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State transitions in a phycobilisome-less mutant of the cyanobacterium *Synechococcus* sp. PCC 7002

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State transitions were investigated in the cyanobacterium *Synechococcus* sp. PCC 7002 in both wild-type cells and mutant cells lacking phycobilisomes. Preillumination in the presence of DCMU induced State 1 and dark-adaptation induced State 2 in both wild-type and mutant cells as determined by 77 K fluorescence emission spectroscopy. Light-induced transitions were observed in the wild-type after preferential excitation of phycocyanin (State 2) or preferential excitation of Chl *a* (State 1). Light-induced transitions were also observed in the phycobilisome-less mutant after preferential excitation of short-wavelength Chl *a* (State 2) or carotenoids and long-wavelength Chl *a* (State 1). We conclude that the mechanism of the light-state transition in cyanobacteria does not require the presence of the phycobilisome. Our results contradict proposed models for the state transition, which require phosphorylation of, and an active role for, the phycobilisome.

Introduction

A variety of photosynthetic organisms containing either LHC Chl *a/b* or phycobilisomes as antenna complexes have the ability to regulate the distribution of excitation energy between PS II and PS I by a process commonly known as the light-state transition [1–3]. The state transition serves to balance the relative activities of PS II and PS I by adjusting the distribution of excitation energy between the photosystems in response to preferential excitation of either one. While the molecular basis for this regulatory response is believed to be understood in the LHC Chl *a/b*-containing organisms (for a review see Ref. 4), the mechanism remains controversial in the phycobilisome-containing red algae and cyanobacteria [5–8]. For a recent review of the mechanism of the state transition in phycobilisome-containing organisms, see Ref. 9.

In LHC Chl *a/b*-containing organisms, reduction of plastoquinone by preferential excitation of PS II is believed to activate a membrane-bound kinase which phosphorylates LHC II. The phospho-LHC II is proposed to migrate laterally away from the appressed membrane regions rich in PS II towards the stroma thylakoid membranes which are rich in PS I. The absorbance cross-section of PS I is thus increased at the direct expense of PS II via the redistribution of the LHC II and the resulting state is called State 2. Preferential excitation of PS I causes oxidation of plastoquinone and deactivation of the kinase, background phosphatase activity cleaves the phospho-LHC II and the LHC II migrates back into the appressed thylakoid regions, resulting in State 1.

The original model for the state transition in phycobilisome-containing organisms, as proposed by Murata [2], differs from that for LHC-containing organisms. The change in energy distribution between PS II and PS I was supposed to be controlled by 'spillover' of excitation energy from PS II Chl *a* to PS I Chl *a*, the rate of spillover being high in State 2 and low in State 1. The role of spillover has been confirmed in a number of later studies [10–12]. The kinetics [13] and energetic requirements [14] of the state transition in phycobilisome-containing organisms are also very different from those observed in LHC-containing organisms (see Ref. 15 for a review). These observations, and experiments to determine the role of phosphorylation in the mechanism, led to the development of a model for the state

Abbreviations: Chl, chlorophyll; PS, Photosystem; LHC, light-harvesting complex; DCMU; 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_{695}, fluorescence yield at 695 nm; F_{720}, fluorescence yield at 720 nm.

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transition in phycobilisome-containing organisms which did not invoke phosphorylation [5].

Another model for the state transition in phycobilisome-containing organisms has been proposed which is analogous to the mechanism generally accepted for the LHC Chl *a/b*-containing organisms [6]. In this model, components of both the phycobilisome and PS II undergo a redox-controlled phosphorylation/dephosphorylation. Upon preferential excitation of PS II, the components become phosphorylated with the kinase(s) most likely triggered by reduction of plastoquinone. The phosphorylation results in a mutual repulsion between the phycobilisome and PS II, which allows the phycobilisome to relocate on the membrane and become directly associated with PS I (State 2). Preferential excitation of PS I results in dephosphorylation, dissociation of the phycobilisome from PS I and subsequent reassociation of the phycobilisome with PS II (State 1).

