl M, Sauer J, Baier T and Forchhammer K (1998) Nitrogen-starvation-induced chlorosis in *Synechococcus* PCC 7942: adaptation to longterm survival. *Microbiol.* 144: 2449-2458

Grimme LH and Boardman NK (1972) Photochemical activities of a particle fraction P_1 obtained from the green alga *Chlorella fusca*. *Biochem. Biophys. Res. Commun.* 49: 1617-1623

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

Helman Y, Tchermov D, Reinhold L, Shibata M, Ogawa T, Schwarz R, Ohad I and Kaplan A (2003) Genes encoding A-type flavoproteins are essential for photoreduction of O_2 in cyanobacteria. *Curr. Biol.* 13: 230-235

Hendrickson L, Furbank RT and Chow WS (2004) A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. *Photosynth. Res.* 82: 73-81

Herranen M, Battchikova N, Zhang P, Graf A, Sirpio S, Paakkarinen V and Aro EM (2004) Towards functional proteomics of membrane protein complexes in *Synechocystis* sp. PCC 6803. *Plant Physiol.* 134: 470-481

Hihara Y, Sonoike K and Ikeuchi M (1998) A novel gene, *pmgA*, specifically

regulates photosystem stoichiometry in the cyanobacterium *Synechocystis* sp. PCC 6803 in response to high light. *Plant Physiol.* 117: 1205-1216

Hihara Y and Sonoike K (2001) in: Aro EM, Andersson B (Eds.), Regulation, inhibition and protection of photosystem I, Regulation of Photosynthesis. *Kluwer Academic Publishers, Dordrecht*: pp. 507-531

Hirano M, Satoh K and Katoh S (1980) Plastoquinone as a common link between photosynthesis and respiration in a blue-green alga. *Photosynth. Res.* 1: 149-162

Hoch G, Owens OH and Kok B (1963) Photosynthesis and respiration. *Arch. Biochem. Biophys.* 101: 171-180

Jallet D, Gwizdala M and Kirilovsky D (2012) ApcA, ApcF and ApcE are not required for the orange carotenoid protein related phycobilisome fluorescence quenching in the cyanobacterium *Synechocystis* PCC 6803. *Biochim. Biophys. Acta* 1817: 1418-1427

Jones LW and Myers J (1963) A common link between photosynthesis and respiration in a blue-green alga. *Nature* 199: 670-672

Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakazaki N, Naruo K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yamada M, Yasuda M and Tabata S (1996) Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res.* 3: 109-136

Kautsky H and Hirsch A (1931) Neue Versuche zur Kohlensäureassimilation. *Naturwissenschaften* 19: 964

Kirilovsky D (2007) Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism. *Photosynth.*

Res. 93: 7-16

Kitajima M and Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochim. Biophys. Acta* 376: 105-115

Krasikov V, Aguirre von Wobeser E, Dekker HL, Huisman J and Matthijs HCP (2012) Time-series resolution of gradual nitrogen starvation and its impact on photosynthesis in the cyanobacterium *Synechocystis* PCC 6803. *Physiol. Plant.* 145: 426-439

Krause GH and Weis E (1984) Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. *Photosynth. Res.* 5: 139-157

Krause GH and Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42: 313-349

Li Q and Canvin DT (1998) Energy sources for HCO_3^- and CO_2 transport in air-grown cells of *Synechococcus* UTEX 625. *Plant Physiol.* 116: 1125-1132

Li H and Sherman LA (2002) Characterization of *Synechocystis* sp. strain PCC 6803 and Δ*nbl* mutants under nitrogen-deficient conditions. *Arch. Microbiol.* 178: 256-266

Maeda S, Badger MR and Price GD (2002) Novel gene products associated with NdhD3/D4-containing NDH-1 complexes are involved in photosynthetic CO_2 hydration in the cyanobacterium, *Synechococcus* sp. PCC7942. *Mol. Microbiol.* 43: 423-435

Manodori A and Melis A (1984) Photochemical apparatus organization in *Anacystis nidulans* (Cyanophyceae). Effect of CO_2 concentration during cell growth. *Plant Physiol.* 74: 67-71

