Phycobilisome Diffusion Is Required for Light-State Transitions in Cyanobacteria¹

Sarah Joshua and Conrad W. Mullineaux*
Department of Biology, University College London, London WC1E 6BT, United Kingdom

Phycobilisomes are the major accessory light-harvesting complexes of cyanobacteria and red algae. Studies using fluorescence recovery after photobleaching on cyanobacteria in vivo have shown that the phycobilisomes are mobile complexes that rapidly diffuse on the thylakoid membrane surface. By contrast, the PSII core complexes are completely immobile. This indicates that the association of phycobilisomes with reaction centers must be transient and unstable. Here, we show that when cells of the cyanobacterium *Synechococcus* sp. PCC7942 are immersed in buffers of high osmotic strength, the diffusion coefficient for the phycobilisomes is greatly decreased. This suggests that the interaction between phycobilisomes and reaction centers becomes much less transient under these conditions. We discuss the possible reasons for this. State transitions are a rapid physiological adaptation mechanism that regulates the way in which absorbed light energy is distributed between PSI and PSII. Immersing cells in high osmotic strength buffers inhibits state transitions by locking cells into whichever state they were in prior to addition of the buffer. The effect on state transitions is induced at the same buffer concentrations as the effect on phycobilisome diffusion. This implies that phycobilisome diffusion is required for state transitions. The main physiological role for phycobilisome mobility may be to allow such flexibility in light harvesting.

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* Corresponding author; e-mail c.mullineaux@ucl.ac.uk; fax 44–20–7679–7096.

Fluorescence recovery after photobleaching (FRAP) can be used to measure the diffusion of thylakoid membrane components in cyanobacteria. The technique involves the use of a highly focused confocal laser spot to selectively bleach the fluorophores in a small region of the cell. The diffusion of the fluorophores can then be monitored by observing the spread and recovery of the bleach (Mullineaux and Sarcina, 1999; Ashby and Mullineaux, 1999; Rakhimbetdieva et al., 2001).

Fluorescence recovery after photobleaching (FRAP) measurements show that the phycobilisomes are highly mobile, with diffusion coefficients typically around $4 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ (Sarcina et al., 2001; Ashby and Mullineaux, 2002). By contrast, PSII appears completely immobile (Mullineaux et al., 1997; Sarcina et al., 2001). This indicates that there is no stable association between phycobilisomes and PSI, and it is likely that the association of phycobilisomes with PSI is also transient. Studies with mutant strains of *Synechococcus* 7942 have shown that the phycobilisome diffusion coefficient is influenced by a number of factors including phycobilisome size and membrane lipid composition (Sarcina et al., 2001) and the oligomerization of PSI (Aspinwall et al., 2004). The physiological role of phycobilisome mobility has remained unclear.

State 1-state 2 transitions (state transitions) are a rapid physiological adaptation of the photosynthetic light-harvesting apparatus, resulting in changes in the distribution of excitation energy between PSI and PSII. State transitions in green plants involve the redistribution of a proportion of the light-harvesting chlorophyll $a/b$-binding protein of PSII (LHCII). This is triggered by LHCII phosphorylation. In State 1, most of the LHCII is associated with PSII complexes in the thylakoid grana. On transition to State 2, a proportion of the LHCII decouples from PSII and reassociates with PSI in the stroma lamellae. Thus, state transitions involve a relatively long-range migration of the LHCII complexes (for review, see Allen and Forsberg, 2001). Although cyanobacteria lack LHCII, they perform state transitions that are analogous to those of green plants (Fork and Satoh, 1983). State transitions in cyanobacteria involve the redistribution of phycobilisome-
absorbed energy between PSII and PSI (van Thor et al., 1998; McConnell et al., 2002), and one effect of the transition to State 2 is the functional decoupling of phycobilisomes from PSII and their reassociation with PSI (Mullineaux, 1992). The biochemical mechanism is not known. However, it is clear that state transitions can be triggered by changes in the redox state of an intersystem electron carrier (Mullineaux and Allen, 1990), and one gene specifically required for state transitions in cyanobacteria has been identified (Emlyn-Jones et al., 1999).

