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Mobilization of Photosystem II Induced by Intense Red Light in the Cyanobacterium *Synechococcus* sp PCC7942

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We use confocal fluorescence microscopy and fluorescence recovery after photobleaching to show that a specific light signal controls the diffusion of a protein complex in thylakoid membranes of the cyanobacterium *Synechococcus* sp PCC7942 in vivo. In low light, photosystem II appears completely immobile in the membrane. However, exposure to intense red light triggers rapid diffusion of up to ~50% of photosystem II reaction centers. Particularly intense or prolonged red light exposure also leads to the redistribution of photosystem II to specific zones within the thylakoid membranes. The mobilization does not result from photodamage but is triggered by a specific red light signal. We show that mobilization of photosystem II is required for the rapid initiation of recovery from photoinhibition. Thus, intense red light triggers a switch from a static to a dynamic configuration of thylakoid membrane protein complexes, and this facilitates the rapid turnover and repair of the complexes. The localized concentrations of photosystem II seen after red light treatment may correspond to specific zones where the repair cycle is active.

INTRODUCTION

The photosystem II (PSII) reaction centers of plants and cyanobacteria are subject to photodamage (Barber and Andersson, 1992). A complex repair cycle operates that involves the partial disassembly of damaged reaction centers, the proteolysis of damaged subunits, and their replacement by newly synthesized subunits (Andersson and Aro, 2001). This repair cycle is of crucial importance for photosynthesis. It is also a beautiful experimental model for the turnover of protein complexes, because damage and turnover can be initiated simply and specifically by exposure to light. For these reasons, the repair cycle has been intensively studied. A key enzyme involved in the early stages of the PSII repair cycle in plants and cyanobacteria was recently identified (Bailey et al., 2002; Silva et al., 2003). However, many questions remain concerning the ways in which the activity of the repair cycle is controlled and the possible localization of different stages of the repair cycle. In green plants, biochemical fractionation studies suggest that key stages of the repair cycle occur in the stroma lamellae and that the migration of photodamaged PSII complexes from the grana to the stroma lamellae plays an important role (Baena-Gonzalez et al., 1999; Andersson and Aro, 2001).

PSII core complexes are naturally fluorescent, raising the possibility that redistribution of PSII complexes during photoinhibition could be observed in vivo, in real time, using fluorescence microscopy. However, the intricate structure of green plant thylakoid membranes makes such studies difficult (Mullineaux, 2004). By contrast, the cyanobacterium *Synechococcus* sp PCC7942 (*Synechococcus* 7942) is an outstanding model system for studies of photosynthetic membrane function in vivo because it has a very simple, regular thylakoid membrane organization, with the thylakoids arranged approximately as concentric cylinders (Sherman et al., 1994; Mullineaux and Sarcina, 2002). When cells are grown in the presence of cell division inhibitors, they are long enough to allow quantitative measurement of the diffusion of thylakoid membrane components using fluorescence recovery after photobleaching (FRAP) (Mullineaux and Sarcina, 2002; Mullineaux, 2004). We previously used FRAP to show that the core complexes of PSII are astonishingly immobile, with no diffusion detectable even on time scales as long as 30 min (Sarcina and Mullineaux, 2004). By contrast, the light-harvesting phycobilisomes diffuse rapidly on the cytoplasmic surface of the thylakoid membrane (Mullineaux et al., 1997; Sarcina et al., 2001), and IsiA, an integral membrane chlorophyll protein, is also mobile (Sarcina and Mullineaux, 2004). Thus, the immobility of PSII indicates that there is a specific anchor preventing its diffusion.

Here, we show that PSII in *Synechococcus* 7942 is not always immobile. After exposure of cells to intense red light, a proportion of PSII centers start to diffuse quite rapidly in the thylakoid membrane. Under some conditions, this leads to a redistribution of PSII within the thylakoid membrane system. We present data suggesting that this process facilitates the rapid initiation of the PSII repair cycle. Our results indicate a novel signal transduction process controlling the dynamics of the thylakoid membrane.