Mi H, Endo T, Schreiber U, Ogawa T and Asada K (1992) Electron donation

from cyclic and respiratory flows to the photosynthetic intersystem chain is mediated by pyridine nucleotide dehydrogenase in the cyanobacterium *Synechocystis* PCC 6803. *Plant Cell Physiol.* 33: 1233-1237

Mi H, Endo T, Schreiber U, Ogawa T and Asada K (1994) NAD(P)H dehydrogenase dependent cyclic electron flow around photosystem I in the cyanobacterium *Synechocystis* PCC 6803: a study of dark-starved cells and spheroplasts. *Plant Cell Physiol.* 35: 163-173

Misumi M, Katoh H, Tomo T and Sonoike K (2016) 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. *Plant Cell Physiol.* 57: 1510-1517

Mullineaux CW and Allen JF (1986) The state 2 transition in the cyanobacterium *Synechococcus* 6301 can be driven by respiratory electron flow into the plastoquinone pool. *FEBS Lett.* 205: 155-160

Mullineaux CW, Tobin MJ and Jones GR (1997) Mobility of photosynthetic complexes in thylakoid membranes. *Nature* 390: 421-424

Murakami A and Fujita Y (1993) Regulation of stoichiometry between PSI and PSII in response to light regime for photosynthesis observed with *Synechocystis* PCC 6714: relationship between redox state of Cyt b_6-*f* complex and regulation of PSI formation. *Plant Cell Physiol.* 34: 1175-1180

Murakami A, Kim S and Fujita Y (1997) Changes in photosystem stoichiometry in response to environmental conditions for cell growth observed with the cyanophyte *Synechocystis* PCC 6714. *Plant Cell Physiol.* 38: 392-397

Muramatsu M and Hihara Y (2012) Acclimation to high-light conditions in cyanobacteria: from gene expression to physiological responses. *J. Plant Res.*125: 11-39

Nanba M and Katoh S (1984) Effects of dibromothymoquinone on

oxidation-reduction reactions and the midpoint potential of the Rieske iron-sulfur center in photosynthetic electron transport of *Synechococcus* sp. *Biochim. Biophys. Acta* 767: 396-403

Ogawa T, Miyano A and Inoue Y (1985) Photosystem-I-driven inorganic carbon transport in the cyanobacterium, *Anacystis nidulans*. *Biochim. Biophys. Acta* 808: 77-84

Ogawa T (1991) A gene homologous to the subunit-2 gene of NADH dehydrogenase is essential to inorganic carbon transport of *Synechocystis* PCC6803. *Proc. Natl. Acad. Sci. USA* 88: 4275-4279

Ogawa T, Harada T, Ozaki H and Sonoike K (2013) Disruption of the *ndhF1* gene affects chlorophyll fluorescence through state transition in the cyanobacterium *Synechocystis* sp. PCC 6803, resulting in apparent high efficiency of photosynthesis. *Plant Cell Physiol.* 54: 1164-1171

Ogawa T and Sonoike K (2015) Dissection of respiration and photosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 by the analysis of chlorophyll fluorescence. *J. Photochem. Photobiol. B: Biol.* 144: 61-67

Ogawa T and Sonoike K (2016) Effects of bleaching by nitrogen deficiency on the quantum yield of photosystem II in *Synechocystis* sp. PCC 6803 revealed by chlorophyll fluorescence measurements. *Plant Cell Physiol.* 57: 558-567

Ohad I, Raanan H, Keren N, Tchernov D and Kaplan A (2010) Light-induced changes within photosytem II protects *Microcoleus* sp. in biological desert sand crusts against excess light. *PLoS One* 5: e11000

Ohkawa H, Pakrasi HB and Ogawa T (2000a) Two types of functionally distinct NAD(P)H dehydrogenases in *Synechocystis* sp. strain PCC6803. *J. Biol. Chem.* 275: 31630-31634

