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Picosecond time-resolved fluorescence emission spectra indicate decreased energy transfer from the phycobilisome to Photosystem II in light-state 2 in the cyanobacterium *Synechococcus* 6301

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Picosecond time-resolved fluorescence emission spectra were recorded for cells of the cyanobacterium *Synechococcus* 6301. Fluorescence decay was measured by single-photon timing and kinetic components were resolved by global data analysis. Time-resolved fluorescence decay components were assigned to PS I, PS II and the phycobilisome terminal emitter by comparing spectra for cells with open PS II centres (fluorescence at F_o) with those for cells with closed PS II centres (fluorescence at F_m) for excitation wavelengths of 620 nm and 670 nm. Time-resolved spectra were recorded under these conditions for cells adapted to state 1 or to state 2. The state 2 transition reduced the amplitude of the PS II fluorescence emission by about 60%, with a complementary increase in the apparent amplitude of the emission from the phycobilisome terminal emitter. Similar changes in amplitude were observed for cells at F_o and at F_m. State transitions had no significant effect on the lifetime of PS II fluorescence decay. These results indicate that state transitions alter the extent of energy transfer from the phycobilisome to PS II.

Introduction

Many photosynthetic organisms, including green plants [1], red algae [2] and cyanobacteria [3], possess mechanisms which rapidly alter the distribution of excitation energy between Photosystems I and II in response to changes in illumination conditions. These mechanisms appear to maximise the efficiency of utilisation of light energy for photochemistry. Exposure to an excess of light preferentially absorbed by PS I induces a transition to state 1, which is characterised by a high efficiency of energy transfer to PS II. Exposure to an excess of light preferentially absorbed by PS II induces a transition to state 2, in which the efficiency of energy transfer to PS II is reduced. In cyanobacteria [4,5] and some green algae [6] the state 2 transition can be induced by respiratory electron flow into the plastoquinone pool as well as by PS II turnover.

In green plants, these light-state adaptations are thought to involve the redistribution of the accessory light-harvesting protein complex LHC-II. In state 1, LHC-II is closely associated with PS II and most of the energy absorbed by LHC-II is transferred to PS II. The transition to state 2 occurs as a result of phosphorylation of LHC-II by a membrane-bound protein kinase which is activated when the plastoquinone pool is reduced [7]. The phosphorylation of LHC-II causes its decoupling from PS II and its lateral migration into the stromal lamellae [8]. Until recently it was assumed that the energy absorbed by LHC-II which is functionally decoupled from PS II is transferred to PS I [9]. However, recent data indicate that the absorption cross-section of PS I is not increased in state 2 [10,11]. In state 2, energy absorbed by LHC-II may be transferred to PS

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem; Chl *a*; chlorophyll *a*; LHC-II, light-harvesting chlorophyll *a*/*b* protein complex of PS II; F_o, fluorescence level when all PS II centres are open; F_m, fluorescence level when all PS II centres are closed; τ, fluorescence lifetime; λ_{max}, fluorescence emission maximum.

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II-β [10] or may be dissipated by non-radiative de-excitation.

Cyanobacteria and red algae lack LHC-II, which is functionally replaced in these organisms by the phycobilisome, an extrinsic phycobiliprotein complex (see Ref. 12 for a review). However, these organisms perform state 1-state 2 transitions which resemble in many ways those seen in green plants [2,3]. As in green plants, the state 1 transition results in increased energy transfer to PS II relative to PS I and the state 2 transition results in decreased energy transfer to PS II relative to PS I [2,3,13]. Also as in green plants, state transitions in the phycobilisome-containing organisms appear to be controlled by the redox level of plastoquinone or an associated electron carrier [4,14,15]. State transitions in the cyanobacterium *Synechococcus* 6301 are accompanied by changes in the phosphorylation state of a number of polypeptides [16–18]. These include a membrane component [16,17] and a component of the phycobilisome [18].

The redistribution of excitation energy in phycobilisome-containing organisms could in principle be accomplished in several different ways. One possibility is that the state 2 transition results from an increase in 'spillover' of excitation energy from PS II to PS I [19,20]. This could result from a conformational change which favours the transfer of energy from PS II to PS I [21]. Alternatively, the state 2 transition could result from the functional decoupling of PS II reaction centres from the phycobilisome [22]. In state 2, energy absorbed by phycobilisomes decoupled from PS II could be transferred primarily to PS I as originally suggested by Allen et al. [17], or could be dissipated by a non-radiative quenching mechanism.

State transitions in phycobilisome-containing organisms have been widely studied by fluorescence emission spectroscopy, both at room temperature [5,22] and at 77 K [2,3,19,20]. The state 2 transition is observed as a decrease in fluorescence emission from PS II [2,3,5,19,20,22]. However, steady-state fluorescence emission spectra do not distinguish between changes in the absorption cross-section of PS II and changes in the extent of spillover from PS II to PS I.

The steady-state fluorescence yield of any component is the product of its fluorescence amplitude and its fluorescence lifetime [23,24]. Fluorescence amplitude is determined by the amount of energy which is absorbed by that component, and hence reflects the absorption cross-section of the attached antenna [23,24]. Fluorescence lifetime is equivalent to the lifetime of the excited state and hence is determined by the rate at which excitation is quenched by processes including photochemistry, fluorescence, non-radiative decay and energy transfer to other components. Changes in fluorescence amplitude can be distinguished from changes in fluorescence lifetime by measuring the fluorescence decay following an excitation pulse on a picosecond time-scale (see Refs. 25, 26 for recent reviews). The observed fluorescence decay can be resolved into components of different lifetimes and amplitudes which come from PS II, PS I and the various light-harvesting antennae [25,26]. Since the different pigment beds have different fluorescence emission maxima, fluorescence decays recorded at different emission wavelengths will contain different proportions of the various decay components: the lifetime of fluorescence from a particular pigment bed can be assumed to be independent of emission wavelength, but its fluorescence amplitude will be strongly dependent on emission wavelength [23,24,26]. Fluorescence decays recorded at different emission wavelengths have conventionally been analysed separately. In the method of global data analysis [10,23], a set of fluorescence decays recorded across the entire fluorescence emission spectrum is analysed in terms of a limited number of exponential components whose amplitudes are each a function of emission wavelength but whose lifetimes are invariant across the spectrum. This greatly improves the accuracy of resolution of multiple fluorescence decay components [10,27].

The various models for the mechanism of state transitions in phycobilisome-containing organisms outlined above can, in principle, be distinguished by time-resolved fluorescence emission spectroscopy, since they predict different effects of state transitions on the lifetimes and amplitudes of the fluorescence decay components. For example, an increase in spillover should decrease the lifetime of the PS II fluorescence decay, without affecting its fluorescence amplitude. By contrast, a decrease in PS II absorption cross-section such as that which would be caused by the decoupling of PS II from the phycobilisome should decrease the amplitude of the PS II fluorescence decay without affecting its lifetime [10,23].

