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P-700 photooxidation in state 1 and state 2 in cyanobacteria upon flash illumination with phycobilin- and chlorophyll-absorbed light

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We have measured the flash yield of P-700 photooxidation in cells of the cyanobacteria *Synechococcus* 6301 and *Nostoc* MAC adapted to light-states 1 and 2. Using excitation at 337 nm, the flash yield of P-700 photooxidation at limiting flash intensity was larger in state 2 in both species, indicating an increased absorption cross-section of PS I in state 2 for light absorbed by both chlorophyll and phycobilin pigments. Using excitation at 532 nm, the flash yield of P-700 photooxidation at limiting intensity was also larger in state 2 in both species, indicating an increased absorption cross-section of PS I for light absorbed specifically by phycocyanin or phycoerythrin. Differences in P-700 re-reduction kinetics between states 1 and 2 were consistently observed following flash excitation at either wavelength. Our results are consistent with a model for redistribution of excitation energy in state 2 that involves decoupling of the phycobilisome from PS II and its functional reassociation with PS I.

Photosynthesis; Light harvesting; Photosystem I; State transition; Phycobilisome; Excitation energy distribution; Cyanobacteria; (*Nostoc* MAC; *Synechococcus* 6301)

1. INTRODUCTION

Photosynthetic organisms that contain two photosystems have the ability to vary the distribution of excitation energy between the photosystems and thereby maximize the overall efficiency of photosynthesis under any given light conditions. This phenomenon was first recognized by Bonaventura and Myers [1] and Murata [2]. They showed that selective excitation of PS I causes a transition to state 1, corresponding to an increased fraction of total absorbed excitation energy that is utilized by the reaction centre of PS II. Selective excitation of PS II causes a transition to state 2, by increasing the fraction of excitation energy that is utilized by PS I.

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Abbreviations: PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

The mechanism in phycobilisome-containing organisms by which the excitation energy is redistributed in state transitions remains controversial. Ley and Butler [3] and Bruce et al. [4] suggested a spillover model by which energy is transferred in the thermodynamically favoured direction from PS II to PS I but not vice versa. This model necessitates a close physical proximity between the antennae of the two photosystems. However, Allen et al. [5] proposed that in state 2 the phycobilisome is detached from PS II and becomes attached to PS I, causing a decrease of the absorption cross-section of PS II and an increase of the absorption cross-section of PS I. Mullineaux and Allen [6] more recently suggested a model by which the detached phycobilisome is not transferred to PS I in state 2 but instead the PS I and PS detached PS II reaction centre cores associate more closely.

Here we report results of experiments designed to address the question of the pathways of excitation energy transfer in the two light states in

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cyanobacteria. We have driven cells of *Synechococcus* 6301 and *Nostoc* MAC into states 1 and 2 and measured P-700 photooxidation using actinic laser flashes of 337 nm and 532 nm to excite chlorophyll *a* and phycobilins, respectively. The results obtained allow us to distinguish between the various models previously proposed.

2. MATERIALS AND METHODS

Nostoc MAC and *Synechococcus* 6301 were grown photoautotrophically at 35°C in BG11 [11] and medium C [7], respectively, under continuous stirring and bubbling with 5% CO_2 in N_2. *Nostoc* MAC cells were subcultured to a concentration of about 4 mg chlorophyll $a \cdot l^{-1}$ and grown for 20–24 h after subculturing. *Synechococcus* 6301 cells were subcultured to 1 mg chlorophyll $a \cdot l^{-1}$ and grown for 6 h after subculturing. Chlorophyll *a* determination was carried out as in [8] following extraction in hot methanol for *Nostoc* MAC and in 80% acetone for *Synechococcus* 6301.

Fluorescence measurements were made in a stirred cuvette at 22°C. Fluorescence was generated using a weak yellow light (light 2) and modulated at 870 Hz. This was provided by an array of yellow LEDs screened by a 650 nm short-pass optical filter (Hansatech, King's Lynn). Fluorescence was detected by a Hansatech photodiode which was screened by a 700 nm interference filter and connected to an amplifier locked-in to the frequency of the modulated light 2 [9]. Light 1 (maximum transmittance at 435 nm) was provided by a combination of Corning 5-60 and Corning 4-96 blue filters. Similar state transitions in *Nostoc* MAC were also obtained using a Perkin-Elmer LS 5 spectrometer at excitation wavelength of 540 nm (light 2) and an emission wavelength of 695 nm, with excitation modulated at 50 Hz (not shown). Light 1 was defined by the same combination of blue Corning filters. Similar results (not shown) were obtained for both species using a 709 nm interference filter to define light 1.

