Biochemical and Biophysical Research Communications 422 (2012) 233–237

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Kinetics and dynamics for light state transition in cyanobacterium *Spirulina platensis* cells

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ARTICLE INFO

Article history:
Received 16 April 2012
Available online 30 April 2012

Keywords:
Cyanobacteria
State transition
Kinetics
Dynamics
Physiological significance

ABSTRACT

Light state transition in oxygenic organisms was defined as the ability to equalize the excitation of the two photosystems for maximal photosynthetic efficiency. In cyanobacteria, extensive researches on state transition have continuously provided new knowledge in the past decades but the molecular mechanism and physiological significance are still ambiguous. In this work, kinetics and dynamics of the transition from state 1 to state 2 in cyanobacterium *Spirulina platensis* cells were studied at different intensity of orange light from 10 to 120 μmol m^{-2} s^{-1}. It was revealed that the state transition worked constantly independent of light intensity while the rates varied. The synchronous fluorescence kinetics for phycobilisome (PBS) and photosystem components indicated that the state transition was entirely regulated by "mobile PBS", and continuously changed fluorescence amplitudes suggested a series of intermediate states were involved between state 1 and state 2. The dynamic property of PBS movement during the state transition was revealed by (1,0) distribution of photo-linkable PBSs, indicating a collective movement of all PBSs. The results suggest that state transition in cyanobacteria possesses not only physiological but also photochemical significance.

1. Introduction

Oxygen evolution and carbon fixation in photosynthetic organisms are the fundamental reactions depending on equal excitation of photosystem II (PSII) and photosystem I (PSI) which have distinct light absorptions. Under a light conditions favoring PSII (state 2) or PSI (state 1), the excitation energy would be redistributed by the mechanism named as light state transition [1,2]. In green plants, state transition is achieved by a directional movement of light-harvesting complex II (LHCII) to PSI or PSII due to a reversible phosphorylation activated by certain protein kinases [3,4]. In comparison, the state transition in cyanobacteria is less understood. For several decades, two different models, "mobile PBS" [5,6] or "energy spillover" from PSII to PSI [7–10], were mainly used to explain the state transitions. It was found that "mobile PBS" was common while the "spillover" only took a part in the state transition when dark condition was involved [11,12]. The two mechanisms were cooperative but not competitive [13]. While PBS mobility is necessary for "mobile PBS", it has never been directly detected in a state transition in cyanobacteria. Fluorescence recovery after photobleaching (FRAP) detected the PBS mobility in a cyanobacterial cell [14] but not the PBS movement in a light state transition. The "damped oscillation" fluorescence fluctuation with selective excitation of PBSs in cyanobacterial cells suggested a dynamic behavior of PBSs searching for the "balanced position" [15]. However, whether the fluorescence fluctuation is resulted by a collective movement of all PBSs or relative change in PBS populations on PSII or PSI could not be answered. State transitions are commonly studied in two fixed states – state 1 and state 2, but the observations on the effect of light dosage or temperature in state transition [16] suggested that light state seems to be a continuous but not discrete variable. The continuous light state is important for understanding the physiological significance of state transition in cyanobacteria. Several years ago, it was indicated that state transitions were physiologically important only at very low light intensities ($\leqslant 2$ μE m^{-2} s^{-1}) and would play no role in protection from photoinhibition for growths of the *Synechocystis rpa*C (Regulator of Phycobilisome Association C) deletion mutant [17]. To investigate the kinetics in various light intensity and dynamics may provide some new insight into state transition.

Abbreviations: PBS, phycobilisome; PSI, photosystem I; PSII, photosystem II; C-PC, C-phycocyanin; APC, allophycocyanin.

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http://dx.doi.org/10.1016/j.bbrc.2012.04.131