We have recorded time-resolved fluorescence emission spectra for cells of *Synechococcus* 6301 adapted to state 1 or to state 2. We have used living cells at their growth temperature of 35 °C. As well as avoiding any artefects due to procedures such as freezing [19,20] or chemical fixation [21], this has allowed us to compare the effects of state transitions when PS II reaction centres are open (fluorescence at F_o) with their effects when PS II reaction centres are closed (fluorescence at F_m). Time-resolved fluorescence decay components were assigned to PS II, PS I and phycobilisome components by comparing spectra recorded with excitation at 620 nm (phycocyanin excitation) and at 670 nm (preferential chlorophyll *a* excitation), for cells with open PS II centres and closed PS II centres. Resolution of fluorescence decay by global data analysis [10,23] has allowed us accurately to determine both the lifetimes and the amplitudes of the fluorescence decay components. This in turn has allowed us to distinguish be-

tween changes in spillover and changes in absorption cross-section accompanying state transitions. We conclude that PS II becomes decoupled from the phycobilisome in the state 2 transition in cyanobacteria.

Materials and Methods

Synechococcus 6301 (*Anacystis nidulans*) (UTEX 625) was grown photoautotrophically under white light at 35 °C in medium C of Kratz and Myers [28]. Cells were grown to late log-phase, diluted with fresh medium C and grown for about 5 h prior to the measurement. The cells were then further diluted with fresh medium C to a total volume of 4 l and a concentration of about 3 μg Chl *a*/ml.

To prevent the laser beam from perturbing the state of the cells, all measurements were performed on cells pumped through a flow cuvette. The time of exposure to the excitation beam was about 0.4 ms. The bulk of the cell suspension was kept in a dark reservoir at 35 °C during the measurement. For the measurements on cells at F_o in state 2, the cells were kept in the dark throughout the measurements apart from the brief exposure to the excitation beam; the dark-adapted state in this organism under these growth conditions is state 2 [15,22]. Cells were adapted to state 1 by exposure to a far-red light defined by a 701 nm interference filter (bandwidth 13 nm, intensity approx. 0.1 $W \cdot m^{-2}$). The cells were exposed to this light in a stirred 500 ml bottle. The average residence time in this bottle was about 1 min. After the illumination with far-red light, the cells were pumped through a black tube for about 3 s before exposure to the excitation beam. This time is sufficient to allow any closed PS II centres to re-open without allowing a significant relaxation towards state 2 [22]. For measurements on cells at the F_m level of fluorescence in state 1 or in state 2 the conditions were as described above except that DCMU was added to the cell suspension to a concentration of 10 μM and the cells were exposed to white light (intensity approximately 60 $W \cdot m^{-2}$) for 0.5 s before the measurement. The exposure to white light should be long enough to close all PS II centres but is too brief to induce a significant transition to state 1 [29]. The delay between the end of the illumination and the measurement was about 30 ms. The flow system used to adapt cells to state 1 or to state 2, at F_o or at F_m, is outlined in Fig. 1.

The effects of a particular change in conditions (e.g., state 1 versus state 2 or F_o versus F_m) were examined by recording time-resolved fluorescence emission spectra for the same batch of cells adapted to each state. No more than two time-resolved emission spectra were recorded for each batch of cells, and all measurements on a particular batch of cells were completed within about 3 h.

Picosecond fluorescence decays were measured by single-photon timing. Picosecond excitation pulses were generated at a frequency of 800 MHz by a synchronously pumped and cavity-dumped dye laser system (Spectra-Physics, Mountain View, CA). The dye laser was tuned to either 620 nm or 670 nm. The excitation pulse width was about 10 ps. The detection system consisted of a monochromator (bandwidth 4 nm) and a multi-channel plate photomultiplier (see Ref. 30 for details). Fluorescence decays at each emission wavelength were averaged over 400 s. Picosecond time-resolved emission spectra were generated by recording fluorescence decays at 5 or 10 nm intervals of emission wavelength and deconvoluting the complete set of fluorescence decays into a limited number of exponential components by a global data analysis procedure [10,23]. The quality of the fits was assessed by global and individual χ^2 values and by plots of weighted residuals [10]. The time-resolved spectra were normalised to steady-state fluorescence emission spectra recorded with the same equipment under identical conditions. It should be noted that the time-resolved spectra as defined here are different from overall time-resolved spectra corresponding to the integrated emission intensity in a specific time-window after excitation. The time-resolved spectra (often referred to as 'decay-associated spectra') as given here are spectra that are associated with a specific fluorescence kinetic component (see Refs. 10,30 for details).

Steady-state fluorescence was monitored with a modulated fluorescence measurement system (Hansatech, King's Lynn). The exitation light was provided by an array of yellow LEDS screened by a 650 nm short-pass filter. The light was modulated at 870 Hz. Fluorescence was detected by a photodiode screened by a 700 nm interference filter and connected to an amplifier locked-in to the modulation frequency of the excitation light.

Results

Adaptation of cells to state 1 or to state 2

Fig. 1 shows the flow system used to adapt cells of *Synechococcus* 6301 to state 1 or to state 2 at the F_o or at the F_m level of fluorescence. On occasion, a second flow cuvette was incorporated into the system shortly after the laser measuring beam. The steady-state fluorescence yield of cells flowing through this cuvette was monitored with a fluorescence measurement system with a weak modulated excitation beam. The addition of the laser excitation beam had no measurable effect on the steady-state fluorescence yield of the cells, showing that the exposure to the laser measuring beam was too brief to perturb the state of the cells significantly. The addition of the white light in the presence of DCMU caused a rapid rise in the steady-state fluorescence characteristic of the transition from F_o to F_m. The

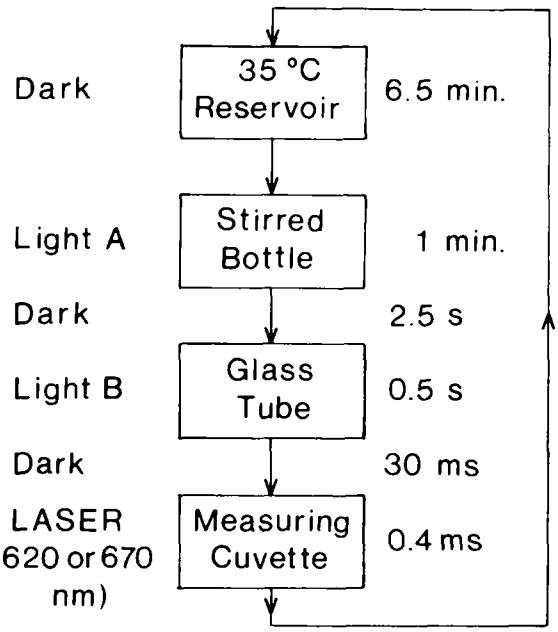

Fig. 1. Illustration of the flow system used to adapt cells of *Synehcococcus* 6301 to state 1 or to state 2, at the F_m or at the F_o level of fluorescence. The illumination conditions at each stage of the cycle are shown on the left and the average time of exposure to each illumination is shown on the right. Cells were adapted to state 1 by exposure to far-red (701 nm) light (light A) and to the F_m level of fluorescence by exposure to a white light (light B). For state 2 cells were kept in darkness instead of being exposed to light A and for the F_o level of fluorescence DCMU was added to the cell suspension to a concentration of 10 μM.

addition of the far-red light caused a slower rise in the steady-state fluorescence which was complete in about 1 min. This is typical of the state 1 transition in this organism [4,29].