Recently, a new version of this phosphorylation model has been presented in which the mutual repulsion between the phosphorylated phycobilisome and phosphorylated PS II again causes their dissociation in State 2 [8]. It is then supposed that PS II moves closer to PS I and that the phycobilisome remains dissociated from both photosystems. The result is an increase in the amount of energy transfer from PS II to PS I by spillover and a decrease in the amount of energy transferred from the phycobilisome to either photosystem. As no increase in phycobilisome fluorescence has been detected in State 2, an unknown fluorescence quencher must be invoked [8]. Preferential excitation of PS I causes dephosphorylation, allowing PS II and the phycobilisome to become reassociated (State 1).

The presence of the phycobilisome is essential to either of these latter models for the state transition, as the basis of the mechanism in both cases is phosphorylation of the phycobilisome and its subsequent dissociation from PS II. In contrast, the phycobilisome is not essential to the mechanism of the original spillover model, and its presence may not be required for the state transition to occur.

The objective of this study was to investigate a phycobilisome-less mutant of the cyanobacterium *Synechococcus* sp. PCC 7002 to determine whether state transitions could occur in the absence of the phycobilisome. Our results show that this mutant has the ability to perform redox-induced and light-induced state transitions (despite the difficulty in preferentially exciting either photosystem). These results contradict the phycobilisome/PS II dissociation models for the state transition in phycobilisome-containing organisms.

Materials and Methods

Synechococcus sp. PCC 7002 (formerly *Agmenellum quadruplicatum* strain PR-6) is a transformable, marine, unicellular cyanobacterium [16,17]. The wild-type organism produces phycobilisomes comprised of phycocyanin and allophycocyanin [18]. A mutant devoid of phycobilisomes and detectable phycobiliproteins, as assayed by fluorescence, was a spontaneous secondary mutant derived from a constructed mutation. The primary mutant carried a deletion of the *apcA* and *apcB* genes, which encode the α and β subunits of allophycocyanin, respectively ($\Delta apcAB$); these genes were replaced by a DNA fragment encoding the *neo* gene of Tn5 which confers resistance to neomycin/kanamycin [18]. A complete description of the primary mutant ($\Delta apcAB$) and secondary mutant ($\Delta apcAB$ *cpc*-) will be published elsewhere (Stirewalt, V.L. and Bryant, D.R., in preparation).

The wild-type and mutant ($\Delta apcAB$ *cpc* −) were grown autotrophically at 32° C on A^+ medium [16] at a light intensity of 25 $\mu E \cdot m^{-2} \cdot s^{-1}$. Both wild-type and mutant cells were harvested during exponential phase by centrifugation, washed and resuspended in growth medium to an optical absorbance of less than 0.1 to avoid self-absorption artifacts in the fluorescence determinations.

In all experiments, cells were brought to State 1 or State 2 at their growth temperature of 32° C. The light-induced transitions were accomplished by a 2 min illumination of a 50 μl algal sample in a capillary cuvette which was then quickly frozen in liquid nitrogen. The wild-type was brought to State 1 by illumination with blue light (Ditric optics 460 nm short-pass filter) at 350 $\mu E \cdot m^{-2} \cdot s^{-1}$ and State 2 by illumination with orange light (combination of a Ditric optics 600 nm short-pass filter and a 580 nm long-pass glass colour filter) at 350 $\mu E \cdot m^{-2}\ s^{-1}$. The mutant was brought to State 1 with either 700 nm light at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ or 520 nm illumination at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$; State 2 was induced with 435 nm at 25 $\mu E \cdot m^{-2} \cdot s^{-1}$ or 670 nm illumination at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. These illumination wavelengths were dispersed by a Jobin Yvon H20 spectrometer (10 nm bandpass). Alternatively, state transitions were induced in cells treated with 1 μM DCMU. These experiments were conducted with stirred 2 ml samples from which 50 μl aliquots were withdrawn into capillary tubes and quickly frozen. Samples were withdrawn sequentially after a 2 min dark-adaptation, 2 min after addition of DCMU in the dark, 2 min after illumination (Ditric optics 460 nm short-pass filter, 100 $\mu E \cdot m^{-2} \cdot s^{-1}$) in the presence of DCMU, and 2 min after a second dark adaptation. In a second experiment, this protocol was repeated with a additional step, a 2 min incubation of the dark-adapted cells with 1 mM KCN prior to the addition of DCMU.