RESULTS

The diffusion of PSII complexes in cells of *Synechococcus* 7942 may be visualized using confocal FRAP with a blue excitation

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light that is mainly absorbed by chlorophyll *a* combined with detection of chlorophyll fluorescence in the red region of the spectrum (Sarcina and Mullineaux, 2004). We use the 457-nm band from an argon laser as the blue excitation light and detect emission wavelengths >665 nm (Sarcina and Mullineaux, 2004). Figure 1 shows a fluorescence emission spectrum for cells of *Synechococcus* 7942 excited at 457 nm. For comparison, a spectrum with excitation at 600 nm (predominantly absorbed by phycocyanin) is shown as well (Figure 1). Spectra were recorded in the presence of DCMU to close PSII reaction centers. This helps to replicate conditions during the confocal measurements, in which the very high intensity of illumination will cause the closure of PSII reaction centers, leading to increased PSII fluorescence. The 680- to 685-nm peak may come either from chlorophyll or from long-wavelength pigments in the phycobilisome core, whereas the 650-nm peak comes only from the phycobilisomes: it originates from phycocyanin in the phycobilisome rods (Sidler, 1994). We used a red glass filter to select longer-wavelength fluorescence (Figure 1). The presence of the 650-nm peak with 600-nm excitation (Figure 1) is indicative of strong excitation of the phycobilisomes. The absence of this peak with 457-nm excitation (Figure 1) indicates that there is little or no excitation of phycobilisomes at this wavelength; thus, the fluorescence we see must all come essentially from chlorophyll.

Chlorophyll fluorescence could come either from PSII or from photosystem I (PSI). PSI fluorescence emission is generally low except at low temperatures, as a result of the very rapid decay of fluorescence in PSI (Turconi et al., 1993). However, PSI fluorescence emission is not completely insignificant in cyanobacteria, in which PSI has a larger chlorophyll light-harvesting antenna and the PSI/PSII ratio is relatively high, typically ~3 in *Synechococcus* 7942 (Joshua and Mullineaux, 2005). Picosecond fluorescence decay measurements give accurate resolution of PSII and PSI fluorescence emission, allowing an estimate of the relative contribution of PSII and PSI fluorescence. Lifetime data with chlorophyll excitation and closed PSII reaction centers indicate that under these conditions, the ratio of PSII to PSI fluorescence emission is ~4; thus, ~80% of the fluorescence observed comes from PSII and 20% comes from PSI (Mullineaux and Holzwarth, 1993). Therefore, to a first approximation, FRAP measurements under our conditions report on the mobility of PSII, although a minor contribution from PSI is also likely.

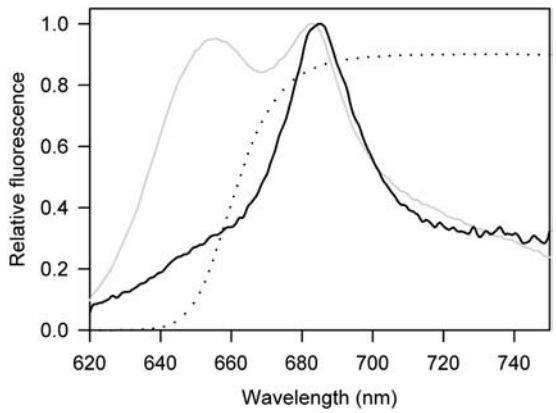

Figure 1. Room Temperature Fluorescence Emission Spectra for Cells of *Synechococcus* 7942 with Closed PSII Reaction Centers.

The cells were excited at 600 nm (gray line) or 457 nm (black line). Spectra are normalized to the peak at 680 to 685 nm. The dotted line shows the transmission spectrum of the RG665 red filter used to select chlorophyll fluorescence.

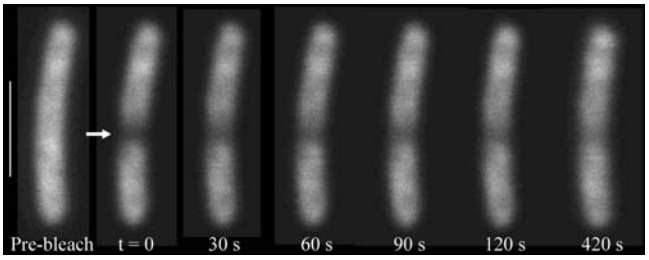

Figure 2. Representative Confocal FRAP Measurement on a Cell of *Synechococcus* 7942 after Pretreatment with Red Light (633 nm).

Chlorophyll fluorescence is visualized, with excitation at 457 nm. A line was bleached across the center of the cell where indicated by the arrow; the subsequent recovery of fluorescence here indicates diffusion. Bar = 5 μm.

For the FRAP measurement, cells are immobilized on an agar surface. The confocal laser spot is used to bleach a line across the center of the cell. Diffusion of fluorescent membrane components may then be visualized by repeatedly imaging the cell and observing the spread and recovery of the bleached line (Mullineaux and Sarcina, 2002; Mullineaux, 2004). We previously used this technique to show that PSII is virtually immobile, with no diffusion detectable even after 30 min. This finding indicates that the PSII diffusion coefficient must be $<2 \times 10^{-13}$ cm^2 s^{-1} (Sarcina and Mullineaux, 2004). By contrast, the phycobilisomes diffuse rapidly, with a mean diffusion coefficient of $\sim 3 \times 10^{-10}$ cm^2 s^{-1} (Sarcina et al., 2001).