This study establishes a direct connection between phycobilisome mobility and state transitions. It has previously been shown that state transitions in cyanobacteria are inhibited when cells are immersed in buffers containing high concentrations of phosphate. Interestingly, treatment with the buffer locks the cells into the state to which they were adapted prior to addition of the buffer, so cells can be locked in either State 1 or in State 2, as judged from fluorescence spectroscopy (Mullineaux, 1993). Here, we use FRAP to show that treatment of cells of *Synechococcus* 7942 with high-phosphate buffers drastically decreases the mobility of phycobilisomes. We further show that this effect is induced at the same phosphate concentrations that are required to lock state transitions. We propose that the buffers lock state transitions because they prevent phycobilisomes from decoupling from reaction centers, and we discuss the reasons this may occur.

RESULTS

Adaptation to State 1 or to State 2 can be monitored by recording fluorescence emission spectra on frozen samples at 77 K. In State 1, there is greater fluorescence emission from PSII relative to the phycobilins and PSI than there is when cells are adapted to State 2. This reflects the higher proportion of excitation energy that is transferred to PSII when cells are adapted to State 1 (Murata, 1969). Figure 1 shows 77 K fluorescence emission spectra for cells of *Synechococcus* 7942 adapted to State 1 or State 2 prior to freezing the cells. The State 1 spectra show greater relative emission from PSII (685–695 nm) as compared to phycocyanin (654 nm) and PSI (720 nm; Fig. 1).

It was shown previously that state transitions in the cyanobacterium *Synechococcus* sp. PCC6301 could be inhibited by immersing cells in K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub> buffers with phosphate concentrations of about 0.2 M or greater. Furthermore, exposure to the buffer had the effect of locking the cells in either State 1 or State 2, depending on the state to which the cells were adapted prior to addition of the buffer (Mullineaux, 1993). Here, we show a similar result with *Synechococcus* 7942. Cells were adapted to State 1 or to State 2 by incubation in respectively red light or dark for 5 min. Phosphate buffer (0.5 M K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>, pH 6.8) was then added, and the suspension was again acclimated to red light (Fig. 2A) or dark (Fig. 2B) before freezing samples in liquid nitrogen for 77 K fluorescence spectroscopy. The spectra in Figure 2A are essentially identical to those in Figure 2B. Therefore, the fluorescence emission spectrum reflects the acclimation of the cells before addition of the buffer: subsequent acclimation to red light or dark has no effect on the spectrum (Fig. 2). The effect is not species specific as a similar result was obtained with the cyanobacterium *Synechocystis* sp. PCC6803 (data not shown).

Exposure to 0.5 M phosphate buffer changes the shape of the fluorescence emission spectrum, with the peak at about 685 nm becoming more prominent (compare Figs. 1 and 2). This effect was not observed when PSII was directly excited at 435 nm (data not shown), indicating that the intrinsic fluorescence emission from PSI is not changed by the buffer. PSII has emission peaks at 685 and 695 nm, but with phycobilin excitation the 685-nm peak comes partly from the terminal emitter pigments of the phycobilisome core (Ashby and Mullineaux, 1999). Therefore, the increase in the 685-nm peak relative to the other peaks, including the shoulder at 695 nm (Figs. 1 and 2), indicates that energy transfer from phycobilisomes to reaction centers becomes slightly less efficient. The effect would be consistent with a structural change leading to slightly slower energy transfer.

