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A proportion of photosystem II core complexes are decoupled from the phycobilisome in light-state 2 in the cyanobacterium *Synechococcus* 6301

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Cells of *Synechococcus* 6301 were briefly exposed to a phycocyanin-absorbed light in the presence of DCMU. PS II trap closure was then estimated from fluorescence induction measurements with excitation light absorbed predominantly either by chlorophyll or by phycocyanin. In cells adapted to light-state 2, the exposure to light absorbed by phycocyanin closed only a proportion of the PS II centres that could be closed by exposure to light absorbed by chlorophyll. This distinction was reduced in cells adapted to light-state 1. We conclude that a proportion of PS II core complexes become decoupled from the phycobilisomes during the transition to light-state 2.

Photosynthesis; Photosystem II; Phycobilisome; Fluorescence induction; State 1-state 2 transition; Light harvesting; (*Synechococcus* 6301)

1. INTRODUCTION

In common with other photosynthetic organisms [1] cyanobacteria possess mechanisms which can rapidly alter the distribution of excitation energy between the photosystems in response to changes in light intensity and quality [2] and to changes in the metabolic state of the cells [3,4]. These changes are known as state 1-state 2 transitions. Light-state 1, which can be induced by exposure to light which predominantly excites PS I, is characterised by a higher efficiency of excitation of PS II relative to PS I [5,6]. Light-state 2, which can be induced by exposure to light which predominantly excites PS II, is characterised by a lower efficiency of excitation of PS II relative to PS I [5,6]. Dark-adapted cyanobacterial cells are normally in state 2 [2,7]. State transitions in cyanobacteria are triggered by changes in the redox state of a component which mediates electron transport between PS I and PS II, probably plastoquinone or a component of the cytochrome b_6f complex [7]. The biochemical basis of state transitions in the phycobilisome-containing organisms remain uncertain, but the phosphorylation of components of the phycobilisome and the thylakoid membrane may be involved [8,9]. This would be a point of resemblance with state transitions in green plants, which are believed to be mediated by the phosphorylation of the light-harvesting Chl *a/b* protein complex of PS II [10].

Phycobilisome-containing organisms are particularly suitable for the investigation of the nature of the changes in excitation energy distribution that accompany state transitions because of the large differences in the excitation spectra of PS I and PS II. The major light-harvesting component of PS II is the phycobilisome, a pigment-protein complex located on the surface of the thylakoid membrane. In *Synechococcus* 6301 the principal light-harvesting component of the phycobilisome is phycocyanin, whose absorption maximum is about 620 nm. In addition, PS II has a small Chl *a*-containing antenna, containing about 50 chromophores per reaction centre [11]. Energy absorbed by the phycobilisome is transferred to the Chl *a* antenna and thence to the PS II reaction centre [11]. By contrast, PS I receives excitation energy almost exclusively from its Chl *a* antenna [12], which contains about 120 chromophores per reaction centre [11].

Conflicting hypotheses have been presented concerning the nature of the change in excitation energy distribution that accompanies state transitions in phycobilisome-containing organisms. It has been suggested that the principal effect of the state 2 transition is an increase in the spillover of excitation energy from PS I to PS II [13,14]. However, fluorescence induction transients [15] and picosecond time-resolved fluorescence emission spectra [16] suggest that the state 2 transition causes a decrease in the population of PS II reaction centres that are functionally coupled to phycobilisomes. If this interpretation is correct, in state 2 there should be a substantial population of PS II reac-

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; F_0, level of fluorescence when all PS II centres are open; F_m, level of fluorescence when all PS II centres are closed; F_v, variable fluorescence ($F_v = F_m - F_0$); PBS, phycobilisome; PS, photosystem

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tion centres that are excited only by light absorbed by Chl. There have been conflicting reports concerning the presence of PBS-decoupled PS II centres in PBS-containing organisms [17,18]. Here we present evidence for such a population of PS II centres and we show that adaptation to state 1 greatly decreases this population.