In the PSII diffusion measurements reported previously, the cells were exposed only to blue light at 457 nm (Sarcina and Mullineaux, 2004). Here, we show that if a similar measurement is performed on cells that have been pretreated with intense red light from a 633-nm HeNe laser, a proportion of chlorophyll fluorescence becomes mobile. Figure 2 shows a typical chlorophyll FRAP measurement performed on a cell of *Synechococcus* 7942 after exposure to 633-nm light. The red light exposure and the FRAP measurement were both performed with light from a laser-scanning confocal microscope. Cells were immobilized by adsorption onto an agar surface and were maintained at their growth temperature of 30°C. In the experiment shown in Figure 2, the cell was exposed to 633-nm light for 3 min by scanning the confocal laser spot rapidly over a field of view of 120×120 μm. This treatment gives an averaged photon flux density of 4.4×10^5 μE·m^{-2}·s^{-1}. After pretreatment with red light, a FRAP measurement was performed with blue excitation at 457 nm, with fluorescence monitored in the red (>665 nm). There is only a partial recovery of fluorescence in the bleached line, indicating that not all PSII complexes are mobile (Figure 2). We considered the possibility that the partial fluorescence recovery might be

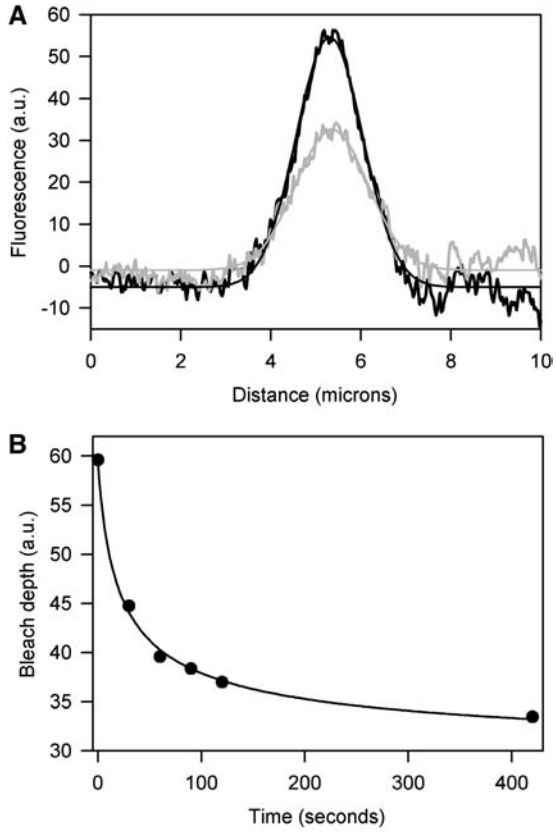

Figure 3. Estimation of the Diffusion Coefficient for Chlorophyll Fluorescence in a Cell of *Synechococcus* 7942 Pretreated with Red Light.

(A) Fluorescence profiles extracted from images like those shown in Figure 2. The profiles show the fluorescence difference between the cell before bleaching and at various times after bleaching. The profiles shown are for time 0 (black line) and 420 s (gray line). The experimental data are fitted to Gaussian curves (shown by the thin lines). a.u., arbitrary units.
(B) Plot of fluorescence bleach depth versus time after bleaching. Black circles show bleach depths obtained by fitting Gaussian curves as in **(A)**. The fitted line shows the predicted fluorescence recovery according to a one-dimensional diffusion equation, with mobile fraction 0.52 and diffusion coefficient $D = 2.3 \times 10^{-10}$ cm^2 s^{-1}.

attributable to processes other than diffusion, such as reversible quenching of fluorescence. Such effects might arise from non-photochemical quenching, for example, although this is generally observed only under iron stress (Cadoret et al., 2004; Bailey et al., 2005). However, total fluorescence from the cell does not increase during the recovery period, and there is no fluorescence recovery when an entire cell is bleached (data not shown). Both these observations show that the bleaching is not reversible; therefore, the recovery we see in the bleached zone must result from diffusion. Figure 3 shows fluorescence profiles extracted from a FRAP image sequence and a fluorescence recovery curve. The evolution of the fluorescence profile (Figure 3A) is what would be expected when there is a significant proportion of immobile fluorescence. The mobile fluorescence redistributes, leading to partial fluorescence recovery in the bleached zone and a slight fluorescence decrease in the remainder of the cell. At the end of the recovery period, a sharp bleached line remains as a result of the immobile proportion of fluorescence (Figure 3A). Under the conditions used in Figure 2, an average of 54% ± 5% of chlorophyll fluorescence was mobile, and we could estimate the mean diffusion coefficient of the mobile fluorescence as $(2.3 \pm 0.4) \times 10^{-10}$ cm^2 s^{-1}.

