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Excitation energy transfer from phycobilisomes to Photosystem I in a cyanobacterium

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Flash-induced absorbance changes at 700 nm were used to determine the absorption cross-section of Photosystem I in whole cells of the cyanobacterium *Synechococcus 6301*. By varying the wavelength of the excitation flash an excitation spectrum for PS I was constructed. The excitation spectrum for PS I in dark-adapted (light-state 2) cells shows a large peak corresponding to absorbance by phycobilins. This peak is absent in phycobilisome-free thylakoid membranes. Adaptation to light-state 1 decreases the absorption cross-section of PS I with phycobilin-absorbed light, but has little effect with chlorophyll-absorbed light. The closure of PS II reaction centres has no detectable effect on the absorption cross-section of PS I, suggesting that energy transfer from phycobilisomes to PS I is direct rather than spillover from PS II. It is proposed that, in cells adapted to light-state 2, a proportion of phycobilisomes are specifically associated with PS I.

Introduction

Although many of the components of the photosynthetic apparatus of cyanobacteria, including the reaction centres and other electron transport components, are very similar to those of green plants, the light-harvesting complexes are strikingly different. Cyanobacteria contain no chlorophyll *b*, and both Photosystem I and Photosystem II have chlorophyll *a*-containing antennae. In the cyanobacterium *Synechococcus* 6301 (*Anacystis nidulans*), it has been estimated that about 120 Chl *a* molecules are associated with each PS I reaction centre [1]. The PS II Chl *a* antenna is considerably smaller: estimates range from 35 [2] to 50 [1] Chl *a* molecules per reaction centre. Under most growth conditions PS I reaction centres outnumber PS II reaction centres, so that the bulk of the Chl *a* in the cell is associated with PS I [1]. In addition to the Chl *a*-containing antennae, cyanobacteria contain phycobilisomes, large complexes of phycobiliproteins bound to the surface of the thylakoid membrane (see [3,4] for reviews). In *Synechococcus* 6301, the principal phycobiliprotein is phycocyanin, which absorbs maximally at about 625 nm [3]. The phycobilisomes of *Synechococcus* 6301 also contain smaller amounts of allophycocyanin [3]. This phycobiliprotein absorbs maximally at about 655 nm and is capable of accepting excitons from phycocyanin and transferring them to the long-wavelength absorbing terminal emitters of the phycobilisomes, which then transfer the excitons to the Chl *a*-containing light-harvesting complexes [3,4]. The structure and light-harvesting function of isolated phycobilisomes have been extensively studied, but less is known about energy transfer from phycobilisomes to the Chl *a*-containing species. A number of different approaches have yielded strong evidence for the association of phycobilisomes with the core complex of PS II:
(a) Measurements of Emerson enhancement have shown that phycobilisome-absorbed light predominantly excites PS II rather than PS I [5];
(b) Time-resolved fluorescence measurements have shown energy transfer from phycobilisomes to PS II [6] (a recent study gave a rate constant of about 5 ns^{-1} for this process at room temperature [7]);
(c) Freeze-fracture electron microscopy has shown a structural association of phycobilisomes and PS II [8,9];

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ΔA_{700}, flash-induced 700 nm absorbance change; ΔA_{max}, saturating flash-induced 700 nm absorbance change; F_0, fluorescence level with all PS II reaction centres open; F_m, fluorescence level with all PS II reaction centres closed; FWHM, full width at half maximum; I_e, flash intensity required to photooxidise a fraction $(1 - 1/e)$ of PS I reaction centres; LHC-II, light-harvesting chl *a*/*b*-binding protein of PS II; PBS, phycobilisome; PS, photosystem.

(d) Functionally active phycobilisome-PS II particles have been isolated [10] and phycobilisomes have been reconstituted in vitro with PS II core complexes [11].

These studies have led to the currently accepted view that phycobilisomes are exclusively associated with PS II. The contribution of phycobilisomes to the action spectrum for PS I excitation [5] has been generally assumed to be due to "spillover" of excitation from phycobilisome-coupled PS II centres to PS I [12,13].

However, this view has been challenged by studies of state 1-state 2 transitions, physiological adaptation mechanisms which allow the rapid modification of the function of the light-harvesting apparatus in response to changes in light-intensity and quality (see Refs. 14,15 for reviews). Picosecond time-resolved fluorescence studies of *Synechococcus* 6301 cells adapted to light-states 1 and 2 indicate that the decreased PS II excitation in state 2 results from a decrease in PS II absorption cross-section rather than an increase in spillover from PS II to PS I [16].