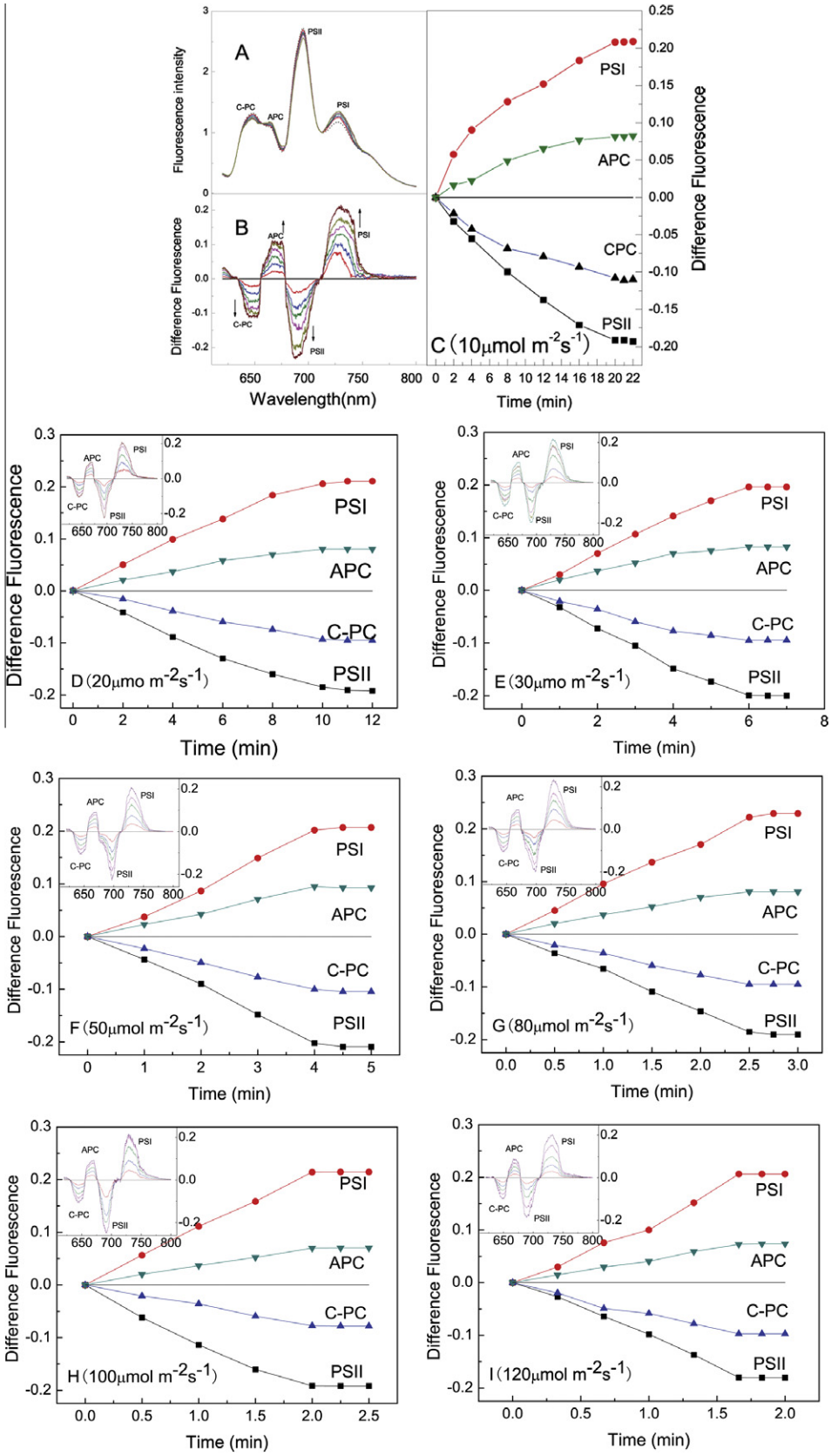

Fig. 1. 77 K fluorescence emission spectra (A), the difference spectra (B) and plots of amplitudes for the four components as denoted to time (C) for state-1 cells under orange light at 10 μmol m^{-2} s^{-1}. Arrows in (B) indicate direction of the changes. (D–F) Are the same to (C) but the light intensity is 20 (D), 30 (E), 50 (F), 80 (G), 100 (H) or 120 (I) μmol m^{-2} s^{-1}, respectively. The difference spectra (insets) derived from 77 K fluorescence spectra (omitted). Excited at 580 nm and normalized to 712 nm.