The mobile fraction of chlorophyll fluorescence depends on the dose of red light (Figure 4). After a 3-s exposure to red light, ~20% of chlorophyll fluorescence is mobile, and the effect saturates within ~15 s. The maximum proportion of PSII that becomes mobile is ~50 to 60%, and longer exposure does not lead to any further increase in the mobile fraction (Figure 4). After comparable pretreatments with blue light (457 nm) and green light (543 nm), with similar exposure times and photon flux densities, there is no detectable PSII diffusion. Figure 5 shows images from a typical FRAP sequence for a cell pretreated with 457-nm light (exposure time of 3 min, average photon flux density of 8.8×10^5 μE·m^{-2}·s^{-1}).

To determine whether the effect could be replicated under more physiological conditions, we exposed cells in bulk liquid culture to bright red light. In this case, cells were incubated in growth medium at 30°C and exposed to broad-band red light (>620 nm) at a photon flux density of 2000 μE·m^{-2}·s^{-1}. Aliquots were then immobilized on agar, and FRAP measurements were performed as before. After preillumination for 30 min, an average of 10% ± 3% of PSII complexes were mobile, although the proportion of mobile complexes is likely to have decreased during the delay required for the preparation of the microscope sample. The mobility of PSII ceased within 30 min of returning the cells to normal growth conditions after red light illumination. A comparable pretreatment with broad-band blue-green light (<560 nm) did not induce any PSII mobility.

In green plants, photodamage has been proposed as the trigger for a series of events leading to the migration of damaged

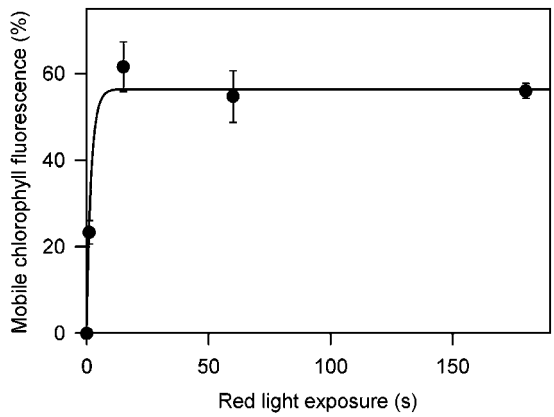

Figure 4. Dependence of the Mobile Fraction of Chlorophyll Fluorescence on Red Light Pretreatment.

Cells were pretreated with intense 633-nm light for the indicated times, before measuring the mobile fraction of chlorophyll fluorescence with FRAP measurements at 457-nm excitation. Data points are means from measurements of three to five cells, with standard errors.

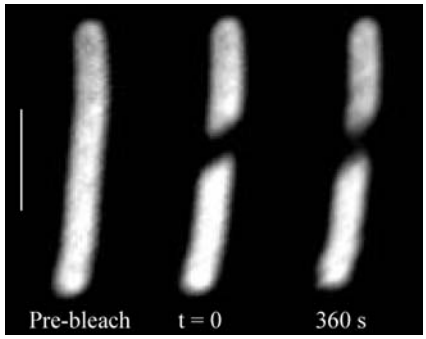

Figure 5. Images from a Representative Confocal FRAP Sequence for a Cell Pretreated with Blue Light (457 nm).

Other conditions were as in Figure 2. Bar = 5 μm.

PSII centers from the grana to the stroma lamellae (Baena-Gonzalez et al., 1999; Andersson and Aro, 2001). Therefore, we considered the possibility that the mobilization of PSII in *Synechococcus* 7942 is a direct result of photodamage. The wavelength specificity of the effect argues against this. Blue light is as effective as red light at inducing photodamage, yet blue light does not induce the mobilization of PSII. To test the possibility further, we applied the red light pretreatment only to a small part of the cell (Figure 6A). The 633-nm confocal laser spot was scanned for 10 s in a line 58 μm long across one end of the cell perpendicular to the long axis. This was sufficient to cause localized photobleaching of chlorophylls (Figure 6A). A FRAP measurement with 457-nm excitation was then performed toward the other end of the cell. As shown in Figure 2, diffusion of chlorophyll fluorescence can be observed, although in this case the PSII complexes in the vicinity of the FRAP bleach had not been exposed to the red light and therefore were not photodamaged before the FRAP experiment. As with light pretreatment of the whole cell (Figures 2 and 5), the effect is wavelength-specific. Pretreatment of one end of a cell with 457-nm light did not induce any PSII mobility at the other end of the cell (Figure 6B).