These results further suggested that a proportion of PS II core complexes becomes decoupled from the phycobilisomes in state 2, a view that was supported by studies of fluorescence induction [17]. However, laser-induced optoacoustic studies showed that energy harvested by phycobilisomes was stored as efficiently in state 2 as in state 1 [18], raising the possibility that, in state 2, a proportion of phycobilisomes transfer energy to PS I rather than to PS II. Such an energy-transfer process would be very difficult to detect by steady-state or time-resolved fluorescence studies because of the rapid decay of excitation in the PS I core complex: excitation decays in PS I with a rate constant of about 25 ns^{-1} [7], which is considerably faster than the putative rate constant for energy transfer from phycobilisomes to PS I of about 5 ns^{-1} [7]. This means that energy transfer from phycobilisomes to PS I would give rise to negligible additional fluorescence from the Chl *a* of PS I [7]. I therefore set out to test the transfer of energy from phycobilisomes to PS I by measuring the absorption cross-section of PS I from flash-induced absorbance changes at 700 nm. By using a continuously flowing sample, it was possible to compare the absorption cross-section of PS I in cells adapted to light-states 1 and 2, and with open and closed PS II reaction centres. I conclude that, in light-state 2, a proportion of phycobilisomes transfer energy directly to PS I.

Materials and Methods

Cells of the cyanobacterium *Synechococcus* 6301 (*Anacystis nidulans*) were grown in liquid culture under white-light illumination (50 $\mu E\ m^{-2}\ s^{-1}$) in medium C of Kratz and Myers [19]. The concentrations of phycocyanin and chlorophyll *a* were estimated from absorption spectra using the formulae of Myers et al. [1]. For the measurement, the cells were diluted where necessary to a Chl *a* concentration of 4–5 μM. The maximum absorbance of the sample in the wavelength region of interest was about 0.4 cm^{-1} (at 625 nm). The optical density of the sample was kept low to minimise artefacts due to self-shading of the sample. The volume of cell suspension used for each measurement was about 500 ml. During the measurement, the bulk of the cell suspension was kept in a stirred flask at room temperature (25°C). This flask was either kept in the dark (cells adapted to state 2) [20] or illuminated with blue light defined by a Corning 5-57 filter at 430 $\mu E\ m^{-2}\ s^{-1}$ (cells adapted to state 1) [20]. A peristaltic pump (type 503S from Watson-Marlow, Falmouth, UK) was used to pump the cell suspension at a speed of 5 ml s^{-1} through a 750-μl flow cuvette (type 175.050 from Hellma, Mülheim/Baden, FRG) where the measurement was performed. The optical path lengths were 10 mm (in the direction of the measuring beam) and 6 mm (in the direction of the flash). The flow speed was sufficiently fast to ensure that no cells were exposed to two successive flashes, but not so fast that the absorption decay kinetics were significantly distorted. The delay between the sample leaving the stirred reservoir and the measurement was about 5 s. For cells at F_m (PS II reaction centres closed) DCMU was added to the cell suspension to a final concentration of 20 μM and the cells were exposed to a brief pre-illumination (exposure time 280 ms) with light defined by a 620 nm interference filter (bandwidth 11 nm) at 50 $\mu E\ m^{-2}\ s^{-1}$ before flowing through a black tube into the measuring cuvette. The delay between the pre-illumination and the measurement was about 370 ms on average.

Absorbance changes were measured at 700 nm. The measuring beam was supplied by a stabilised light-source (CUEL, Kenilworth, UK) and defined by a 700-nm interference filter (bandwidth 12.3 nm). The intensity of the measuring beam at the sample was 14 $\mu E\ m^{-2}\ s^{-1}$. At this intensity, the measuring beam caused no detectable photooxidation of P700 prior to the excitation flash. The light passed through the measuring cuvette and then through a second 700 nm interference filter. The signal was detected with a red-sensitive photomultiplier tube (9558QB from EMI, Ruislip, Middlesex, UK). The optics of the system were designed to minimise detection of fluorescence from the sample. The signal from the photomultiplier was amplified with an offset current amplifier (Type 427 from Keithley Instruments, Cleveland, OH, USA) with a rise-time of 10 μs and stored with a computer-linked transient recorder (R2000 from Rapid Systems Inc., Seattle, USA). The excitation flash was supplied by a xenon flashlamp (Type 4.50XAD from Noblelight, Cambridge, UK) triggered simultaneously with the transient recorder by a repetitive flash unit (Model 445 from Applied Photophysics, London, UK). The dura-

tion of the flash was 4 μs (FWHM) and the repetition rate was 1.25 Hz.