We have used 77 K fluorescence emission spectra to quantify the extent of fixation of light-state. The ratio of fluorescence at 685 nm to fluorescence at 654 nm (*F<sub>685</sub>/F<sub>654</sub>*) gives an indication of light state, with a higher *F<sub>685</sub>/F<sub>654</sub>* indicating adaptation to State 1 (Fig. 1). The extent of fixation in State 1 may be quantified as (LD−DD)/(LL−DD), where LD is the *F<sub>685</sub>/F<sub>654</sub>* for cells adapted to red light and then readapted to dark after addition of phosphate buffer, DD is
Figure 2. The 77 K fluorescence emission spectra for cells of Synechococcus 7942 in 0.5 M phosphate buffer. Fluorescence spectra recorded with excitation at 600 nm and normalized to the phycocyanin fluorescence peak (654 nm). Cells were adapted to red light (black line) or to dark (gray line) before addition of phosphate buffer. A, Cells readapted to red light after addition of phosphate buffer. B, Cells readapted to dark after addition of phosphate buffer.

Figure 3. Fixation of cells in State 1 and State 2 as a function of phosphate concentration. Cells were adapted to State 1 (black) or to State 2 (gray) before addition of phosphate buffer. Cells were then adapted to the opposite light regime before being frozen for 77 K fluorescence spectroscopy. Fixation of light state is calculated from $F_{685}/F_{654}$ for cells adapted to dark both before and after addition of phosphate buffer, and LL is $F_{685}/F_{654}$ for cells adapted to red light both before and after addition of phosphate buffer. If the cells were completely fixed in State 1, then dark adaptation after addition of phosphate would have no effect on the spectrum. Then LD would be equal to LL and (LD−DD)/(LL−DD) would be 1. Conversely, if there was no fixation of light state, then LD would be equal to DD and (LD−DD)/(LL−DD) would be 0. The extent of fixation in State 2 can be quantified in a similar way as (DL−DD)/(LL−DD), where DL is the $F_{685}/F_{654}$ for cells adapted to dark and then readapted to red light after addition of phosphate buffer. Figure 3 shows the extent of fixation in State 1 and State 2 in Synechococcus 7942 as a function of phosphate concentration. Fixation increases with increasing phosphate concentration.

There is no fixation of light state at 0.1 M phosphate: the negative values for fixation at this concentration indicate that state transitions are slightly enhanced (Fig. 3). State transitions are partially inhibited at 0.2 to 0.3 M phosphate, and cells can be completely locked in either state at 0.4 M phosphate or above. As observed previously in Synechococcus 6301 (Mullineaux, 1993), the concentration of phosphate required to fix cells in State 1 appears slightly lower than that required to fix cells in State 2 (Fig. 3). Figure 4 shows 77 K fluorescence emission spectra for cells treated with 0.2 M phosphate. At this concentration, there is significant fixation of cells in State 1 but not in State 2. Therefore, when cells are readapted to dark after addition of phosphate buffer, cells initially adapted to State 1 remain close to State 1 (Fig. 4B). However, when cells are readapted to red light after addition of buffer, cells initially in State 2 revert to State 1 (Fig. 4A).

We have also examined the effect of phosphate buffers on the kinetics of state transitions, monitored by fluorescence time courses at room temperature. Cells were adapted to State 2 in the dark. Phosphate buffer was then added, and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added to block electron transport at the acceptor side of PSII. Cells were then illuminated with bright light, and red fluorescence was monitored over a time course of a few minutes. The transition to State 1 results in a fluorescence rise on a timescale of a few seconds to a few minutes (Schluchter et al., 1996; Aspinwall et al., 2004). The bright illumination ensures that the kinetics of the state transition are not limited by the kinetics of electron transport, and in the presence of DCMU, PSII centers remain closed, so that the fluorescence kinetics are not complicated by changes in PSII trap closure (Schluchter et al., 1996; Aspinwall et al., 2004). The results are shown in Figure 5. The State 1 transition is not inhibited at 0.1 M phosphate, and in fact the fluorescence rise is more rapid in 0.1 M phosphate than in growth medium. At 0.2 M phosphate, the fluorescence rise becomes much smaller and slower. At 0.3 M phosphate and above, there is apparently complete inhibition of the State 1 transition, and fluorescence falls slightly during illumination (Fig. 5).

