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Conformational changes in photosynthetic pigment proteins on thylakoid membranes can lead to fast non-photochemical quenching in cyanobacteria

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A high non-photochemical quenching (NPQ) appeared below the phase transition temperature when *Microcystis aeruginosa* PCC7806 cells were exposed to saturated light for a short time. This suggested that a component of NPQ, independent from state transition or photo-inhibition, had been generated in the PSII complex; this was a fast component responding to high intensity light. Glutaraldehyde (GA), commonly used to stabilize membrane protein conformations, resulted in more energy transfer to PSII reaction centers, affecting the energy absorption and dissipation process rather than the transfer process of phycobilisome (PBS). In comparison experiments with and without GA, the rapid light curves (RLCs) and fluorescence induction dynamics of the fast phase showed that excess excitation energy was dissipated by conformational change in the photosynthetic pigment proteins on the thylakoid membrane (PPPTM). Based on deconvolution of NPQ relaxation kinetics, we concluded that the fast quenching component (NPQ_f) was closely related to PPPTM conformational change, as it accounted for as much as 39.42% of the total NPQ. We hypothesize therefore, that NPQ_f induced by PPPTM conformation is an important adaptation mechanism for *Microcystis* blooms under high-intensity light during summer and autumn.

chlorophyll fluorescence, photoprotection, *Microcystis aeruginosa*, non-photochemical quenching, thylakoid membrane proteins

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In various cyanobacteria, harvested light energy absorbed by phycobilisomes (PBS) is transferred from L_{CM} to the chlorophylls of photosystem II (PSII) and photosystem I (PSI) [1,2]. Some excitons are formed in the reaction center (RC) and are then deactivated through three pathways: photochemical reaction, fluorescence, and thermal dissipation or non-photochemical quenching (NPQ) [3,4]. NPQ is an indispensable pathway of deactivation and plays an important role in protecting PSII from photo-inhibition or photo-damage when exposed to stress conditions [5,6]. Rapid relaxation of NPQ in darkness suggests that algae have a strong vitality and adaptability to high intensity light. NPQ can be deconvoluted into three components based on the different relaxation times in dark periods after exposure to high intensity light [7–9]. These three components of NPQ were found to be (i) a fast quenching component (NPQ_f), which refers to the ΔpH-dependent process or high-energy state (qE); (ii) a medium quenching component (NPQ_m), related to the quenching of state transition process (qT); and (iii), a low quenching component (qI), resulting from photo-inhibition of photosynthesis.

In cyanobacteria there is no qE dependence on a trans-thylakoid proton gradient [10], although this is the predominant component of NPQ in higher plants [11]. Since

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*q*E is a fast component of NPQ that appears in a few seconds in higher plants when exposed to high-intensity light [7,8], the rapid response of PSII to saturated light might be affected due to *q*E deficiency in cyanobacteria. However, our survey in Lake Taihu, China, showed that most *Microcystis* biomass, including some large colonies, was floating on the water surface [12]. In addition, under conditions of wind-wave disturbance, some *Microcystis* were randomly brought to the surface and exposed to high light during summer and autumn. Previous researchers [13] have reported that light intensity was highest at 13:00 on sunny days, and was approximately 2000 μmol photons s^{-1} m^{-2}. As *Microcystis* can grow well under these high light conditions, we had reason to believe that *Microcystis* must have developed an efficient quenching mechanism to dissipate excess absorbed energy. We hypothesized that a close relationship might exist between adaptation to high intensity light and the special structure of the PSII complex. In addition, El-Bissati *et al*. [14] reported that an undiscovered mechanism of NPQ induced by strong light in *Synechocystis* PCC 6803 was different from state transition or photo-inhibition. We aimed, therefore, to reveal some key mechanisms of NPQ_f in cyanobacteria, which lead to rapid dissipation of excess energy in the PSII reaction center (RC).