The intensity and wavelength of the flash were controlled by combinations of coloured and neutral density filters. Bandpass interference filters were from Ealing Electro-Optics (Watford, UK). Broad-band blue (Chl *a*-absorbed) light was defined by an Ealing 540 nm short-pass filter. Broad-band yellow (phycobilin-absorbed) light was defined by a combination of Corning 3-67 and Ealing 660 nm short-pass filters. This filter combination gave high transmission between 550 nm and 660 nm.

In whole-cell measurements, either 256 or 512 signals were averaged. This large number of repeats was necessary because of a poor signal/noise ratio due to a high degree of light-scattering and turbulence from the pumping of the sample. A signal recorded with no measuring beam was subtracted to eliminate the fluorescence signal from the sample and other flash artefacts.

For measurements of flash-induced fluorescence yields, the 700-nm filter in front of the detector was replaced with a 680-nm interference filter (bandwidth 11.5 nm) and the optics on the detector side were adjusted to improve detection of fluorescence. Continuous fluorescence measurements were made at the measuring cuvette with a Modulated Fluorescence Measurement System (Hansatech, King' s Lynn, UK). Fluorescence emission was detected at 700 nm. Excitation was with yellow light (maximum at 583 nm).

Phycobilisome-free thylakoid membranes were prepared by a method based on that of England and Evans [21]: cells of *Synechococcus* 6301 were harvested by centrifugation and resuspended to 150 μM Chl *a* in a medium containing 25% (v/v) glycerol, 10 mM $MgCl_2$, 50 mM Hepes (pH 7.5) and deoxyribonuclease 1 (1 μg/ml). The suspension was passed through a French pressure cell at 100 MPa and centrifuged for 15 min at 8000 $\times g$ (2–4°C) to pellet unbroken cells and cell debris. The supernatant was centrifuged for 45 min. at 100000 $\times g$ (2–4°C). The phycobilin-containing supernatant was discarded and the pellet resuspended in a buffer containing 15% (v/v) glycerol, 10 mM $MgCl_2$ and 25 mM Hepes (pH 7.5). For the measurement of flash-induced absorbance changes, the suspension was diluted to a Chl *a* concentration of 5 μM. Sodium ascorbate (10 mM) and phenazine methosulphate (10 μM) were added as electron donors to PS I. The measurement was performed as for whole cells except that the sample was stationary and the number of repeats was decreased to 16.

Results

Flash-induced transient absorption changes at 700 nm probe the photooxidation and the kinetics of re-reduction of P700, the primary electron donor of PS I [22]. Fig. 1 shows such a transient for dark-adapted cells of the cyanobacterium *Synechococcus* 6301. This transient was induced by broad-band blue (Chl *a*-absorbed) light at saturating intensity. As was previously observed for cyanobacterial cells [23], the re-reduction of PS I proceeds in at least two phases. The first phase has a lifetime ($1/e$) of about 200 μs. The second phase has a lifetime in the millisecond time-range.

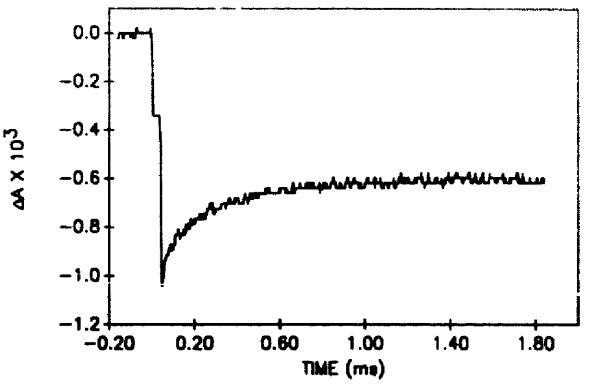

Fig. 1. Flash-induced 700 nm absorbance changes for dark-adapted (state 2) cells of *Synechococcus* 6301. A 4μs (FWHM) flash was given at time $t = 0$. The flash was with blue (Chl *a*-absorbed) light and was at saturating intensity. The signal was summed from 512 repeats.

At saturating flash-intensity, the initial amplitude of the absorbance change indicates the population of active PS I reaction centres in the sample [22]. Information on the absorption cross-section of PS I (the size of the light-harvesting antenna feeding excitons into each PS I reaction centre) may be obtained from the amplitude of the absorbance change at sub-saturating light intensities. The larger the absorption cross-section of PS I, the lower is the flash intensity required to induce any given level of photooxidation of P700 and the steeper is the increase in the absorbance change with increasing flash intensity. Fig. 2 shows plots, on a semi-logarithmic scale, of the fraction of photooxidised P700 versus the intensity of the exciting flash. These results were obtained for dark-adapted cells, which were therefore in light-state 2 [20]. Plots are shown for two wavelengths of the flash: 680 nm, which is predominantly absorbed by Chl *a*, and 630 nm which is only weakly absorbed by Chl *a* but strongly absorbed by phycocyanin. Over the intensity range shown, the semi-logarithimic plots are linear at both excitation wavelengths. The gradient of the semi-logarithmic plot is a measure of the average relative absorption cross-section of PS I. This is only slightly less for 630 nm light than for 680 nm light, suggesting that, for cells in state 2, phycobilisomes as well as Chl *a* can transfer energy to PS I (Fig. 2).