2. MATERIALS AND METHODS

Cells of the cyanobacterium *Synechococcus* 6301 were grown at 30°C under white light in inorganic liquid medium. The cells were bubbled with 3% CO_2 in air. Cells were grown to late-logarithmic phase and then diluted to a concentration of about 2 μg Chl $a \cdot ml^{-1}$ and grown under the same conditions for about 5 h. The cells were then harvested by centrifugation and resuspended to a concentration of 10 μg Chl $a \cdot ml^{-1}$ in fresh growth medium. All the results presented here were obtained from the same batch of cells, within about 3 h. The bulk of the cell suspension was kept in the dark while measurements were in progress.

Fluorescence induction (the time-evolution of fluorescence following the addition of an excitation light) was measured with a home-made apparatus incorporating a very small measuring cuvette (3 × 3 × 3 mm) to minimise distortion of the signal due to shading of the sample. The excitation light was defined by a monochromator set to either 435 nm or 620 nm (bandwidth 10 nm). Fluorescence was detected by a photomultiplier screened by a Schott RG685 filter. Where appropriate, cells were exposed for 1 s to 633 nm light from a He-Ne laser prior to the fluorescence induction measurement. The laser beam was dispersed to ensure even illumination of the sample. The intensity of illumination was controlled with neutral density filters. Both the pre-illumination light and the light used for fluorescence induction were controlled with electronic shutters opening in about 2 ms. The delay between the end of the pre-illumination and the recording of the emission was 68 ms.

For state 2, cells were dark-adapted for 3 min before the measurement [7]. Fluorescence induction was recorded in the presence of DCMU at 20 μM. For state 1, cells were exposed for 3 min in a stirred cuvette to a far-red light [7] defined by a Schott RG695 filter at an intensity of 0.5 $W \cdot m^{-2}$. The light was extinguished, DCMU was added to 20 μM and the cells were pumped into the measuring cuvette where the fluorescence induction was recorded. The delay between the removal of the far-red light and the recording of the transient was 20 s.

All measurements were performed at 25°C.

3. RESULTS AND DISCUSSION

We have recorded fluorescence induction transients (see [19] for a review) with two alternative excitation wavelengths, 435 nm and 620 nm. 435 nm light is strongly absorbed by Chl *a* and may therefore be expected to excite all active PS II centres. 620 nm light selectively excites phycocyanin, and will therefore strongly excite only those PS II centres that are functionally coupled to phycobilisomes; other PS II centres will be only weakly excited. Any PBS-decoupled PS II centres will contribute to the emission obtained with 435 nm excitation, but will make no significant contribution to the emission obtained with 620 nm excitation, since under the latter excitation they will be closed slowly and will fluoresce little. Fluorescence induction transients were recorded in the presence of the herbicide DCMU, which prevents the re-opening of PS II centres due to electron transfer to the plastoquinone pool. DCMU prevents the redox state of the plastoquinone pool from having any direct effect on PS II fluorescence yield.

A 1 s, 633 nm pre-illumination was used in order selectively to close those PS II centres that are functionally coupled to phycobilisomes. The pre-illumination had no effect on the F_m level of fluorescence, indicating that it was too brief to change the light-state of the cells (not shown). This also indicates that both excitation lights used were sufficiently bright for F_m to be attained by the end of the 1 s measuring time. As the intensity of the pre-illumination was increased, F_v was reduced due to the closure of PS II reaction centres prior to the recording of the emission. About 8% of F_v could not be removed by the pre-illumination, however intense (not shown). This indicates that a small proportion of PS II centres reopened during the 68 ms dark-interval between the pre-illumination and the recording of the emission.

Fig.1 shows the effect of various intensities of the pre-illumination on F_v obtained with 435 nm excitation and 620 nm excitation. These results were obtained from dark-adapted cells which are close to light-state 2 [2]. At low intensites of the pre-illumination, the proportional decrease in F_v caused by the pre-illumination is less with 435 nm excitation than with 620 nm (fig.1). This pattern was consistently observed in measurements on many cell samples. This suggests that there is a population of PS II centres that are not functionally coupled to phycobilisomes, but which can be excited by Chl-absorbed light. A proportion of the F_v obtained with 435 nm light can therefore be removed only by high intensities of 633 nm pre-illumination (fig.1).