Some studies have focused on thermal dissipation of absorbed energy in cyanobacteria. Arteni *et al*. [15] reported that hemiellipsoidal PBS have relatively flexible conformation, and that PBS arrangement on the thylakoid membranes of the red algae *Porphyridium cruentum* changed when it was transferred from low to high light conditions; however, the photosynthetic mechanism of this light-inducing change was not specified. Also, a decoupling of PE from PBS in red algae was induced by a green laser, which led to less excitation energy reaching the photosynthetic reaction center, and prevented the photosystem from photo-damage [16]. It has been reported that IsiA, acting as an additional and efficient light-harvesting complex (LHC) for PSI, could increase the absorptional cross-section of PSI [17,18], and that IsiA aggregates induced by high light were in a strongly quenched state, suggesting that they were efficient dissipation pathways of absorbed energy [19,20]. In addition to IsiA, HLIPs, high light-induced proteins related to LHCs [21], were synthesized in cyanobacterial cells under high light conditions and seemed to protect the photosynthetic reaction center by heat dissipation of excess excitation energy [22]. Recently, many researchers have reported that orange carotenoid protein (OCP) plays a key role in the photoprotective mechanism in various cyanobacteria [23–25]. It has been demonstrated that absorbed light can induce structural changes in OCP in the carotenoid, and convert the protein from its resting orange form into a red, relatively unstable, active form [23]. Gwizdala *et al*. [24] used an *in vitro* reconstitution system to show that cyanobacteria thermally dissipated excess absorbed energy through interactions between OCP, fluorescence recovery protein (FRP), and PBS.

Since PBS, OCP, FRP, IsiA, and HLIPs are all photosynthetic pigment proteins in the thylakoid membrane (PPPTM), and can act as efficient quenchers of absorbed energy under high light conditions, we hypothesized that some energy dissipation mechanism might be related to the conformational changes in PPPTM induced by high light. In previous studies, stable and flexible PPPTM conformation could be obtained with or without 1% (v/v) glutaraldehyde (GA) [16]. Therefore, using GA, we attempted to reveal the relationship between PPPTM conformational change and NPQ, and to quantify this quenching mechanism. Our findings offer an explanation as to why cyanobacterial blooms avoid photo-inhibition and grow rapidly under the high intensity light conditions present during the summer.

1 Materials and methods

1.1 Cyanobacterial strain and culture conditions

Microcystis aeruginosa PCC 7806 was originally obtained from the Pasteur Culture Collection of Cyanobacteria (PCC) in France, and was kindly provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), of Chinese Academy of Sciences. *Microcystis* was grown in BG_{11} medium [26] under a 12:12 LD (light: dark) cycle with an intensity of 60 μmol photons s^{-1} m^{-2} provided by cool white fluorescent tubes at (25±1)°C. Cultures were harvested during the exponential growth phase and concentrations of cells were adjusted to yield approximately 5 μg of chlorophyll a mL^{-1} in the measurement of all the following photosynthetic parameters.

1.2 Phase-transition temperature of the thylakoid membrane

The phase-transition temperature of the thylakoid membrane in intact cells was measured following the method reported by Murata *et al*. [27], with a few modifications. The maximum relative electron transport rate ($rETR_{max}$) plotted against different temperatures (30, 28, 26, 24, 22, 20, 18, 16, 14, 12, 10, and 8°C) showed a discontinuity point caused by change in the physical phase of the thylakoid membrane. Also, the maximum photochemical efficiency of PSII (F_v/F_m) was plotted against decreasing temperature, where the temperature of the highest F_v/F_m value was used to suggest the phase-transition temperature of the thylakoid membrane.

1.3 PPPTM conformational changes

Glutaraldehyde (GA) has been used as a stabilizer for phycobilisome (one kind of PPPTM) conformations [15,28].

Therefore, stable and flexible PPPTM conformations can be acquired with or without GA. Before the measurement of chlorophyll fluorescence parameters, fluorescence emission spectra, and fast-phase fluorescence induction dynamics, the *Microcystis* cells were dark-adapted for one hour and then added to a small Eppendorf tube containing varying concentrations of GA (Sigma Chemical Co., St. Louis, MO, USA). Tubes were kept for 2 min in the dark, during which the infiltration of GA into cells was primarily dependent upon the diffusion rate.

