

## Fluorescence induction transients indicate altered absorption cross-section during light-state transitions in the cyanobacterium *Synechococcus* 6301

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**State 1-State 2 transitions in the cyanobacterium *Synechococcus* 6301 were observed using a lock-in amplifier to detect the fluorescence generated by a modulated excitation beam. Millisecond fluorescence induction transients were recorded for cells in State 1 and State 2. Comparison of the transients suggests that excitation energy distribution in this cyanobacterium is regulated by changes in the absorption cross-section of Photosystem II.**

Plants and cyanobacteria can adapt to changes in the spectral quality of actinic light by regulating the distribution of absorbed excitation energy between PS I and PS II so as to maximise the efficiency of light-energy utilisation [1-3]. In green plants light-state adaptation involves the redistribution of LHC II between PS I and PS II, i.e., a change in the absorption cross-section of the two photosystems [4-6]. The process requires protein phosphorylation which is catalysed by a kinase whose activity is controlled by the redox state of the plastoquinone pool [7,8].

This model cannot, without modification, be applied to the cyanobacteria or the red algae, since these organisms accomplish light-harvesting largely by extrinsic phycobiliprotein complexes called phycobilisomes [9], rather than by an LHC

II. Furthermore, the thylakoid membranes of these organisms do not exhibit the partial appression and lateral heterogeneity which have been postulated to play a crucial role in light-state adaptation in higher plants [10,11].

Biggins and his co-workers have proposed a model for light-state adaptation in phycobilisome-containing organisms which differs radically from that proposed for higher plants in that it does not require protein phosphorylation or other covalent modification and in that light-energy distribution is regulated by adjusting the extent of spill-over of excitation energy from PS II to PS I rather than by changing the absorption cross-sections of the two photosystems [12]. However, it has recently been shown that changes in the phosphorylation state of several polypeptides do accompany state transitions in the cyanobacterium *Synechococcus* 6301 [13,14].

Here we show that the time-scale of state transitions in this organism is consistent with a protein phosphorylation reaction, contrary to the results of Biggins and Bruce with *Porphyridium cruentum* [15]. We also show that the absorption cross-section

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea; LHC II, light-harvesting chlorophyll *a/b* protein complex; PS, Photosystem; PQ, plastoquinone;  $F_0$ , initial level of fluorescence;  $F_m$ , maximal level of fluorescence;  $F_t$ , fluorescence at time *t*; light 1, a light preferentially exciting PS I; light 2, a light preferentially exciting PS II.

tion of PS II is greater in State 1 than State 2.

State transitions in *Synechococcus* 6301 were observed by a method similar to that recently used with intact leaves [5]. A weak, modulated, yellow light was used both to drive State 2 transitions and to excite PS II fluorescence, which was detected by a photodiode with lock-in amplifier [16]. The fluorescence yield of PS II is partly dependent both on absorption cross-section and on the extent of spillover, and hence can reflect the extent of light-state adaptation in the cells [17]. State 1 transitions were driven by superimposing a stronger, continuous, blue light, whilst using the modulated yellow light as a measuring beam.

A typical result is shown in Fig. 1. When cells adapted to State 2 were exposed to the blue light 1, there was a small drop in fluorescence due to the oxidation of the PQ pool by PS I and the consequent net opening of PS II reaction centres. The amplitude of the fluorescence drop was frequently greater than that observed in the experiment of Fig. 1. The drop was followed by a slower rising phase most easily interpreted as being due to a state transition involving redistribution of excitation energy from PS I to PS II. When the blue light was extinguished, these effects were reversed. These fluorescence changes were consistently observed, although their magnitude varied with growth conditions. Under the conditions used, the half-time for the State 2 transition was about 50 s, while that for the State 1 transition was about 20 s. Addition of DCMU to cells in State 2 caused a biphasic fluorescence rise as photochemical quenching relaxed (fast phase) and as the cells were driven to State 1 (slow phase). The slow phase was absent from cells in State 1 (results not shown).

Information on the nature of state transitions can be obtained by comparing the relative values of  $F_0$ , the minimum fluorescence yield of PS II observed when all the centres are open, and  $F_m$ , the maximal fluorescence yield of PS II when all the centres are shut [17]. If a change in spill-over is involved, the increase in  $F_0$  on transition from State 2 to State 1 should be small in comparison with the increase in  $F_m$ . If, however, a change in absorption cross-section is involved, as in the model of Allen et al. [13],  $F_0$  and  $F_m$  should increase in proportion, so the ratio  $F_0/F_m$  should

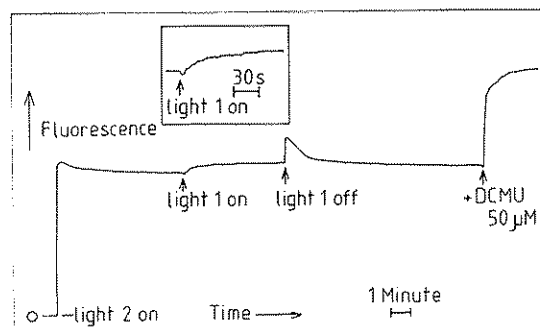


Fig. 1. State 1–State 2 transitions in cells of *Synechococcus* 6301 (*Anacystis nidulans*). Cells were grown as in Ref. 13 and taken in early log-phase (5–7 h after subculturing). ‘Light 2’ was provided by an array of yellow LEDs (Hansatech, Kings Lynn) screened with a short-pass optical filter blocking the transmission of any wavelengths greater than 650 nm, and was modulated at 870 Hz. Fluorescence was detected with a Hansatech photodiode which was screened by a 700 nm interference filter and which was connected to an amplifier locked-in to the frequency of the modulated light 2 [16]. ‘Light 1’ was defined by a Corning 5-60 blue filter. The experiment was performed in a stirred cuvette at 22°C. The chlorophyll *a* concentration (measured as in Ref. 18) was 4  $\mu\text{g}/\text{ml}$ . The inset shows the effect of addition of light 1 at a scale expanded proportionately on both axes.

remain constant, as it does under physiological conditions in green plants [4–6].