Fig. 2 shows steady-state fluorescence emission spectra for cells of *Synechococcus* 6301 adapted to state 1 or to state 2, at F_0 or at F_m as indicated in Fig. 1. The steady-state fluorescence emission spectrum with excitation at 620 nm has two main peaks: that at 650 nm comes from phycocyanin, whereas that at 680 nm contains elements from the terminal emitter of the phycobilisome and from PS II. Both the state 1 transition and the transition from F_o to F_m increase the fluorescence yield from PS II, but have little effect on phycocyanin fluorescence emission at 650 nm. The effects of state transitions and PS II trap closure on the steady-state fluorescence emission spectra shown in Fig. 2 are similar to those previously reported for this organism [5,22].

Comparison of 620 nm and 670 nm excitation

Fig. 3 shows time-resolved fluorescence emission spectra for the same sample of cells of *Synechococcus* 6301 with excitation at 670 nm and at 620 nm. The cells were dark adapted and are therefore at the F_o level of fluorescence and in state 2 [3,15,22]. Excitation light at 620 nm (Fig. 3b) is absorbed mainly by the phycobilisome [12,31]. Excitation light at 670 nm is strongly absorbed by the Chl *a* antennae of PS I and PS II, but there is also absorption by components of the phycobilisome core at this wavelength [31]. Blue light in the region of 435 nm is absorbed almost exclusively by Chl *a*, but the laser system did not allow us to generate an excitation beam of this wavelength for time-resolved measurements.

Fluorescence decays with excitation at 670 nm at F_o (Fig. 3A) could normally be resolved satisfactorily into four components. One of these components has a lifetime of 1.5–2.0 ns and is of very low amplitude. A similar component has been observed under comparable conditions in green plants and assigned to either a small proportion of closed PS II reaction centres or to a very small amount of 'dead' Chl [10]. The component of largest amplitude with excitation at 670 nm has a lifetime of about 40 ps. The emission spectrum of this component is slightly red-shifted with respect to the other components: the emission maximum is at 690 nm. This component is seen only with Chl-absorbed excitation (Fig. 3A). It is not seen when fluorescence is excited at wavelengths absorbed predominantly by the phycobilisome (Fig. 3B and Table I). We therefore assign this component to PS I, which is directly excited only by Chl-absorbed light [32]. The time-resolved fluorescence emission spectrum of PS I in green algae is similar to the 40 ps component which we observe here: it has a fluorescence emission maximum at 690 nm and a much shorter lifetime than the other fluorescence decay components [24]. PS I would be expected to have the largest fluorescence amplitude with excitation at 670 nm, since most of the Chl *a* in the cell is associated with PS I [31].

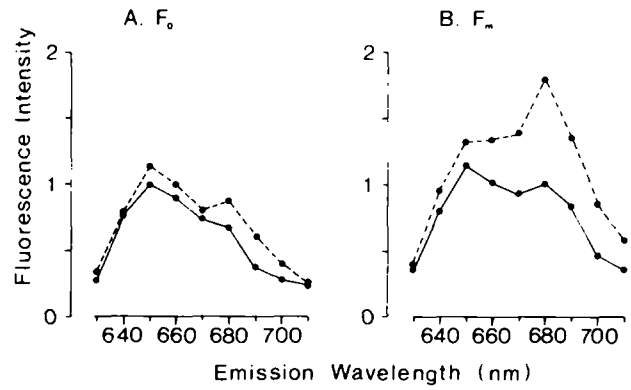

Fig. 2. Steady-state fluorecence emission spectra of *Synechococcus* 6301 cells. Solid line: cells in state 2. Broken line: cells in state 1. The steady-state fluorescence intensities are obtained as the sum of the products of the fluorescence amplitudes and the fluorescence lifetimes of the components of the time-resolved fluorescence emission spectra [24]. Fluorescence intensities are expressed relative to the fluorescence intensity at 650 nm for cells at F_o in state 2. Excitation was at 620 nm.

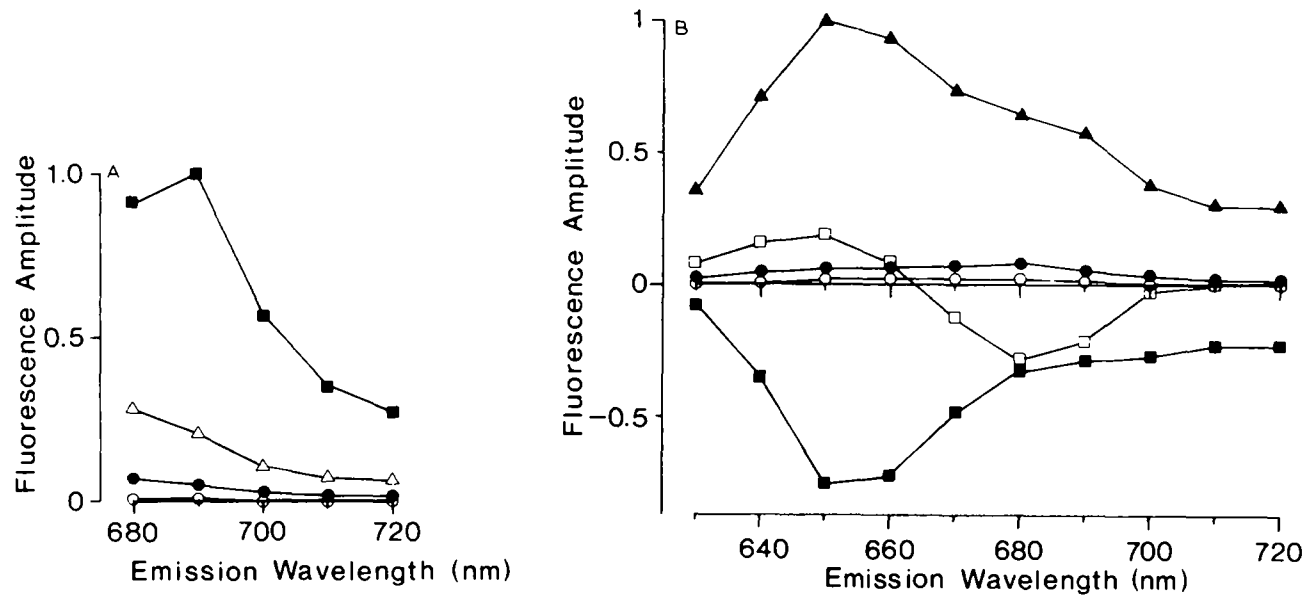

Fig 3. Time-resolved fluorescence emission spectra of *Synechococcus* 6301 cells obtained by global analysis of fluorescence decays: comparison of 620 nm and 670 nm excitaton.