We have investigated the effect of adaptation to light-state 1 on the population of PS II centres that are

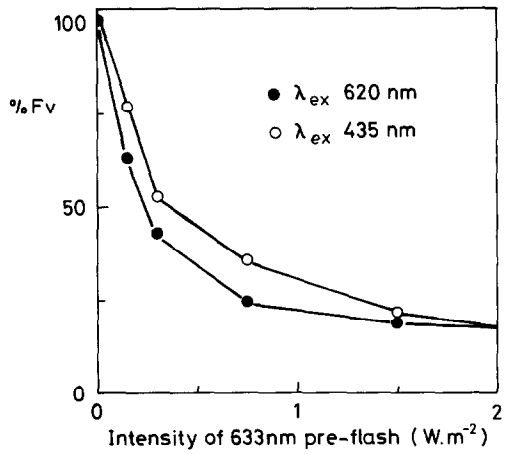

Fig.1. Effect of various intensities of a 633 nm pre-illumination on F_v for 435 nm or 620 nm excitation. "% F_v" is relative to the F_v obtained with the appropriate excitation wavelength with no pre-illumination. Signals were obtained from dark-adapted cells (state 2) and were summed from 5 repeats.

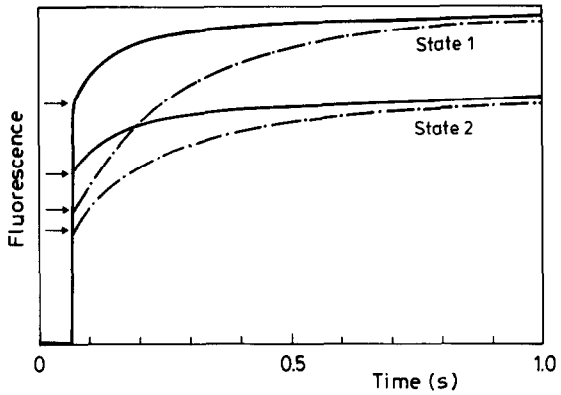

Fig.2. Effect of a 633 nm pre-illumination (intensity 0.3 $W \cdot m^{-2}$) on fluorescence induction transients obtained with 435 nm excitation for cells in state 1 and in state 2. Signals were obtained from single shots. Arrows indicate F_0. Solid line, with pre-illumination; broken line, without pre-illumination.

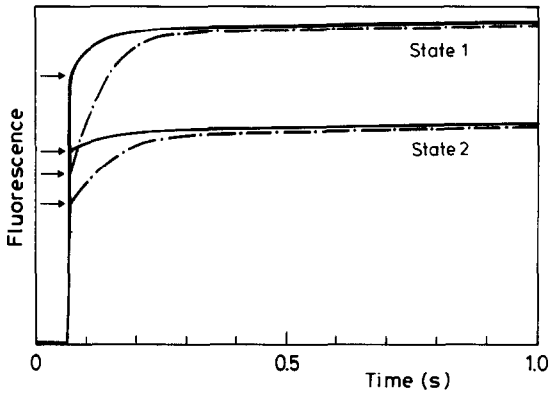

Fig.3. Effect of a 633 nm pre-illumination (intensity 0.3 $W \cdot m^{-2}$) on fluorescence induction transients obtained with 620 nm excitation for cells in state 1 and in state 2. Signals were obtained from single shots. Arrows indicate F_0. Solid line, with pre-illumination; broken line, without pre-illumination.

not coupled to phycobilisomes. Fig.2 shows fluorescence induction transients recorded with Chl-absorbed (435 nm) light in cells adapted to light-states 1 and 2. The signals show the characteristic effects of state transitions as observed by fluorescence generated by light absorbed by Chl; the state 2 transition results in a substantial decrease in F_m and a comparatively small decrease in F_0 [15]. The pre-illumination with PBS-absorbed light consistently reduces F_v to a greater extent in state 1 than in state 2 (fig.2 and table 1), suggesting that adaptation to state 1 increases the proportion of PS II centres that are coupled to phycobilisomes. The effect was observed at two intensities of the pre-illumination (table 1).

