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Fluorescence induction transients indicate dissociation of Photosystem II from the phycobilisome during the State-2 transition in the cyanobacterium *Synechococcus* 6301

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Cells of *Synechococcus* 6301 were adapted to State 1 or to State 2. Fluorescence induction transients on a millisecond time-scale were then recorded in the presence of DCMU, using excitation of different wavelengths primarily in order to excite either the phycobilisome or the chlorophyll *a* antenna of Photosystem II (PS II). The transients recorded with the phycobilisome-absorbed light indicate a reduction in PS II absorption cross-section during the transition to State 2. The transients recorded using chlorophyll *a*-absorbed light indicate increased energy transfer away from PS II in State 2, possibly due to increased spill-over of energy from PS II to PS I in State 2. We suggest that the basis of the State-2 transition in phycobilisome-containing organisms is a decreased excitation-energy transfer to PS II reaction centres that results from detachment of PS II from the phycobilisome.

Introduction

In all photosynthetic organisms that contain two photosystems, different populations of light-harvesting pigments are associated with PS I and PS II. As a result, some wavelengths of light preferentially excite PS II, while others preferentially excite PS I. This means that under some light regimes there is an imbalance in the rates of turnover of PS I and PS II, which leads to a reduction in the efficiency of the utilisation of light energy to drive linear electron transport. Many photosynthetic organisms, including green plants [1], red algae [2] and cyanobacteria [3], possess mechanisms which alter the distribution of excitation energy between the two photosystems in response to changes in the spectral quality of light. These mechanisms appear to maximise the efficiency of utilisation of light energy.

In green plants, light-state adaptation is thought to involve the redistribution of LHC II between PS I and PS II. This occurs as a result of protein phosphorylation catalysed by a membrane-bound kinase which is activated when the plastoquinone pool is reduced [4]. This model cannot, without modification, be applied to the cyanobacteria and the red algae, which lack LHC II. In these organisms, this pigment-protein complex is functionally replaced by the phycobilisome, an extrin-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; λ_{ex}, excitation wavelength; F_0, fluorescence yield when all PS II centres are open; F_m, fluorescence yield when all PS II centres are closed; LHC II, light-harvesting chlorophyll *a*/*b*-protein complex of PS II; PS, Photosystem.

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sic phycobiliprotein complex (see Ref. 5 for a review). The principal light-harvesting component in the phycobilisome of the cyanobacterium *Synechococcus* 6301 is phycocyanin, whose absorption maximum is at 620 nm. Energy absorbed by phycocyanin is transferred, via allophycocyanin in the phycobilisome core, to the terminal emitter of the phycobilisome which in turn transfers energy to the chlorophyll *a* antenna of PS II [6]. Both PS I and PS II have chlorophyll *a* antennae. The antenna of PS I contains about 120 chlorophyll molecules, whereas the antenna of PS II contains only about 50 chlorophyll molecules [7]. Under normal growth conditions, PS I reaction centres outnumber PS II reaction centres [7]. It follows that the bulk of the chlorophyll in the cell is associated with PS I and therefore any light which is absorbed primarily by chlorophyll *a* will preferentially excite PS I; this is particularly true of far-red light at wavelengths longer than about 680 nm, which excites almost exclusively PS I. Light which is absorbed by the phycobilisome preferentially excites PS II [8].

Phycobilisome-containing organisms perform state transitions which resemble in many ways those seen in green plants. The State-1 transition results from exposure to light which preferentially excites PS I; the State-2 transition results from exposure to light which preferentially excites PS II [3,9,10]. In cyanobacteria, the State-2 transition can also be induced by respiratory electron flow [11,12]. As in green plants [1], the State-2 transition involves an increase in energy transfer to PS I relative to PS II [9,13–16]. Also as in green plants [4], state transitions in the phycobilisome-containing organisms appear to be controlled by the redox level of plastoquinone or an associated electron carrier [10,11,17]. State transitions in the cyanobacterium *Synechococcus* 6301 appear to be accompanied by changes in the phosphorylation state of a number of polypeptides [18,19]. These include a membrane component [18,19] and a component of the phycobilisome [20]. The way in which energy is redistributed during state transitions in phycobilisome-containing organisms remains controversial; both a change in spill-over of excitation energy from PS II to PS I [13,15] and a change in the absorption cross-section of PS II [19,21] have been proposed.

We have investigated the mechanism by which excitation energy is redistributed during the State-2 transition in the cyanobacterium *Synechococcus* 6301 by recording fluorescence induction transients on a millisecond time-scale, in the presence of the herbicide DCMU, in cells pre-adapted to State 1 or to State 2. These transients result from the closure of PS II centres during exposure to a bright actinic light, causing a rise in fluorescence from an initial level, F_0, when all the centres are open, to a final level, F_m, when all the centres are closed. The presence of DCMU ensures that the re-opening of PS II centres is negligible on the time-scale of the transient. The shape of the transient is influenced by the absorption cross-section of PS II, the extent of spillover of excitation energy from PS II to PS I, and the degree of co-operativity among PS II centres [22]. Changes in absorption cross-section can be distinguished from changes in spillover by their effect on the ratio F_m/F_0 [23]; a decrease in absorption cross-section will reduce F_m and F_0 in the same proportion. An increase in spill-over will reduce F_m proportionately more than F_0 because spill-over is more likely to occur when PS II centres are closed [24].