1.4 Chlorophyll fluorescence measurements in PSII

Chlorophyll fluorescence parameters were measured using a pulse-amplitude modulated fluorescence monitoring system (Phyto-PAM, Walz, Effeltrich, Germany). After the dark-adaptation period and treatment with 0, 0.5%, 1% and 2% (v/v) GA, F_o (original fluorescence) and F_m (maximum fluorescence) were measured using a low measuring light at 0.15 μmol photons m^{-2} s^{-1} and a saturation light pulse of about 3000 μmol photons m^{-2} s^{-1} for 0.8 s, respectively. F_s (instantaneous steady state fluorescence) and F_m were measured under active light conditions of 256 μmol photons m^{-2} s^{-1}. The F_v/F_m value, the actual photochemical efficiency of PSII (Φ_{PSII}), the relative electron transport rate (rETR), and non-photochemical quenching (NPQ) were calculated as follows: $F_v/F_m=(F_m-F_o)/F_m$ [29]; $\Phi_{PSII}=(F_{m'}-F_s)/F_{m'}$ [30]; The rETR=$(F_{m'}-F_s)/F_{m'}\times0.42\times$PPFD [31] (PPFD, photosynthetic photo flux density); qP =$(F_{m'}-F_s)/(F_m-F_{o'})$; NPQ=$1-(F_v/F_v)$ [32]. A RLC was used to measure the rETR as a function of irradiance. F_s and $F_{m'}$ were measured under different light intensities at every step of actinic light exposure. The RLC included 11 increments of actinic irradiance: 1, 32, 64, 128, 256, 384, 512, 640, 832, 1088, and 1344 μmol photons m^{-2} s^{-1}, and a saturation light pulse of about 3000 μmol photons m^{-2} s^{-1} with a 10 s interval between any two adjacent steps, was applied to determine $F_{m'}$ [5]. Some parameters of RLC such as F_o, F_m, F_v/F_m, Φ_{PSII}, initial slope of RLC (α), maximum relative electron transport rate ($rETR_{max}$), and half-saturated intensity of illumination light (I_k) were automatically analyzed and recorded using the Phytowin v1.47 software installed in the Phyto-PAM.

1.5 Fluorescence emission spectra

Fluorescence emission spectra were determined using a steady-state spectrofluorimeter (QuantaMaster 4CM, Photon Technology International (PTI), New Jersey, USA) under conditions of 0 and 0.5% (v/v) GA. Samples were dark-adapted at room temperature (25°C) for 30 min prior to measurement. Using a computerized nonlinear optimization approach, the fluorescence spectra were separated into their respective components, which were imitated with Gaussian distributions [33]. Light intensity was controlled by excitation slit width. Low light and high light were relative values, obtained under 0.25 and 0.5 nm slit widths, respectively.

1.6 Chlorophyll fluorescence induction dynamics of fast-phase

A plant efficiency analyzer (PEA) (Handy PEA, Hansatech, England) was used to monitor chlorophyll fluorescence induction dynamics for dark-adapted (1 h) cultures (each treatment in triplicate) under conditions of 0 and 0.5% (v/v) GA. Fast-phase dynamic curves (O-J-I-P transient) were analyzed using a JIP test [34]. The JIP test translated the original fluorescence data into biophysical parameters that quantified the flow of energy through PSII. All of the parameters related to the onset of fluorescence induction (Table 1).

1.7 Deconvolution of NPQ relaxation kinetics

The *Microcystis* cultures were exposed for 30 min at a PPFD of 1344 μmol photons m^{-2} s^{-1} provided by the actinic light in the Phyto-PAM. The actinic light was then turned off and the algae were kept in the dark to measure the relaxation kinetics of NPQ by applying saturating pulses at different times from the beginning of the dark period (2, 5, 10, 15, 20, 30, and 40 min). The dark recovery data for NPQ

Table 1 Summary of the JIP test parameters calculated using data extracted from the fast fluorescence transient. Parameter definitions are given in the text

Formulae and terms	Illustrations
$V_J=(F_J-F_o)/(F_m-F_o)$	Relative variable fluorescence intensity at the J-step
$M_o=4(F_{300\,\mu s}-F_o)/(F_m-F_o)$	Approximated initial slope of the fluorescence transient
$\Phi_{Po}=TR_o/ABS=[1-(F_o/F_m)]$	Maximum quantum yield for primary photochemistry (at t=0)
$\Phi_{Eo}=ET_o/ABS=[1-(F_o/F_m)]*\psi_o$	Quantum yield for electron transport (at t=0)
$\psi_o=ET_o/TR_o=(1-V_J)$	Probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at t=0)
Specific energy fluxes (per Q_A-reducing PSII reaction center (RC))	
$ABS/RC=M_o*(1/V_J)*(1/\Phi_{Po})$	Absorption flux per RC
$TR_o/RC=M_o*(1/V_J)$	Trapped energy flux per RC (at t=0)
$ET_o/RC=M_o*(1/V_J)*\psi_o$	Electron transport flux per RC (at t=0)
$DI_o/RC=(ABS/RC)-(TR_o/RC)$	Dissipated energy flux per RC (at t=0)
Phenomenological energy fluxes (per excited cross section (CS))	
$ABS/CS_o\approx F_o$	Absorption flux per CS (at t=0)
$TR_o/CS_o=\Phi_{Po}*(ABS/CS_o)$	Trapped energy flux per CS (at t=0)
$ET_o/CS_o=\Phi_{Eo}*(ABS/CS_o)$	Electron transport flux per CS (at t=0)
$DI_o/CS_o=(ABS/CS_o)-(TR_o/CS_o)$	Dissipated energy flux per CS (at t=0)