Treatment with the 633-nm confocal laser spot not only mobilizes chlorophyll fluorescence but also causes it to rapidly redistribute within the cell (Figure 7). Chlorophyll fluorescence is initially distributed very evenly along the length of the cell (Figure 7A). However, after red light treatment, it becomes concentrated in localized zones within the thylakoid membranes (Figure 7B). In small cells, these are usually at the poles of the cell, whereas longer cells have additional concentrations throughout the length of the cell (Figure 7B). This effect could easily be induced with short, very intense red light treatments with the confocal microscope. However, when cells in liquid culture were exposed to red light (of much lower intensity), it could be induced only by very prolonged treatment. Exposure of a liquid culture to red light at 1200 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 3 h was required to induce localized concentrations of chlorophyll fluorescence (data not shown). Localized concentrations of chlorophyll fluorescence could not be induced by 457-nm light treatment with the confocal microscope (data not shown).

The wavelength specificity of the red light effect allows us to test the possibility that the mobilization of chlorophyll complexes plays a role in the response of the cells to photoinhibitory conditions. Figure 8 compares oxygen evolution during photoinhibitory treatments with red or blue-green light. No electron acceptors were added; therefore, oxygen evolution reports on the activity of the entire photosynthetic electron transport chain. Lincomycin inhibits de novo protein synthesis and therefore blocks the PSII repair cycle. The response of the cells to photoinhibitory treatment may be gauged by comparing oxygen evolution during photoinhibitory light treatments in the presence and absence of lincomycin. Higher oxygen evolution in the absence of lincomycin indicates an active PSII repair cycle (Silva et al., 2003). In the presence of lincomycin, oxygen evolution declines to zero in ~50 min in both blue-green light (Figure 8A) and red light (Figure 8B). Thus, both light treatments are effective at inducing photodamage. In blue light in the absence of lincomycin, oxygen evolution initially declines almost as fast as when lincomycin is present. Thus, there is initially little activity of the repair cycle, although it becomes significant after 20 to 30 min. In red light in the absence of lincomycin, there is initially a significant increase in oxygen evolution. This is not seen when lincomycin is present, indicating an adaptation process requiring protein synthesis. Oxygen evolution eventually declines during the photoinhibitory treatment, but it always remains significantly higher than in the presence of lincomycin, indicating that repair and adaptation processes operate efficiently from the start under these conditions (Figure 8B).

The difference between the response of the cells to photoinhibition under red and blue light is confirmed by *t* tests performed for the data shown in Figure 8 at 15 min after the

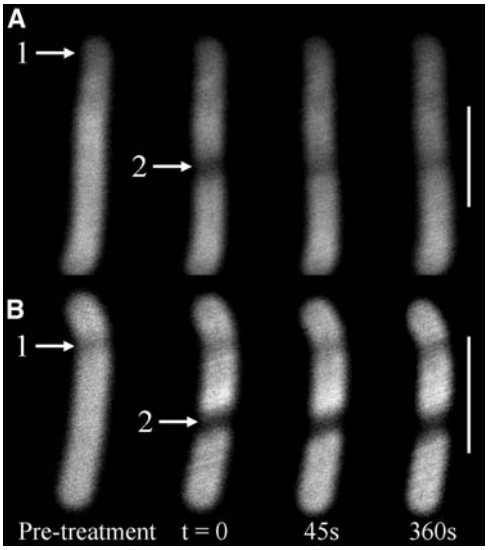

Figure 6. PSII Mobilization Induced by a Localized Light Pretreatment.

(A) A red light (633 nm) pretreatment was applied by scanning the confocal laser spot across the cell where indicated (1). A FRAP measurement was then performed with 457-nm excitation as in Figure 1, bleaching across the cell as indicated (2). Bar = 5 μm.

(B) A similar experiment except that the pretreatment (1) was with 457-nm light. Bar = 5 μm.