We have recorded fluorescence induction transients using two different lights, a yellow-green light which primarily excites the phycobilisome, and a blue light which primarily excites chlorophyll *a*. We have also recorded fluorescence emission spectra for cells in State 1 or in State 2, using excitation wavelengths selective for either the phycobilisome or the chlorophyll-containing light-harvesting components. These methods have enabled us to compare the ways in which phycobilisome-absorbed light and chlorophyll-absorbed light are redistributed during the State-2 transition. Our results suggest that the State-2 transition involves the detachment of PS II reaction centres from the phycobilisome, and that increased PS I turnover in State 2 may be caused by spill-over of excitation energy from these detached PS II centres to PS I.

Materials and Methods

Synechococcus 6301 (*Anacystis nidulans*) (UTEX 625) was grown photoautotrophically at

35 °C in medium C of Kratz and Myers [25]. Cells were grown to middle log-phase, subcultured and grown for about 5 h under white light at 15 $W \cdot m^{-2}$. They were then harvested by centrifugation and resuspended in fresh medium C to a concentration of 5 μg chlorophyll *a* per ml.

Fluorescence induction transients were recorded for cells in a stirred 1 cm glass cuvette at 25 °C. Cells were adapted to State 1 by 5 min exposure to a far-red light defined by an Ealing 709 nm interference filter at an intensity of 30 $W \cdot m^{-2}$. The cells were then incubated in the dark for intervals of up to 5 min before the recording the transient. DCMU was added to 50 μM about 5 s before the transient was recorded, and the stirrer was switched off immediately before the transient was recorded. The induction transients were obtained using either yellow-green light centred at 570 nm (defined by a combination of Corning 4-96 and Ealing 560 nm long-wavelength band-pass filters, at an intensity of 2.4 $W \cdot m^{-2}$), or a blue light centred at 425 nm (defined by a combination of Corning 5-60 and Corning 4-96 filters, at an intensity of 9 $W \cdot m^{-2}$). The light was controlled by a Uniblitz electronic shutter opening in about 1.5 ms. Fluorescence was detected by a photodiode (Hansatech, King's Lynn) screened by a Schott RG-665 filter. The amplified signal was recorded using a digital storage oscilloscope and *X*–*Y* plotter (Farnell, Wetherby).

Fluorescence emission spectra were recorded with a Perkin-Elmer LS-5 luminescence spectrometer. Spectra were recorded at 25 °C with cells at a concentration of 5 μg chlorophyll *a* per ml. The slit width for the excitation beam was 15 nm; the detector slit width was 2.5 nm. The cells were adapted to State 1 by illumination with far-red light as described above, or were adapted to the weak excitation light, which did not measurably perturb the cells from their dark-adapted state. Spectra for cells at the F_m level of fluorescence were recorded in the presence of 50 μM DCMU.

The concentration of chlorophyll *a* was determined as in Ref. 26. Phycobilins were isolated from cells of *Synechococcus* 6301. Cells were suspended in a medium containing 100 mM NaCl and 5 mM Na_2HPO_4/KH_2PO_4 at pH 7.0 and broken in a French pressure cell at 100 MPa. The broken cells were centrifuged at 100 000 × *g* for 45 min and the phycobilins were purified from the supernatant by adsorption onto a hydroxylapatite column [6]. The absorption spectrum of this preparation showed no detectable chlorophyll contamination (results not shown).

Results

Fluorescence induction transients

Cyanobacterial cells revert to State 2 in the dark [3] due to respiratory electron flow into the plastoquinone pool [11,12]. We have recorded fluorescence induction transients for *Synechococcus* 6301 cells at various stages during the transition from State 1 to State 2 in the dark, using either a light which predominantly excites the phycobilisome or a light which predominantly excites chlorophyll *a*. Cells were initially adapted to State 1 by exposure to a far-red light, which excites almost exclusively PS I. The State-1 transition can also be driven by exposure to blue light [3], which excites predominantly PS I but is also absorbed by the chlorophyll antenna of PS II. We used a far-red light in preference to a blue light because a blue light might cause some closure of PS II reaction centres, making an accurate determination of F_0 impossible. The far-red light caused no measurable PS II trap closure in the absence of DCMU. Since DCMU was added only after the light was extinguished, the initial point of each induction transient gives an accurate value for F_0.

If an accurate value for F_m is to be obtained from transients recorded for cells in State 2, it is important that the rise in fluorescence from F_0 to F_m be kinetically distinguishable from the onset of the State-1 transition which will occur when cells are illuminated in the presence of DCMU [11,21]. Although the illumination time required to induce state transitions in phycobilisome-containing organisms can be very brief [27] and can be as short as 30 ms [28], the actual time required for the state transition to occur is considerably longer [27,28]. Reported half-times for the State-1 transition in phycobilisome-containing organisms vary from 1.3 s [28] to about 15 s [11,21,27]. Under our conditions, the half-time for the State-1 transition in the presence of DCMU is 10–15 s [11]. This means that during the 200–300 ms time-course of