were deconvoluted into exponentially decaying components, based on a modified procedure reported by Walters and Horton [9]. Semi-logarithmic values of NPQ versus recovery times were plotted and NPQ parameters associated with each phase were calculated by linear regression of three exponentially decaying components. The absolute value, percent value, and half-time of the three NPQ components (NPQ_f, NPQ_m, and NPQ_s) were calculated based on the equations reported by D'Ambrosio *et al*. [6].

2 Results

2.1 Phase-transition temperature of the thylakoid membrane in *Microcystis*

The photosynthetic activity indicated by chlorophyll a fluorescence parameters had a very close relationship with the physical phase state of the thylakoid membrane. The linear correlation of photosynthetic activity with $1/T$ might be different between the solid state and the liquid crystalline state of the thylakoid membrane. When $rETR_{max}$ was plotted with linear regression versus different temperatures, there were two obvious lines of $rETR_{max}$ and a flex point appeared at about 18°C (Figure 1A). Also, the highest F_v/F_m value was observed at 21°C in the fitted curve of F_v/F_m versus temperature (Figure 1B). Based on these two results, we concluded that the phase transition temperature of the thylakoid membrane (cultured under the experimental conditions described above) was below 18°C. Therefore, the thylakoid membrane undoubtedly was in a solid state at 15°C; consequently, some of the following experiments were performed at 15°C.

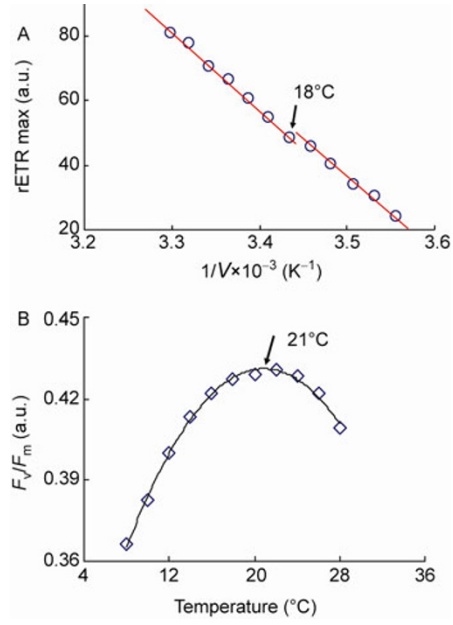

Figure 1 Changes in chlorophyll fluorescence parameters with decreasing temperature. The maximum rETR ($rETR_{max}$) plotted against the reciprocal of absolute temperature (A) and the fitted curve of F_v/F_m versus temperature (B).

2.2 Effect of PPPTM conformation on NPQ below the phase-transition temperature

NPQ significantly increased from 0.177±0.006 to 0.263±0.015 after the addition of GA at room temperature (25°C). During transfer from 25 to 15°C, the NPQ in the PSII of *Microcystis* rapidly dropped by 20.9%. In the presence of GA, NPQ increased by 48.6% at 25°C, while it increased by 19.3% at 15°C (Figure 2).

2.3 Effect of PPPTM conformational change on energy transport between PBS and PSII

The *Microcystis* cells were illuminated with 436 and 532 nm lasers at different intensities of light, and the relative intensities of fluorescence emission were recorded from 600 to 800 nm in wavelength. The fluorescence emission peaks at 662, 685, and 720 nm originated from PBS, PSII, and PSI, respectively. When compared with GA-free samples excited with a 436 nm laser, the relative intensities of fluorescence emission at wavelengths of 662, 685, and 720 nm in the presence of GA, did not significantly increase under low light conditions, but rose markedly when under high light (Figure 3A). The details of the increasing ratios are shown in Table 2. There were however, virtually no changes in fluorescence intensities at wavelengths of 662, 685, and 720 nm, after the addition of 0.5% (v/v) GA under both high and low light conditions (Figure 3B).

