Changes in the transthylakoid proton gradient are caused by the movement of phycobilisomes in the cyanobacterium *Synechocystis* sp. strain PCC 6803

MA WeiMin^{1*}, GAO FuDan¹, MI HuaLing² & SHEN YunGang²

¹ College of Life and Environmental Sciences, Shanghai Normal University, Shanghai 200234, China;

² Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

Received July 1, 2010; accepted November 12, 2010

Phycobilisomes (PBSs) are the main accessory light-harvesting complexes in cyanobacteria and their movement between photosystems (PSs) affects cyclic and respiratory electron transport. However, it remains unclear whether the movement of PBSs between PSs also affects the transthylakoid proton gradient (ΔpH). We investigated the effect of PBS movement on ΔpH levels in a unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803, using glycinebetaine to immobilize and couple PBSs to photosystem II (PSII) or photosystem I (PSI) by applying under far-red or green light, respectively. The immobilization of PBSs at PSII inhibited decreases in ΔpH , as reflected by the slow phase of millisecond-delayed light emission (ms-DLE) that occurs during the movement of PBSs from PSII to PSI. By contrast, the immobilization of PBSs at PSI inhibited the increase in ΔpH that occurs when PBSs move from PSI to PSII. Comparison of the changes in ΔpH and electron transport caused by the movement of PBSs between PSs indicated that the changes in ΔpH were most likely caused by respiratory electron transport. This will further improve our understanding of the physiological role of PBS movement in cyanobacteria.

phycobilisome movement, ΔpH , *Synechocystis* sp. strain PCC 6803

Citation: Ma W M, Gao F D, Mi H L, et al. Changes in the transthylakoid proton gradient are caused by the movement of phycobilisomes in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Chinese Sci Bull, 2011, 56: 285–289, doi: 10.1007/s11434-010-4303-0

A balanced distribution of the excitation energy absorbed by light-harvesting complexes to the complexes of photosystems I and II (PSI and PSII) is considered to be one of the most important factors for ensuring optimal photosynthetic efficiency [1–3]. In response to fluctuating light conditions, the photosynthetic machinery regulates the distribution of excitation energy between the 2 photosystems (PSs) [4]. This dynamic and rapid process of achieving energy balance is called “state transition” [5,6]. Because phycobilisome (PBS) movement is a prerequisite for cyanobacterial state transitions [7,8], “mobile PBSs” are believed to play a key role in allowing state transitions in cyanobacteria. A recent study indicated that the movement of PBSs between PSII and PSI affects both cyclic and respiratory electron transport [9]. However, it remains unclear whether the movement of

PBSs between PSs also affects transthylakoid proton gradient (ΔpH) levels in cyanobacterial cells.

The aim of this study was to investigate the effect of PBS movement between the 2 PSs on ΔpH levels in cyanobacteria. It is difficult to probe ΔpH levels *in vivo* as the system relaxes rapidly [10,11]. Millisecond-delayed light emission (ms-DLE) originates from the reverse reaction of the photoact in PSII, and is composed of one fast (within 0.1 s of the onset of measuring flashes) and one slow phase [12,13]. Crofts and his colleagues concluded that the intensities of the fast and slow phases of ms-DLE are stimulated by the membrane potential (ΔE) and the ΔpH , respectively [14–16]. Further characteristics and properties of ms-DLE were studied and confirmed in subsequent research [17–21]. It was found that ΔpH levels can be probed *in vivo* by measuring the slow phase of ms-DLE.

*Corresponding author (email: wma@shnu.edu.cn)

The movement of PBSs is inhibited by glycinebetaine (GB) in the cyanobacterium *Synechocystis* sp. strain PCC 6803 (hereafter *Synechocystis* 6803) regardless of changes in spectral quality and light intensity [9]. We applied GB to cells to immobilize and couple PBSs to PSII or PSI under light which preferentially excites PSI or PSII, respectively. Comparison of the ΔpH levels in untreated and GB-treated cells enabled us to study the physiological role of PBS movement in cyanobacteria.

1 Materials and methods

1.1 Culture conditions

Synechocystis 6803 cells were cultured at 30°C in BG-11 medium [22], buffered with Tris-HCl (5 mmol/L, pH 8.0) bubbled with 2% (v/v) CO_2 in air, under continuous illumination with fluorescent lamps ($40 \mu\text{E m}^{-2} \text{s}^{-1}$).