Prior to measuring P-700 photooxidation the cells were adapted to either light 1 or light 2. Light 1 was defined by the same combination of blue filters as described earlier. Light 2 was provided by a combination of Corning 4-96 and Ealing 560 filters for *Synechococcus* 6301 and a broad-band green and Corning 4-96 glass filters for *Nostoc* MAC.

Absorption measurements at 820 nm were made using a laser flash spectrophotometer as described by Mansfield et al. [10], except that the measuring device was a large area silicon photodiode type UDT 10D (United Detector Technology, from Optilas, UK). Samples were excited at either 337 nm with a 800 ps flash supplied by a N_2 laser (LN 1000, Photochemical Research Associates, USA) or 532 nm with a 6 ns flash using a Nd:YAG laser (DCR-11 from Spectra-Physics Ltd, UK).

3. RESULTS

The fluorescence emission of PS II is dependent in part on the absorption cross-section of PS II and hence can be used as an indicator of light state transitions [13]. Fig.1a shows characteristic state 1 and state 2 transitions in *Nostoc* MAC using a modulated fluorescence measurement system as described in [12]. The addition of light 1 shows a fast fluorescence decrease due to opening of the PS II traps by increasing PS I turnover. A slow rising phase is also observed that can be attributed to an increase in the absorption cross-section of PS II and to the relaxation of photochemical quenching of PS II fluorescence because of the increase in PS II turnover. These changes are characteristic of the state 1 transition [2]. When light 1 is extinguished there is a rapid rise in fluorescence due to the closure of PS II traps. This is followed by a slow falling phase which is caused by the PS II absorption cross-section decrease and by PS II traps reopening, characteristic of the state 2 transition [2].

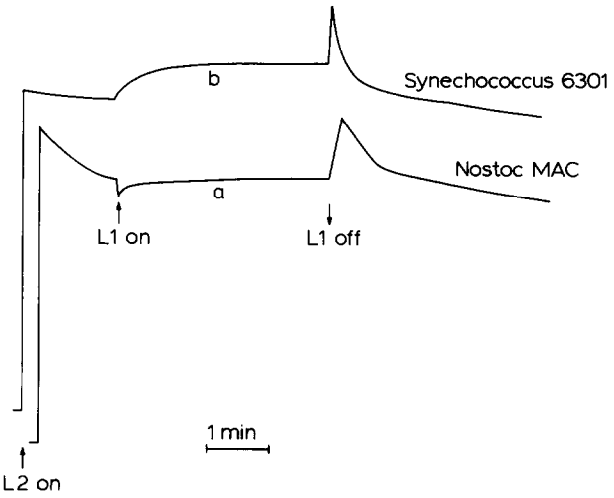

Fig.1. State 1-state 2 transitions in cells of *Nostoc* MAC and of *Synechococcus* 6301. Modulated fluorescence signal showing effects of background light 1 as described in the text.

Fig.1b shows characteristic state transition in *Synechococcus* 6301 as described in [20]. Light 1 induces a slow rise in fluorescence which we also interpret as a state 1 transition. When light 1 is extinguished there are a rapid rise and a slow falling phase most easily interpreted as a state 2 transition.

The light absorbed by PS I is proportional to the incident light intensity and to the effective absorption cross-section of PS I reaction centres [14]. We have measured the flash-induced absorbance change at 820 nm (ΔA_{820}) which in the microse-

cond time range has been attributed essentially to P-700$^+$ [15].

Fig.2 shows the absorption change at 820 nm induced by a 337 nm laser when the cells are adapted into state 1 or state 2. These data demonstrate that in state 2 there is an increase in the absorption cross-section of PS I for light at 337 nm. The absorption decay at 820 nm following a laser flash during state 1 is monophasic ($t_{1/2}$ of about 6 ms) in both species. However, multiphasic kinetics are observed in state 1, $t_{1/2}$ = 140 μs (60% of initial A amplitude) and $t_{1/2}$ = 2–3 ms (40%) for *Nostoc* MAC and $t_{1/2}$ = 105 μs (50%) and $t_{1/2}$ = 8 ms (50%) for *Synechococcus* 6301.