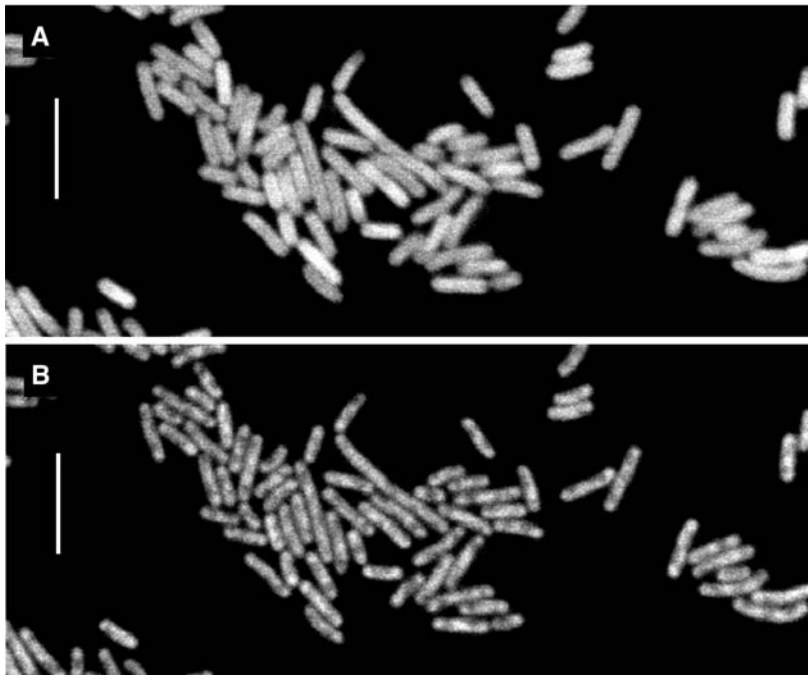

Figure 7. PSII Redistribution Induced by Red Light.

PSII chlorophyll fluorescence is visualized, with excitation at 457 nm. Bars = 10 μm.
(A) Before red light treatment.
(B) After 8 min of red light treatment (633 nm, 4.4×10^5 $\mu E \cdot m^{-2} \cdot s^{-1}$).

onset of photoinhibition. In the presence of lincomycin, oxygen evolution declines on average to 80% ± 4% in blue light and to 84% ± 3% in red light. This difference is not significant ($P = 0.53$), showing that blue and red light are equally effective at inducing photoinhibitory damage. In the absence of lincomycin, mean oxygen evolution is 86% ± 3% in blue light and 108% ± 2% in red light. Oxygen evolution under red light is significantly higher ($P = 3 \times 10^{-6}$). The presence of lincomycin makes no significant difference under blue light ($P = 0.27$), but oxygen evolution is significantly lower when lincomycin is present under red light ($P = 10^{-6}$).

DISCUSSION

A proportion of chlorophyll fluorescence in the cyanobacterium *Synechococcus* 7942 begins to diffuse after exposure of the cells to intense red light (Figure 2). Depending on the dose of red light, up to 50 to 60% of chlorophyll fluorescence becomes mobile under these conditions (Figure 4). The majority of chlorophyll fluorescence (~80%) comes from PSII. Therefore, PSII reaction centers must be diffusing under these conditions, although it is possible that a part of the diffusion that we see comes from PSI. Prolonged exposure to intense red light also leads to a very clear redistribution of chlorophyll complexes within the thylakoid membrane system. Under normal conditions, chlorophyll fluorescence is rather evenly distributed within the thylakoid membranes (Figure 7A), but after exposure to intense red light, there are obvious concentrations at the poles of the cell and at other points in long cells (Figure 7B).

The diffusion of chlorophyll complexes is triggered by red light (633 nm) but not by comparable doses of blue light (457 nm) (Figure 5) or green light (543 nm) (data not shown). The wavelength specificity of the effect suggests that it is caused by light perception and signal transduction rather than being a simple consequence of photodamage. This is confirmed by an experiment showing that a red light signal at one end of the cell triggers diffusion at the other end (Figure 6), indicating that the reaction centers that become mobile are not simply those that were photodamaged by the light pretreatment. There are two possible explanations for this. (1) The red light may induce a redox signal, or a specific kind of photodamage, as a result of its preferential absorption by the phycobilisomes. This could then trigger a signal transduction pathway leading to the mobilization of reaction centers. (2) The red light may be perceived by a specific photoreceptor, initiating a signal transduction pathway leading to the mobilization of PSII throughout the cell. The nature of the possible photoreceptor is unknown, but it could plausibly be a phytochrome. Phytochromes are generally considered to be low-irradiance sensors, but there are indications that plant phytochromes can also act as high-irradiance sensors of red light (Shinomura et al., 2000). One phytochrome-like protein has been characterized in *Synechococcus* 7942 (Mutsuda et al., 2003), and other possible phytochromes can be detected in the *Synechococcus* 7942 genome sequence (U.S. Department of