1.2 Immobilization of PBSs

Cells cultured for 4 d ($A_{730} = 0.6\text{--}0.8$) that showed the highest photosynthetic activity [23] were harvested by centrifugation ($5000\times g$ for 5 min at 25°C) and suspended in fresh BG-11 medium buffered with Tris-HCl (5 mmol/L, pH 8.0) at a chlorophyll *a* concentration of $20 \mu\text{g mL}^{-1}$. After exposure to far-red light (FR, $5.2 \mu\text{E m}^{-2} \text{s}^{-1}$; Ditric Optics 705 nm long pass filter) or green light (GL, $15 \mu\text{E m}^{-2} \text{s}^{-1}$; Ditric Optics 520 nm long pass and 546 nm short pass filter) for 20 min, the cells were treated with 1 mol/L GB under the same FR or GL illumination for 20 min to immobilize and couple PBSs to the stromal surface of the PSII or PSI core complex in the thylakoid membrane.

1.3 Measurements of chlorophyll fluorescence yield

The yield of chlorophyll fluorescence was monitored at several indicated time points with a pulse-amplitude-modulated (PAM) chlorophyll fluorometer (Walz, Effeltrich, Germany) and an emitter-detector-cuvette assembly (ED-101US) with a 101ED unit, as described elsewhere [9,24]. Cells were subjected to darkness for 2 min before measurements were made. The intensity of the modulated measurement light was lower than $0.2 \mu\text{E m}^{-2} \text{s}^{-1}$ to avoid photosynthetic electron transport. Maximum fluorescence levels (F_m or F_m') were determined using saturating light pulses ($3200 \mu\text{E m}^{-2} \text{s}^{-1}$, 600 ms duration), which were produced by a KL-1500 lamp (Schott, Germany). The intensities of FR and GL were $5.2 (>705 \text{ nm, through a home-made filter})$ and $15 \mu\text{E m}^{-2} \text{s}^{-1} (\sim 540 \text{ nm, through another home-made filter})$, respectively.

1.4 Measurements of ms-DLE

The ms-DLE signal was measured using a lab-made

phosphoroscope. A sample, in a polymethylmethacrylate cuvette, was irradiated with light passing through a 2-cm thick layer. The holes on the rotating wheels were arranged such that the measuring process might be divided into a series of 5.6 ms cycles for the excitation measurement, with 1-ms excitation with light of $1500 \mu\text{E m}^{-2} \text{s}^{-1}$ followed by 4.6-ms darkness. The delayed light observed between 2.8 and 3.8 ms after each flash was measured with an EMI9558B photomultiplier with a red glass filter. The signal passing through an amplifier was recorded continuously with a SC-16 light beam oscillograph [17].

2 Results

2.1 The slow phase of ms-DLE reflects *in vivo* ΔpH

It has been previously reported that the intensity of fast and slow phases of ms-DLE closely correlates with ΔE and ΔpH levels, respectively [14–16]. To confirm the relationship between ΔpH and the slow phase of ms-DLE, an uncoupler, nigericin (Nig), which decreases ΔpH by stimulating exchanges of H^+ and K^+ between the two sides of the thylakoid, was used. As shown in Figure 1, Nig treatment resulted in a considerable decrease in the slow phase of ms-DLE but the same effect was not observed for the fast phase, indicating that the slow phase of ms-DLE indeed reflects changes of ΔpH *in vivo* in the unicellular cyanobacterium *Synechocystis* 6803.

2.2 Light-induced state transitions are dependent on PBS movement

The small molecule, GB, has previously been used to lock PBSs into one position, rendering them unable to diffuse

Figure 1 Effect of nigericin on the intensity of the slow phase of ms-DLE in *Synechocystis* 6803. A, control; B, 50 $\mu\text{mol/L}$ nigericin (Nig).

through the thylakoid membrane surface [9,25,26]. In this study, we used 1 mol/L GB to immobilize and couple PBSs to PSII or PSI in *Synechocystis* 6803. Figures 2 and 3 show

the changes in chlorophyll fluorescence yield at room temperature in untreated and GB-treated cells of *Synechocystis*

Figure 2 Effect of switching from FR to GL on state transition and ms-DLE in untreated and GB-treated cells. PAM fluorescence kinetic traces of untreated cells (a) and GB-treated cells in which PBSs were immobilized and coupled to PSII (c) under FR. Sample in (a) was illuminated with FR as indicated by arrow 1, and was then switched to GL (arrow 2). During the switch from FR to GL, the ms-DLE was measured in untreated (b) and GB-treated cells (d) at several indicated time points. Subsequently, the changes in slow phase intensity of ms-DLE were analyzed (e). The experiments were repeated at least six times and standard errors were calculated.