Source-corrected 77 K fluorescence excitation spectra were determined with a SPEX Industries spectrofluorimeter as described previously [5]. Detector-corrected 77 K fluorescence emission spectra were obtained with

a spectrofluorimeter based on a Jarrel Ash 1/4 meter spectrograph and EG&G diode array detector (1420R) controlled by an EG&G detector interface (1461) accessed by an IBM AT-compatible computer. Excitation light (10 nm bandwidth) was supplied by a 100 W tungsten halogen lamp dispersed by a Jobin Yvon H20 spectrometer.

For the determination of 77 K emission spectra, the capillary tube sample cuvettes were mounted in a spinning sample-holder driven by compressed air (Varian A60-A high-resolution NMR spinning assembly) [19]. The capillary tubes were spun at approx. 1000 rpm during the 20 s exposure time used to obtain the emission spectra, resulting in a signal averaging of the fluorescence emission over the capillary tube surface. This technique corrects for the random scattering and sample inhomogeneities that interfere with fluorescence yield determination from frozen samples. The spinning sample-holder dramatically improved the reproducibility of fluorescence yield determinations and gave more consistent results than the use of added fluorophores as internal fluorescent standards. Repeated fluorescence yield determinations from identical samples were always within 4%.

Absorption measurements were made with an Aminco DW-2 spectrometer which was interfaced to an IBM AT-compatible computer by OLIS (GA, U.S.A.).

O_2-exchange measurements were made at 30 °C with a Yellow Springs O_2 electrode.

Results

Absorbance spectra for the wild-type and mutant (*ΔapcAB cpc-*) cells of *Synechococcus* sp. PCC 7002 are shown in Fig. 1. The wild-type cells (dashed line) show a significant contribution by phycocyanin and allophycocyanin in the 580–650 nm region, in contrast to the mutant (solid line) which shows only the Chl *a* Q_x (0,0) transition at 585 nm and the Q_y (0,1) transition at 624 nm in this region. The mutant cells show higher absorption in the 450–530 nm region than the wild-type

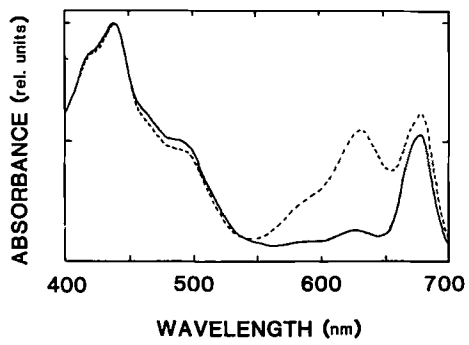

Fig. 1. Room-temperature absorbance spectra of intact wild-type and phycobilisome-less mutant cells of *Synechococcus* sp. PCC 7002. The dashed line is for the wild-type and the solid line for the mutant.

which reflects an increase in the carotenoid to Chl *a* ratio.

The low-temperature fluorescence-emission spectra of the wild-type cells in States 1 and 2 are shown in Fig. 2. Panel A shows the fluorescence emission spectra of 50 μl aliquots taken from a 2 ml stirred sample as described in Materials and Methods; the actinic wavelength was chosen to excite phycocyanin (590 nm). The light solid line is for dark-adapted cells, the dashed line is for cells after the addition of DCMU in the dark and the heavy solid line is after a 2 min blue-light preillumination of the DCMU-treated cells. There is a large change in the relative yield of PS II Chl *a* and PS I Chl *a* emission (F_{695}/F_{720}) indicative of a state transition between dark-adapted cells (State 2) and cells preilluminated in the presence of DCMU (State 1). The addition of DCMU in the dark slightly increased F_{695}/F_{720} but the emission curve more closely resembles that of dark-adapted cells (state 2) than cells preilluminated in the presence of DCMU. A control experiment showed that the change in fluorescence yield of the phycocyanin peak at 650 nm, seen upon addition of DCMU in the dark, was due to the addition of 0.5% ethanol with the DCMU (data not shown). However, the addition of ethanol alone did not increase F_{695}/F_{720} (data not shown). The cells could be cross-linked with glutaraldehyde, as described previously [20], into a high F_{695}/F_{720} state (State 1) or low F_{695}/F_{720} state (State 2) if glutaraldehyde was added during preillumination in the presence of DCMU or during dark-adaptation of cells, respectively (data not shown). The addition of DCMU and subsequent preillumination did not alter the fluorescence emission spectrum of dark-adapted cells which had been cross-linked with glutaraldehyde (data not shown).