2.4 Effect of PPPTM conformation on the activity of the PSII reaction center

Effects of GA on PSII reaction centers were estimated using

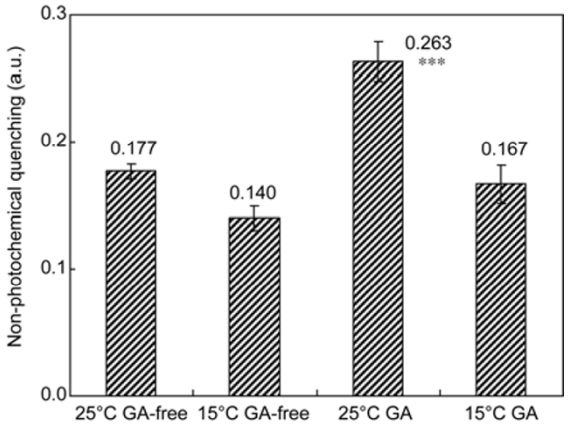

Figure 2 NPQ values measured at room temperature (25°C) and the temperature below the membrane transition temperature (15°C), in the presence and absence of GA, respectively (n=3).

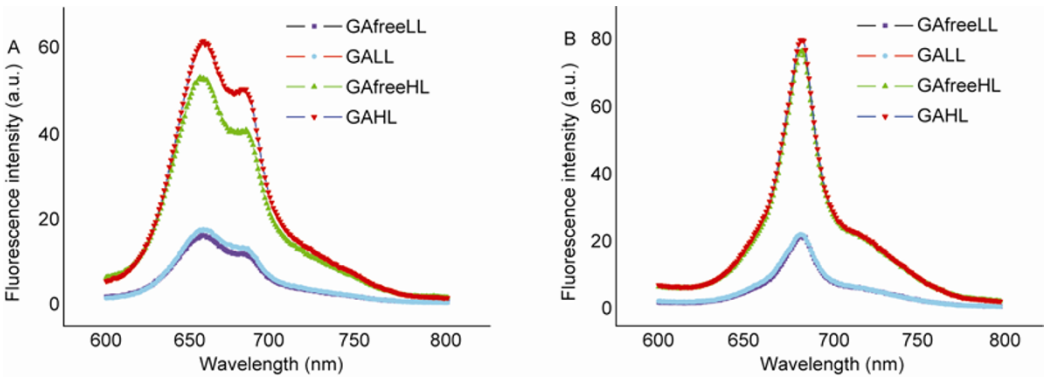

Figure 3 Fluorescence spectra of the complex measured under different intensities of excitation light, in the presence or absence of GA. A, Excitation at 532 nm. B, Excitation at 436 nm. GAfreeLL, GALL, GAfreeHL, and GAHL denote various emission spectra with GA at low light, without GA at low light, with GA at high light, and without GA at high light, respectively.

Table 2 Relative fluorescence intensities for the three components of NPQ under high light illumination[a)]

Component	Excitation at 532 nm			Excitation at 436 nm		
	–GA	+GA	Increase (%)	–GA	+GA	Increase (%)
PBS (662 nm) ($\times10^5$ a.u.)	5.034	5.940	18.0	2.642	2.769	0.48
PSII (685 nm) ($\times10^5$ a.u.)	3.873	4.786	23.6	7.548	7.990	0.59
PSI (720 nm) ($\times10^5$ a.u.)	1.136	1.307	15.1	2.126	2.148	0.10

a) –GA, in the absence of glutaraldehyde; +GA, in the presence of glutaraldehyde; a.u., arbitrary unit.

the chlorophyll fluorescence parameters measured by the Phyto-PAM. With increasing concentrations of GA, F_o quickly increased (by 25.10% at 0.5% GA), while F_m and photosynthetic activities only decreased (by 6.17% at 0.5% GA) (Table 3). In the presence of 0.5% GA, F_v/F_m and Φ_{PSII} decreased to 0.25 and 0.05, respectively. Photosynthetic activity of PSII was almost completely lost in 1% GA conditions. The absorption and utilization of excitation energy in PSII were revealed by three parameters; namely, α, $rETR_{max}$, and I_k in RLC (Table 3). In comparison with the control (GA-free), α, calculated during the initial low light phase, decreased slightly by 13.5%, while $rETR_{max}$, measured under saturated light, decreased significantly by 87.1% in 0.5% (v/v) GA. In addition, I_k distinctly decreased from 423.2 to 69.2.