Figure 3 Effect of switching from GL to FR on state transition and ms-DLE in untreated and GB-treated cells. PAM fluorescence kinetic traces of untreated cells (a) and GB-treated cells in which PBSs were immobilized and coupled to PSI (c) under GL. Sample in (a) was first illuminated with GL (arrow 1), which was switched to FR as indicated by arrow 2. During the switch from GL to FR, the ms-DLE was determined in untreated (b) and GB-treated cells (d) at several indicated time points. The changes in the slow phase intensity of ms-DLE were then analyzed (e) and standard errors were calculated from at least six independent experiments.

6803 upon switching from FR ($5.2 \mu\text{E m}^{-2} \text{s}^{-1}$, $>705 \text{ nm}$) to GL ($15 \mu\text{E m}^{-2} \text{s}^{-1}$, 540 nm ; Figure 2(a) and (c)) or from GL to FR (Figure 3(a) and (c)). The transition from state 1 (2) to state 2 (1) was clearly observed in untreated cells by switching from FR (GL) to GL (FR), and the process was complete within 1–2 min (Figures 2(a) and 3(a)). The transition was strongly inhibited when the PBSs were immobilized by GB (Figures 2(c) and 3(c)). These results clearly show that light-induced state transitions depend predominantly on the movement of PBSs [7,25].

2.3 Changes in the slow phase of ms-DLE are caused by the movement of PBSs

To investigate the effect of PBS movement between PSs on ΔpH levels, the intensity of ms-DLE was compared between untreated and GB-treated cells of *Synechocystis* 6803. Switching from FR to GL considerably decreased the intensity of the slow phase of ms-DLE in untreated cells but not in GB-treated cells (Figure 2(b), (d) and (e)). By contrast, switching from GL to FR increased the amplitude of the slow phase of ms-DLE in untreated cells but only slightly in GB-treated cells (Figure 3(b), (d) and (e)). Further, the movement of PBSs between PSs did not significantly influence the fast phase of ms-DLE (Figures 2 and 3). Therefore, the movement of PBSs between PSs affects the slow phase of ms-DLE but has no effect on the fast phase. Taking these combined results together, we conclude that the intensity of the slow phase of ms-DLE is high when PBSs are associated with PSII, but low when PBSs are associated with PSI.

3 Discussion

It has been shown previously that the speed of PBS movement depends on the intensity of the inducing light [27]. Under $5.2 \mu\text{E m}^{-2} \text{s}^{-1}$ FR and $15 \mu\text{E m}^{-2} \text{s}^{-1}$ GL, the movement of PBSs occurred rapidly and was complete within 1–2 min (Figures 2 and 3). No significant changes in linear electron transport were observed during this period [9]. However, the activity of respiration was high when cells were in state 1 (PBSs associated with PSII) and low when cells were in state 2 (PBSs associated with PSI). By contrast, the activity of cyclic electron transport was high when PBSs were coupled to PSI and low when coupled to PSII [9]. The results of this study indicated that ΔpH levels, as reflected by the intensity of the slow phase of ms-DLE, were high when PBSs were associated with PSII but low when PBSs were associated with PSI (Figures 2 and 3), which is consistent with the changes observed in respiration, and not cyclic, electron transport caused by the movement of PBSs [9]. These findings suggest that changes in ΔpH , caused by the movement of PBSs between PSs, most likely depend on the rate of respiratory electron transport.

It has been reported previously that NADPH dehydrogenase (NDH-1)-mediated respiration is absent in *ndh* gene inactivation mutants ΔndhB (M55) and $\Delta\text{ndhD1/D2}$ (D1/D2) [28–30]. However, NDH-1-dependent cyclic electron transport was enhanced in D1/D2 cells and reduced in M55 cells, when compared to the wild-type *Synechocystis* 6803 (WT) [28–30]. Under low photon flux densities ($5\text{--}6 \mu\text{E m}^{-2} \text{s}^{-1}$), ΔpH levels, as determined by the intensity of the slow phase of ms-DLE, declined considerably in both M55 and D1/D2 cells, when compared to those of WT cells (data not shown). Under these light conditions, linear electron transport significantly declined, and was therefore not a major contributor for the establishment of ΔpH . The NDH-1-dependent respiratory electron transport was therefore found to be responsible for the establishment of ΔpH , which is consistent with previous observations [31]. This result supports the contribution of respiration to the changes in ΔpH during the movement of PBSs between the two PSs.