(A) Excitation at 670 nm: τ =
- ■ 40 ps
- △ 150 ps
- ● 470 ps
- ○ 1900 ps

(B) Excitation at 620 nm: τ =
- ■ 20 ps
- □ 70 ps
- ▲ 150 ps
- ● 370 ps
- ○ 1500 ps

In (A) fluorescence amplitudes are expressed relative to the τ = 40 ps component at 690 nm. In (B) fluorescence amplitudes are expressed relative to the τ = 150 ps component at 650 nm.

Fig. 3B shows the time-resolved fluorescence emission spectrum for dark-adapted *Synechococcus* cells with excitation at 620 nm, which is predominantly absorbed by the phycobilisome. This spectrum is resolved into five components. We were unable to resolve into more components with the quality of data available. In fact, five components are clearly insufficient to resolve this spectrum fully: the 150 ps component in particular contains two components, one with an emission maximum at about 650 nm and one with an emission maximum at about 680 nm. We were sometimes able to resolve these components separately (see Fig. 6). The 150 ps component with a maximum at 650 nm must come from phycocyanin: the fluorescence emission maximum correlates with the phycocyanin fluorescence emission maximum seen in the steady-state fluorescence emission spectrum [12]. A component of similar lifetime and emission maximum has been observed in isolated

TABLE I

Lifetimes of fluorescence decay components of cells of Synechococcus 6301

Data taken from the time-resolved fluorescence emission spectra in Figs. 3 and 4. + indicates that a component could be detected that had a significant amplitude under the specified conditions. − indicates that the component could not be observed or was of very low amplitude. The assignment of kinetic components is explained in the Results section. Component (c) was normally resolved only as a shoulder on the spectrum of component (b). Component (d) appeared to have a slightly longer lifetime with excitation at 670 nm than with excitation at 620 nm. Fluorescence lifetimes and amplitudes are accurate to within about ± 10%.

Component	τ (ps)	λ_{max} (nm)	Excitation at 670 nm	Excitation at 620 nm	F_o	F_m	Assignment
a	40	690	+	−	+	+	PS I
b	150	650	−	+	+	+	phycocyanin
c	170	680–685	+	+	+	+	terminal emitter
d	370–490	680–685	+	+	+	−	PS II (open)
e	560	680–685	+	+	−	+	PS II (closed)
f	1500	680–685	+	+	−	+	PS II (closed)

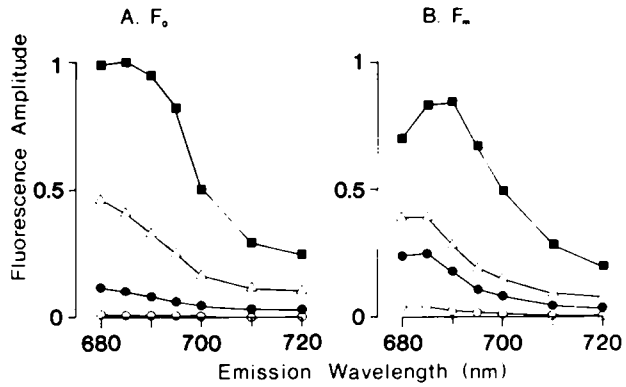

Fig. 4. Time-resolved fluorescence emission spectra of *Synechococcus* 6301 cells obtained by global analysis of fluorescence decays: comparison of F_0 and F_m.

(A) Cells at F_0: τ =	(B) Cells at F_m: τ =
■ 40 ps	■ 40 ps
△ 170 ps	△ 190 ps
● 490 ps	● 560 ps
○ 1700 ps	○ 1500 ps

Fluorescence decays were recorded with excitation at 670 nm. Fluorescence amplitudes are expressed relative to the τ = 40 ps component at 685 nm at F_0.

phycobilisomes and attributed to the overall energy transfer time from the phycocyanin-containing rods to the phycobilisome core [33]. The 150 ps component with a maximum at about 680 nm appears to correspond to the 160 ps component seen with excitation at 670 nm (Fig. 3A). The 370 ps component with an emission maximum at 680 nm (Fig. 3B) appears to correspond to the 470 ps component seen with excitation at 670 nm (Fig. 3A). Since both these components are seen both with excitation which is absorbed mainly by phycocyanin (Fig. 3B) and with excitation which is absorbed by Chl *a* and the phycobilisome core (Fig. 3A) they could come either from PS II or from the terminal emitter of the phycobilisome. Both these components have steady-state emission maxima at about 680 nm [12].

Two short-lifetime components with negative peaks representing fluorescence rise-terms are seen with excitation at 620 nm (Fig. 3B). These must arise from energy transfer processes within the phycobilisome since similar rise-terms are seen in isolated phycobilisomes [33,34].

Comparison of cells at F_o and at F_m

Fig. 4 shows time-resolved fluorescence emission spectra for cells of *Synechococcus* 6301 at the F_0 level of fluorescence (open PS II centres) and at the F_m level of fluorescence (PS II centres closed by the photochemcial reduction of the acceptor Q). The closure of PS II centres should increase the lifetime of the PS II fluorescence [26]. Both spectra were recorded for cells adapted to state 2, and both spectra were recorded with excitation at 670 nm, which is absorbed both by Chl *a* and by the phycobilisome core [31]. Both spectra are resolved into four components as in Fig. 3A.

The closure of PS II centres has little effect on either the amplitude or the lifetime of the 170–190 ps decay component (compare Figs. 4A and 4B). We therefore assign this component to an emitter in the phycobilisome core rather than to PS II. It may come from the terminal emitter of the phycobilisome which is believed to mediate energy transfer from the phycobilisome core to PS II [12].

The closure of PS II centres causes the 470–490 ps component seen at F_0 (Figs. 3A and 4A) to disappear. At F_m it is replaced by two components, one of 560 ps and one of 1.5 ns (Fig. 4B). We accordingly assign these components to PS II, since their presence depends on the state of the PS II reaction centres (Table I). The changes in the fluorescence amplitude and lifetime of PS II which we observe to accompany the closure of PS II centres would increase the steady-state PS II fluorescence yield by a factor of about 3. This is comparable to the F_m/F_0 ratio for cells of *Synechococcus* 6301 obtained from fluorescence induction transients [22].