Ohkawa H, Price GD, Badger MR and Ogawa T (2000b) Mutation of *ndh* genes leads to inhibition of CO_2 uptake rather than HCO_3^- uptake in *Synechocystis*

sp. strain PCC 6803. *J. Bacteriol.* 182: 2591-2596

Omata T, Price GD, Badger MR, Okamura M, Gohta S and Ogawa T (1999) Identification of an ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *Proc. Natl. Acad. Sci. USA* 96: 13571-13576

Oxborough K and Baker NR (1997) Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components - calculation of qP and Fv'/Fm' without measuring Fo' . *Photosynth. Res.* 54: 135-142

Ozaki H, Ikeuchi M, Ogawa T, Fukuzawa H and Sonoike K (2007) Large-scale analysis of chlorophyll fluorescence kinetics in *Synechocystis* sp. PCC 6803: identification of the factors involved in the modulation of photosystem stoichiometry. *Plant Cell Physiol.* 48: 451-458

Ozaki H and Sonoike K (2009) Quantitative analysis of the relationship between induction kinetics of chlorophyll fluorescence and function of genes in the cyanobacterium *Synechocystis* sp. PCC 6803. *Photosynth. Res.* 101: 47-58

Peschek GA and Schmetterer G (1982) Evidence for plastoquinol-cytochrome *f*/b_{563} reductase as a common electron donor to P700 and cytochrome oxidase in cyanobacteria. *Biochem. Biophys. Res. Commun.* 108: 1188-1195

Pfündel EE, Klughammer C, Meister A and Cerovic ZG (2013) Deriving fluorometer-specific values of relative PSI fluorescence intensity from quenching of Fo fluorescence in leaves of *Arabidopsis thaliana* and *Zea mays*. *Photosynth. Res.* 114: 189-206

Price GD, Maeda S, Omata T and Badger MR (2002) Modes of active inorganic carbon uptake in the cyanobacterium, *Synechococcus* sp. PCC7942. *Funct. Plant Biol.* 29: 131-149

Price GD, Woodger FJ, Badger MR, Howitt SM and Tucker L (2004)

Identification of a SulP-type bicarbonate transporter in marine cyanobacteria. *Proc. Natl. Acad. Sci. USA* 101: 18228-18233

Price GD, Badger MR, Woodger FJ and Long BM (2008) Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): functional components, genetic regulation and prospects for engineering into plants. *J.Exp. Bot.* 59: 1441-1461

Renger G and Renger T (2008) Photosystem II: The machinery of photosynthetic water splitting. Photosynth. Res. 98: 53-80

Salomon E, Bar-Eyal L, Sharon S and Keren N (2013) Balancing photosynthetic electron flow is critical for cyanobacterial acclimation to nitrogen limitation. *Biochim. Biophys. Acta* 1827: 340-347

Sato H, Fujimori T and Sonoike K (2008) *sll1961* is a novel regulator of phycobilisome degradation during nitrogen starvation in the cyanobacterium *Synechocystis* sp. PCC 6803. *FEBS Lett.* 582: 1093-1096

Sauer J, Schreiber U, Schmid R, Völker U and Forchhammer K (2001) Nitrogen starvation-induced chlorosis in *Synechococcus* PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. *Plant Physiol.* 126: 233-243

Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O and Hankamer B (2008) Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenerg. Res.* 1: 20-43

Schluchter WM, Zhao J and Bryant DA (1993) Isolation and characterization of the *ndhF* gene of *Synechococcus* sp. strain PCC 7002 and initial characterization of an interposon mutant. *J. Bacteriol.* 175: 3343-3352

Schreiber U (1986) Detection of rapid induction kinetics with a new type of high-frequency modulated chlorophyll fluorometer. *Photosynth. Res.* 9: 261-272

Schreiber U, Schliwa U and Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* 10: 51-62

Schreiber U, Endo T, Mi H and Asada K (1995) Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. *Plant Cell Physiol.* 36: 873-882

Shibata M, Ohkawa H, Kaneko T, Fukuzawa H, Tabata S, Kaplan A and Ogawa T (2001) Distinct constitutive and low-CO_2-induced CO_2 uptake systems in cyanobacteria: genes involved and their phylogenetic relationship with homologous genes in other organisms. *Proc. Natl Acad. Sci. USA* 98: 11789-11794