In the cyanobacterium *Synechocystis* 6803, the respiratory electron transport that is mainly mediated by NDH-1 plays an important role in donating electrons to the plastoquinone (PQ) pool, thereby establishing the ΔpH [31,32]. It therefore seems likely that high respiration rates induce a rise in H^+ concentration on the lumen side, resulting in a high ΔpH level, and *vice versa*.

In higher plants, ΔpH levels, as reflected by ms-DLE measurements, show biphasic behavior during the transition from state 1 to state 2, with the ΔpH transiently increasing to its maximal level within 0.5 min, and then gradually decreasing from the maximal value [33,34]. However, in cyanobacteria, biphasic behavior was not observed during the movement of PBSs between PSII and PSI (Figures 2 and 3). This may be attributed to the different mechanisms of state transition in cyanobacteria and higher plants.

Under FR- and GL-preillumination, the addition of 1 mol/L GB to the cultures reduced markedly the intensity of the slow phase of ms-DLE (Figures 2 and 3). Similarly, treatments with 1 mol L^{-1} phosphate, sucrose and potassium chloride solutions, which are also inhibitors of PBS movement [7,35], also significantly reduced the intensity of the slow phase of ms-DLE (data not shown). Taken together, it appears that the movement of PBSs on the surface of the thylakoid membrane is responsible for the effective establishment of ΔpH in cyanobacteria.

In conclusion, our results, which were obtained with a unicellular cyanobacterium, increase our knowledge of the physiological role of PBS movement, indicating that ΔpH levels are high when PBS associates with PSII but low when PBS associates with PSI. The changes in ΔpH caused by the movement of PBSs between PSs most likely depend on the rate of respiratory electron transport. While the working mechanism underlying such changes remains uncertain, our findings will help in understanding the physiological role of PBS movement in cyanobacteria.

This work was supported by the National Natural Science Foundation of China (30770175), the National Basic Research Program of China (2009CB118500), the Key Project of the Chinese Ministry of Education (209045), the Shanghai Leading Academic Discipline Project (S30406), and the Leading Academic Discipline Project of Shanghai Normal University (DZL808).