We found that fixation is not influenced by small changes in pH; similar results were obtained with phosphate buffers at pH 6.0 and pH 8.0 (data not
shown). We also found cells could be fixed in either state with sucrose (Suc) solutions or with high concentrations of potassium chloride (data not shown). As with phosphate, low concentrations of Suc and potassium chloride enhanced state transitions rather than inhibiting them (data not shown). Thus, the effects are not specific to phosphate and are presumably related to osmotic strength rather than to any more specific chemical properties of the buffer. The minimum concentrations of Suc and potassium chloride required to inhibit the State 1 transition were estimated using kinetic experiments of the type shown in Figure 5. The minimum concentration of Suc required for complete inhibition of the State 1 transition was about 0.8 M. This compares with a critical concentration of 0.3 M potassium phosphate (Fig. 5). The higher critical concentration of Suc is consistent with an osmotic effect: a potassium phosphate solution will exert a greater osmotic effect than an equimolar solution of Suc because potassium phosphate dissociates into anions and cations in solution. At the intermediate Suc concentration of 0.5 M, 77 K fluorescence measurements of the type shown in Figures 2 and 4 show that fixation of cells in State 1 is 0.97 ± 0.16, but the fixation of cells in State 2 is only 0.17 ± 0.09. The critical concentration of potassium chloride was also about 0.8 M, again higher than for potassium phosphate. In this case both the anion and the cation are small, so the solute is likely to leak across the plasma membrane, reducing the osmotic effect. Comparison of the critical concentrations of potassium phosphate and potassium chloride confirms that this is not an electrostatic effect dependent on ionic strength in the cytoplasm. If this were the case, then potassium chloride would be more effective than potassium phosphate because potassium chloride will penetrate the plasma membrane more easily.

In view of the effect of high osmotic strength buffers on state transitions, it is of interest to see if these buffers affect the diffusion of phycobilisomes. We therefore carried out FRAP measurements using a laser-scanning confocal microscope as described previously (Sarcina et al., 2001; Aspinwall et al., 2004). A 633-nm red He-Ne laser was used to selectively excite phycocyanin, and fluorescence emission was monitored at >665 nm. At room temperature, most fluorescence at these wavelengths comes from the phycobilisome cores, so these settings can be used to monitor the diffusion of intact phycobilisomes (Sarcina et al., 2001). Prior to the measurement, cells were grown in the presence of 0.5% dimethylsulfoxide; this generates elongated cells that are more suitable for quantitative FRAP measurements (Aspinwall et al., 2004). The elongated cells do not exhibit any changes in thylakoid membrane structure as judged by electron microscopy and fluorescence imaging, and their photosynthetic properties as judged by fluorescence spectroscopy and oxygen-electrode measurements are normal (Mullineaux and Sarcina, 2002). Where appropriate, cells were dark adapted and then treated with phosphate buffer. Cells were then immobilized by adsorption onto agar containing growth medium or phosphate buffer. The confocal spot was scanned across the center of a cell for 2 s to bleach the phycobilins. The laser power was then reduced by a factor of 8 to prevent further bleaching, and the cell was repeatedly imaged. Phycobilisome diffusion causes the bleached line to spread and fill in, and the phycobilisome diffusion coefficient can be calculated from the rate of recovery of fluorescence at the center.

Figure 4. The 77 K fluorescence emission spectra for cells of Synechococcus 7942 in 0.2 M phosphate buffer. Fluorescence spectra recorded with excitation at 600 nm and normalized to the phycocyanin fluorescence peak (654 nm). Cells were adapted to red light (black line) or to dark (gray line) before addition of phosphate buffer. A, Cells readapted to red light after addition of phosphate buffer. B, Cells readapted to dark after addition of phosphate buffer.