In this work, 77 K fluorescence spectra were monitored at a series of time for *Spirulina platensis* cells initially at state 1 illuminated by orange light of different intensity, from which kinetics of the state transition at various light intensity was determined. In

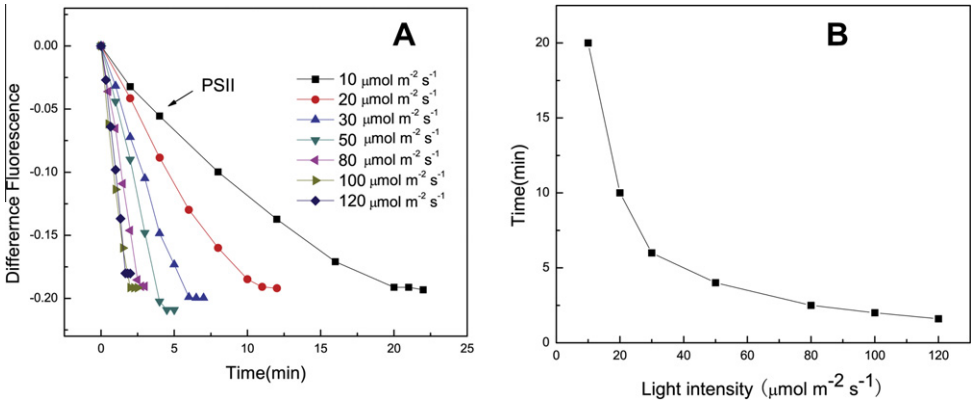

Fig. 2. Plots of the amplitudes for the PSII fluorescence (695 nm) to illumination time (A) collected from Fig. 1 and plot of the time needed for the state transition to light intensity (B).

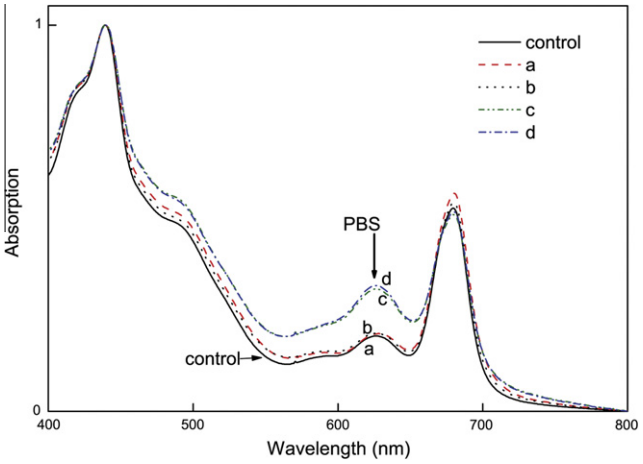

Fig. 3. Absorption spectra of Triton X-100 (1%)-washed PBS-thylakoid membrane complexes isolated from the cells which were previously fixed to state 2 by 25 μM DBMIB (a) or by betaine (b) or state 1 by 150 μM PBQ (c) or by betaine (d) and irradiated by 635 nm laser for 2 h, with the spectrum for the thylakoid membrane complexes isolated from untreated cells as the control (solid black line). All the spectra were normalized to 440 nm.

addition, taking advantage of specific photo-linkage of PBSs to PSII [18], time-dependent distribution of photo-linked PBS amount in (0,1) manner revealed the microscopic dynamics of PBS movement in the state transition. The kinetics and dynamics are also significant for understanding the physiological significance of light state transition in cyanobacteria.

2. Materials and methods

2.1. Culture and growth conditions

S. platensis FACHB-900 cells were cultured in AB medium (pH 9.0, 1 L) at 25 °C with continuous shaking and illumination by three 40-W fluorescence lamps. Ten-day cultures were harvested by centrifugation, washed and re-suspended in fresh growth medium. Cells of the same generation were used for all of the experiments.

2.2. State transitions

For the orange-light induced transition, the cells were previously induced to state 1 by illuminating the cells by blue light (Ditric Optics 410-nm long-pass and 460-nm short-pass filter) of 100 μmol m^{-2} s^{-1} for 10 min and were then illuminated by orange light (Ditric Optics 580-nm long-pass and 600-nm short-pass filter) at 10, 20, 30, 50, 80, 100 or 120 μmol m^{-2} s^{-1} for various times. The light intensity was measured by a UV-radiometer.

2.3. Spectral measurements

Absorption spectra were recorded on a UV-1601 ultra–vis spectrophotometer (Shimadzu, Japan). Fluorescence emission spectra were recorded on an F7000 spectrofluorimeter (Hitachi, Japan). Both of the excitation and emission slit widths were 5 nm. The chlorophyll concentration of the intact cell samples was adjusted to no more than 5 μg Chl *a* mL^{-1}, estimated by the absorbance at 665 nm in methanol extracts [19]. For 77 K fluorescence measurements, the samples were frozen into liquid nitrogen immediately after a certain time of illumination. The spectra presented in this work are the averages from five independent measurements.