To check that the effect of the state transition on the extent to which PS II centres are closed by the 633 nm pre-illumination is not a trivial consequence of a faster rate of PS II trap closure in state 1, we have compared fluorescence induction transients with 620 nm excitation for cells in state 1 and in state 2, with and without a 633 nm pre-illumination (fig.3). The signals show the characteristic effects of state transitions as observed by fluorescence generated by light absorbed by phycocyanin; the state 2 transition decreases both F_m and F_0 [15]. The pre-illumination with 633 nm light consistently reduces F_v to a similar extent in state 1 and in state 2 (fig.3 and table 1), in contrast to the result obtained with 435 nm excitation (fig.2 and table 1). We therefore

Table 1

Effect of state transitions and a 633 nm pre-flash on fluorescence induction transients obtained with Chl- and PBS-absorbed light.

Light-state	Pre-flash intensity ($W \cdot m^{-2}$)	Excitation wavelength (nm)	F_0	F_m	F_v	% F_v
1	0	435	0.41	1.00	0.59	100
1	0.3	435	0.75	1.01	0.27	45
1	0.75	435	0.86	1.01	0.14	24
2	0	435	0.35	0.75	0.39	100
2	0.3	435	0.53	0.76	0.23	58
2	0.75	435	0.62	0.77	0.15	38
1	0	620	0.53	1.00	0.47	100
1	0.3	620	0.84	1.00	0.16	34
1	0.75	620	0.91	1.00	0.09	19
2	0	620	0.44	0.69	0.25	100
2	0.3	620	0.60	0.69	0.09	36
2	0.75	620	0.64	0.69	0.05	22

Fluorescence levels are expressed relative to F_m in state 1 with the appropriate excitation wavelength. "% F_v" is relative to the F_v obtained under the same conditions with no pre-illumination. "F_0" in pre-illuminated cells is not a true F_0 since some PS II centres were closed. F_0 with 620 nm excitation includes a contribution from phycobilisome components [15].

conclude that the differential effect of the 633 nm preflash on induction transients recorded with 435 nm excitation for cells in state 1 and in state 2 reflects a difference in the proportion of PS II centres that are coupled to phycobilisomes. On transition to state 1, the proportion of phycobilisome-decoupled PS II centres is reduced. However, a residual population of decoupled centres remains: this is indicated by the fact that the 633 nm pre-illumination reduces F_v obtained with 435 nm excitation to a smaller extent than F_v obtained with 620 nm excitation even in state 1 (table 1). It is possible that a complete adaptation to state 1 would cause the coupling of all PS II centres to phycobilisomes. In our state 1-adapted cells, there was an unavoidable 20 s delay between the end of the far-red illumination and the recording of the transient, it is likely that some reversion to state 2 would occur during this time [15].

Diner [17] found that about half the PS II centres were not coupled to phycobilisomes in the red alga *Cyanidium caldarium* but others have found no evidence for PBS-decoupled PS II centres [18]. We suggest that the discrepancies may be the result of differences in the light-state of the cells, resulting from different growth conditions and experimental protocols.

The decoupling of PS II centres from the PBS may completely account for the effects of the state 2 transition as observed with PBS-absorbed light [15]. However, the state 2 transition also affects the distribution of light absorbed by chlorophyll [6,15]. This cannot directly be accounted for by the decoupling of PS II centres from phycobilisomes. The effect of state transitions on F_v as observed with Chl-absorbed light is smaller than that observed with PBS-absorbed light (see [15] and table 1). The effects of state transitions as observed with Chl-absorbed light may result from the altered environment of the PBS-decoupled PS II centres. One possibility is that PBS-decoupled PS II centres may be in closer contact with PS I, leading to greater spillover of energy from PS II to PS I [15]. Alternatively, changes in membrane environment or conformation could affect the rate constants for the electron-transfer processes in PS II; these processes are very sensitive to factors such as the transmembrane electrochemical potential [20].

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