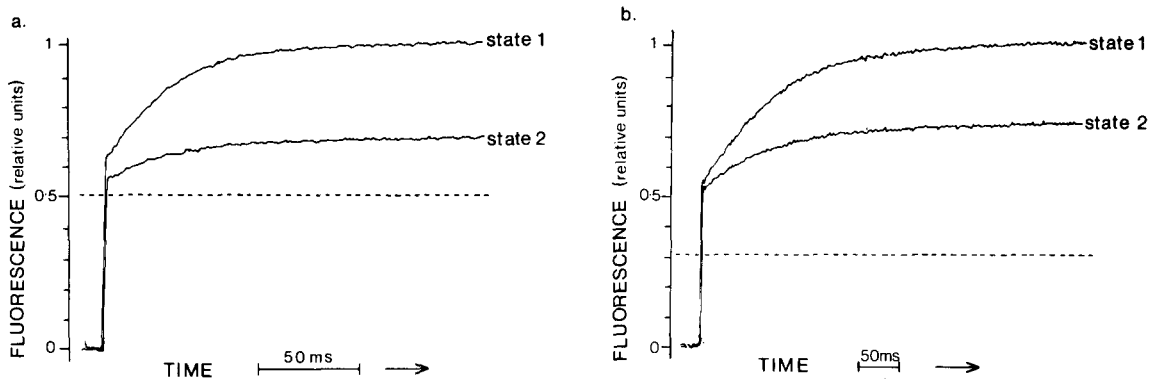


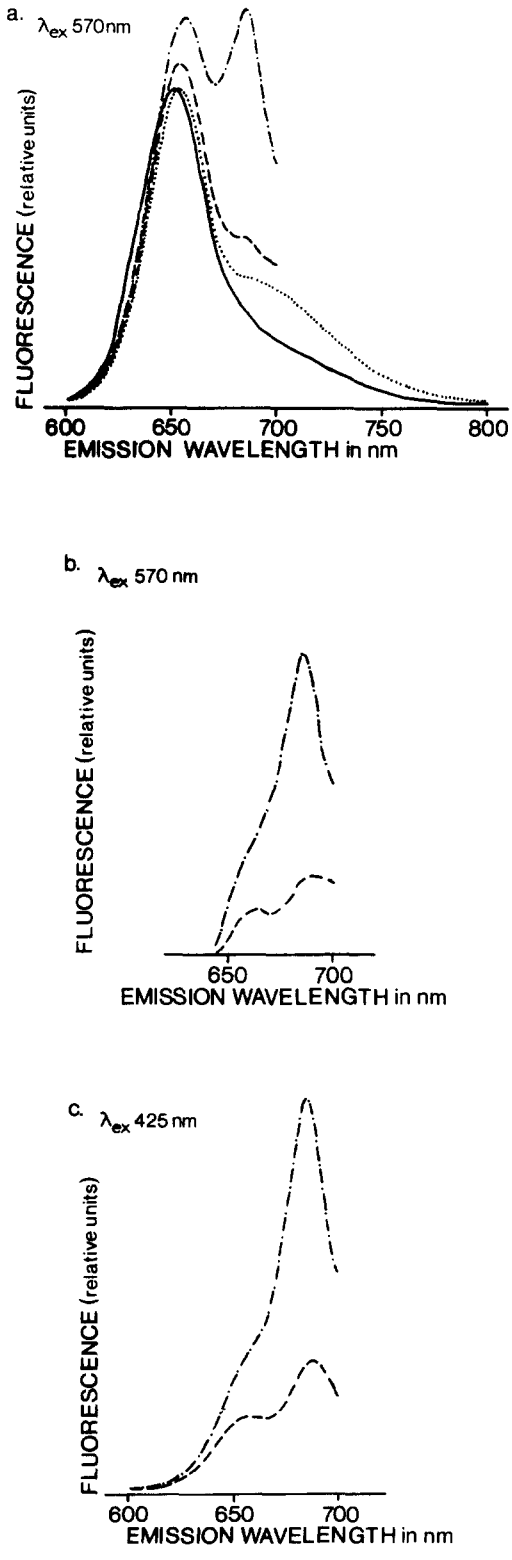
Fig. 1. Fluorescence induction transients of *Synechococcus* 6301 cells in State 1 and in State 2: (a) transients recorded with phycobilisome-absorbed light; (b) transients recorded with chlorophyll-absorbed light. Cells were adapted to State 1 by 5 min exposure to far-red light. They were then incubated in the dark for 10 s (State 1) or for 5 min (State 2) before the transient was recorded. DCMU was added to 50 μM about 5 s before, and the stirrer wa switched off immediately before the transient was recorded. The fluorescence scales are relative to F_m in State 1; the transients in (b) are amplified about threefold relative to the transients in (a). The corrected base levels for PS II chlorophyll fluorescence (see Results section) are indicated by the broken lines.

our fluorescence induction transients (Fig. 1a and b) the extent of the transition towards State 1 will be less than 2%. Thus it is possible to obtain accurate values for F_m whether the cells are in State 1 or in State 2.

The transients were recorded at room temperature, so only fluorescence from PS II and the phycobilisome was detected; the fluorescence yield of PS I is negligible, except at low temperature [22]. Fig. 1a and b shows transients recorded for cells in the two extreme states, with phycobilisome-absorbed light and chlorophyll-absorbed light, respectively. The State-1 transients were recorded 10 s after extinguishing the far-red light; transients recorded after shorter time delays did not show significantly higher F_m levels. Both pairs of transients appear to show F_m/F_0 ratios much lower than those normally seen in green plants [23]. This is because a high proportion of the apparent F_0 does not come from PS II fluorescence. With chlorophyll-absorbed light (Fig. 1b), the PS II fluorescence yield is so low that much of the apparent F_0 comes from scattered light. With phycobilisome-absorbed light (Fig. 1a) the proportion of scattered light detected is negligible, but a high proportion of the observed F_0 comes from the phycocyanin fluorescence which is centred at 650 nm. State transitions appear to have little effect on the phycocyanin fluorescence yield. The presence of this unchanging component of the observed F_0 will reduce the apparent proportional change in F_0 as compared to the change in F_m. It is therefore essential to correct for phycocyanin fluorescence and for scattered light if any comparison of F_m/F_0 ratios in State 1 and State 2 is to be made. In order to determine the proportion of detected fluorescence which comes from PS II chlorophyll we have recorded fluorescence emission spectra for *Synechococcus* 6301 cells using phycobilisome-absorbed and chlorophyll-absorbed excitation lights.