2.5 Effect of PPPTM conformation on chlorophyll a fluorescence induction dynamics

Excitation of dark-adapted *Microcystis aeruginosa* PCC7806 with a saturated light pulse induced a typical Kautsky fluorescence induction (O-J-I-P transients) for cultures, both in the presence and absence of GA, under temperatures of 25 and 15°C (Figure 4A). All details of the electron transport chain in PSII were shown in a more visible way after O-J-I-P transient curves were normalized relative to total variable fluorescence. Fluorescence values significantly increased at steps J (2 ms) and I (30 ms), after 0.5% GA addition at both temperatures (Figure 4B). Results of the JIP test also showed that more energy was fluxed to PSII. This was indicated by the values of ABC/RC, DI_o/RC, ABS/CS_o and DI_o/CS_o, which had increased significantly by 16.3%, 31.3%, 12.3%, and 28.7%, respectively, at temperatures of 25°C (Figure 5A and B).

2.6 Deconvolution of NPQ components in dark relaxation kinetics

The fast quenching component (NPQ_f) was 39.42%, which took a predominate role in NPQ, and the half-time of NPQ_f was 1.01 min (Table 4). When PPPTM conformation was stabilized by GA, NPQ significantly increased from 0.228 to 1.362 (Table 5). However, even more remarkably, GA caused NPQ_f to drop to a very low level (5.26%) and re-

Table 3 Effect of GA on chlorophyll fluorescence parameters in RLCs

Concentration of GA (v/v)	F_o (Rel. units)	F_m (Rel. units)	F_v/F_m	Φ_{PSII}	α	$rETR_{max}$	I_k
0	1323	2366	0.44	0.33	0.170	72.1	423.2
0.5%	1655	2220	0.25	0.05	0.147	9.3	69.2
1%	1856	1964	0.05	0	0.021	0.5	14.5
2%	1792	1936	0.07	0	0.019	0.3	13.0

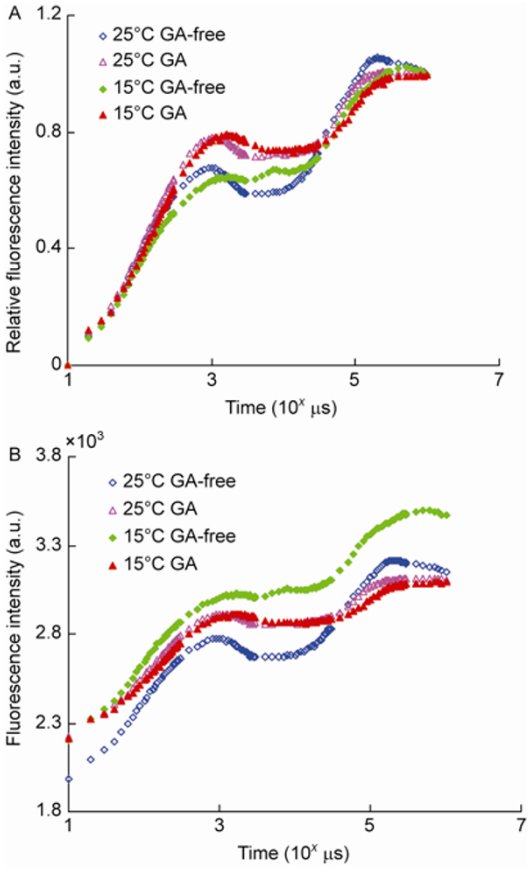

Figure 4 Average O-J-I-P Chl a fluorescence transients (n=3) at room temperature (25°C) and below the membrane transition temperature (15°C), in the presence and absence of GA, respectively. A, Curves are the actual data from fluorescence signals. B, Curves were normalized relative to the total variable fluorescence.