Table I characterises the fluorescence decay components which we have observed in cells of *Synechococcus* 6301 and summarises our assignment of these kinetic components to the various pigment-protein complexes.

Changes in time-resolved fluorescence emission spectra accompanying state transitions

We have examined the effect of state 1-state 2 transitions on the time-resolved fluorescence emission spectra of *Synechococcus* 6301 for cells at the F_m and at the F_0 level of fluorescence. Fig. 5 shows time-resolved fluorescence emission spectra for cells adapted to state 1 (Fig. 5A) and to state 2 (Fig. 5B) with excitation at 620 nm which is absorbed predominantly by the phycobilisome. These spectra are recorded for cells at the F_0 level of fluorescence. We have analysed these spectra in two ways. Firstly, we have analysed both the spectra together in terms of the same set of lifetime components (Fig. 5). According to this analysis, the principal effect of the state 2 transition is a decrease of about 70% in the amplitude of the PS II fluorescence decay (τ = 370 ps). There is a complementary increase in the observed amplitude of the fluorescence decay which we have assigned to the phycobilisome terminal emitter (τ = 150 ps) at 680 nm (Fig. 5 and Table II). We were unable to resolve the 20 ps component previously seen under similar conditions (Fig. 3B). This component may, however, contribute to the 60 ps component seen here (Fig. 5).

This analysis depends on the assumption that there is no change in the lifetime of any component. We have therefore also analysed the state 1 and state 2 spectra

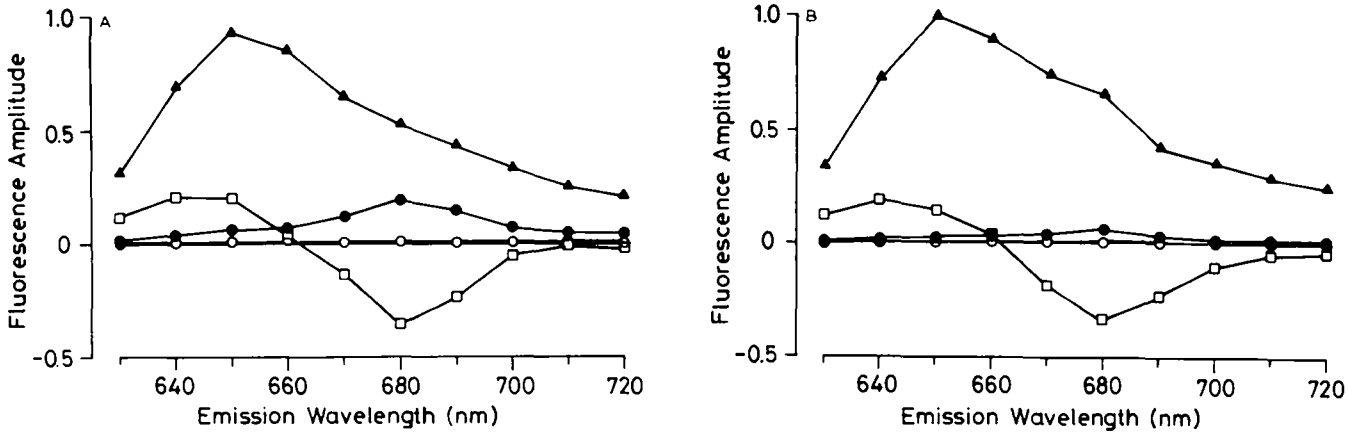

Fig. 5. Time-resolved fluorescence emission spectra of *Synechococcus* 6301 cells obtained by global analysis of fluorescence decays: comparison of state 1 and state 2 at F_o with excitation at 620 nm.

(A) Cells in state 1: τ =
□ 60 ps
▲ 150 ps
● 370 ps
○ 1600 ps

(B) Cells in state 2: τ =
□ 60 ps
▲ 150 ps
● 370 ps
○ 1600 ps

Fluorescence amplitudes are expressed relative to the $\tau = 150$ ps component at 650 nm in state 2. The spectra in (A) and (B) were resolved together on the assumption that the same fluorescence lifetimes were present in each spectrum.

separately, making no assumptions about the lifetime components present in each case. These analyses appeared to show a significant increase in the amplitude of the phycocyanin fluorescence ($\tau \approx 150$ ps, $\lambda_{max} = 650$ nm) to accompany the state 2 transition. However, this was probably an artefact due to mixing of the $\tau = 150$ ps component with the faster ($\tau \approx 60$ ps) component. The PS II fluorescence decay was still clearly resolved in both state 1 and state 2. The principal effect of the state 2 transition was still a decrease in the amplitude of the PS II fluorescence decay, with increase in the observed amplitude of the fluorescence decay from the phycobilisome terminal emitter (Table II). The state 2 transition also appears to reduce the lifetime of the PS II fluorescence decay slightly (Table II). However, this change in lifetime is within the limits of accuracy of the analysis and is proportionately much smaller than the change in amplitude (Table II).

TABLE II

Effect of state 1-state 2 transitions on time-resolved fluorescence emission spectra of cells of Synechococcus 6301 with excitation at 620 nm

Data taken from the time-resolved fluorescence spectra in Figs. 5 and 6. Decay components were assigned to PS II and the phycobilisome terminal emitter as described in the Results section and summarised in Table I. Fluorescence amplitudes (A) are for emission at 680 nm. Amplitudes are expressed relative to the amplitude of fluorescence from phycocyanin ($\tau = 150$ ps) at 650 nm in state 2. The spectra for cells at F_o have been analysed both individually and together in terms of the same set of fluorescence lifetimes. The F_m spectra have been analysed together. At F_o, only one PS II fluorescence decay component could be resolved. Fluorescence lifetimes and amplitudes are accurate to within about ±10%.

		PS II (fast)		PS II (slow)		Terminal emitter	
		A	τ (ps)	A	τ (ps)	A	τ (ps)
F_o	state 1			0.14	400	0.57	160
F_o	state 2			0.08	330	0.63	140
F_o	state 1 [a]			0.20	370	0.54	150
F_o	state 2 [a]			0.06	370	0.67	150
F_m	state 1 [a]	0.35	550	0.08	1400	0.41	200
F_m	state 2 [a]	0.13	550	0.02	1400	0.68	200

[a] Combined global analysis of decays in state 1 and state 2.

