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ScienceDirect
Biochimica et Biophysica Acta 1767 (2007) 15–21
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbabio

Demonstration of phycobilisome mobility by the time- and space-correlated fluorescence imaging of a cyanobacterial cell

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Received 8 June 2006; received in revised form 4 October 2006; accepted 8 November 2006
Available online 17 November 2006

Abstract

The cell-wide mobility of PBSs was confirmed by synchronously monitoring the fluorescence recovery after photobleaching (FRAP) and the fluorescence loss in photobleaching (FLIP). On the other hand, a fluorescence recovery was still observed even if PBSs were immobile (PBSs fixed on the membranes by betaine and isolated PBSs fixed on the agar plate) or PBS mobility was unobservable (cell wholly bleached). Furthermore, it was proved that some artificial factors were involved not only in FRAP but also in FLIP, including renaturation of the reversibly denatured proteins, laser scanning-induced fluorescence loss and photo-damage to the cell. With consideration of the fast renaturation component in fluorescence recovery, the diffusion coefficient was estimated to be tenfold smaller than that without the component. Moreover, it was observed that the fluorescence intensity on the bleached area was always lower than that on the non-bleached area, even after 20 min, while it should be equal if PBSs were mobile freely. Based on the increasing proportion of the PBSs anti-washed to Triton X-100 (1%) with prolonged laser irradiation to the cells locked in light state 1 by PBQ, it was concluded that some PBSs became immobile due to photo-linking to PSII.

Keywords: Mobility; Phycobilisome; FRAP; FLIP; Photosystem I; Photosystem II

1. Introduction

To keep the excitation energy distribution between the photosystem I (PSI) and the photosystem II (PSII) in balance is one of the most important factors for photosynthetic efficiency of green plants and cyanobacteria, while how to regulate the energy distribution is related to the light state transition mechanism. In green plants, the major light-harvesting complex (LHCII) in the membrane can shuttle between PSI and PSII to balance the distribution of the excitation energy [1,2]. Similarly, it is believed that phycobilisome (PBS), the light-harvesting complex attached to the stromal surface of the thylakoid membrane in cyanobacteria, could transfer the excitation energy to PSI and PSII alternatively [3–5], although other possibilities have been proposed [6–9]. Recently, PBS mobility was proved to be a prerequisite for light state transition [10,11], while it had never been experimentally observed until 1997 when the fluorescence recovery of PBSs on the photobleached area of a cyanobacterial cell was observed by the use of a technique called FRAP (fluorescence recovery after photobleaching), from which the time-related shallowing and widening of the bleaching profile, ascribed to PBS mobility, was clearly observed [12]. However, later, the fluorescence recovery was suspected of some artificial results caused by the high-energy laser [13]. In fact, the phycobiliproteins in PBSs, also known as a kind of photosensitizers, could photosensitively produce highly reactive oxygen species or free radicals [14–16], which in turn may induce successive photochemical processes.

Abbreviations: PBS, phycobilisome; PSI, photosystem I; PSII, photosystem II; LHCII, light-harvesting complex II; FRAP, fluorescence recovery after photobleaching; FLIP, fluorescence loss in photobleaching; PBQ, phenyl-1, 4-benzoquinone

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0005-2728/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2006.11.012

Therefore, some complexities may be involved in FRAP. Alternatively, the fluorescence loss in photobleaching (FLIP) could be used to monitor the synchronous fluorescence loss on the non-bleached region [17], which would certainly demonstrate PBS mobility even if FRAP itself might be suspected of any artifact. In this work, by jointly using FRAP and FLIP, a cell-wide mobility of PBSs was definitely demonstrated in a cyanobacterium *Thermosynechococcus elongates* cell (about 1.5 μm across and 8 μm long). It was also found that some artificial factors induced by the full-power laser were involved in both FRAP and FLIP, i.e., the renaturation of the reversibly-denatured proteins contributing to the fluorescence recovery and the laser-scanning to the fluorescence loss. Furthermore, under prolonged irradiation to the cells by the full-power laser, photo-crosslink of PBSs, most likely to PSII, was observed.

2. Materials and methods

2.1. Cell culture and sample preparation

Cells of the thermophilic cyanobacterium *Thermosynechococcus elongatus* were grown in liquid culture in BG-11 medium and bubbled with 5% CO_2 in air [18]. Cells were grown at 45 °C under illumination with white fluorescence lamp at an intensity of 50 μmol m^{-2} s^{-1}. The cells harvested at the middle-logarithmic phase of growth were used for the experiments. Cells were pre-illuminated with blue light (to state 1) or orange light (to state 2) and then glycine betaine was added to a final concentration of 0.5 M (to lock the cells in the pre-induced states). For observation on the laser confocal fluorescence microscopy, the cells were immobilized onto 1.5% (w/w) agar plates containing growth medium or 0.5 M betaine buffer (for the betaine-treated cells) on a slide covered with a glass slip and located under objective lens. For measurements of the diffusion coefficients at different temperatures, the samples were placed on a temperature-controlled holder connected with circulated water bath to keep the samples at 25 °C and 45 °C respectively.