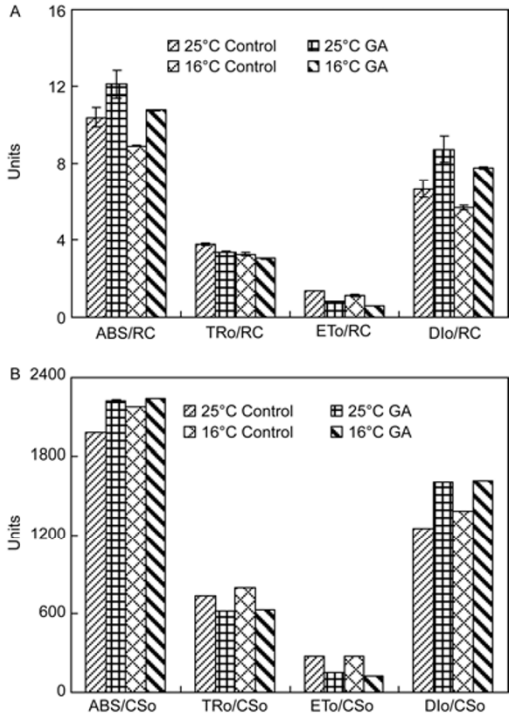

Figure 5 The effects of GA on the parameters in O-J-I-P curves. A, Effects of GA on specific fluxes in *Microcystis* cells. The specific fluxes are *ABS/RC*, absorption; TR_o/RC, trapping; ET_o/RC, electron transport; and DI_o/RC, dissipation, at the level of the antenna chlorophylls. B, Effects of GA on phenomenological energy fluxes in *Microcystis* cells. The phenomenological energy fluxes per excited cross section (CS) are *ABS/CS*, absorption flux per *CS*; TR_o/CS_o, trapped energy flux per CS; ET_o/CS_o, electron transport flux per CS and DI_o/CS_o, dissipated energy flux per CS, n=3. Error bars are standard deviations.

sulted in a shorter half-time (0.93 min). Meanwhile, the ratio of the slow quenching component (NPQ_s) in NPQ rose from 39.88% to 85.98% after addition of 0.5% GA (Table 5).

3 Discussion

After chlorophyll a (Chl a) molecules absorbed solar energy, they switched to an excited state. The excited chlorophyll molecule has several ways to return to the ground state, namely by excitation transfer to a neighboring pigment, photochemical reaction, fluorescence emission, or non-photochemical quenching (NPQ) [35]. NPQ can usually be deconvoluted into three components, namely qE, qT, and qI in higher plants [9]. However, it has been reported that the ΔH-dependent qE is not included in NPQ in cyanobacteria [10], and qT, which is closely related to the fluidity of the thylakoid membrane, will be inhibited in a solid membrane state below the phase transition temperature [14]. Moreover, a short, one-minute exposure of saturated light did not cause a significant qI induced by D_1-protein damage. Therefore, theoretically, NPQ should be very low, below the phase transition temperature when exposed to saturated light for a short time. However, a high NPQ appeared under this controlling condition (Figure 2), suggesting that a component of NPQ, independent from state transition or photo-inhibition, existed in the PSII complex, and was a fast component under high light conditions.

GA has commonly been used to stabilize the PPPTM conformation such as PBS conformation [15,28], and GA also can stabilize all kinds of PPPTM including OCP. NPQ significantly increased at room temperature (25°C) in the presence of GA (Figure 2). This might be attributable to the transfer of more energy from PBS to PSII reaction centers under conditions of more stable PPPTM conformation and high light. However, the addition of GA could not significantly increase NPQ when measured below the phase transition temperature (15°C), suggesting that the PPPTM conformation-related NPQ was based on the fluidity of the thylakoid membrane.

Excitation energy transferring between PBS and the PSII reaction center (RC) was measured by fluorescence emission spectra (Figure 3). Since there was a back transfer of excitation energy from chlorophyll molecules to PBS when excited at 436 nm [36], a depressed fluorescence at 685 nm

Table 4 Three NPQ components and their regression equations in the absence of GA

NPQ components	Regression equation of each component	Absolute values	Relative values (%)	Half times (min)
NPQ_f	y=–0.112x+0.5717 (R^2=1)	0.219±0.008	39.42±0.14	1.01±0.03
NPQ_m	y=–0.0098x+0.3463 (R^2=0.95)	0.118±0.006	20.69±0.44	6.04±0.13
NPQ_s	y=–0.0033x+0.2280 (R^2=0.99)	0.228±0.005	39.88±0.58	34.55±2.59

Table 5 Three NPQ components and their regression equations in the presence of GA