Fluorescence emission spectra

Fig. 2a shows fluorescence emission spectra for *Synechococcus* cells recorded with excitation at 570 nm, which corresponds to the maximum transmission of the filter combination with which we have defined our phycobilisome-absorbed light. Time-courses of fluorescence yield at 685 nm showed that the weak excitation light did not measurably perturb the state of the cells; exposure to this light did not cause any measurable PS II trap closure in the absence of DCMU, nor did it alter the extent of the state transition which occurred when the cells were exposed to far-red light (result not shown). Cells adapted only to the excitation light are therefore at F_0 in State 2; this spectrum (Fig. 2a) corresponds to the initial point in the State-2 fluorescence induction transient in Fig. 1a. Cells adapted to the superimposed far-red



light are at F_0 in State 1; this spectrum (Fig. 2a) corresponds to the initial point of the State-1 fluorescence induction transient in Fig. 1a. Cells adapted to far-red light in the presence of 50 μM DCMU are at F_m in State 1; this spectrum (Fig. 2a) corresponds to the end of the State-1 fluorescence induction transient in Fig. 1a. No spectrum for cells at F_m in State 2 (corresponding to the end of the State-2 fluorescence induction transient in Fig. 1a) could be recorded because this state cannot be maintained; in the presence of DCMU any light strong enough to give complete closure of PS II reaction centres will tend to drive a State-1 transition [11,21].

For cells at F_0 in State 2, when the PS II chlorophyll fluorescence is at a minimum, the chlorophyll fluorescence, centred at about 685 nm, is reduced to a shoulder on the phycocyanin fluorescence peak (Fig. 2a). The filter with which we have screened our detector will transmit light of wavelength longer than about 665 nm and will, therefore, transmit a high proportion of phycocyanin fluorescence. The emission spectrum of a chlorophyll-free preparation of phycobilins is shown for comparison (Fig. 2a). Both the State-1 transition and the transition from F_0 to F_m increase the PS II chlorophyll fluorescence centred at 685 nm, but have little effect on the phycocyanin fluorescence centred at about 650 nm. The shape of the 650 nm peak appears to be similar in whole cells and in our phycobilin preparation, apart from a slight red shift of about 3 nm in the whole cells (Fig. 2a). By subtracting the phycobilin fluorescence spectrum (normalised to the 650 nm peak for cells at F_0 in State 2) from the cell

Fig. 2. Fluorescence emission spectra of *Synechococcus* 6301 cells: (a) spectra recorded with phycobilisome-absorbed light (570 nm); (b) difference spectra constructed by subtracting the phycobilins spectrum in (a) from the cell spectra in (a); (c) spectra recorded with chlorophyll-absorbed light (425 nm). All spectra were recorded at 25° C. Cells were adapted to the weak excitation light (F_0, State 2), or were adapted to far-red light (F_0, State 1). To obtain F_m in State 1, cells were adapted to far-red light in the presence of 50 μM DCMU. Fluorescence could not be measured at wavelengths longer than 700 nm in the presence of the far-red light. The spectra in (c) are amplified threefold relative to the spectra in (a) and (b). ———, phycobilins preparation; ·····, cells, F_0, State 2; - - - - -, cells F_0, State 1; ·–·–·, cells, F_m, State 1.

spectra we have obtained difference spectra which show the PS II chlorophyll fluorescence. Fig. 2b shows these corrected spectra for cells at F_0 in State 1 and at F_m in State 1. These spectra indicate that the ratio F_m/F_0 for cells in State 1 is about 3.9 when fluorescence is excited with phycobilisome-absorbed light. This ratio is comparable to that seen in green plants [23]. Because of the spectral shift in the phycocyanin fluorescence peak, the corrected spectra (Fig. 2b) are still slightly contaminated by phycocyanin fluorescence. This contamination is proportionately greater in the F_0 spectrum than in the F_m spectrum. For this reason, the F_m/F_0 ratio for cells in State 1 which we have estimated from Fig. 2b is likely to be a slight underestimate; the true ratio F_m/F_0 for cells in state 1 may be slightly greater than 3.9, but it cannot be significantly less.

Fig. 2c shows fluorescence emission spectra for cells at F_0 and at F_m in State 1 recorded with a 425 nm light of similar spectral quality to the blue chlorophyll-absorbed light used for the fluorescence induction transients in Fig. 1b. Like the 570 nm light used for the spectra in Fig. 2a, this light was weak enough to be essentially non-perturbing for cells in State 1; it did not cause measurable PS II trap closure in the absence of DCMU, nor did it affect the extent of adaptation to State 1 in the presence of the far-red light. The 425 nm light did, however, induce a partial State-1 transition in cells pre-adapted to State 2 both in the presence and in the absence of DCMU. We were therefore unable to record spectra for cells in State 2 either at F_0 or at F_m using this excitation light.