2.4. Photo-linkages of PBSs during the state transition

The cyanobacterium cells suspended in BG-11 medium were led to a fixed state 1 or state 2 by PBQ (150 μM) or DBMIB (25 μM) respectively according to literatures [20]. The cells in a fixed state were irradiated by intense laser (635 nm, 100 mW) for 2 h. PBS-thylakoid membrane complexes were isolated from the laser-irradiated cells or untreated cells. The complexes were further treated by Triton X-100 (1%) before measurement of the absorption spectra, as reported previously [18]. To measure quantity of photo-linked PBSs during orange light illumination, glycine betaine (0.5 M) was added immediately after a certain time of illumination. Then the cells were irradiated by the 635 nm laser for 2 h and after that glycine betaine was removed by dialysis against BG-11 medium. Then the isolation of PBS-thylakoid membrane complexes and the treatment by Triton X-100 (1%) were in the same way as mentioned above before measurement of the absorption spectra.

3. Results and discussion

3.1. Time-dependent fluorescence responses of the cells at different intensity of orange light

Fig 1(A) showed 77 K fluorescence spectra measured at a series of time when the state-1 cells were illuminated by 10 μmol m^{-2} s^{-1} orange light. And the difference spectra were derived as shown in Fig. 1(B). In the difference spectra, four bands with the peaks at

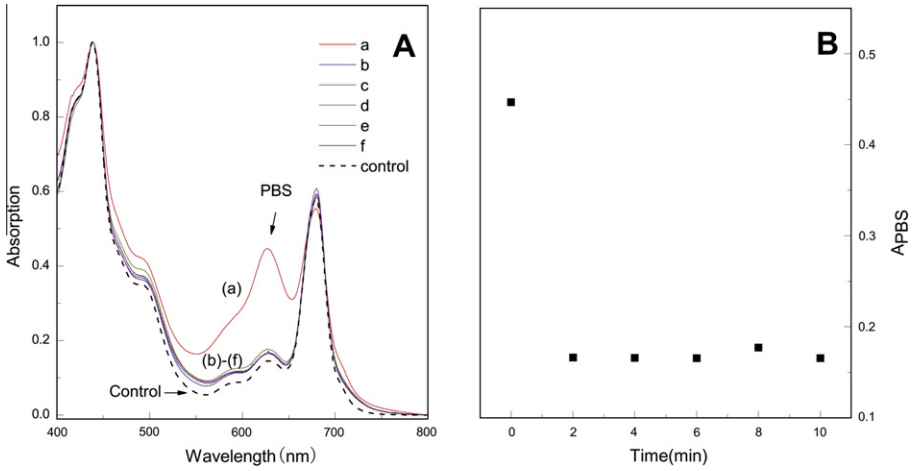

Fig. 4. (A) The absorption spectra of the Triton X-100 (1%)-washed PBS-thylakoid membrane complexes isolated from the cells which were previously induced to state 1 and then illuminated by orange light (20 μmol m^{-2} s^{-1}) for 0 (a), 2 (b), 4 (c), 6 (d), 8 (e) or 10 (f) min and irradiated by 635 nm laser for 2 h after adding betaine at each time. The spectrum for thylakoid membrane complexes isolated from untreated cells used as the control (dash black line). (B) Plot of the absorbance at 627 nm to the illumination time. All the spectra were normalized to 440 nm.

645, 660, 695 and 730 nm are ascribed to emissions of PBS rod (C-PC), PBS core (APC), PSII and PSI respectively [21–24]. The time-dependent amplitudes for the four components were shown in Fig. 1(C). It could be learnt that at the light condition, the state transition completed in 20 min when the fluorescence amplitudes became invariable. From time zero to 20 min, the fluorescence increased or decreased monotonously, implying that a series of immediate states were involved in the state transition. During the state transition, the synchronous changes in the PBS and photosystem components indicated that the state transition was entirely regulated by "mobile PBS". And the fluorescence decrease in PBS rod (and PSII) and increase in the PBS core (and PSI) was a character of the state 1 to 2 transition [11,12], which suggested the migration of PBSs toward PSI. Similarly, the state-1 cells were induced to state 2 by orange light at a series of intensities (20, 30, 50, 80, 100 and 120 μmol m^{-2} s^{-1}), 77 K fluorescence spectra (figures omitted) were measured and the difference spectra and the time-dependent amplitudes were derived, as shown in Fig. 1(D–I) .