Fig. 3 shows the variation of PS I relative absorption cross-section with excitation wavelength; in effect this

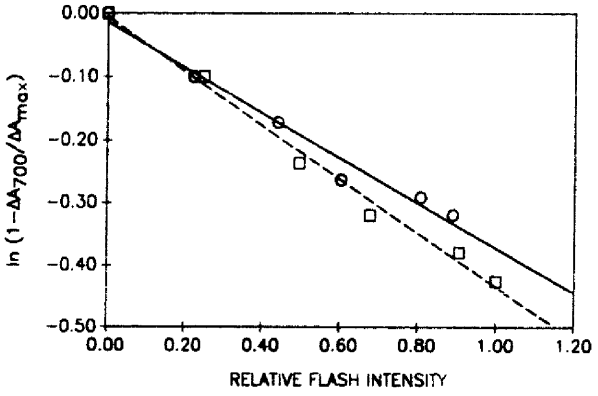

Fig. 2. Dependence of the 700 nm absorbance change on flash intensity. The wavelength of the flash was controlled with interference filters (bandwidth about 11 nm FWHM). ΔA_{max} was determined using broad-band blue light. □——□, 630 nm excitation; □- - - - - -□, 680 nm excitation. The relative light intensities at the two wavelengths are scaled according to the relative number of photons in each flash.

gives the excitation spectrum for PS I. PS I relative absorption cross-section is normalised to 680 nm to allow a comparison between whole cells (adapted to state 2) and phycobilisome-depleted thylakoid membranes, which scattered light to a lesser extent. As well as the peak at 680 nm corresponding to the absorption maximum of Chl *a*, the cells show a prominent peak at 630 nm corresponding to the absorption maximum of phycocyanin. This peak is absent in phycobilisome-free thylakoid membrane fragments (Fig. 3). In the cells, the ratio of PS I absorption cross-section at 630 nm to that at 680 nm is 0.84. This compares to a ratio

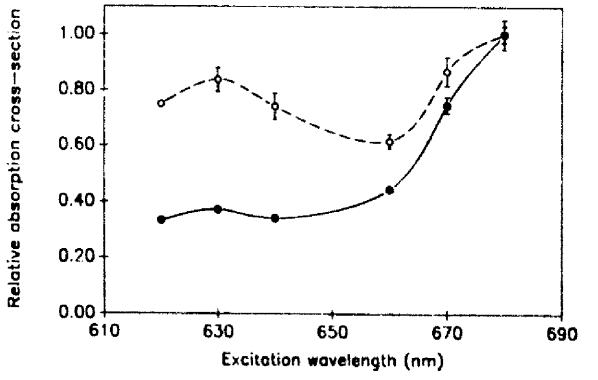

Fig. 3. Action spectra for PS I. The absorption cross-sections of PS I for different wavelengths of light were determined from the gradients of plots such as those in Fig. 2 and are expressed relative to the absorption cross-section at 680 nm. The wavelength of the flash was controlled with interference filters (bandwidth about 11 nm). As in Fig. 2, relative light intensities at different wavelengths were scaled according to the relative number of photons in the flash when calculating these results. ○- - - - - -○, cells of *Synechococcus* 6301 (state 2, PS II reaction centres open); ●——●, phycobilisome-free thylakoid membranes from *Synechococcus* 6301.

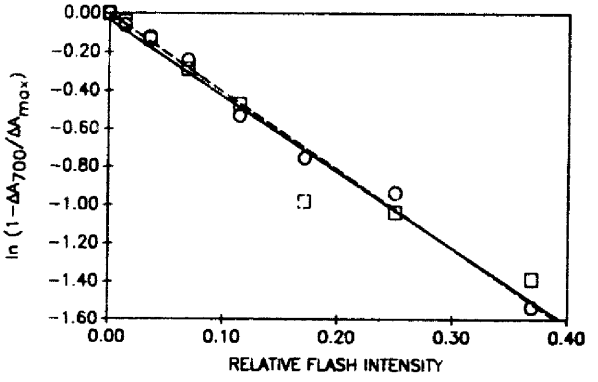

Fig. 4. Effect of state transitions on PS I absorption cross-section with broad-band blue light (predominantly absorbed by Chl *a*). □——□: cells in state 1. ○- - - - - -○: cells in state 2.