Panel B of Fig. 2 shows a typical light-induced state transition measured in the absence of DCMU. The heavy solid line shows cells preilluminated with blue light and the light solid line, orange light as described in Materials and Methods.

Panel C of Fig. 2 compares the fluroescence emission spectra for 435 nm actinic illumination of dark-adapted cells (light solid line) to cells preilluminated with blue light in the presence of DCMU (heavy solid line). It is clear by comparing panel A and panel C that the change in F_{695}/F_{720}, indicative of the state transition, was apparent whether the actinic wavelength used to measure the emission spectra was chosen to excite phycocyanin or Chl *a*.

Fig. 3 shows 77 K fluorescence emission spectra of 50 μl aliquots of phycobilisome-less mutant cells taken sequentially from a 2 ml sample as described in Materials and Methods. Due to the absence of phycobilins in these organisms the actinic wavelength used was 435 nm, exciting both PS I and PS II Chl *a*. Spectra obtained with excitation at 590 nm were similar in

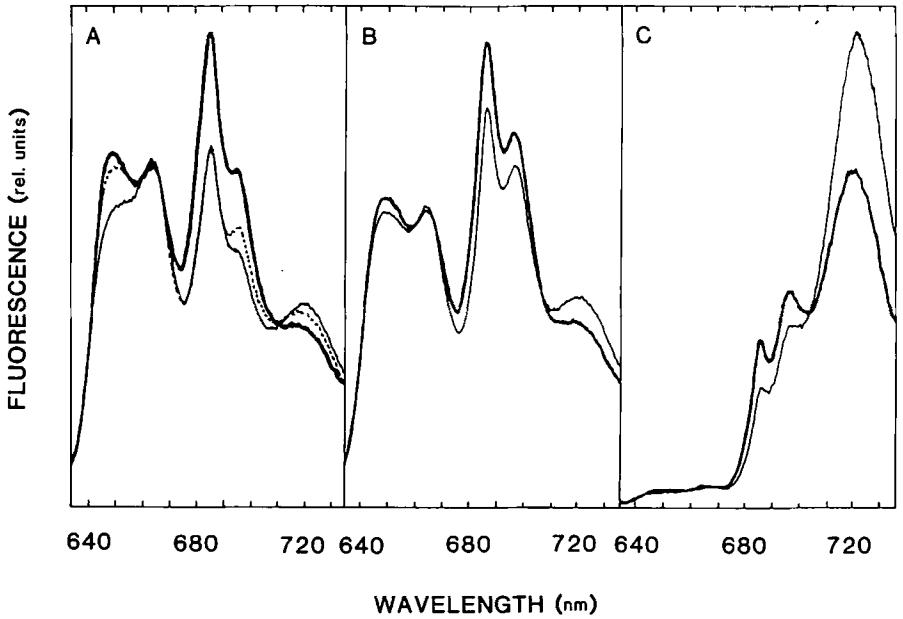

Fig. 2. 77 K fluorescence emission spectra of wild-type cells of *Synechococcus* sp. PCC 7002. In panel A, the light solid line is for dark-adapted cells, the dashed line for dark-adapted cells with DCMU and the heavy solid line DCMU-poisoned cells illuminated for 2 min (see text for experimental details). The excitation wavelength was 590 nm. In panel B, the light solid line is for cells preilluminated for 2 min with orange light and the heavy solid line cells preilluminated with blue light (see Materials and Methods). The excitation wavelength was 590 nm. In panel C, the light solid line is for dark-adapted cells and the heavy solid line for cells preilluminated with blue light in the presence of DCMU. The excitation wavelength was 435 nm. The spectra were normalized to emission at 660 nm.

shape but the fluorescence yield was very low (data not shown). In both panels A and B the light solid lines are for dark-adapted cells and show a low F_{695}/F_{720} ratio; the heavy solid lines are for cells preilluminated with blue light in the presence of DCMU and show a high F_{695}/F_{720} ratio. The cells in panel B had been previously treated with 1 mM KCN to inhibit cytochrome oxidase