The phycocyanin fluorescence peak is relatively small when fluorescence is excited with chlorophyll-absorbed light (Fig. 2c); the spectrum is dominated by the PS II chlorophyll fluorescence emission peak at 685 nm. It does not therefore appear necessary to correct for phycocyanin fluorescence when determining F_m/F_0 ratios from these spectra. The spectra in Fig. 2c indicate that the ratio F_m/F_0 for cells in State 1 is about 2.9 when fluorescence is excited with chlorophyll-absorbed light. Note that there is no significant detection of scattered light in the spectrofluorimeter; the F_m/F_0 ratio obtained from these spectra can therefore be used to correct for the scattered light which contributes to the apparent F_0 in the fluorescence induction transients recorded with chlorophyll-absorbed light (Fig. 1b).

Changes in F_m/F_0 during the State-2 transition in Synechococcus 6301 cells

By applying the ratios for F_m/F_0 in State 1 obtained from fluorescence emission spectra (Fig. 2) to the induction transients recorded for cells in State 1 (Fig. 1), it is possible to determine the proportion of the apparent F_0 which comes from PS II chlorophyll fluorescence, and hence to correct for the phycocyanin fluorescence and scattered light which reduce the observed F_m/F_0 ratios in the fluorescence induction transients. The corrected base levels for PS II chlorophyll fluorescence are indicated in Fig. 1.

Fig. 3 shows the corrected F_m/F_0 ratios obtained from fluorescence induction transients recorded for both phycobilisome-absorbed light and chlorophyll-absorbed light. This figure shows that the effect of the State-2 transition on the ratio F_m/F_0 depends on the spectral quality of the light used to induce the induction transient. With phycobilisome-absorbed light, the ratio F_m/F_0 remains approximately constant during the State-2 transition. It is important to note that our results are unequivocally inconsistent with a fall in the ratio F_m/F_0 during the State-2 transition; this

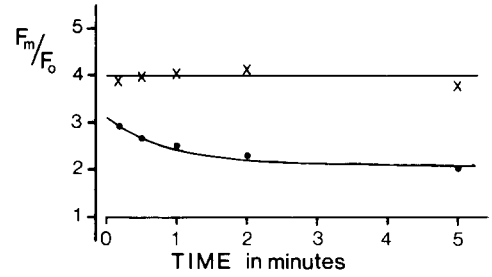


Fig. 3. Changes in F_m/F_0 during the State-2 transition in *Synechococcus* 6301 cells. F_m/F_0 ratios were obtained from fluorescence induction transients recorded as in Fig. 1. Cells were adapted to State 1 as described in the caption to Fig. 1, and were then incubated in the dark for varying periods before adding DCMU to 50 μM and recording the transient. The time axis indicates the length of the dark incubation. Values for F_m and F_0 have been corrected for phycobilin fluorescence and scattered light as described in the Results section. × ——— ×, F_m/F_0 from transients recorded with phycobilisome-absorbed light; ● ——— ●, F_m/F_0 from transients recorded with chlorophyll-absorbed light.

TABLE I

SUMMARY OF CHANGES IN FLUORESCENCE INDUCTION TRANSIENTS ACCOMPANYING THE STATE-2 TRANSITION IN CELLS OF *SYNECHOCOCCUS* 6301

Data from the fluorescence induction transients shown in Fig. 1. Fluorescence levels are expressed relative to F_m in State 1; the fluorescence scales are therefore different for chlorophyll excitation and phycobilisome excitation. $t_{1/2}$ is the half-time for the fluorescence rise from F_0 to F_m during the induction transient. All values for F_0 and F_m have been corrected for phycobilin fluorescence and scattered light as described in the Results section.

	F_0	F_m	F_m/F_0	$t_{1/2}$ (ms)
Chlorophyll excitation				
State 1	0.34	1.00	2.92	69
State 2	0.30	0.62	2.04	64
Phycobilisome excitation				
State 1	0.26	1.00	3.89	25
State 2	0.10	0.38	3.73	22

would require the contribution of phycocyanin fluorescence to the observed F_0 (represented by the broken line in Fig. 1a) to be significantly lower than our estimate. This cannot be the case because, as we have explained above, the ratio F_m/F_0 for cells in State 1 which we have used to calculate the contribution of phycocyanin fluorescence to the observed F_0 may be a slight underestimate of the true F_m/F_0, but cannot be an overestimate.

With chlorophyll-absorbed light, the ratio F_m/F_0 decreases during the State-2 transition from about 2.9 in State 1 to about 2.0 in State 2 (Fig. 3). With phycobilisome-absorbed light, both F_m and F_0 decrease by about 62% on transition to State 2 (Table I). With chlorophyll-absorbed light, F_m decreases by about 38% on transition to State 2, whereas F_0 decreases by only about 12% (Table I). This indicates that the State 2 transition has different effects on the distribution of phycobilisome-absorbed light and chlorophyll-absorbed light. The unchanged F_m/F_0 ratio with phycobilisome-absorbed light indicates a decrease in the absorption cross-section of PS II on transition to State 2. Any increase in spillover from phycobilisome-attached PS II centres to PS I would cause a decrease in the F_m/F_0 ratio [24] observed with phycobilisome-absorbed light. The absence of such a decrease during the State 2 transition indicates that the State-2 transition does not involve any measurable increase in spillover from phycobilisome-attached PS II reaction centres. The reduction in F_m/F_0 for chlorophyll-absorbed light which accompanies the State-2 transition is consistent with an increase in spillover from PS II to PS I [24]. Since this effect is not detected with phycobilisome-absorbed light, it must involve a population of PS II reaction centres which are not functionally coupled to the phycobilisome.