A_{630}/A_{680} of 1.1 in the absorption spectrum of the cells, and indicates efficient energy transfer from phycobilisomes to PS I.

Figs. 4 and 5 show the effect of state transitions on the absorption cross-section of PS I. For these measurements I have used broad-band excitation light to enable higher excitation flash intensities-to be attained. Cells were adapted to dark (state 2) or exposed to blue light to induce state 1, as described in Materials and Methods. Exposure to the blue light produced an increase of about 10% in the steady-state fluorescence yield of the cells with a half-time of about 30 s; this is typical of the state 1 transition in this organism [24]. Since most of the fluorescence observed under these conditions comes from the phycobilins and does not vary with state transitions [25], the actual increase in PS II fluorescence must be much greater than 10%. Adaptation to state 1 did not significantly alter the 700 nm absorbance change induced by saturating flash intensities. This indicates that adaptation to state 1 neither changed the population of active PS I reaction

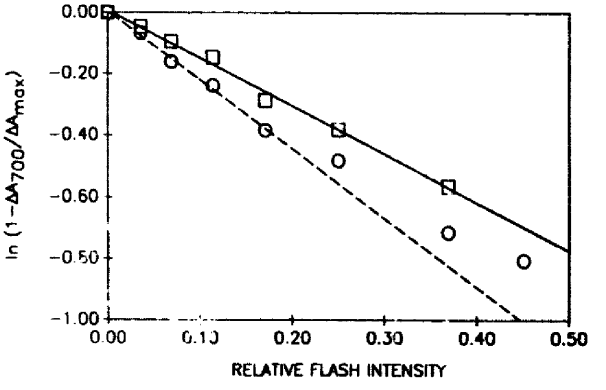

Fig. 5. Effect of state transitions on PS I absorption cross-section with broad-band yellow light (predominantly absorbed by phycobilins). ΔA_{max} was determined using blue light at saturating intensity. □——□: cells in state 1. ○- - - - - -○: cells in state 2.

centres nor caused the photooxidation of a significant proportion of PS I reaction centres prior to the flash. With light absorbed predominantly by Chl *a*, adaptation to state 1 caused no significant change in the absorption cross-section of PS I (Fig. 4 and Table I). With light absorbed predominantly by phycobilins, adaptation to state 1 decreased the average absorption cross-section of PS I by about 30% (Fig. 5 and Table I). Note that the plot in Fig. 5 for cells in state 2 is biphasic with a slower second phase of similar gradient to the linear plot for cells in state 1.

If energy transfer from phycobilisomes to PS I proceeds by spillover of energy from phycobilisome-coupled PS II centres to PS I, as has been frequently suggested [12,13,26], the closure of PS II reaction centres should increase the effective absorption cross-section of PS I [27]. In principle, this could be tested by comparing the intensity-dependence of the flash-induced 700 nm absorbance change under two conditions:

(a) PS II centres open;

(b) PS II centres closed by a pre-illumination.

A complication is that the excitation flash itself will inevitably cause the closure of PS II reaction centres as well as the photooxidation of PS I reaction centres. The extent of spillover should increase when the excitation flash is sufficiently bright for a significant proportion of PS II centres to absorb two photons: the first photon is required to close the PS II reaction centre and the second photon then has an increased chance of being transferred to PS I. The closure of PS II reaction centres may be monitored by the PS II

TABLE I

Effect of state transitions and PS II trap closure on PS I absorption cross-section

The average PS I absorption cross-section was determined from the initial gradient of the plots of $\ln(1 - \Delta A_{700}/\Delta A_{max})$ versus flash intensity shown in Figs. 4, 5 and 7. Gradients were determined by linear regression and are expressed as $1/I_e$, where I_e is the flash intensity at which $\ln(1 - \Delta A_{700}/\Delta A_{max}) = -1.0$. The excitation flash was with broad-band phycobilisome-absorbed light (PBS) or Chl *a*-absorbed light (Chl). $1/I_e$ is in relative units. values are not comparable for PBS and Chl-absorbed light.