Fig.3 shows that when using a 532 nm laser (light predominantly absorbed by the phycobilisome) the absolute yield of P-700 photooxidation is increased in state 2. These data suggest that there is an increase in the absorption cross-section of PS I for phycobilisome-absorbed

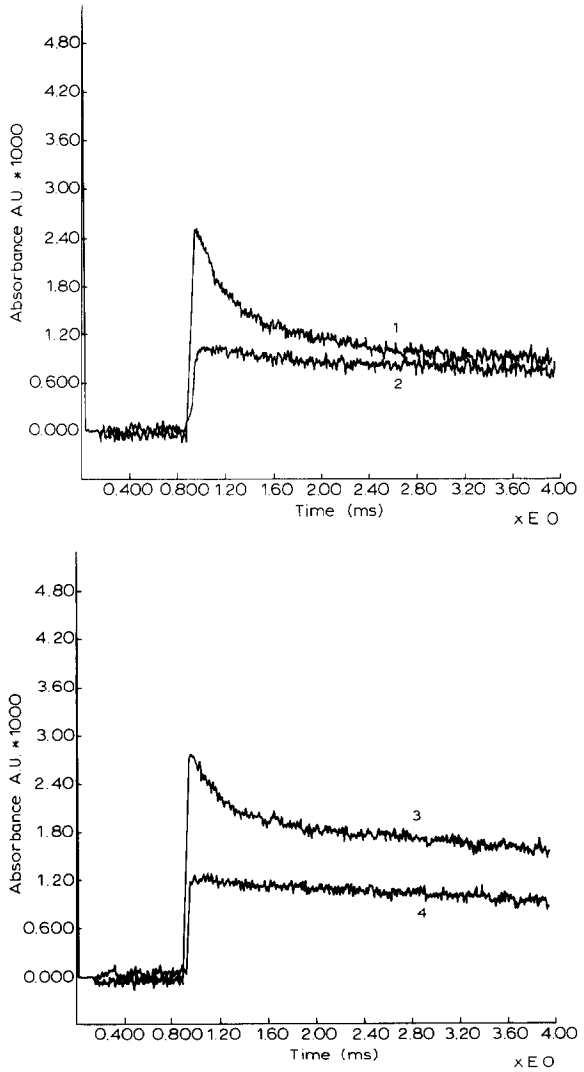

Fig.2. Absorption changes at 820 nm following laser excitation at 337 nm in *Nostoc* MAC in state 2 (1) and state 1 (2) and in *Synechococcus* 6301 in state 2 (3) and state 1 (4). Each measurement is the average of 32 flashes, using a path length of 10 mm at 20°C. The laser flash intensity was 2.5 mJ per pulse. Chlorophyll concentration was 5 $\mu g \cdot ml^{-1}$ for *Nostoc* MAC and 2.5 $\mu g \cdot ml^{-1}$ for *Synechococcus* 6301.

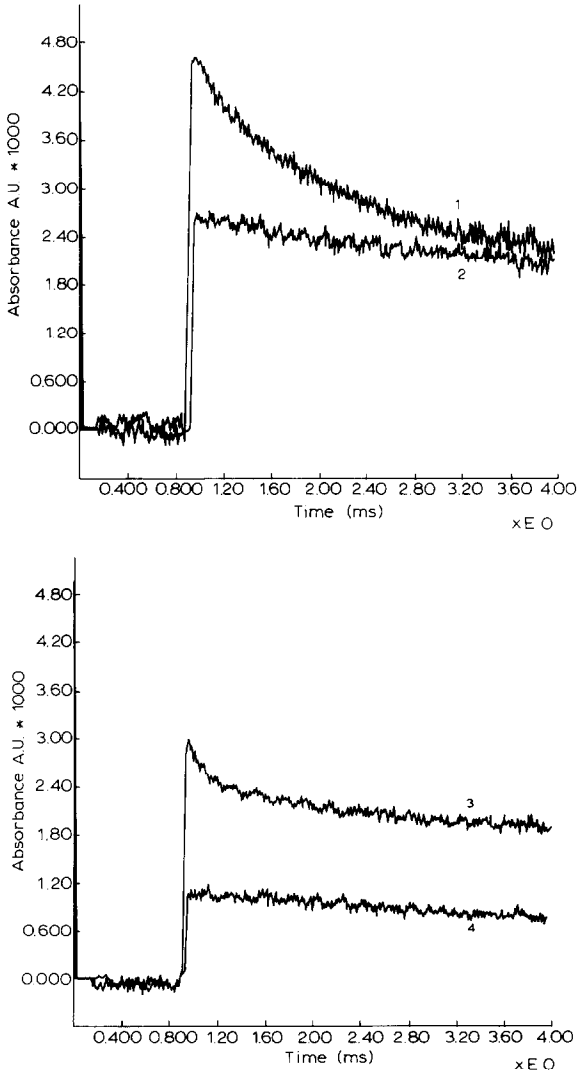

Fig.3. Effect of state transitions on the absorption change at 820 nm following laser excitation at 532 nm in *Nostoc* MAC in state 2 (1) and state 1 (2) and in *Synechococcus* 6301 in state 2 (3) and state 1 (4). Conditions are similar to those in fig.2.

light. The absorption decay (fig.2) after the 532 nm flash is similar to that after the 337 nm flash (fig.3).