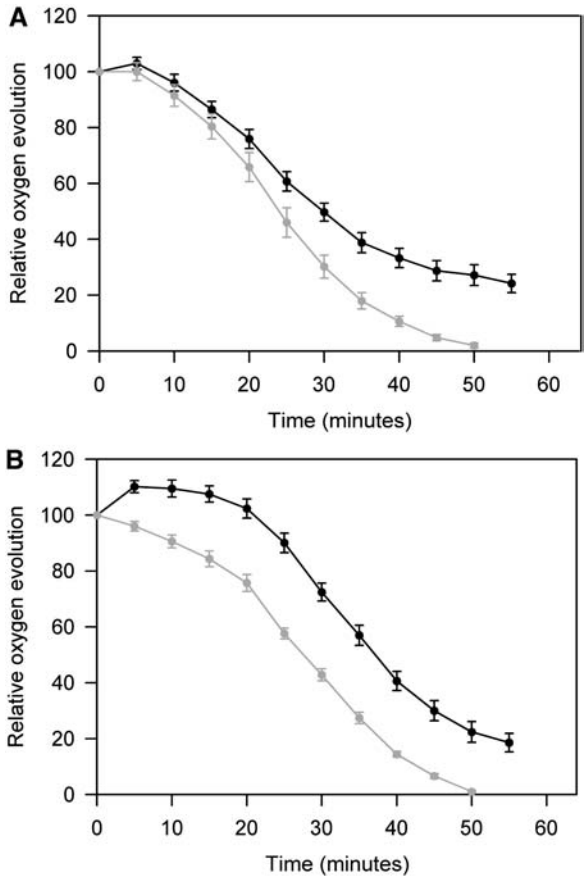

Figure 8. Photoinhibition of *Synechococcus* 7942 as Monitored by Oxygen Evolution.

(A) Photoinhibition with blue-green light (2000 $\mu E \cdot m^{-2} \cdot s^{-1}$).
(B) Photoinhibition with red light (2000 $\mu E \cdot m^{-2} \cdot s^{-1}$).
Black circles indicate treatment without lincomycin, and gray circles indicate treatment with lincomycin. Oxygen evolution is shown relative to the rate before photoinhibition, and rates are means of 11 to 20 replicates, with standard errors.

Energy Joint Genome Institute: http://genome.jgi-psf.org/draft_microbes/synel/synel.home.html).

Mobilization of chlorophyll complexes is induced only by light at intensities considerably greater than those required to saturate photosynthesis. This suggests that the physiological role of the effect is somehow to minimize photoinhibition. There are two obvious possibilities for this. (1) Mobilization of chlorophyll complexes allows redistribution into localized concentrations within the cell (Figure 7). When this occurs, overall light absorption will be decreased and thus photodamage could be reduced. (2) Mobilization of PSII could facilitate the PSII repair cycle, allowing the rapid turnover and repair of photodamaged PSII. Other adaptation and repair processes could also be facilitated by switching the thylakoid membrane to a more dynamic state, allowing the diffusion of protein complexes.

Possibility 1 seems unlikely because concentrations of PSII are formed rapidly only after treatment with photon flux densities of red light far greater than anything likely to be encountered in nature. At more physiologically realistic light intensities, they are seen only after very prolonged high-light treatment, which has already caused major photodamage. Therefore, this cannot be an efficient mechanism for minimizing photodamage by reducing light absorption. Thus, we favor possibility 2, that the mobilization of PSII facilitates the PSII repair cycle. The wavelength specificity of the effect allows us to test this possibility. PSII becomes mobile when cells are exposed to photoinhibitory doses of red light but not blue or green light. Therefore, we predict that the response of the cells to photoinhibition should be more effective during exposure to red light than to blue light. We find that this is the case (Figure 8). As a result, we suggest that the mobilization of PSII is required to allow rapid initiation of the repair cycle and possibly other responses involving de novo protein synthesis. However, it is clear that the PSII repair cycle does eventually become active under blue light when no PSII diffusion is induced (Figure 8). Further studies will be required to determine whether this represents a slower induction of essentially the same repair process or perhaps an alternative mechanism requiring the de novo synthesis of repair enzymes. The data shown in Figure 8 indicate that the rapid initiation of repair significantly enhances photosynthetic performance during short-term high-light treatments.

In green plants, the phosphorylation of PSII core proteins may facilitate the diffusion of photodamaged PSII complexes from the grana to the stroma lamellae (Baena-Gonzalez et al., 1999). However, PSII core proteins do not appear to be phosphorylated in cyanobacteria (Pursiheimo et al., 1998). Further studies will be required to establish the molecular mechanism of PSII mobilization and the reasons why PSII is normally immobile.