Flash	State	PS II	$1/I_e$ (relative)	Standard error
Batch 1				
Chl	2	open	4.0	0.1
Chl	1	open	4.1	0.1
Batch 2				
PBS	2	open	2.25	0.02
PBS	1	open	1.57	0.02
Batch 3				
PBS	2	open	2.48	0.02
PBS	2	closed	2.51	0.02

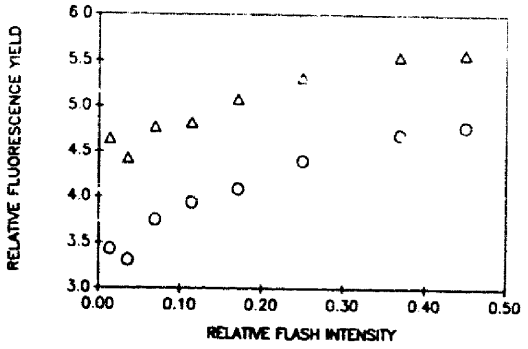

Fig. 6. Dependence of flash-induced fluorescence yield at 680 nm on flash intensity. The flash was with broad-band yellow light (predominantly absorbed by phycobilins). Flash intensity is expressed on the same scale as in Fig. 5. *Synechococcus* 6301 cells were in state 2. ○: PS II centres open prior to the flash. △: PS II centres closed prior to the flash by a 620 nm pre-illumination in the presence of DCMU.

fluorescence yield [27]. Since fluorescence, like spillover, is a process that competes with PS II photochemistry [27], the yield of PS II fluorescence from the flash should be a precise indicator of the increased probability of spillover as a result of PS II trap closure. Fig. 6 shows the variation of fluorescence yield at 680 nm with flash intensity over the same range of intensities that were used for the measurements of PS I absorption cross-section (Fig. 5). At the lowest flash intensities used it may be assumed that most PS II are open and the fluorescence yield is close to F_0 (Fig. 6). Under these conditions, much of the fluorescence observed comes from the phycobilins rather than from the Chl *a* of PS II [25]. The increasing fluorescence yield at higher flash intensities indicates the closure of PS II centres (Fig. 6). This process is still not saturated at the highest flash intensities used (Fig. 6).

If the absorption cross-section of PS I increases with increasing PS II closure, we would expect the gradient of the plot for cells in state 2 in Fig. 5 to increase gradually with increasing flash intensity. This is not observed: in fact the gradient of this plot is lower at high intensities (Fig. 5).

A more direct test of the influence of PS II closure on PS I absorption cross-section is to compare the intensity-dependence of the flash-induced 700-nm absorbance change with initially open PS II centres and with PS II centres closed by a pre-illumination in the presence of DCMU. The effect on PS II of this pre-treatment is shown in Fig. 6: the PS II fluorescence yield is considerably increased at all the flash intensities used. An important condition for this experiment is that the pre-illumination should close PS II centres without causing P700 to become photo-oxidised prior to the measuring flash. This is made possible by the different timescales for re-opening of PS II and re-reduction of P700. First, a brief 620-nm pre-illumination

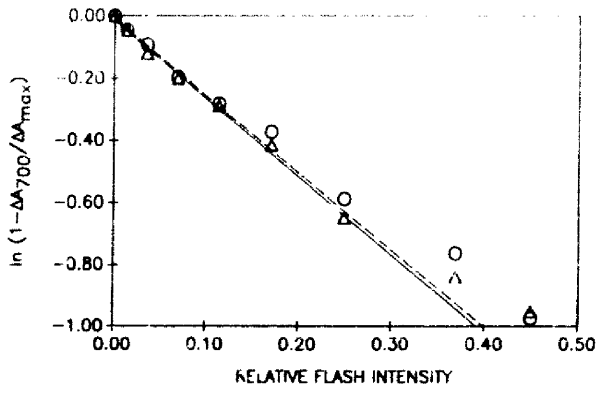

Fig. 7. Effect of PS II trap closure on PS I absorption cross-section. Cells were adapted to state 2 and excitation was with broad-band yellow light (predominantly absorbed by phycobilins). ΔA_{max} was determined using blue light at saturating intensity. Flash intensity is expressed on the same scale as in Figs. 5 and 6. ------ : PS II centres open prior to the flash. ○, ——— △: PS II centres closed prior to the flash by a 620 nm pre-illumination in the presence of DCMU.

is used to close those reaction centres that are functionally coupled to phycobilisomes. There is then a delay of about 370 ms as the cells flow into the measuring cuvette. In the presence of DCMU there is only a partial re-opening of PS II centres during this time, as is indicated by the high fluorescence yield at low flash intensities (Fig. 6). However, P700 is re-reduced much more rapidly (Fig. 1), so that all P700 should be re-reduced by the time the cells enter the measuring cuvette. The pre-illumination decreased the 700 nm absorbance change at saturating light-intensity by only 2%, confirming that the delay between the pre-illumination and the measurement was sufficient to allow virtually all P700 to be re-reduced.