Nature of the change in PS II absorption cross-section accompanying the state transition

A change in PS II absorption cross-section is detected only with phycobilisome-absorbed light; with chlorophyll-absorbed light the reduction in F_0 accompanying the State-2 transition is very small (Fig. 1b and Table I), and could be accounted for solely by increased spillover in State 2. This indicates that the decreased PS II absorption cross-section in State 2 results from decreased transfer of energy from the phycobilisome to PS II and not from decreased transfer of energy from the chlorophyll antenna of PS II to the reaction centre.

A decrease in energy transfer from the phycobilisome to PS II could result from a decoupling of a proportion of PS II reaction centres from phycobilisomes leading to the formation of a population of PS II centres which are not excited by phycobilisome-absorbed light. Alternatively, a competing acceptor for excitation energy harvested by the phycobilisome could reduce the probability of energy transfer from the phycobilisome to PS II without functionally disconnecting any PS II centres from phycobilisomes. Both these possibilities are consistent with the unchanged F_m/F_0 ratios which we observe with phycobilisome-absorbed light (Fig. 1a and Table I). The two possibilities can, however, be distinguished by their effect on the rate of the fluorescence rise during the induction transient. The rate of the fluorescence rise indicates the rate at which PS II reaction centres are closed during exposure to the actinic light and hence the rate of energy transfer to PS II. A reduction in the proportion of energy absorbed by the phycobilisome which is transferred to PS II would therefore be expected to increase the half-time for the fluorescence rise in

induction transients recorded with phycobilisome-absorbed light. Note, however, that the rate of the fluorescence rise seen in Fig. 1a gives information only for PS II reaction centres which are functionally connected to the phycobilisomes; any detached PS II reaction centres will not fluoresce significantly when excited by phycobilisome-absorbed light.

Although the State-2 transition causes a 62% decrease in F_m, the half-time for the fluorescence rise during the induction transient does not increase (Table I). This suggests that the decreased PS II fluorescence in State 2 results from the detachment of a population of PS II reaction centres from the phycobilisome; this could reduce the overall absorption cross-section of PS II without reducing the absorption cross-section of the remaining attached PS II reaction centres, and hence would reduce the amplitude of the fluorescence rise during the induction transient without increasing its half-time.

Rate of the fluorescence changes during the State-2 transition

Fig. 4 shows time-courses for the decrease in F_m during the State-2 transition for phycobilisome-absorbed light and for chlorophyll-absorbed light. The two effects occur at similar rates; both have half-times of about 45 s. This is comparable to half-times for the State-2 transition in phycobilisome-containing organisms previously reported [11,21,28]. The fact that the time-courses for the decrease in F_m are so similar with phycobilisome-absorbed light and chlorophyll-absorbed light suggests that both effects are manifestations of the same molecular rearrangement in the thylakoid membrane.

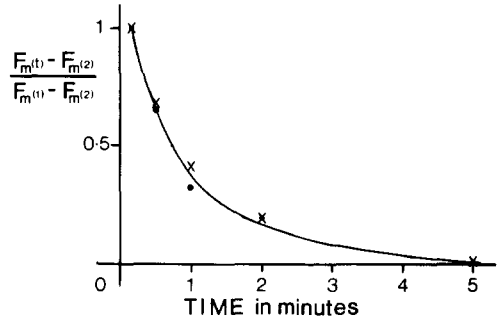


Fig. 4. Decrease in F_m during the State-2 transition in *Synechococcus* 6301 cells. F_m values were obtained from transients recorded at various stages during the dark State-2 transition as described in the caption to Fig. 3. Changes in F_m are expressed relative to the total change in F_m on transition from State 1 to State 2 so that the rates of the State-2 transition as observed with phycobilisome-absorbed light and chlorophyll-absorbed light can be directly compared. $F_m(1)$ and $F_m(2)$ represent F_m in State 1 and in State 2, respectively. $F_m(t)$ represents F_m after dark incubation for time t following adaptation to State 1. ×——×, data from transients recorded with phycobilisome-absorbed light; ●——●, data from transients recorded with chlorophyll-absorbed light.

Discussion

State transitions in phycobilisome-containing organisms alter the distribution of both phycobilisome-absorbed light and chlorophyll-absorbed light between PS II and PS I [12,13]. This has been thought to rule out the possibility that state transitions in these organisms involve a change in the association of the phycobilisome with PS II [12], since such a change would be expected to alter only the distribution of phycobilisome-absorbed light without altering the distribution of chlorophyll-absorbed light. By contrast, a change in spillover of excitation energy from PS II to PS I would be expected to have the same effect on energy distribution whether the energy was initially absorbed by the phycobilisome or by the chlorophyll antenna of PS II.

Our results indicate that the State-2 transition in the cyanobacterium *Synechococcus* 6301 has two distinct effects on the distribution of excitation energy. One effect alters the distribution of phycobilisome-absorbed energy, and the other effect alters the distribution of chlorophyll-absorbed energy. The two changes differ both in their amplitude and in their effect on the ratio F_m/F_0 (Table I). However, both changes occur with a similar time-course during the State-2 transition (Fig. 4), which suggests that both changes are manifestations of the same molecular rearrangement in the thylakoid membrane.