Shibata M, Ohkawa H, Katoh H, Shimoyama M and Ogawa T (2002) Two CO_2 uptake systems in cyanobacteria: four systems for inorganic carbon acquisition in *Synechocystis* sp. strain PCC 6803. *Funct. Plant Biol.* 29: 123-129

Sonoike K, Hihara Y and Ikeuchi M (2001) Physiological significance of the regulation of photosystem stoichiometry upon high light acclimation of *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.* 42: 379-384

van Kooten O and Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.* 25: 147-150

Vass I, Kirilovsky D and Etienne AL (1999) UV-B radiation-induced donor- and acceptor-side modifications of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803. *Biochemistry* 38: 12786-12794

Wilson A, Ajlani G, Verbavatz JM, Vass I, Kerfeld CA and Kirilovsky D (2006) A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria. *Plant Cell* 18: 992-1007

References

Zhang P, Battchikova N, Jansen T, Appel J, Ogawa T and Aro EM (2004) Expression and functional role of the two distinct NDH-1 complexes and the carbon acquisition complex NdhD3/NdhF3/CupA/Sll1735 in *Synechocystis* sp. PCC 6803. *Plant Cell* 16: 3326-3340

CyanoBase, <http://www.kazusa.or.jp/cyano/>

Fluorome, <http:///www.photosynthesis.jp/flurome/>

早稲田大学　博士（理学）　学位申請　研究業績書

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○	[3] **Takako Ogawa** and Kintake Sonoike (2015) Dissection of respiration and photosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 by the analysis of chlorophyll fluorescence. Journal of Photochemistry and Photobiology B: Biology 144: 61-67 [4] **Takako Ogawa**, Tetsuyuki Harada, Hiroshi Ozaki and Kintake Sonoike (2013) Disruption of the *ndhF1* gene affects chlorophyll fluorescence through state transition in the cyanobacterium *Synechocystis* sp. PCC 6803, resulting in the apparent high efficiency of photosynthesis. Plant & Cell Physiology 54: 1164-1171
講演	※発表者に★印を付す。 **（国際会議・口頭）** [1] ★**Takako Ogawa**, Kenta Suzuki and Kintake Sonoike “Respiration affects photosynthesis through the reducing side of photosystem I in cyanobacterium *Synechocystis* sp. PCC 6803” Finnish-Japanese symposium 2016 (Saariselkä, Finland), 09/2016 **（国際会議・ポスター）** [1] ★**Takako Ogawa** and Kintake Sonoike “Problems and its exploitation of chlorophyll fluorescence measurement in cyanobacterium *Synechocystis* sp. PCC 6803” 15th International Symposium on Phototrophic Prokaryotes (Tübingen, Germany), 08/2015 **（国内学会・口頭）** [1] ★小川敬子、鈴木健太、園池公毅「シアノバクテリア *Synechocystis* sp. PCC 6803 における翻訳関連遺伝子の破壊がクロロフィル蛍光に与える影響」 第 57 回日本植物生理学会年会（岩手大学上田キャンパス）、2016 年 3 月 [2] ★小川敬子、鈴木健太、園池公毅「クロロフィル蛍光測定から見えるシアノバクテリアの代謝系相互作用」 第 56 回日本植物生理学会年会（東京農業大学世田谷キャンパス）、2015 年 3 月