A requirement of PSII mobility for rapid PSII repair suggests that photodamaged PSII complexes are not readily repaired in situ but must instead migrate to specialized repair zones, where the necessary enzymes are concentrated. The localized concentrations of PSII seen after brief, very intense red light treatment (Figure 7) may correspond to such repair zones. We propose that the red light signal leads to the mobilization of a proportion of PSII centers and that this allows photodamaged centers to diffuse to repair zones. When photodamage is faster than repair, PSII will accumulate in the repair zones. Concentrations of PSII are not observed during less intense red light photoinhibitory treatments, such as those used in Figure 8B, although PSII is mobilized under these conditions. However, under these conditions, there is little net photoinhibition, indicating that PSII repair keeps pace with PSII photodamage (Figure 8B). Therefore, we would not expect PSII to accumulate in the repair zones. We observe the concentrations of PSII only after extremely intense red light treatments or after very prolonged treatments at more physiologically realistic light intensities. In the first case, the very intense red light treatment will cause the rapid production of photodamaged PSII, overwhelming the cell's capacity for repair. In the second case, the prolonged high-light treatment compromises the cell's capacity for PSII repair, so that we observe very sustained photoinhibition under these conditions (data not shown). Under both of these extreme conditions, damaged PSII centers may accumulate in the repair zones faster than they can be turned over, allowing us to visualize the repair zones as concentrations of PSII.

Cell fractionation studies suggest that the early stages of PSII biosynthesis in cyanobacteria may occur in the plasma membrane rather than in the thylakoids (Zak et al., 2001; Keren et al., 2005). Therefore, a role for the plasma membrane in PSII turnover is plausible, although the nature of any contact or exchange between the plasma and thylakoid membranes is unclear. The PSII concentrations may be in areas where the thylakoid and plasma membranes interact.

Intense red light treatment leads to the mobilization of up to ~50 to 60% of PSII centers (Figure 4), and mobility may not be confined to photodamaged centers (Figure 6). Because the thylakoid membranes are densely packed with protein complexes (Mullineaux, 1999), it may be necessary to mobilize undamaged complexes, to allow the damaged complexes to migrate rapidly to repair zones. Membrane protein diffusion is severely restricted at high protein densities (Kirchhoff et al., 2004), and a large population of immobile PSII centers would hinder the diffusion of other proteins. The red light signal triggers a switch from a rather static configuration of integral membrane proteins (which may be optimal for photosynthesis under low light) to a dynamic state (which may be required to allow the rapid repair of photodamaged reaction centers). It remains to be seen whether comparable mechanisms are involved in the PSII repair cycle in green plants.

METHODS

Cell Growth and Sample Preparation

The cyanobacterium *Synechococcus* sp PCC7942 was obtained from the Pasteur Culture Collection and grown in liquid cultures in an illuminated orbital shaking incubator in BG11 medium (Castenholz, 1988) supplemented with 10 mM $NaHCO_3$. Growth was at 30°C under white light at 10 $\mu E \cdot m^{-2} \cdot s^{-1}$. Before FRAP measurements, cells were grown for 16 h in the presence of 0.5% DMSO to induce cell elongation (Aspinwall et al., 2004). For measurements with the confocal microscope, drops of cell culture were adsorbed onto an agar surface (1.5% Difco Bacto-agar containing BG11 medium). Blocks of agar were placed in the well of a laboratory-built, temperature-controlled sample holder and covered with glass cover slips. All measurements were performed at 30°C.

Fluorescence Spectroscopy

Cells were diluted in growth medium to a chlorophyll concentration of ~1 μM and placed in a 3-mL fluorescence cuvette. DCMU was added to a final concentration of 50 μM. Emission spectra were recorded at room temperature with a Perkin-Elmer LS55 luminescence spectrometer fitted with a red-sensitive photomultiplier. Excitation and emission slit widths were 5 nm, and the spectrum was corrected for the spectral response of the detector.

Confocal Microscopy and FRAP

A Nikon PCM2000 laser scanning confocal microscope was used. PSII chlorophyll fluorescence was visualized with excitation at 457 nm and emission at >665 nm defined by a Schott RG665 red glass filter (Sarcina and Mullineaux, 2004). Red light pretreatments were applied by scanning the confocal spot from a 633-nm HeNe laser over the sample. Comparable pretreatments with blue and green light were applied using the 457-nm line of an argon laser and a 543-nm green HeNe laser. FRAP measurements were performed as described previously (Sarcina and Mullineaux, 2004). Data analysis was as described (Mullineaux et al., 1997; Mullineaux, 2004), except that the diffusion equation included an additional term to allow for the possibility of an immobile fraction.

Photoinhibition and Oxygen Evolution

Cell cultures at a chlorophyll concentration of 8 μM were placed in the light- and temperature-controlled housing of an oxygen electrode (Oxy-Lab2; Hansatech) and maintained at 30°C. Photoinhibitory light was supplied by an Intralux 6000-1 cold light source. Red light was defined by an Ealing 620-nm long-pass filter. Blue-green light was defined by an Ealing 560-nm short-pass filter. In both cases, the photon flux density was 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$. Light-saturated oxygen evolution was measured using the same light sources. Where indicated, lincomycin was present at a concentration of 100 μg/mL.

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