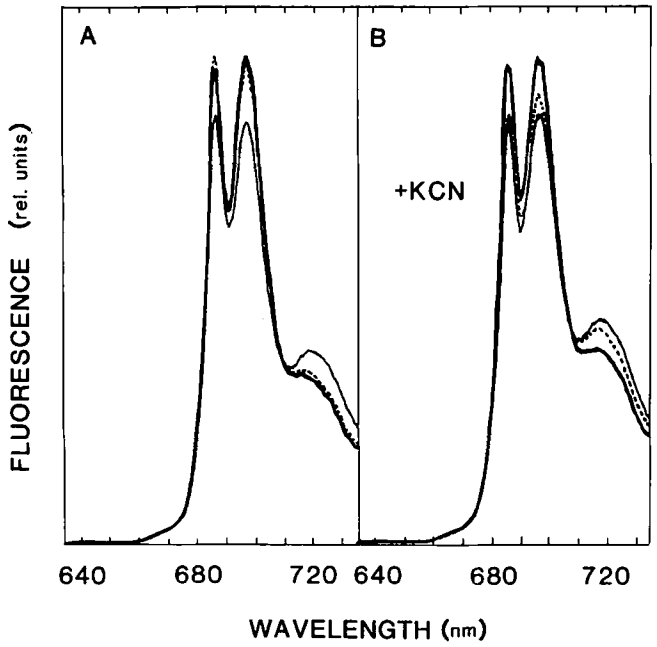

Fig. 3. 77 K fluorescence emission spectra of phycobilisome-less mutant cells of *Synechococcus* sp. PCC 7002. In both panel A and panel B, the light solid line is for dark-adapted cells, the dashed line for dark-adapted cells treated with DCMU, and the heavy solid line for cells preilluminated with blue light for 2 min in the presence of DCMU, see text for experimental details. In panel B, the cells were treated with 1 mM KCN. The excitation wavelength was 435 nm.

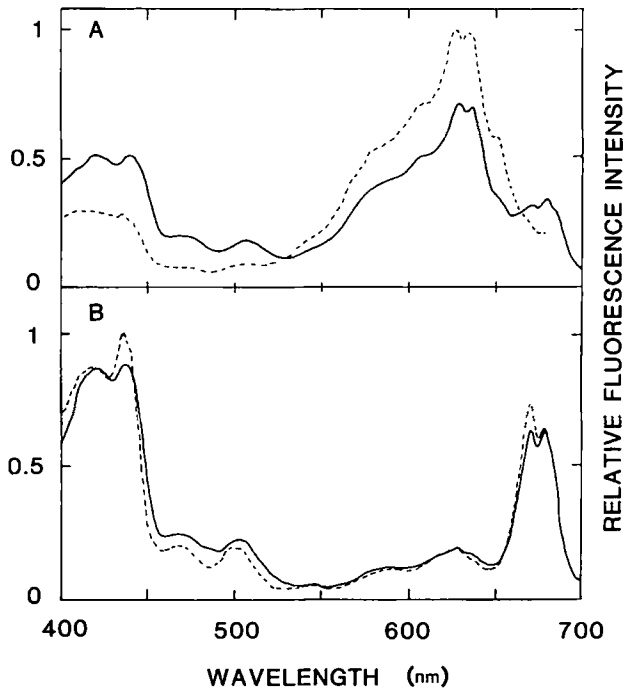

Fig. 4. Excitation spectra for 77 K fluorescence emission from PS2 (695 nm), dashed line, and PS I (720 nm), solid line. Wild-type cells are shown in panel A and PBS-less mutant cells in panel B. The spectra in panel B were normalized at 680 nm.

activity. In panel A the addition of DCMU in the dark (dashed line) results in an increase in F_{695}/F_{720} and the spectrum is very similar to that for cells preilluminated in the presence of DCMU. However, in panel B the KCN-treated cells show only a slight increase in F_{695}/F_{720} after the addition of DCMU in the dark (dashed line) similar to the response of the wild-type cells shown in Fig. 2. The preillumination/dark-adaptation-induced changes in F_{695}/F_{720} in the presence of DCMU and KCN were completely reversible in the mutant, as were the preillumination/dark-adaptation-induced changes in the presence of DCMU in the wild-type.