Phycobilisomes were isolated from the cells as reported previously [19] with some modifications. The cells (1 g) suspended in 10 ml 0.75 M K-phosphate (pH 7.0) buffer were ultrasonically broken in ice bath. Triton X-100 was immediately added to the broken cells to a final concentration of 2% (w/w), and the mixture was incubated for 20 min with stirring at room temperature. Large fragments and cell debris were removed by centrifugation at 25,000×*g* for 30 min. Medium supernatant samples were collected by centrifugation twice for 30 min at 50,000×*g*. PBSs were concentrated by further centrifugation at 250,000×*g* for 2 h. Isolated PBSs were immobilized as described above for observation on the laser confocal fluorescence microscopy at room temperature (25 °C).

2.2. FRAP-FLIP Measurements

Olympus FV500 laser scanning confocal fluorescence microscope with a 60× oil-immersion objective lens was employed with a He–Ne red laser (633 nm, 10 mW) for photobleaching PBSs selectively. The laser light was passed through a 100-μm pinhole and focused onto the sample with a 60× oil-immersion objective lens. The vertical (*Z*) resolution was 0.27 μm and the resolution in the *x*–*y* plane was 0.20 μm. Fluorescence emission from the sample was separated from the excitation light (633 nm) by a 660 nm long-pass filter, passing through a 100-μm pinhole and detected by a cooled photomultiplier. The fluorescence image processing was implemented in MATLAB (MathWorks, Inc.). For synchronous measurements of FRAP and FLIP, a 1-μm-wide region at one end of cell was photobleached across the long axis of the cell and through the entire depth with 100% power laser for 12.5 s, and then the whole cell was imaged with 1% power laser, with that before photobleaching as a control. The images were recorded at 3-s intervals in the first 300 s and at 30-s intervals after then. The photobleaching at one end of the cell instead of that in the middle was for better observation of FRAP and FLIP.

2.3. FRAP measurements for determination of diffusion coefficient

For determination of diffusion coefficients, a 1-μm-wide strip in the middle of the cell was selectively bleached with 100% power laser for 1.2 s. Then, the fluorescence images were recorded at 3-s intervals with 1% power laser until 300 s, with the image recorded before photobleaching as a control. The width of the strip was verified by optical parameters. A cell was wholly photobleached by 100% power laser across the long axis of the cell line by line repeated 5, 15, 30 or 50 times to reach a 50%, 70%, 85% or 95% bleach depth respectively. Recovery of fluorescence was monitored by scanning the cell with 1% power laser at 3-s intervals until the fluorescence intensity reached a plateau.

2.4. Calculation of the diffusion coefficient

The lateral diffusion coefficient of PBSs was estimated by analysis of the recovery profile. The fluorescence recovery of the photobleached strip was fitted by Eq. (1), an empirical formula agrees within 5% with the solution of the diffusion equation in one dimension [20], while Eq. (2) contains both of the diffusion term and an exponential one.

$$I_{(t)} = I_{\text{final}}\left(1 - w^2(w^2 + 4\pi Dt)^{-1}\right)^{1/2} \quad (1)$$

$$I_{(t)} = I_r(1 - e^{-t/\tau}) + (I_{\text{final}} - I_r)\left(1 - w^2(w^2 + 4\pi Dt)^{-1}\right)^{1/2} \quad (2)$$

here, $I_{(t)}$ is the intensity as time *t*, with the time zero defined as the midpoint of the photobleaching; I_r and $(I_{\text{final}} - I_r)$ are the amplitudes of the two terms respectively with I_{final} defined as the maximal intensity reached after recovery; τ is the lifetime of the exponential term; *w* is the strip width and *D* is effective diffusion coefficient. For determination of the diffusion coefficients, "non-linear curving fitting" program in Origin software was used to fit the fluorescence intensities data to Eqs. (1) or (2).

2.5. Photo-crosslink of PBSs

Cells suspended in growth medium in the presence of PBQ (150 μM) were exposed to intense laser (635 nm, 100 mW) for 10, 30, 60, 90 and 120 min. The PBS-thylakoid membrane complexes were isolated from unexposed and exposed cells and washed by Triton X-100 according to the previously reported method [11]. Absorption spectra were recorded on a UV-1601 ultra-vis spectrophotometer (Hitachi, Japan) before and after the laser irradiation.

3. Results and discussion

3.1. Cell-wide mobility of PBSs

To observe FRAP and FLIP at the same time, the cell-wide fluorescence images were recorded from the time immediately after photobleaching to about 20 min. Fig. 1 shows the

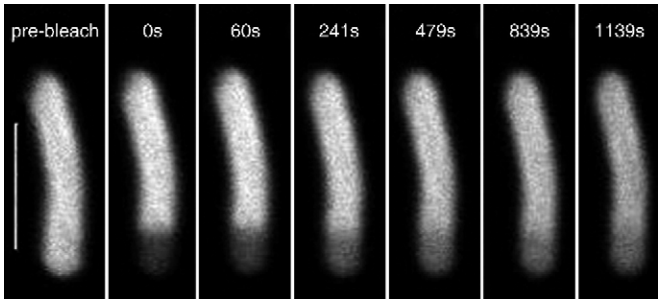

Fig. 1. The fluorescence images for a cell of thermophilic cyanobacterium *Thermosynechococcus elongatus* at selected times before and immediately after photobleaching of PBSs on one end of the cell. Scale bar: 5 μm.