Fig. 6 shows the effect of state 1-state 2 transitions on the time resolved fluorescence emission spectra for *Synechococcus* cells at the F_m level of fluorescence. Fig. 6a shows spectra for cells in state 1 and Fig. 6b shows spectra for cells in state 2. As for the F_o spectra (Fig. 5), excitation was at 620 nm. Because of the complexity of the PS II fluorescence decay at F_m (Fig. 4), we were unable to resolve these spectra satisfactorily when they were treated individually. However, we were able to resolve these spectra adequately in terms of five components when the spectra were analysed together in terms of the same set of lifetime components (Fig. 6). As at F_o (Fig. 5) the principal effect of the state 2 transition at F_m (Fig. 6) is a decrease of about 60% in the amplitudes of the PS II fluorescence decay components ($\tau \approx 550$ ps and $\tau \approx 1.4$ ns) with a complementary increase in the amplitude of the fluorescence decay from the phycobilisome terminal emitter ($\tau \approx 200$ ps). The apparent increase (Fig. 6) in the amplitude of the 20 ps rise-term

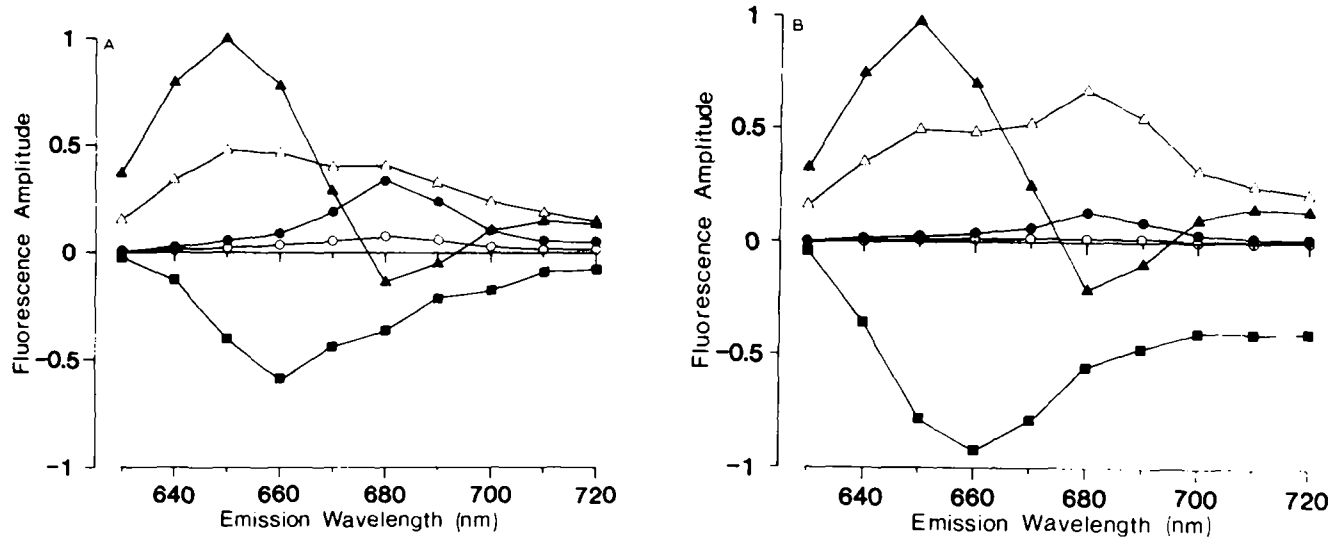

Fig. 6. Time-resolved fluorescence emission spectra of *Synechococcus* 6301 cells obtained by global analysis of fluorescence decays: comparison of state 1 and state 2 at F_m with excitation at 620 nm.

(A) Cells in state 1: τ =	(B) Cells in state 2: τ =
■ 20 ps	■ 20 ps
▲ 120 ps	▲ 120 ps
△ 200 ps	△ 200 ps
● 550 ps	● 550 ps
○ 1400 ps	○ 1400 ps

The spectra in (A) and (B) were resolved together on the assumption that components with the same fluorescence lifetimes were present in each spectrum. Fluorescence amplitudes are expressed relative to the $\tau = 120$ ps component at 650 nm in state 1.

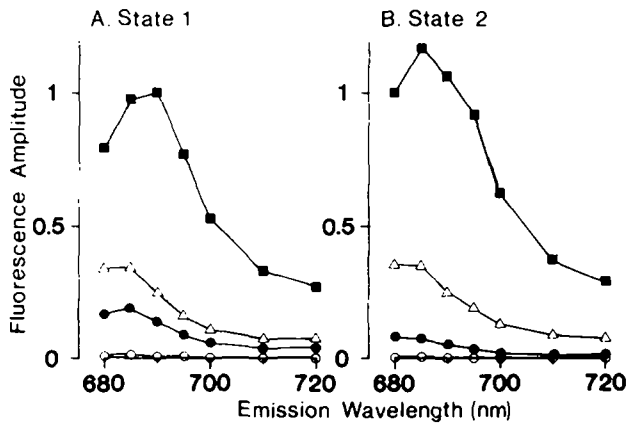

Fig. 7. Time-resolved fluorescence emission spectra of *Synechococcus* 6301 cells obtained by global analysis of fluorescence decays: comparison of state 1 and state 2 at F_o with excitation at 670 nm.

(A) Cells in state 1: τ =	(B) Cells in state 2: τ =
■ 40 ps	■ 40 ps
△ 180 ps	△ 170 ps
● 490 ps	● 470 ps
○ 1500 ps	○ 1700 ps

Fluorescence amplitudes are expressed relative to the 40 ps component at 670 nm in state 1.

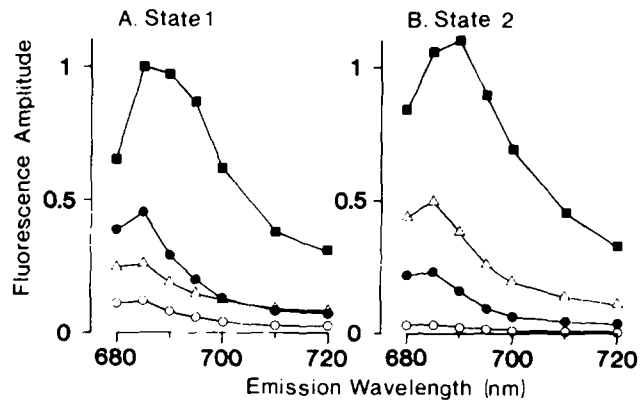

Fig. 8. Time-resolved fluorescence emission spectra of *Synechococcus* 6301 cells obtained by global analysis of fluorescence decays: comparison of state 1 and state 2 at F_m with excitation at 670 nm.

(A) Cells in state 1: τ =	(B) Cells in state 2: τ =
■ 40 ps	■ 40 ps
△ 180 ps	△ 190 ps
● 600 ps	● 530 ps
○ 1300 ps	○ 1400 ps

Fluorescence amplitudes are expressed relative to the 40 ps component at 685 nm in state 1.

in state 2 may not be significant: the amplitude of this component is difficult to determine accurately because its lifetime is so short.