Generally, the fluorescence fluctuation profiles at various intensities are similar, suggesting that state transition is a conservative physiological behavior of cyanobacteria. However, the time for state transition is variable. To demonstrate it clearly, plots of the PSII component amplitudes to time at all the light intensities were selectively shown in Fig. 2(A). Taking the time at the fluorescence saturation point as that needed for state transition, plot of the time to light intensity was shown in Fig. 2(B).

The difference fluorescence amplitude at the saturated illumination time reflects the extent of state transition at a light intensity. It can be seen from Fig. 2 that in different light intensity, the extent is almost the same but the rate is variable. It should be indicated that the light intensities used in this work are well in the range for normal photosynthesis in cyanobacteria [25]. The conservative extent and variable rate suggested that cyanobacterial cells can sensitively sense any imbalance of the excitation and response immediately by state transition. Therefore, state transition in cyanobacteria possesses not only a physiological but also a photochemical significance.

It was reported that state transition in cyanobacteria was physiologically important only at low light intensity (2 μE m^{-2} s^{-1}) based on observation of the effect of light intensity on growth of the *Synechocystis rpa*C mutant [17]. In the *rpa*C mutant, PBS mobility was inhibited [26], however, it does not necessarily means that state transition is absent because "mobile PBS" is the one but is not the only one mechanism for state transition in cyanobacteria [11] [24].

3.2. Dynamics of PBS movement in light state transition

As mentioned above, orange light induced state transition was entirely regulated by "mobile PBS". However, it is not clear whether "mobile PBS" is corresponding to a collective migration of all PBSs away from PSII or a relative change in PBS populations on the two photosystems. It was reported that PBSs could be specifically photo-linked to PSII but not other membrane proteins [18], which may be helpful to elucidate the microscopic dynamics of PBS movement during the state transition. It is imaginable that amount of photo-linked PBSs will be a continuous variable if the light state is regulated by relative change in PBS population on PSI and PSII, while it would be 1 or 0 if light state is regulated by a collective movement of all PBS. After the cyanobacterial cells were locked at state 2 by DBMIB [20,27] or state 1 by PBQ [20,27] or betaine [11], the cells were irradiated by full-power 635 nm laser, and the PBS-thylakoid membrane complexes were quantitatively isolated and then washed by a detergent Triton X-100. As the photo-linked PBSs could not be washed off thylakoid membranes, amount of photo-linked PBSs could be measured by absorption spectra. As shown in Fig. 3, PBSs were completely washed off from the state-2 cells, suggested by almost identical absorbance at 627 nm to the control, while PBS absorbance remains for the state-1 cells, confirming the specific photo-linkage of PBSs to PSII.

As the photo-linkage occurs only between two closely connected reactants, PBS photo-linkage will not occur if a PBS moves away from the docking site in PSII [28,29]. Similarly, after the cyanobacterial cells were locked to state 1, state 2 or any intermediate state by betaine and irradiated by full-power 635 nm laser, the PBS-Thylakoid membrane complexes were isolated from the cells and further washed by Triton X-100 and then the absorption spectra were measured, as shown in Fig. 4.

Fig 4 showed that PBSs were photo-linked at state 1 but not at state 2 or any other intermediate state, indicating that all the PBSs moved collectively away from PSII during the state transition. As PBSs in the state-1 cells are mainly connected to PSII, orange light leads to an over-excitation of PSII. Imaginably, if a light state is regulated by a relative change in PBS population on PSI or PSII, there would be some PSIIs keeping connected to PBSs. It is logically

unreasonable for those PSIIs always remain over-excited under orange light. The collective model proposed in this work is more reasonable.

This study shows that the extent of state transition is conservative but the rates vary in quite a large range of light intensities, implying that cyanobacterial cells can sensitively sense any imbalance of the excitation and immediately response by state transition. And during the state transition all of PBSs move collectively, indicating that a light state, corresponding to a fluorescence fluctuation amplitude, reflects a moving scale for all the PBSs but not redistribution of PBS population on the photosystems.

Acknowledgments

The research supported by the National Natural Science Foundation of China (NSFC, No. 30970676) and Chinese Academy of Sciences.

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