Figure 5. Effect of phosphate buffers on the kinetics of state transitions. Cells were dark adapted (State 2) prior to addition of phosphate buffer. Cells were then illuminated in the presence of DCMU as described in “Materials and Methods.” The faster phase of the fluorescence rise (appearing immediate on this timescale) has been subtracted. Fluorescence is expressed relative to this initial fluorescence for cells with no added phosphate.
of the bleach (Mullineaux et al., 1997; Mullineaux and Sarcina, 2002). Figure 6 shows a typical result for a cell in growth medium. Under these conditions the fluorescence in the center of the bleach recovers on a timescale of seconds, and we measured a mean phycobilisome diffusion coefficient of $(4.3 \pm 1.7) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, which is comparable with that previously obtained (Sarcina et al., 2001; Aspinwall et al., 2004). By contrast, when cells are treated with 0.5 M phosphate buffer, no fluorescence recovery is seen on these short timescales, and only partial recovery is seen after 20 min (Fig. 7). Under these conditions, we estimated the mean phycobilisome diffusion coefficient to be $(9.8 \pm 0.6) \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$. Thus, exposure to the buffer decreases the rate of diffusion of the phycobilisomes by a factor of about 40 to 50. Figure 8 shows the dependence of the mean phycobilisome diffusion coefficient on phosphate concentration. At 0.1 M phosphate, phycobilisomes diffuse slightly faster on average than in growth medium, but we cannot be sure that this is significant due to the substantial variation in diffusion coefficient from cell to cell. At 0.2 M phosphate, there is a significant decrease in the diffusion coefficient, and a further significant decrease at 0.3 M phosphate.

We found that the effect of phosphate buffer on phycobilisome mobility is reversible. Cells were treated with 0.5 M phosphate buffer and then adsorbed onto agar containing growth medium rather than 0.5 M phosphate. Under these conditions, the phosphate will be diluted by the growth medium in the agar. The rapid diffusion of phycobilisomes is then restored. The mean diffusion coefficient was $(6.0 \pm 3.3) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ with no significant difference from untreated cells ($t$ test $P = 0.3$).

The FRAP data presented above (Figs. 7 and 8) were obtained from cells that were dark adapted prior to addition of phosphate buffer. At high phosphate concentrations, the cells will therefore be fixed in State 2 (Figs. 2 and 3). We obtained similar results with cells fixed in State 1 with 0.5 M phosphate buffer (data not shown), and we could not detect any significant differences between State 1- and State 2-adapted cells at lower phosphate concentrations (data not shown). However, Suc solutions also affected the phycobilisome diffusion coefficient. When we carried out FRAP measurements on cells in 0.5 M Suc, a concentration at which cells are efficiently fixed in State 1 but not in State 2 (see above), we found a significant difference depending on adaptation prior to addition of Suc. For cells adapted to State 1, the mean phycobilisome diffusion coefficient was $(6.5 \pm 0.3) \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$, whereas for cells adapted to State 2 it was $(4.7 \pm 0.3) \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$. The difference is significant ($t$ test, $P = 0.019$). Thus, at this Suc concentration, the rate of phycobilisome diffusion is much more strongly affected when cells are adapted to State 1.