When fluorescence is excited with a phycobilisome-absorbed light a decrease in the amount of energy which is transferred from phycobilisomes to PS II is observed on transition to State 2 (Fig. 1a and Table I). This change is consistent with

decoupling of a population of PS II reaction centres from phycobilisomes. When fluorescence is excited with chlorophyll-absorbed light, an increase in the transfer of energy away from PS II reaction centres is observed on transition to State 2 (Fig. 1b and Table I). This is most easily explained as an increase in spill-over of excitation energy from PS II to PS I. The latter effect appears not to involve those PS II reaction centres which are functionally connected to phycobilisomes.

We suggest that both effects could result from the detachment of PS II reaction centres, with their chlorophyll *a* antennae, from phycobilisomes. The association of PS II with the phycobilisome appears to restrict the mobility of PS II reaction centres in the thylakoid membrane; PS II reaction centres are normally ordered in rows in the membrane, but are randomly arranged in phycobilisome-deficient mutants [29]. It is conceivable that the PS II-containing domains in the thylakoid membranes of phycobilisome-containing organisms, which are defined by the rows of phycobilisomes on the membrane surface, may in some ways be analogous to the granal membrane domains of green plants, which are highly enriched in PS II, and whose structure is thought to be maintained by electrostatic interactions between LHC II molecules [30]. In particular, it is conceivable that the phycobilisome imposes a spatial separation between PS II and PS I which reduces the extent of spillover of excitation energy from PS II to PS I. The radius of the hemidiscoidal phycobilisome of *Synechococcus* 6301 is about 18 nm [32]; a spatial separation of this magnitude will drastically decrease the probability of energy transfer [33]. In this case, the detachment of PS II reaction centres from phycobilisomes could allow the detached PS II reaction centres to associate more closely with PS I, causing an increase in spillover from PS II to PS I which would be observed only with chlorophyll-absorbed light (Fig. 1b and Table I). Our model is illustrated in Fig. 5. It does not seem necessary to postulate a specific association between phycobilisome-decoupled PS II and PS I to explain increased spill-over from PS II to PS I in State 2; in green plants treatments which reduce the extent of granal stacking are thought to increase spill-over

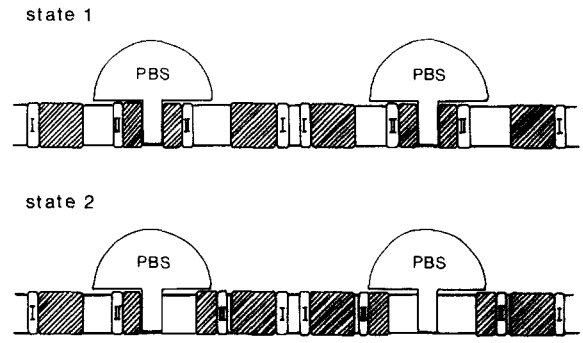


Fig. 5. Model for State 1–State 2 transitions in phycobilisome-containing organisms. The thylakoid membrane contains the PS II and PS I reaction centres, each with their associated chlorophyll *a* antennae (shaded). The State-2 transition results in the detachment of PS II reaction centres from the phycobilisomes (PBS), allowing the detached centres to associate more closely with PS I. This allows increased spillover of excitation energy from PS II to PS I in State 2. The phycobilisomes remain attached to the membrane by the 75 kDa anchor polypeptides, which may act as quenchers of excitation energy when no PS II centre is attached.

simply because they reduce the spatial separation between PS I and PS II [30].

We find the ratio F_m/F_0 to be lower when fluorescence is excited with chlorophyll-absorbed light than when fluorescence is excited with phycobilisome-absorbed light (Fig. 3 and Table I). This is the case even for cells in State 1. This is a prediction of the model illustrated in Fig. 5 if it is assumed that there is a residual population of phycobilisome-uncoupled PS II centres [31] in State 1. These phycobilisome-uncoupled centres will contribute to PS II fluorescence when fluorescence is excited with a chlorophyll-absorbed light, but not when fluorescence is excited with phycobilisome-absorbed light. Our model predicts that the phycobilisome-uncoupled centres will give a lower F_m/F_0 ratio because of increased spillover to PS I [24]. The overall F_m/F_0 ratio will therefore be lower when fluorescence is excited with chlorophyll-absorbed light than when fluorescence is excited by phycobilisome-absorbed light.

Several previous studies of state transitions in phycobilisome-containing organisms have concluded that there is no change in the absorption cross-section of PS II and that the State-2 transition is purely an increase in spillover from PS II to PS I. We do not think that the results presented in