In order to test this point, millisecond fluorescence transients of cells in State 1 and State 2 were recorded in the presence of DCMU (Fig. 2a). In the case of cells in State 2, a 3-s preillumination with the blue light 1 was necessary before the addition of the DCMU, in order to ensure that all the PS II centres were open and a true  $F_0$  was observed. This brief illumination serves to open all the centres without significantly driving the cells towards State 1 (see Fig. 1).

$F_0$  was significantly higher in State 1 than in State 2, indicating that the absorption cross-section of PS II is greater in State 1 than in State 2. However, the ratio  $F_0/F_m$  did not remain constant, being consistently slightly lower in State 1. It is possible that a general realignment of light-harvesting pigments and reaction centres in the membrane results in a change of spill-over in addition to a change in cross-section, causing a disproportionately large change in  $F_m$  as compared with the change in  $F_0$ . The measured  $F_0$  may

include a significant component of fluorescence from allophycocyanin; we have no reason to suppose that such a component would change significantly with light state. This would also reduce the

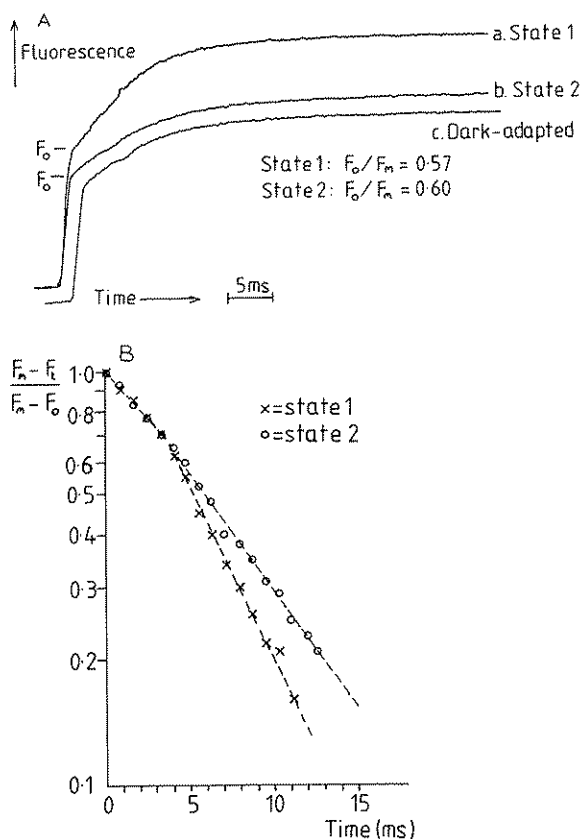


Fig. 2. (A) Fluorescence induction transients of *Synechococcus* 6301 in State 1, State 2 and dark adapted. Cells were put into State 1 or State 2 under the conditions described in the caption to Fig. 1. (a) Cells were illuminated with light 2 for 10 min and then for 10 min with light 1 before measurement of the transient. (b) Cells were illuminated with light 2 for 10 min and then for 3 s with light 1 before measurement of the transient. (c) Cells were dark-adapted for 30 min before measurement of the transient. (Transient off-set on both time and fluorescence axes.) In all cases DCMU was added to 50  $\mu$ M at the end of the pre-illumination. After 10 s dark incubation the stirrer was switched off and the cells were then exposed to a light defined by a Corning 4-96 filter and controlled by a Uniblitz electronic shutter opening in approx. 1.5 ms. Fluorescence was detected with a Hansatech photodiode screened by an Ealing 680 nm interference filter. The amplified signal was recorded using a digital storage oscilloscope and X-Y plotter (Farnell, Wetherby). (B) The data of (A) are plotted semi-logarithmically. Note that the plot for the State 1 transient is more biphasic than that for State 2, indicating a markedly more sigmoidal transient for State 1.

apparent change in  $F_0$  as compared with the change in  $F_m$  and is consistent with the high ratio  $F_0/F_m$  which we observed in this organism: the ratio observed in green plants is considerably lower [4-6]. Fork and Satoh have observed a similarly high ratio  $F_0/F_m$  in the thermophilic cyanobacterium *Synechococcus lividus* [3]. Semi-log plots of the induction transients (Fig. 2b) reveal that the transient in State 1 is markedly more sigmoidal than that in State 2, indicating greater co-operativity and hence connectivity of PS II reaction centres in State 1. This co-operativity appears to result from the association of two PS II reaction centres with each phycobilisome [19]. The loss of co-operativity on transition to State 2 therefore suggests that at least some PS II reaction centres become dissociated from the phycobilisome in State 2. The fluorescence induction transient for dark adapted cells was similar to that for cells in State 2, indicating that the dark state under these conditions is State 2 (Fig. 2a).

We conclude that State 1-State 2 transitions occur on comparable time-scales in cyanobacteria and green plants, and that a change in absorption cross-section occurs during state-transitions in both groups of organisms. Our results are inconsistent with a purely spill-over model for cyanobacterial state-transitions [12], but are consistent with a protein phosphorylation model involving mobility of the light-harvesting phycobilisome [13,20].

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