KCN did inhibit respiratory O_2-uptake in the phycobilisome-less mutant. After a 2 min incubation with 1 mM KCN, the rate of O_2 uptake in the phycobilisome-less mutant was decreased to 20% of the control rate (data not shown). In contrast, the rate of O_2 evolution was only inhibited to 60% of the control rate by the same treatment (data not shown).

Excitation spectra at 77 K for PS I and PS II Chl *a* fluorescence emission of the wild-type and mutant were recorded to compare the pigment complements of PS II and PS I (Fig. 4). The spectra for dark-adapted wild-type cells are shown in panel A. Wavelengths in the 560–660 nm region excite PS II preferentially (dashed line) due to the presence of phycocyanin and allophycocyanin associated with PS II. Absorption by Chl *a* in the Soret at 435 nm and Q_y (0,0) transitions at 680 nm excite PS I preferentially, solid line, showing that most of the Chl *a* is associated with PS I. In contrast to the wild-type, the excitation spectra for PS II and PS I emission, normalized to the 680 nm CHl *a* peak, are very similar in the mutant, panel B. However, some preferential contributions to PS II or PS I are apparent. The 435 and 670 nm peaks contribute relatively more to PS II emission and the 470 and 505 nm peaks relatively more to PS I emission. The fluorescence excitation spectra were normalized to show differences in the components of PS II and PS I, but are not a reliable measure of relative antenna size as the trapping efficiency of PS II and PS I are not necessarily equal.

With the excitation spectra of the mutant in mind, four wavelengths were chosen for preillumination, two as light 1 (520 and 700 nm) and two as light 2 (435 and 670 nm). Table I compares the F_{695}/F_{720} values for these preilluminations to the values of dark-adapted cells, cells preilluminated with blue light in the presence of DCMU and dark-adapted KCN-treated cells. Preillumination at 520 and 700 nm resulted in 77 K emission spectra with a significantly higher F_{695}/F_{720} ratio than the spectra for cells preilluminated at 435 and 670 nm, indicative of a successful light-induced state transition. Comparison of these ratios with those for dark-adapted cells and cells preilluminated with blue light in the presence of DCMU indicate that the changes observed in the light-induction experiments were within the limits of the larger changes observed in the redox-induced experiments. Table I also shows that the addition of KCN to dark-adapted cells caused a decrease in the F_{695}/F_{720} ratio.

TABLE I

PS II/PS I 77 K fluorescence emission ratio (F_{695}/F_{720}) for the phycobilisome-less mutant of Synechococcus sp. PCC 7002 as a function of pretreatment or preillumination wavelength

Dark adaptation and all preilluminations were for 2 min. Cells were incubated with DCMU or KCN for 2 min. DCMU was added to a final concentration of 1 μM; KCN to a final concentration of 1 mM. Preillumination in the presence of DCMU was with blue light. See text for further details. The values for F_{695}/F_{720} have an uncertainty of ±0.02.

Treatment	F_{695}/F_{720}	Illumination wavelength	F_{695}/F_{720}
Dark	2.70	light 2, 435 nm	2.78
Dark + DCMU	3.42	light 1, 520 nm	2.85
Illuminated + DCMU	3.53	light 2, 670 nm	2.73
Dark + KCN	2.32	light 1, 700 nm	2.91

Discussion

In confirmation of earlier studies [21–23], we show that illumination after DCMU poisoning in cyanobacteria induces a transition to State 1, while the dark state is similar to State 2. It has been suggested that the dark state in cyanobacteria is State 2 as a result of respiratory electron transport reducing the plastoquinone pool, a proposed trigger for transition to State 2 [23]. Large redox-induced state transitions have been reported in cyanobacteria under conditions designed to reduce or oxidize plastoquinone. An extreme State 2 is reached under dark anaerobic conditions (used to inhibit plastoquinone oxidation by cytochrome oxidase) and an extreme State 1 in cells preilluminated in the presence of DCMU [23]. Dominy and Williams [24] have shown a strict correlation between light-induced and redox-induced state transitions in *Synechococcus* sp. PCC 6301 which is best explained by a competition between PS I and cytochrome oxidase for electrons from plastoquinone and/or the cytochrome b_6-*f* complex.