NPQ components	Regression equation of each component	Absolute values	Relative values (%)	Half times (min)
NPQ_f	y=–0.0446x+1.5845 (R^2=1)	0.080±0.004	4.99±0.39	0.91±0.04
NPQ_m	y=–0.0096x+1.5011 (R^2=0.97)	0.142±0.005	8.82±0.08	7.17±0.08
NPQ_s	y=–0.0023x+1.3623 (R^2=0.92)	1.387±0.035	86.19±0.30	279.30±23.84

under an excitation of 436 nm was considered strong evidence for the decoupling of PBS from the reaction centers [33]. When excited at 532 nm, more excitation energy was absorbed by PBS and then fluxed to the PSII reaction center in the presence of GA. However, the fluorescence emission spectra excited at 436 nm showed that the transfer paths of excitation energies among PBS, PSII, and PSI were not significantly changed by the addition of GA. These results suggested that only the energy absorption and dissipation process of PBS, rather than the transfer paths, were affected by GA. GA is usually used as a fixative and crosslinker for proteins, and the effective concentration is usually 2%. However, some researchers have used 1% (v/v) GA as a stabilizer of protein structures [16]. Thus we thought that GA might play different roles in different doses. Some researchers reported that F_m could reflect the status of the electronic pass through the PSII [37,38]. GA therefore had almost no significant effects on fluorescence quenching and the photosynthetic reaction center, as evidenced by only a small decrease in F_m (6.17%) in low concentrations of 0.5% GA. However, F_o rapidly increased by 25.10%. Campbell *et al*. [39] proposed that phycobiliproteins contained in cyanobacterial cells could contribute to the F_o, so we thought that this increase in F_o might be attributable to some structural changes induced by 0.5% GA. Therefore, based on all these characteristics of fluorescence parameters after GA addition, we conclude that low concentrations (0.5%) of GA would not lead to multiple effects on biological processes. This can be confirmed by the relatively high photosynthetic activity (F_v/F_m=0.25, Table 3).

RLC provides detailed information on the electron transport system as well as the overall photosynthetic performance of higher plants or cyanobacteria [5,40]. The ability of plants to use light is usually indicated by the photosynthetic parameter α at the low light phase in RLC, and the photo-inhibitory extent is denoted by $rETR_{max}$ at the high light phase in RLC [5]. We have shown that with increasing concentrations of GA, the ability of *M. aeruginosa* PPC 7806 to use light gradually decreased, while the photo-inhibitory extent gradually increased (Table 3). When in the presence of 0.5% GA, $rETR_{max}$ significantly decreased while α slightly decreased. This suggested that under high light conditions, excessive excitation energy can be dissipated by PPPTM conformational change. Through this energy-quenching mechanism, *Microcystis* can alleviate the inhibitory effects of high light. Since more excitation energy transferring to PSII reaction centers can cause $Q_A^-Q_B$ and $Q_A^-Q_B^-$ to accumulate at PSII acceptor sites [41], the increase in both energy absorption and NPQ in the presence of GA was further confirmed by increased "J" and "I" steps in the fluorescence induction dynamics (Figure 4). The addition of GA resulted in an increase in ABS/RC and DIo/RC ratios, indicating that partial NPQ resulted from PPPTM conformational change.

In higher plants, *q*E, a fast fluorescence quenching component, usually plays a major role in NPQ under high light [6]. We found that the fast fluorescence quenching component of NPQ was also very important for *Microcystis* when exposed to high light. The analysis of NPQ components under GA addition showed that the fast quenching component (NPQ_f) significantly decreased while the slow quenching component (NPQ_s) significantly increased, in comparison with those in the absence of GA (Tables 4 and 5). This suggested that the fast quenching component (NPQ_f) was closely related to PPPTM conformational change, accounting for as much as 39.42% of the total NPQ. We assume therefore that NPQ_f induced by PPPTM conformation is an important adaptation mechanism for *Microcystis* blooms under high intensity light during summer and autumn.

4 Conclusion

We have shown that a component of NPQ, independent from state transition or photo-inhibition, is generated in the PSII complex of the cynanobacterium *Microcyctis*; this is a fast component responding to high intensity light. Stabilization of PPPTM conformation appears to result in the transfer of more energy from PBS to PSII reaction enters. Furthermore, PPPTM conformational change can cause dissipation of partial excessive excitation. Based on the deconvolution of NPQ relaxation kinetics, we conclude that the

fast quenching component (NPQ_f) is closely related to PPPTM conformational change through which *Microcystis* can efficiently alleviate the inhibitory effects of high light.

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