Figs. 7 and 8 show the effect of state transitions on time-resolved fluorescence emission spectra recorded with excitation at 670 nm. All these spectra have been analysed individually, making no prior assumptions about the lifetime components present. The effects of the state transitions on these spectra are similar to those observed with excitation at 620 nm (Fig. 5 and 6). At F_o (Fig. 7) the state 2 transition reduces the amplitude of the PS II fluorescence decay component ($\tau \approx 490$ ps) by about 60%. At F_m (Fig. 8), the state 2 transition reduces the amplitudes of both PS II fluorescence decay components ($\tau \approx 600$ ps and $\tau \approx 1300$ ps). There is no significant change in the lifetime of any component under either of these conditions. At F_m, the state 2 transition also increased the amplitude of the 180 ps component which we have assigned to the phycobilisome terminal emitter (Fig. 8). At F_o (Fig. 7) we saw no increase in the amplitude of this component, in contrast to all the other conditions examined (Figs. 5, 6 and 8). This might be because the 180 ps component seen in Fig. 7 comes in part from a faster PS II fluorescence decay component, which would be more prominent in relation to the phycobilisome core fluorescence decay under these conditions than under any other conditions examined. Such a component would be predicted on the basis of studies on isolated PS II particles [35,36]. A decrease in the amplitude of this component could compensate for an increase in the amplitude of the terminal emitter fluorescence.

TABLE III

Effect of state 1-state 2 transition on time-resolved fluorescence emission spectra of cells of Synechococcus 6301 with excitation at 670 nm

Data taken from the time-resolved fluorescence emission spectra in Figs. 7 and 8. Decay components were assigned to PS II, PS I and the phycobilisome terminal emitter as described in the Results section and summarised in Table I. Fluorescence amplitudes (A) are for emission at 685 nm. Amplitudes are expressed relative to the amplitude of fluorescence from PS I ($\tau = 40$ ps) at 690 nm in state 1. At F_o, only one PS II fluorescence decay component could be resolved. Fluorescence lifetimes and amplitudes are accurate to within about ±10%.

		PS II (fast)		PS II (slow)		Terminal emitter	
		A	τ (ps)	A	τ (ps)	A	τ (ps)
F_o	state 1			0.19	490	0.34	180
F_o	state 2			0.08	470	0.35	170
F_m	state 1	0.48	600	0.13	1300	0.26	180
F_m	state 2	0.24	530	0.03	1400	0.51	190

Discussion

The state 2 transition in phycobilisome-containing organisms results in a decrease in the fluorescence yield of PS II [2,3,19,20,22] with a corresponding decrease in the yield of PS II photochemistry [13,19]. Here we report on the nature of the decrease in PS II fluorescence yield caused by the state 2 transition by comparing time-resolved fluorescence emission spectra for cells of the cyanobacterium *Synechococcus* 6301 adapted to state 1 and to state 2.

Tables II and III summarise the effect of state 1-state 2 transitions on time-resolved fluorescence decay components from PS II, PS I and the phycobilisome terminal emitter. The state 2 transition causes a decrease of 60–70% in the amplitudes of the PS II fluorescence decay components both when PS II reaction centres are open (F_o) and when PS II reaction centres are closed (F_m). This decrease is seen both with excitation at 620 nm (Table II) which is absorbed principally by phycocyanin and with excitation at 670 nm (Table III) which is absorbed both by Chl *a* and by the phycobilisome core [31]. The state 2 transition has no significant effect on the lifetime of the PS II fluorescence emission under any of these conditions. At F_m we observe two PS II fluorescence decay components of different lifetimes (Figs. 4, 6 and 8 and Table I). The state 2 transition reduces the amplitude of both these components roughly in proportion (Figs. 6 and 8, Tables II and III) so that there is no change in the overall lifetime of the PS II fluorescence decay. With 620 nm excitation at the F_m level of fluorescence (Fig. 6) we found it necessary to analyse the spectra for cells in state 1 and in state 2 together in terms of the same set of fluorescence decay lifetimes. Hence we cannot rule out changes in decay lifetimes under these conditions. However, it should be noted that the proportional decrease in PS II fluorescence observed at F_m is similar to that observed at F_o (Table II) which makes it unlikely that there is any extra effect at F_m in addition to the changes in PS II fluorescence amplitude observed at F_o (Fig. 5 and Table II).

The effects of the state 2 transition on PS II fluorescence decay are consistent with a decrease in the absorption cross-section of PS II. This would be expected to reduce the amplitude of the PS II fluorescence decay without changing its lifetime, since the change is in the proportion of absorbed energy which is transferred to PS II rather than in the efficiency of de-excitation of the PS II antenna. We find no evidence for a change in the efficiency of direct 'spillover' of excitation energy from PS II to PS I [19,20]. This would decrease the lifetime of PS II fluorescence in state 2. We find that state transitions have no significant effect on the lifetime of PS II fluorescence (Tables II and III).

Our interpretation of the effects of state transitions on time-resolved fluorescence emission spectra is supported by the fact that the proportional decrease in PS

II fluorescence amplitude on transition to state 2 is similar at F_o and at F_m (Tables II and III). This confirms results previously obtained from fluorescence induction transients [22]. A decrease in PS II absorption cross-section would be expected to reduce PS II fluorescence yield at F_o and F_m in proportion [37]. By contrast, a change in 'spillover' of excitation energy from PS II to PS I would be expected to have a greater effect on PS II fluorescence yield at F_m than at F_o because of competition from PS II photochemistry at F_o [38].

A second effect of the state 2 transition is an increase in the amplitude of the component with $\tau \approx 170$ ps and an emission maximum at 680–685 nm (Figs. 5–8, Tables II and III). This increase was seen under all conditions examined except at F_o with 670 nm excitation (Fig. 7, Table II). This component appears to come from the phycobilisome core rather than from PS II, and for the purposes of this discussion we have assigned it to the terminal emitter of the phycobilisome (Table I). When PS II is excited by energy transferred from the phycobilisome, PS II fluorescence will not be at a maximum immediately after the exciting flash. Instead, there will be a delay (or rise-term) corresponding to the time required to transfer energy from the phycobilisome to PS II. This rise term will have a lifetime corresponding to the fluorescence decay from the phycobilisome core [33,34], but its amplitude will be of opposite sign since it represents a fluorescence rise rather than a fluorescence decrease. Since the rise-term for PS II fluorescence will have the same lifetime and a similar emission maximum to the fluorescence decay component from the phycobilisome core, the two components will not be separately resolvable. The rise-term for PS II fluorescence will simply reduce the observed amplitude of the fluorescence decay from the phycobilisome core. The increase in the amplitude of this component which we find to accompany the state 2 transition (Tables II and III) is therefore consistent with the decoupling of PS II core complexes from the phycobilisome. This would decrease the amplitude of the rise-term due to energy transfer from the phycobilisome to PS II and therefore increase the observed amplitude of the fluorescence from the phycobilisome core. We therefore suggest that the decrease in the absorption cross-section of PS II which we find to accompany the sate 2 transition (Tables II and III) results from the decoupling of a proportion of PS II core complexes from the phycobilisome.