The changes in 77 K fluorescence emission spectra we have reported between dark-adapted cells and cells preilluminated in the presence of DCMU indicate that both the wild-type and the phycobilisome-less mutant are capable of large redox-induced state transitions. In both wild-type and mutant cells we found the dark-adapted state to be characterized by a low F_{695}/F_{720}, indicative of State 2. This would suggest, in terms of a model for the state transition involving control by the redox state of plastoquinone and/or the cytochrome b_6f complex, that these electron carriers were reduced in the

dark. Cytochrome *f* has been shown to be reduced in the dark in *Synechococcus* sp. PCC 7002 [25]. The dark reduction was proposed to be by respiratory electron transport via an NADH and/or NADPH dehydrogenase and the rate of oxidation of plastoquinone and/or cytochrome b_6-*f* via cytochrome oxidase was assumed to be slower than the reduction rate [25]. In the same study it was found that DCMU partially inhibited the dark reduction of cytochrome *f*. An inhibition of the dark-reduction pathway by DCMU would allow the cytochrome oxidase activity to oxidize partially plastoquinone and/or cytochrome b_6-*f*. This is in agreement with our finding that the addition of DCMU to wild-type cells in the dark causes a slight increase in F_{695}/F_{720}.

Further support for redox control of the state transitions comes from our studies with KCN, a potent inhibitor of many cytochrome oxidases. The decrease in F_{695}/F_{720} observed following the addition of KCN to dark-adapted phycobilisome-less mutant cells may be explained by inhibition of plastoquinone and/or cytochrome b_6-*f* oxidation via cytochrome oxidase activity. The KCN treatment did effectively inhibit O_2 uptake in the cells; however, the inhibition required a high KCN concentration and required a 2 min incubation. The other potential inhibitory site of KCN is plastocyanin, which would also impede oxidation of plastoquinone and/or cytochrome b_6-*f*. However, there is no evidence for the occurrence of plastocyanin in this organism (Bryant, D.A., unpublished data).

A comparison of dark-adapted cells and cells preilluminated in the presence of DCMU suggests the operation of a redox-induced state-transition mechanism in the phycobilisome-less mutant cells. However, the addition of DCMU to dark-adapted cells caused an increase in F_{695}/F_{720} to the same level as that in illuminated, DCMU-poisoned cells. This result is in contrast to that for wild-type cells which only showed a partial transition to state 1 after DCMU treatment in the dark. One possible explanation in keeping with a redox control of the state transition is a lower reduction rate or higher oxidation rate of plastoquinone and/or cytochrome b_6-*f* in the mutant as compared to the wild type. Under these conditions, the partial DCMU inhibition of the reduction rate could be enough to make the reduction rate slower than the oxidation rate in the dark. This idea is supported by the experiments with KCN-poisoned phycobilisome-less cells. In the presence of the cytochrome oxidase inhibitor, the addition of DCMU in the dark no longer causes the cells to go to a high F_{695}/F_{720} state (State 1). In contrast, the KCN-poisoned and DCMU-poisoned phycobilisome-less cells respond in a fashion similar to the DCMU-poisoned wild-type cells.

These results indicate that *Synechococcus* sp. PCC 7002 does not require the presence of the phycobilisome to undergo redox-induced state transitions. More convincing, however, are the light-induced state transitions we observed in the mutant. Even though the excitation spectra show that the pigments contributing to the PS II and PS I antenna were very similar it was possible to observe small reversible light-induced changes in F_{695}/F_{720} by careful selection of preillumination wavelength.

It was expected that preillumination of the mutant should lead to State 1 as the absorbance cross-section of PS II with the phycobilisome should be much lower than that of PS I. However, preillumination resulted in an intermediate state which moved toward State 1 or State 2, if the preillumination wavelength was selected to preferentially excite PS I or PS II, respectively. These preillumination results argue against a large difference in absorbance cross-section between PS II and PS I in the mutant. This idea is supported by comparing the fluorescence emission spectra of the mutant (Fig. 3) and wild-type (Fig. 2C) for excitation of Chl *a* at 435 nm. The fluorescence yield of PS II relative to PS I is much higher in the mutant than in the wild-type. Although the increase in fluorescence yield may result from a larger PS II Chl *a* antenna in the mutant, it may also reflect a lower PS II trapping efficiency.