Using 337 nm and 532 nm laser flashes an increase in the absolute yield of P-700$^+$ in state 2 in both species was observed (figs 2,3). At 532 nm only phycobilins can absorb and the increased P-700 photooxidation is therefore consistent with a mobile phycobilisome being transferred to PS I in state 2. At 337 nm chlorophyll *a* absorbs as well as phycocyanin, so this wavelength is not very specific for excitation of chlorophyll *a*. Thus our results at this wavelength could also be explained by the mobile phycobilisome model. However, we were unable to test the proposal that there is a spillover of energy from PS II to PS I. To test this will require a laser excitation beam in the blue region of spectrum where chlorophyll *a* selectively absorbs.

4. DISCUSSION

From fluorescence induction transients [6,12] and time-resolved fluorescence emission spectra [16], it has been previously shown that the state transitions in cyanobacteria involve changes in the efficiency of the energy transfer from the phycobilisome to PS II. In state 2 a proportion of PS II core complexes become functionally decoupled from the phycobilisome [5]. The effects of chlorophyll-absorbed light (fig.2) and phycobilisome-absorbed light (fig.3) on P-700 photooxidation are therefore difficult to reconcile with the spillover model of the excitation energy distribution in light states. Also, our observations are not obviously compatible with the earlier model of Mullineaux and Allen [6] which involves decoupling of PS II from the phycobilisome state 2 and functional reassociation of PS II chlorophyll with PS I.

However, our results are in agreement with the predictions of the model proposed by Allen et al. [5]. Thus in state 2 the phycobilisome becomes detached from PS II and the detached phycobilisome then functionally associates with PS I thereby increasing the absorption cross-section of PS I for phycobilisome-absorbed light. Recent results of Rehm et al. [17] with two species of red algae show a decrease in the quantum yield of PS I after illumination with light 1. We believe that this is consistent with the operation in red algae of the mechanism [5] supported by the results we present here for cyanobacteria.

The mechanism by which state transitions are controlled in phycobilisome-containing organisms remains controversial. Murata [2], Duysens [18] and also Ried and Reinhardt [19] proposed that state transitions in red algae are controlled by the redox state of an inter-photosystem electron carrier, a conclusion supported by the results of Mullineaux and Allen [21] and consistent with the accepted mechanism for green plant chloroplasts [22,23]. However, Satoh and Fork [20] proposed that state transitions in cyanobacteria are controlled by cyclic electron transport around PS I, state 1 being induced by high rates of cyclic electron transport. Biggins et al. [24] suggested that state 1 is induced by localised electrochemical gradients around PS I which cause a small conformational change. Biggins [25] also reported an increase in cytochrome *f* oxidation by broad-band actinic illumination with light 2 in state 2 in *Porphyridium cruentum*, but no changes were observed by illumination with light 1, in apparent contrast with the results of Rehm et al. [17].

The differences in kinetics of P-700 re-reduction that we have observed between state 2 and state 1 allow us also to draw conclusions about the mechanism that drives state transitions in cyanobacteria, as follows. Our data show that in state 2 the P-700 re-reduction kinetics are biphasic while in state 1 they are monophasic, with no fast phase (fig.2). This difference in kinetics could be attributed to the redox state of an electron carrier which is located in the donor site of PS I: reduction of this carrier in state 2 causes the biphasic kinetics, while oxidation of the electron carrier in state 1 causes the monophasic kinetics. Haehnel et al. [26] also observed biphasic kinetics of P-700 re-reduction in intact chloroplasts upon pre-illumination at 655 nm (in terms of light states, light 2) and monophasic kinetics upon far-red pre-illumination (light 1). It is also now known that in PS I linear electron transport occurs according to the following scheme [27–29]:

$$PQ \longrightarrow Cyt f \longrightarrow PC \longrightarrow P\text{-}700$$

Finally we conclude that state transitions in cyanobacteria are regulated by the redox state of an intermediate electron carrier, possibly plasto-

quinone [21]. The reduction of this electron carrier may trigger activation of a protein kinase causing the phosphorylation of light-harvesting polypeptides [30–32] as is the case in chloroplasts [22,23]. Whatever the biochemical basis for redistribution of excitation energy in phycobilisome-containing organisms, it is now likely that it involves redox control of the absorption cross-section of PS II and PS I by detachment of PS II from the phycobilisome and reattachment of the phycobilisome with PS I. The relevance of these results to the question of absorption cross-section changes in green plants is under further investigation [33–35].

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