Bruce et al. have previously reported time-resolved fluorescence emission spectra for cells of *Anacystis nidulans* and *Porphyridium cruentum* frozen in state 1 and state 2 at 77 K [20,39]. These authors found that the principal effect of the state 2 transition was a decrease in the lifetime of fluorescence emission from PS II and therefore concluded that the state 2 transition involves an increase in spillover from PS II to PS I. However, this interpretation of the results of Bruce et al. [20,39] depends on the assumption that the fluorescence observed at each peak of the 77 K fluorescence emission spectrum comes from a single component. The non-exponential nature of the fluorescence decays which were observed indicate that this is not necessarily the case. The data of Bruce et al. [20,39] do not therefore distinguish between a change in the fluorescence lifetime of a single component or a change in the relative amplitudes of two or more components. Such a change in relative amplitude could occur simply because a decrease in the amplitude of a particular component would increase the proportion of fluorescence at that wavelength which comes from spectrally overlapping components. Such results give no information on any changes in the overall fluorescence amplitude at any emission wavelength because all the decays are normalised to the same fluorescence amplitude. It is very difficult to compare the absolute fluorescence yields of frozen samples. The data of Bruce et al. are not therefore inconsistent with a change in the coupling of PS II to the phycobilisome. In order to distinguish between changes in spillover and changes in absorption cross-section it is essential to resolve both the lifetimes and the amplitudes of individual fluorescence decay components [23].

The 60% decrease in energy transfer from the phycobilisome to PS II which we observe to accompany the state 2 transition in *Synechococcus* 6301 (Table II) seems adequate to account for the decreased yield of PS II fluorescence [2,3,19,20,22] and photochemistry [2,19] observed in state 2 with phycobilisome-absorbed light. However, the state 2 transition also reduces the fluorescence yield of PS II observed with Chl-absorbed excitation light [5,19,22]. This cannot be the direct result of decoupling of PS II from the phycobilisome, which would not affect energy transfer from the Chl antenna of PS II to the reaction centre. However, the change in the distribution of Chl-absorbed energy is different in nature from the change in the distribution of phycobilisome-absorbed energy. Fluorescence induction transients recorded with Chl-absorbed light suggest that the decreased PS II fluorescence in state 2 observed with Chl-absorbed light is due to spillover of excitation energy from a population of phycobilisome-uncoupled PS II centres rather than to a change in absorption cross-section [22]. Mullineaux and Allen have proposed that the state 2 transition is a dissociation of Chl-containing PS II core complexes from the phycobilisome [22]. They also proposed that these phycobilisome-uncoupled PS II core complexes can associate more closely with PS I, thus causing an increase in spillover to PS I which is observed only with Chl-absorbed light.

In the present study we confirm by independent means that the state 2 transition in *Synechococcus* 6301 involves the functional decoupling of PS II reaction

centres from the phycobilisome. However, we saw no significant decrease in the lifetime of PS II fluorescence in state 2 with 670 nm excitation, which will excite phycobilisome-uncoupled PS II centres as well as phycobilisome-coupled centres (Figs. 7 and 8; Table III). This casts some doubt on the hypothesis that there is increased spillover from phycobilisome-uncoupled PS II to PS I [22]. However, a definitive test of this proposal by time-resolved fluorescence emission spectroscopy will require a laser excitation beam in the blue region of the spectrum where there is strong selective absorption of light by Chl *a*.

Surprisingly, no significant change in the fluorescence lifetime of the terminal emitter of the phycobilisome seems to accompany the state transition (Tables II and III). This indicates that there must be an efficient sink for the phycobilisome-absorbed energy that is directed away from PS II in state 2. Without such a sink the fluorescence lifetime of the phycobilisome terminal emitter would be much greater in state 2 than in state 1. One possibility would be that energy absorbed by the phycobilisome is dissipated by a non-radiative quenching mechanism when no PS II centre is attached [40]. A serious problem for this interpretation is the lack of any indication as to the nature of this quencher, and this explanation remains at present unsatisfactory. It is of interest, however, that the fluorescence decay from the terminal emitter of the phycobilisome remains rapid in a cyanobacterial mutant lacking PS II reaction centres [41]. An alternative possibility is that the phycobilisome could transfer energy primarily to PS I in state 2. Our results do not distinguish between these possibilities because any PS I fluorescence decay component arising from energy transfer from the phycobilisome would probably not be detectable under our conditions due to the very short lifetime of PS I fluorescence (Table I). Becuase PS I fluorescence decays much faster than fluorescence from the phycobilisome terminal emitter (Table I), a PS I fluorescence decay component arising from energy transfer from the phycobilisome to PS I would be of very low amplitude. Such a component would also have a decay lifetime corresponding to that of the phycobilisome terminal emitter and could therefore not be resolved easily from the $\tau \approx 170$ ps component. At present this latter possibility might seem the more reasonable one, but this question requires further study.

Recent studies of the kinetics of energy transfer and exciton trapping in cyanobacterial PS II particles indicate that PS II fluorescence decays biexponentially both at F_o and at F_m [35,36]. In the present study with whole cells we were unable to resolve a faster PS II fluorescence decay component at F_o (Table I). Such a component may, however, be present but mixed with other decay components. For the purpose of the present discussion, we have assumed that energy transfer from the phycobilisome to PS II is essentially irreversible. This is justifiable as a first approximation, but in reality there will be a finite probability for reverse energy transfer from PS II to the phycobilisome. The decoupling of PS II from the phycobilisome could then lead to small changes in the fluorescence lifetimes of both PS II and the phycobilisome components. The possibility of such effects is currently under investigation.

The decrease in energy transfer from the phycobilisome to PS II which we observed to accompany the state 2 transition requires in principle only a small conformational change in the phycobilisome-PS II complex, since the efficiency of energy transfer is very sensitive to the spatial separation and relative alignment of the donor and accpetor pigment molecules [42]. However, Olive et al. [43] have published freeze-fracture electron micrographs of the thylakoid membranes of the cyanobacterium *Synechocystis* 6714 which indicate that state transitions in cyanobacteria involve a large-scale realignment of PS II centres in the membrane: PS II reaction centres are arranged in rows in state 1, but are more randomly arranged in State 2. Since it is the association of PS II with the phycobilisome which causes PS II reaction centres to be arranged in rows [44,45], the randomisation of PS II reaction centres in state 2 is consistent with the detachment of PS II from the phycobilisome. We suggest that the detachment of a proportion of PS II centres from the phycobilisomes in state 2 and the subsequent randomisation of these centres in the thylakoid membrane fully accounts for the decrease that we report in energy transfer from the phycobilisome to PS II.

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