No claim is made concerning the physiological significance of the state transition in the phycobilisome-less mutant. The pigment complements of PS II and PS I are so similar that preferential excitation of either one is extremely difficult to accomplish and would not likely occur in a natural environment. However, even though the autotrophic growth rates of the phycobilisome-less mutant are slow compared to the wild-type, the mutant is photosynthetically competent and the mechanism of the state transition appears intact.

The ability of the phycobilisome-less mutant to undergo reversible, light-induced state transitions is convincing evidence that the phycobilisome is not required in the mechanism of the state transition in *Synechococcus* sp. PCC 7002. These data do not support models for the state transition in phycobilisome-containing organisms, which depend on phosphorylation of the phycobilisome and its subsequent dissociation from PS II [6,8].

The mobile phycobilisome model proposed by Allen et al. [6] suggests that there is a change in the direct absorption cross-section of PS I as a result of the phycobilisome becoming attached to PS I in State 2. This model predicts that energy absorbed by the phycobilisome should be regulated by the state transition while energy absorbed by Chl *a* would not. As shown previously in cyanobacteria [24] and red algae [10] we show in this report that the state transition in *Synechococcus* sp. PCC 7002 effectively redistributes light absorbed by either the phycobilisome or by Chl *a*. This evidence disproves the simple mobile phycobilisome model.

The mobile phycobilisome model has recently been revised by Mullineaux and Allen [8], who have now proposed that upon phosphorylation the phycobilisome and PS II dissociate and PS II moves closer to PS I, increasing the rate of spillover from PS II to PS I. The phycobilisome remains dissociated and the probability of energy absorbed by the phycobilisome being transferred to either photosystem decreases. This model predicts that transition to State 2 should decrease the contribution of both the phycobilisome and PS II Chl *a* to PS II activity but increases only the contribution of PS II Chl *a* to PS I. It has been shown in *Porphyridium cruentum,* however, that the rate of PS I oxidation driven by light absorbed by the phycobilisome is dependent on the state transition and does increase in State 2 [26,27]. There is neither evidence for, nor is there a requirement to suppose that, the dissociation of the phycobilisome from PS II occurs during the transition to State 2.

Evidence against the two phosphorylation models is convincing. The models are inconsistent with the observed changes in energy distribution accompanying the state transition and neither of the models can account for the ability of the phycobilisome-less mutant to regulate the distribution of excitation energy. Both models require phosphorylation of the phycobilisome to effect the mobility of either the phycobilisome itself or PS II. Without revision, neither model can be correct.

The model of Biggins et al. [5] proposes that there is a conformational change in which the phycobilisome-PS II complex moves in relation to PS I, changing the amount of spill-over from PS II Chl *a* to PS I Chl *a*. As this model does not suggest that the phycobilisome is actively involved in the mechanism of the state transition, it is consistent with the data presented in this report. The spillover model also correctly predicts that energy absorbed by both the phycobilisome and by PS II Chl *a* is redistributed by the state transition. For a further discussion of this point, see Refs. 9 and 24.

Our results do not prove or disprove a role for phosphorylation of PS II in the state transition but they do disprove a requirement for phosphorylation of the phycobilisome. Few investigations on protein phosphorylation in bilin-containing organisms have been carried out to this point, primarily due to the lack of an in vitro preparation which undergoes state transitions. Biggins et al. [5] did not find any changes in the whole cell phosphorylation profile of *P. cruentum* in State 1 and State 2. They concluded from these results that protein phosphorylation did not play a role in the mechanism of the state transition in this organism. In contrast to these studies, Allen and co-workers [6] reported the light-dependent phosphorylation of two polypeptides in *Synechococcus* sp. PCC 6301 following long-term labelling in vivo. However, upon repetition of these experiments by Biggins and Bruce [7], it was found that the phosphorylation was not light-dependent over the time-scale of a state transition, nor was it correlated with changes in excitation energy distribution in the in vitro preparation. In a recent paper, Sanders and Allen have confirmed their observation of the light-induced phosphorylation of a 15-kDa polypeptide but have retracted their claim that the phosphorylation was correlated to fluorescence changes indicative of a state transition [28]. In an attempt to clarify the role of phosphorylation, experiments are now underway to determine in both the wild-type and mutant of *Synechococcus* sp. PCC 7002 whether any phosphorylation changes occur in vivo in response to a state transition.

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