**DISCUSSION**

Previous FRAP studies have shown that the phycobilisomes are highly mobile complexes, diffusing rapidly on the thylakoid membrane (Mullineaux et al., 1997; Sarcina et al., 2001). Figure 6 shows the rapid diffusion of phycobilisomes under physiological con-
conditions in vivo. However, we show here that when cells of *Synechococcus* 7942 are immersed in concentrated phosphate buffers, the rate of diffusion of phycobilisomes is drastically decreased (Figs. 7 and 8). This appears to be a rather nonspecific osmotic effect, since it is pH independent and we obtained similar results with Suc solutions. The most plausible cause of such an extreme decrease in the diffusion coefficient is a strong interaction with another, immobile component in the membrane (Zhang et al., 1993). FRAP studies have shown that PSII is completely immobile under normal conditions (Mullineaux et al., 1997; Sarcina et al., 2001). We have no direct information on the diffusion of PSI, but its diffusion is likely to be very slow in the crowded environment of a cyanobacterial thylakoid membrane (Mullineaux, 1999). Therefore, we suggest that high osmotic strength buffers greatly increase the stability of phycobilisome-reaction center complexes and that this restricts the diffusion of the phycobilisomes. It is notable that high osmotic strength buffers are essential for the isolation of membranes and reaction centers with functionally coupled phycobilisomes. The buffer developed for this purpose by Gantt and co-workers contains 0.5 M phosphate and 0.5 M Suc (Katoh and Gantt, 1979; Gantt et al., 1988). It is possible that high osmotic strength buffers stabilize phycobilisome-reaction center complexes by decreasing the availability of water molecules around the thylakoid membranes. Consistent with this, we found the effect on the phycobilisome diffusion coefficient to be fully reversible and to be pH independent. The 0.5 M phosphate buffer changes the shape of the 77 K fluorescence spectrum, with the 685-nm peak becoming more prominent (Figs. 1 and 2). This is consistent with an altered structural interaction between phycobilisomes and reaction centers.

It was previously shown that state transitions in cyanobacteria are inhibited when cells are immersed in high-phosphate buffers. Furthermore, cells are locked in the state to which they were adapted prior to addition of the buffer (Mullineaux, 1993). Our current results confirm this effect for *Synechococcus* 7942 (Fig. 2). We found similar effects with Suc and potassium chloride solutions, and glycine (Gly) beta-tetaine also has this effect (Li et al., 2004). Thus, the effect on state transitions also appears to depend on the osmotic strength of the buffer. We tested the effect of increasing concentrations of phosphate (Figs. 3–5). The 0.1 M phosphate does not inhibit state transitions (Fig. 3), which actually occur more rapidly in 0.1 M phosphate than in growth medium (Fig. 5). At 0.2 M phosphate, the transition to State 2 is partially inhibited (Fig. 3). There is little effect on the transition to State 1 as judged from 77 K fluorescence spectra recorded on cells frozen after 5 min of adaptation (Figs. 3 and 4). However, the kinetics of the State 1 transition as judged from room-temperature fluorescence time courses are strongly affected (Fig. 5). At 0.3 M phosphate and above, both the State 1 and the State 2 transitions are very strongly inhibited (Figs. 3 and 5). The phosphate concentration dependence of the state transition effect correlates with the phosphate concentration dependence of the phycobilisome diffusion coefficient (Fig. 8). The 0.1 M phosphate slightly increases the mean phycobilisome diffusion coefficient, although the variation from cell to cell means that this may not be significant (Fig. 8). Phycobilisomes diffuse significantly slower at 0.2 M phosphate, and the diffusion coefficient becomes minimal at 0.3 M phosphate and above (Fig. 8). Therefore, we propose that the two effects are linked.