any of these reports preclude the model illustrated in Fig. 5. Ley and Butler [13] concluded on the basis of low-temperature fluorescence spectra and action spectra for oxygen evolution that state transitions in a red alga are solely the result of changes in spillover from PS II to PS I. This paper [13] has been widely cited in support of the spillover hypothesis for state transitions in phycobilisome-containing organisms (e.g., in Refs. 14, 15 and 28). However, the deconvoluted 77 K fluorescence spectra which Ley and Butler present [13] show that state transitions have little effect on the amplitude of the PS I fluorescence emission peak at F_m with phycobilisome-absorbed excitation. This is consistent with the model which we illustrate in Fig. 5, but not with an increase in spill-over from phycobilisome-coupled PS II to PS I. Ley and Butler postulate an increase in spillover which is of significant amplitude only at F_0. This seems implausible; any change in rate constant for spill-over will inevitably have a greater effect on the yield for spillover at F_m than on the yield for spillover at F_0, where there is competition from photochemistry [24]. The method by which Ley and Butler calculate changes in the yield of spill-over from PS II to PS I from 77 K fluorescence spectra depends on the prior assumption that there are no changes in the absorption cross-sections of PS II and PS I. The demonstration by these authors that the relative absorption cross-sections of the photosystems are unchanged by the state transition depends on the values for the yield of spillover obtained in this way and is therefore meaningless. Ley and Butler [13] also show that the state transition has little effect on the shape of the normalised action spectrum for modulated oxygen evolution. This is not incompatible with the model which we present in Fig. 5, which predicts that the State-2 transition will result in a decrease in the yield for oxygen evolution with both chlorophyll-absorbed light and phycobilisome-absorbed light.

Bruce et al. [15] have examined the picosecond fluorescence decay kinetics of cells of a cyanobacterium and a red alga frozen in State 1 and in State 2 at 77 K. They excited fluorescence with a phycobilisome-absorbed light. They interpret their results to indicate a decrease in the lifetime of PS II fluorescence emission in State 2, and they conclude that the State-2 transition results from increased spillover from PS II to PS I. However, their interpretation depends on the assumption that each peak in the 77 K fluorescence emission spectrum comes from a single component; the non-exponential nature of the fluorescence decays which they observe shows this not to be the case. It is not possible from their data to distinguish between the change in the lifetime of a single component which they assume and a change in the relative amplitudes of two or more components of different lifetimes. Their data do not therefore distinguish between a change in spill-over and a change in absorption cross-section of the type illustrated in Fig. 5. To do this, it is essential to resolve both the amplitudes and the lifetimes of the components of the fluorescence emission spectrum [34,35]. Furthermore, the interpretation of Bruce et al. predicts a slow component in the rise of PS I fluorescence in State 2 due to the transfer of energy from PS II to PS I. Kinetic analysis shows that the lifetime of this rise-term should correspond to the lifetime of PS II fluorescence decay, regardless of the efficiency of energy transfer from PS II to PS I. Bruce et al. find the lifetime of PS II fluorescence to be 330 ps in State 2 [15]. They do not, however, find any delay in the rise of PS I fluorescence in State 2, although a rise-term with a lifetime of 330 ps would be easily observable with the equipment used. The data of Bruce et al. [15] are therefore not incompatible with the model which we illustrate in Fig. 5.

We have previously shown that the State-2 transition in *Synechococcus* 6301 results in a decrease in the sigmoidicity of the fluorescence induction transient [21]. Since this sigmoidicity results from the association of two PS II centres with each phycobilisome [36], this loss of sigmoidicity is consistent with the detachment of at least some PS II reaction centre from the phycobilisome. Olive et al. have shown that PS II reaction centres are ordered in rows in the thylakoid membrane of the cyanobacterium *Synechocystis* 6714 in State 1, but are more randomly arranged in State 2 [37]. Since it is the association of PS II with the phycobilisome which causes PS II reaction centres to be arranged in rows [29], the randomisation of PS II reaction centres in State 2 is consistent with the detachment of PS II from

the phycobilisome and with the model illustrated in Fig. 5. There is evidence for a population of PS II reaction centres which are not functionally coupled to PS II in *Cyanidium caldarium* [31]. Changes in the association of PS II with the phycobilisome appear to form part of a long-term adaptive response to growth under different light regimes in *Synechococcus* 6301 [38].

Our results give little indication as to the fate of the proportion of excitation energy absorbed by the phycobilisome that is diverted away from PS II in State 2. This energy does not appear to be dissipated as fluorescence, since this would result in an increase in the 685 nm fluorescence from the phycobilisome terminal emitter, which is not observed either at room temperature (Fig. 2a) or at 77 K [15]. It would also result in an increase in F_0 level of fluorescence on transition to State 2, whereas we observe a decrease (Fig. 1a). One possibility is that the phycobilisome transfers energy primarily to PS I in State 2, as originally proposed by Allen et al. [19]. Alternatively, the excitation energy from phycobilisomes with no attached PS II reaction centres could be dissipated by non-radiative decay; Manodori and Melis [38] have suggested that the 75 kDa terminal acceptor polypeptide of the phycobilisome may act as a fluorescence quencher when no PS II reaction centre is attached. In this case, the State-2 transition in phycobilisome-containing organisms could be primarily a mechanism for protecting PS II from excess excitation rather than for diverting energy from PS II to PS I.

The phosphorylation of a number of polypeptides, including a component of the soluble fraction, is known to accompany the State-2 transition in *Synechococcus* 6301 [18,19]. The soluble phosphoprotein has been identified as a component of the phycobilisome [20]. The phosphorylation of a 15 kDa membrane polypeptide also accompanies the State-2 transition [19]. If this protein is a PS II component, as suggested in Ref. 19, then the phosphorylation of the two proteins could cause the detachment of PS II from the phycobilisome by their mutual electrostatic repulsion or conformational dissociation. The mechanism underlying the State-2 transition in cyanobacteria could therefore be similar to that encountered in regulation of photosynthetic unit function in purple photosynthetic bacteria and in green plants [39].

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