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講演	**（国内学会・口頭）** [3] ★小川敬子、園池公毅「クロロフィル蛍光測定によるシアノバクテリアの呼吸および CO_2 取込み能の解析」 第 55 回日本植物生理学会年会（富山大学五福キャンパス）、2014 年 3 月 [4] ★小川敬子、小川晃男、池内昌彦、原田哲行、園池公毅「シアノバクテリアの NADH 脱水素酵素複合体がプラストキノンプールの酸化還元状態に与える影響」 日本植物学会第 76 回大会（兵庫県立大学姫路書写キャンパス）、2012 年 9 月 **（国内学会・ポスター）** [1] ★小川敬子、鈴木健太、園池公毅「シアノバクテリアにおける翻訳関連遺伝子の破壊の影響は光化学系 I 還元側を通してクロロフィル蛍光に反映される」 日本植物学会第 80 回大会（沖縄コンベンションセンター）、2016 年 9 月 [2] ★小川敬子、鈴木健太、園池公毅「シアノバクテリアにおけるタンパク質翻訳の阻害は呼吸活性を上昇させる」 第 7 回日本光合成学会年会および公開シンポジウム（東京理科大学葛飾キャンパス）、2016 年 5 月 [3] ★小川敬子、園池公毅「フィコビリン量によらずシアノバクテリアの光化学系Ⅱ量子収率をクロロフィル蛍光により見積もる方法」 藍藻の分子生物学 2015（かずさアカデミアホール）、2015 年 11 月 [4] ★小川敬子、園池公毅「窒素欠乏条件下におけるシアノバクテリアの光合成量子収率の新しい評価法による解析」 第 6 回日本光合成学会年会および公開シンポジウム（岡山国際交流センター）、2015 年 5 月 [5] ★小川敬子、園池公毅「シアノバクテリアの NPQ の光強度依存性を決める要因の解析」 第 5 回日本光合成学会年会および公開シンポジウム（近畿大学農学部奈良キャンパス）、2014 年 5 月 [6] ★小川敬子、園池公毅「シアノバクテリアの *ndhF1* 遺伝子の破壊は光合成速度を見かけ上高くする」 第 4 回日本光合成学会年会および公開シンポジウム（名古屋大学）、2013 年 5 月

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その他	（国際会議・口頭） [1] Masahiro Misumi, **Takako Ogawa**, Hiroshi Katoh, Tatsuya Tomo and ★Kintake Sonoike “Probing cyanobacterial redox and energy distribution by chlorophyll fluorescence” Finnish-Japanese symposium 2016 (Saariselkä, Finland), 09/2016 [2] Masahiro Misumi, **Takako Ogawa**, Hiroshi Katoh, Tatsuya Tomo and ★Kintake Sonoike “Variation of redox state of plastoquinone pool in cyanobacteria revealed by photochemical and non-photochemical quenching of chlorophyll fluorescence” International Conference Photosynthesis Research for Sustainability (Pushchino, Russia), 06/2016 [3] **Takako Ogawa**, Kenta Suzuki, Ayaka Aoki, Masahiro Misumi and ★Kintake Sonoike “Effects of metabolic modification on the redox state of photosynthetic electron transfer in cyanobacteria” The German-Japanese Binational Seminar 2015 "Harvesting Light: From light to biotechnological products" (Atami, Japan), 05/2015 [4] **Takako Ogawa**, Teruo Ogawa, Masahiko Ikeuchi and ★Kintake Sonoike “Effect of cyanobacterial NDH complex on the redox state of plastoquinone pool” Japanese-Finnish Seminar 2012 “Photosynthetic Research for Sustainable Energy Production” (Naantali, Finland), 09/2012 [5] ★Kintake Sonoike, **Takako Ogawa**, Tetsuyuki Harada and Hiroshi Ozaki “Probing metabolic interactions in cyanobacterial cells by chlorophyll fluorescence measurements” Binational Seminar Germany-Japan “Microalgal Products: From Metabolic Fundamentals to Promising Applications” (Freiburg, Germany), 10/2011 （国際会議・ポスター） [1] **Takako Ogawa**, Masahiro Misumi and ★Kintake Sonoike “Estimation of photosynthesis by chlorophyll fluorescence measurements of cyanobacteria and eukaryotic algae” 17th International Congress on Photosynthesis Research: Photosynthesis in a Changing World (MECC Maastricht, The Netherlands), 08/2016 （国内学会・口頭） [1] ★園池公毅、三角将洋、鈴木健太、小川敬子、加藤浩、鞆達也「シアノバクテリアと藻類における呼吸と光合成の相互作用」 日本植物生理学会第 57 回年会（岩手大学上田キャンパス）、2016 年 3 月

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