A number of models for the mechanism of state transitions in cyanobacteria have been proposed (Allen and Holmes, 1986; Bruce et al., 1989; Meunier et al., 1997). Recent work has led to a consensus that state transitions involve changes in the relative energy transfer from phycobilisomes to the PSI and PSII reaction centers (Mullineaux, 1994; Schluchter et al., 1996; McConnell et al., 2002). This is usually accompanied by a redistribution of chlorophyll-absorbed energy, although the two effects can be separated (Emlyn-Jones et al., 1999; McConnell et al., 2002). The structural basis of state transitions is still controversial. The mobility of phycobilisomes (Mullineaux et al., 1997) suggests that state transitions may involve the physical decoupling of phycobilisomes from one type of reaction center and their reassociation with the other, as originally proposed by Allen and co-workers (Allen and Holmes, 1986). The transition to State 1 occurs more rapidly in mutants lacking Psal, the subunit required for trimerization of PSI (Schluchter et al., 1996; Aspinwall et al., 2004). Phycobilisomes also diffuse more rapidly in Psal mutants, which is consistent with the idea that state transitions involve phycobilisome mobility (Aspinwall et al., 2004). However, other models that do not involve the movement of phycobilisomes have been proposed (Schluchter et al., 1996; McConnell et al., 2002).
Our current results indicate that phycobilisome mobility is critical for state transitions: the same conditions that immobilize phycobilisomes also lock cells into the light state to which they were adapted. Under physiological conditions, the association between phycobilisomes is unstable and transient, and a phycobilisome will frequently detach from a reaction center, diffuse, and reassociate with another reaction center (Mullineaux et al., 1997; Sarcina et al., 2001). We propose that high osmotic strength buffers prevent phycobilisomes from decoupling from reaction centers and that this prevents any redistribution of phycobilisomes between PSII and PSI.

With 0.1 M phosphate, state transitions are enhanced (Figs. 3 and 5) and the mean phycobilisome diffusion coefficient increases, although this may not be significant (Fig. 8). It is therefore possible that under these conditions, phycobilisome-reaction center interactions actually become more labile than in growth medium. At intermediate osmotic strengths (0.2 M phosphate or 0.5 M Suc), cells are fixed in State 1 much more efficiently than they are fixed in State 2 (Figs. 3 and 4). The difference is particularly pronounced in 0.5 M Suc, and at this Suc concentration, phycobilisome mobility is reduced significantly more when cells are adapted to State 1 prior to addition of Suc. This suggests that the phycobilisome-PSII complex is stabilized at slightly lower osmotic strength than the phycobilisome-PSI complex.

CONCLUSIONS

Phycobilisome mobility is critical for state transitions in cyanobacteria. Our data support a model in which excitation energy distribution from phycobilisomes to reaction centers is governed by a dynamic equilibrium in which PSII and PSI reaction centers compete to bind phycobilisomes. State transitions change the position of the equilibrium by changing the binding constant of phycobilisomes with one or both of the reaction centers, although the biochemical mechanism is not known. High osmotic strength buffers stabilize phycobilisome-reaction center association, and this has the effect of drastically slowing the diffusion of phycobilisomes and preventing any redistribution of the phycobilisomes between PSI and PSII. The major physiological role of phycobilisome mobility may be to allow flexibility in light harvesting.

MATERIALS AND METHODS

Strains and Culture Conditions

Wild-type Synechococcus sp. PCC7942 was obtained from the Pasteur Culture Collection. Cells were grown in liquid culture in BG11 medium (Castenholz, 1988) supplemented with 10 mM NaHCO3. Cells were grown in batch culture in an incubated orbital incubator at 30°C under white light at 9 μE m−2 s−1. For FRAP studies, the growth medium was supplemented with dimethylsulfoxide at 0.5% (v/v) for 3 d prior to the experiment, as this induces cell elongation (Aspinwall et al., 2004).

Adaptation of Cells to State 1 or to State 2

Cells were harvested by centrifugation and resuspended in growth medium to a final chlorophyll concentration of 5 μg μL−1. Chlorophyll concentrations were determined by methanol extraction (Porra et al., 1989). Aliquots of the cell suspension were injected into quartz capillary tubes (2.5 mm internal diameter). For State 2, the sample was dark adapted for 5 min, and for State 1 the sample was adapted for 5 min to a red light defined by a Schott RG665 glass filter (transmitting wavelengths longer than about 665 nm). The light intensity was 20 μE m−2 s−1.