Fig. 7 shows the intensity-dependence of $\Delta A_{700}/\Delta A_{max}$ for cells in state 2 with PS II centres initially open (F_0) or closed (F_m). Excitation was with phycobilin-absorbed light, as in Fig. 5. The closure of PS II reaction centres has no significant effect on the absorption cross-section of PS I, as determined from the initial gradient of the semi-logarithmic plot (Table 1).

Discussion

In this study I set out to test the extent of energy transfer from phycobilisomes to PS I in the cyanobacterium *Synechococcus* 6301, and the possible control of this process by state 1-state 2 transitions. The results presented here clearly show that, for cells adapted to light-state 2, the phycobilisomes make a major contribution to the light-harvesting capacity of PS I (Fig. 3). On transition to light-state 1, the absorption cross-section of PS I for phycobilin-absorbed light is decreased (Fig. 5 and Table 1). However, the absorption cross-section of PS I for Chl *a*-absorbed light is apparently unchanged (Fig. 4 and Table 1). This conflicts with the conclusions of Tsinoremas et al. [23] who found that the state 1 transition decreased the absorption cross-section of PS I both with Chl-absorbed light and with phycobilisome-absorbed light. However, the Chl-absorbed light used by these authors was in the near-UV (wavelength 337 nm) [23]. It is likely that this was less selective for Chl *a* than the blue light used in this study. The results presented here indicate that the transition to state 1 specifically decreases the extent of energy transfer from phycobilisomes to PS I.

In principle, energy transfer from phycobilisomes to PS I could result either from the tight coupling of a limited population of PS I to phycobilisomes or from a less selective energy transfer process giving a finite probability of energy transfer from phycobilisomes to all PS I reaction centres. My results suggest the former possibility. For cells in state 2 excited with phycobilin-absorbed light, the saturation curve for P700 photooxidation is biphasic: a rapid initial phase is followed by a slower second phase (Fig. 5). This suggests a heterogeneity in PS I antenna sizes. The initial gradient indicates the average absorption cross-section of all PS I, while the final gradient indicates the absorption cross-section of the PS I population with smaller antenna size (the PS I with larger antenna size will have been selectively photooxidised at lower light intensities). This heterogeneity is not seen with Chl *a*-absorbed light (Fig. 4) indicating that it results specifically from the coupling of phycobilisomes to PS I. The simplest explanation would be that the slower second phase seen in Figs. 5 and 7 comes from PS I that are not coupled to phycobilisomes and thus receive excitation energy only from their Chl *a* antennae. Although the broad-band yellow light used for these measurements is most strongly absorbed by phycobilins, there will also be significant absorption by Chl *a*. The faster initial phase seen in Figs. 5 and 7 would then include a population of PS I coupled to phycobilisomes. On transition to state 1, the heterogeneity is no longer detectable with phycobilin-absorbed light and the gradient of the saturation curve is similar to that of the second phase in state 2 (Fig. 5). This suggests that the population of phycobilisome-coupled PS I is very low in state 1. On transition to state 2, a limited population of PS I become coupled to phycobilisomes. In principle, it should be possible to determine the proportion of phycobilisome-coupled PS I centres from the intercept of the second phase of the saturation curve with the *y*-axis. In practice, it is impossible to do this accurately with the quality of data available. The proportion of phycobilisome-coupled PS I centres appears to be quite small, even in state 2, however. Values of about 10–20% would be consistent with the data in Fig. 5. Note, however, that since PS I centres generally

outnumber phycobilisomes by a factor of 5–6 [2,28] the proportion of phycobilisomes involved could be considerably higher depending on the number of PS I that can be associated with each phycobilisome.

Energy transfer from phycobilisomes to PS I could proceed by two routes: (a) spillover from phycobilisome-coupled PS II centres to PS I; (b) direct energy transfer from phycobilisomes to PS I.

The former possibility has been postulated in the literature on the basis of the deconvolution of low-temperature fluorescence emission spectra [12,29]. A traditional diagnosis for spillover has been the effect of PS II trap closure on PS I absorption cross-section: PS II should compete with PS I for excitons and, therefore, the closure of PS II reaction centres should increase the absorption cross-section of PS I [27]. Time-resolved fluorescence measurements have confirmed that the closure of PS II reaction centres by the reduction of Q_A greatly increases the lifetime of excitons in the PS II antenna [30,31], which should correspondingly increase the probability for spillover. However, the closure of PS II reaction centres has no influence on PS I absorption cross-section (Fig. 7, Table I). This suggests that energy is transferred directly from phycobilisomes to PS I rather than as spillover from PS II. A further implication is that the changes in excitation energy distribution associated with state transitions occur as a result of changes in the proportions of PS II and PS I reaction centres coupled to phycobilisomes, as originally suggested by Allen et al. [32]. This conflicts with the conclusions of other authors who have proposed changes in the rate constant for spillover [12,13,15,26]. However, I do not believe that these authors' results conclusively distinguish between changes in spillover and changes in absorption cross-section. See Ref. 25 for a discussion of the data in Refs. 12 and 13.