- 1 Haldrup A, Jensen P E, Lunde C, et al. Balance of power: A view of the mechanism of photosynthetic state transitions. *Trends Plant Sci*, 2001, 6: 301–305
- 2 Wollman F A. State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *EMBO J*, 2001, 20: 3623–3630
- 3 Allen J F. State transition – A question of balance. *Science*, 2003, 299: 1530–1532
- 4 Yu L, Zhao J, Bryant D A, et al. Psae is required for *in vivo* cyclic electron flow around photosystem I in the cyanobacterium *Synechococcus* sp. PCC 7002. *Plant Physiol*, 1993, 103: 171–180
- 5 Bonaventura C, Myers J. Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. *Biochim Biophys Acta*, 1969, 189: 366–383
- 6 Murata N. Control of excitation energy transfer in photosynthesis: I. Light-induced change of chlorophyll *a* fluorescence in *Porphyridium cruentum*. *Biochim Biophys Acta*, 1969, 172: 242–251
- 7 Joshua S, Mullineaux C W. Phycobilisome diffusion is required for light-state transitions in cyanobacteria. *Plant Physiol*, 2004, 135: 2112–2119
- 8 Zhang R, Li H, Xie J, et al. Estimation of relative contribution of “mobile phycobilisome” and “energy spillover” in the light-dark induced state transition in *Spirulina platensis*. *Photosynth Res*, 2007, 94: 315–320
- 9 Ma W, Ogawa T, Shen Y, et al. Changes in cyclic and respiratory electron transport by the movement of phycobilisomes in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Biochim Biophys Acta*, 2007, 1767: 742–749
- 10 Kramer D M, Cruz J A, Kanazawa A. Balancing the central roles of the thylakoid proton gradient. *Trends Plant Sci*, 2003, 8: 27–32
- 11 Kramer D M, Avenson T J, Edwards G E. Dynamic flexibility in the light reactions of photosynthesis governed by both electron and proton transfer reactions. *Trends Plant Sci*, 2004, 9: 349–357
- 12 Lavorel L. Luminescence. In: Govindjee, ed. *Bioenergetics of Photosynthesis*. New York: Academic Press, 1975. 223–317
- 13 Malkin S. Delayed luminescence. In: Trebst A, Arvon M, eds. *Photosynthesis I. Encyclopedia of Plant Physiology*. Heidelberg/New York: Springer-Verlag, 1977. 473–491
- 14 Wraight C A, Crofts A R. Delayed fluorescence and the high-energy state of chloroplasts. *Eur J Biochem*, 1971, 19: 386–395
- 15 Evans E H, Crofts A. The relationship between delayed fluorescence and the H^+ gradients in chloroplast. *Biochim Biophys Acta*, 1973, 292: 130–139
- 16 Bowes L M, Crofts A R, Itoh S. Effect of pH on reactions of the donor side of photosystem II. *Biochim Biophys Acta*, 1979, 547: 336–346
- 17 Xu C, Shen Y. Relationship between the changes of the fast phase of millisecond delayed light emission and the proton released by the oxidation of water. *Sci China Chem*, 1983, 27: 37–47
- 18 Li D, Shen Y. Relationship between components of proton motive force and photosynthesis. *Chinese Sci Bull*, 1995, 40: 66–70
- 19 Wei J, Shi J, Xu C, et al. Studies on the relation between the fast phase of millisecond delayed light emission and the proton released from oxidation of water in spinach chloroplast. *Photosynth Res*, 1998, 57: 317–322
- 20 Tang Q, Wei J. Contribution of ΔpH and ΔE to photosynthesis of *Chlamydomonas reinhardtii*. *Photosynthetica*, 2001, 39: 127–129
- 21 Wang H, Mi H, Ye J, et al. Low concentrations of $NaHSO_3$ increase cyclic photophosphorylation and photosynthesis in cyanobacterium *Synechocystis* PCC6803. *Photosynth Res*, 2003, 75: 151–159
- 22 Allen M M. Simple conditions for growth of unicellular blue-green algae on plates. *J Phycol*, 1968, 4: 1–4
- 23 Ma W, Mi H. Expression and activity of type-1 NAD(P)H dehydrogenase at different growth phases of cyanobacterium, *Synechocystis* PCC 6803. *Physiol Plant*, 2005, 125: 135–140
- 24 Schreiber U, Endo T, Mi H, et al. Quenching analysis of chlorophyll fluorescence by the saturation pulse method: Particular aspects relating to the study of eukaryotic algae and cyanobacteria. *Plant Cell Physiol*, 1995, 36: 873–882
- 25 Li D, Xie J, Zhao J, et al. Light-induced excitation energy redistribution in *Spirulina platensis* cells: “Spillover” or “mobile PBSs”? *Biochim Biophys Acta*, 2004, 1608: 114–121
- 26 Yang S, Su Z, Li H, et al. Demonstration of phycobilisome mobility by the time- and space-correlated fluorescence imaging of a cyanobacterial cell. *Biochim Biophys Acta*, 2007, 1767: 15–21
- 27 Yang S, Zhang R, Hu C, et al. The dynamic behavior of phycobilisome movement during light state transitions in cyanobacterium *Synechocystis* PCC6803. *Photosynth Res*, 2009, 99: 99–106
- 28 Ogawa T. A gene homologous to the subunit-2 gene of NADH dehydrogenase is essential to inorganic carbon transport of *Synechocystis* PCC 6803. *Proc Natl Acad Sci USA*, 1991, 88: 4275–4279
- 29 Ohkawa H, Pakrasi H B, Ogawa T. Two types of functionally distinct NAD(P)H dehydrogenases in *Synechocystis* sp. strain PCC 6803. *J Biol Chem*, 2000, 275: 31630–31634
- 30 Ma W, Wei L, Wang Q. The response of electron transport mediated by active NADPH dehydrogenase complexes to heat stress in the cyanobacterium *Synechocystis* 6803. *Sci China Life Sci*, 2008, 51: 1082–1087
- 31 Mi H, Endo T, Schreiber U, et al. Electron donation from cyclic and respiratory electron flows to the photosynthetic intersystem chain is mediated by pyridine nucleotide dehydrogenase in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol*, 1992, 33: 1233–1237
- 32 Mi H, Endo T, Ogawa T, et al. Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic electron transport in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol*, 1995, 36: 661–668
- 33 Su J, Shen Y. Change of proton motive force across thylakoid membrane in soybean leaf during state transitions. *Chinese Sci Bull*, 2003, 48: 652–657
- 34 Su J, Shen Y. Influence of state-2 transition on the proton motive force across the thylakoid membrane in spinach chloroplasts. *Photosynth Res*, 2005, 85: 235–245
- 35 Mullineaux C W. Inhibition by phosphate of light-state transitions in cyanobacterial cells. *Photosynth Res*, 1993, 38: 135–140

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.