Fluorescence Spectroscopy

Cell suspensions at 5 μg chlorophyll in growth medium or buffer in quartz capillary tubes were frozen by dropping the tube into liquid nitrogen. Fluorescence emission spectra were recorded at 77 K in a Perkin-Elmer (Foster City, CA) LS50 luminescence spectrometer equipped with a liquid-nitrogen sample housing and a red-sensitive photomultiplier. The excitation wavelength was 600 nm, and emission was scanned from 620 to 750 nm. Excitation and emission slitwidths were 5 nm. Because the absolute amplitudes of low-temperature spectra are unreliable, spectra were routinely normalized to the phycocyanin fluorescence emission peak at 654 nm. Fluorescence ratios were averaged from spectra obtained from five samples.

Treatment with High Osmotic Strength Buffer

The phosphate buffers used were K2HPO4/KH2PO4 solutions at phosphate concentrations of 0.1 to 0.5 M. The pH was 6.8 unless otherwise specified. An aliquot of cell suspension at a chlorophyll concentration of 50 μg μL−1 was placed in a stirred beaker and preadapted to State 1 or to State 2 using the illumination conditions described above. Nine volumes of buffer were then added, and the illumination conditions were maintained for a further 5 min. For fluorescence spectroscopy, an aliquot of the cell suspension was then injected into a quartz capillary tube and adapted for a further 5 min to either red light or dark before freezing and recording fluorescence spectra as described above.

Kinetics of State Transitions

Fluorescence transients were recorded at room temperature using a laboratory-built fluorimeter (Peter Rich, University College London, UK). Cells were resuspended in growth medium at a chlorophyll concentration of 30 μg μL−1 and dark adapted for 5 min. Nine volumes of phosphate buffer were then added, and the suspension was kept in the dark for a further 5 min. DCMU was then added to a final concentration of 50 μM. The cells were then illuminated with phycobilin-absorbed light defined by a combination of Schott RG610 and Ealing 660-nm short-pass filters. The illumination was controlled by an electronic shutter opening in about 1 ms and was at an intensity of 100 μE m−2 s−1. Fluorescence was detected by a photomultiplier screened by a Schott RG695 red glass filter.

FRAP Measurements

Cells grown in the presence of 0.5% dimethylsulfoxide, as described above, were used. Where specified, cells were pretreated with phosphate buffer as described above. In this case, cells were preadapted to State 2 by dark incubation unless otherwise specified. Cell suspensions were spotted onto 1.5% agar plates (Difco Bacto-Agar). The agar was made up either with growth medium or buffer at the appropriate concentration, unless otherwise specified. Aliquots of cells were injected into a quartz capillary tube and adapted for a further 5 min to either red light or dark before freezing and recording fluorescence spectra as described above.


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filter and scanned over a sample region of 20.5 × 20.5 μm. Cells aligned in the Y-direction were used for FRAP measurements. After recording a prebleach image by scanning the laser in the XY-plane, the microscope was switched to X-scanning mode, the laser intensity was increased 8-fold by removing the neutral density filter, and the confocal spot was scanned across the cell for 2 s to bleach a line across the cell. The laser intensity was then decreased again and images were recorded every 3 s for 30 s. Where appropriate, further images were recorded every 5 min up to 30 min after the bleach.

FRAP Data Analysis

Diffusion coefficients were obtained from the image series as described previously (Mullineaux et al., 1997; Sarcina et al., 2001; Mullineaux and Sarcina, 2002; Aspinwall et al., 2004). Optimas 5.2 image analysis software was used to obtain a one-dimensional fluorescence profile along the long axis of the cell, summing pixel values across the cell in the X-direction. Fluorescence profiles for bleached cells were corrected by subtracting the prebleach profile and then fitted to Gaussian curves using SigmaPlot software (Jandel Scientific, San Rafael, CA). Diffusion coefficients were obtained by plotting bleach depth versus time according to a one-dimensional diffusion equation (Mullineaux et al., 1997; Aspinwall et al., 2004; Mullineaux, 2004). Values shown are means from six cells, with sds.

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