A model for the structural effects of state transitions is summarised in Fig. 8. For the purposes of the model, it is assumed that each phycobilisome has two reaction centre binding sites. This is consistent with the stoichiometry of phycobilisomes to PS II [28] and the two-fold symmetry of the *Synechococcus* 6301 phycobilisome core [3]. In state 1, most PS II core complexes are bound to phycobilisomes and the population of phycobilisome-coupled PS I is negligible. On transition to state 2, about 60% of PS II core complexes become decoupled from phycobilisomes [16] and PS I core complexes become bound to phycobilisomes in their place. Since PS I reaction centres normally outnumber PS II reaction centres by a factor of about 2.5 [1,2], the proportion of PS I that become coupled to phycobilisomes on transition to state 2 will be smaller than the proportion of PS II centres that become decoupled (Fig. 8). The model is consistent with the results reported here and with observations from a wide range of experimental approaches: (a) Picosecond time-resolved fluorescence measurements indicate decoupling of PS II from phycobilisomes on transition to state 2 [7,16]; (b) Fluorescence induction studies indicate a population of phycobilisome-decoupled PS II centres in cells adapted to state 2 [17]; (c) Laser-induced optoacoustic studies show that there is no additional dissipation of energy in state 2. Energy is redistributed between the photosystems rather than being quenched [18]; (d) Freeze-fracture electron microscopy shows randomisation of PS II particles on transition to state 2 [33]; (e)PS I particles isolated from the cyanobacterium *Chlorogloea fritschii* contained small amounts of functionally coupled allophycocyanin [34].

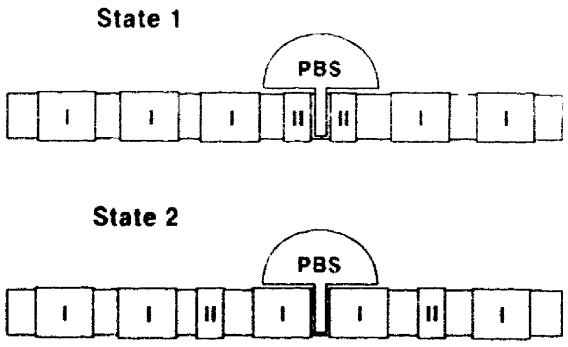

Fig. 8. Model for state transitions in *Synechococcus* 6301. In state 1, most PS II centres are coupled to phycobilisomes and most PS I centres are decoupled from phycobilisomes. On transition to state 2, most PS II centres become decoupled from phycobilisomes and a proportion of PS I centres becomes coupled to phycobilisomes in their place. A part of the PS II Chl *a* antenna may be transferred to PS I together with the phycobilisome. It is assumed that each phycobilisome has two reaction centre binding sites.

One problem that has been recently highlighted [26] is that the state 2 transition in cyanobacteria decreases the PS II fluorescence yield with chlorophyll-absorbed light as well as with phycobilin-absorbed light. A previous suggestion was that this could result from spillover as a result of increased contact between phycobilisome-decoupled PS II centres and PS I [25]. However, no change in PS II lifetimes could be detected with 670-nm excitation, which should be strongly absorbed by Chl *a* [16]. This suggests that the effect observed with Chl-absorbed light is a change in PS II absorption cross-section, not a change in spillover. One solution would be that on transition to state 2 PS II loses a part of its Chl *a* antenna in addition to the phycobilisome. The Chl *a*-binding protein involved could act as a link between phycobilisomes and reaction centres and would be transferred to PS I together with the phycobilisome. Since the amount of Chl *a* associated with PS I is far greater than that associated with PS II [1], such a process could significantly reduce the Chl *a*-antenna size of PS II without significantly increasing the Chl *a*-antenna size of PS I. According to this hypothesis, state transitions would involve changes in the coupling

between two Chl-binding proteins in a manner analogous to state transitions in green plants, where protein phosphorylation causes the decoupling of LHC-II from PS II [35]. Although this hypothesis is rather speculative at this stage, I believe it to be consistent with all current data on state transitions in phycobilisome-containing organisms, including the observation that a process resembling state transitions occurs in a phycobilisome-free cyanobacterial mutant [36]. Further progress in the understanding of the molecular basis of state transitions in the phycobilisome-containing organisms may require detailed structural and biochemical studies in addition to spectroscopy.

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