P-700 PHOTOOXIDATION IN STATE 1 AND IN STATE 2 IN CYANOBACTERIA UPON FLASH ILLUMINATION WITH PHYCOBILIN AND CHLOROPHYLL ABSORBED LIGHT.

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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. Bonaventura and Myers [1] and Murata [2] showed that selective excitation of PS I causes the transition to state 1 and selective excitation of PS II causes the transition to state 2.

The mechanism in phycobilisome-containing organisms by which the excitation energy is redistributed in state transitions remains controversial. Ley and Butler [3] suggested a spillover model by which energy is transferred from PS II to PS I but not vice versa. However Allen et al. [4] proposed that in state 2 the phycobili- some is detached from PS II and becomes attached to PS I, causing a decrease of the absorption cross-section of PS I and an increase in the absorption cross-section of PS I. Mullineaux and Allen [5] more recently suggested a model by which the detached phycobilisome does not couple to PS I but instead the PS I and the detached PS II reaction centre cores associate more closesly.

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

2. MATERIALS AND METHODS

Nostoc MAC and Synechococcus 6301 were grown as in [6]. Fluorescence measurements were made in a stirred cuvette at 22 °C as in [7]. Absorption measurments at 820 nm were made using a laser flash spectrophotometer as described in [8], except that the measuring device was a large area silicon photodiode type UDT 10D. Samples were excited at either 337 nm with a 800 ps flash supplied by a N_2 laser or 532 nm with 6 ns flash using a Nd:YAG laser.

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3. RESULTS AND DISCUSSION

The fluorescence emission of PS II is dependent in part on the absorption cross-section of PS I and hence can be used as an indicator of light state transitions.

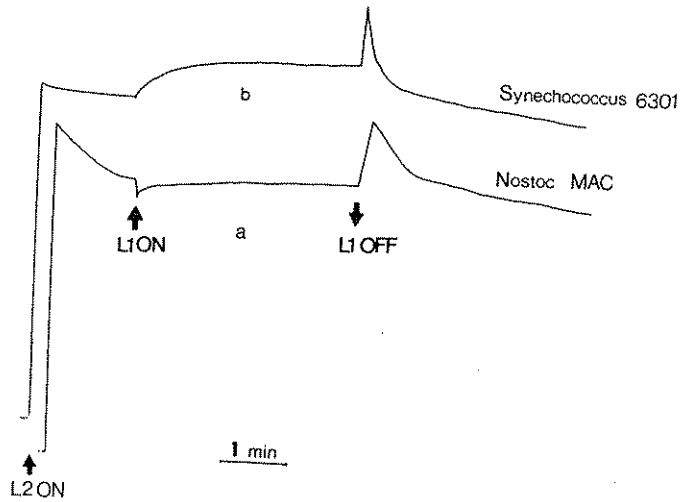


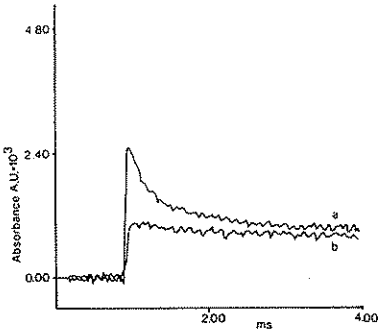
FIG.1

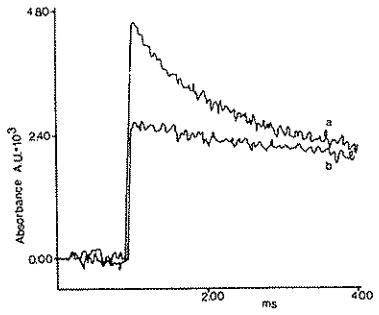
Fig. 1a shows characteristic state 1 and state 2 transitions in Nostoc MAC using a modulated fluorescence measurement system as described in [6]. Light 1 induces a fast fluorescence decrease and a slow rising phase which are characteristic of the state 1 transition [2]. When light 1 is extinguished there is a rapid rise of fluorescence followed by a slow falling phase and these are interpreted as the state 2 trasition [2].Fig. 1b shows characteristic state transitions in Synechococcus 6301.

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 [9]. We have measured the flash-induced absorbance change at 820 nm which in microsecond time range has been attributed essentially to $P\text{-}700^{+}$ [10]. Fig. 2 shows absorption changes at 820 nm following laser excitation at 337 nm in Nostoc MAC in state 2 (a) and in state 1 (b) and for Synechococcus 6301 in state 2 (a) and state 1 (b). These data demonstrate that in state 2 there is an increase in the absorption cross-section of PS I for light at 337 nm.

Fig. 3 shows that when using a 532 nm laser (light predominantly absorbed by the phycobilisome) in Nostoc MAC in state 2 (a) and state 1 (b) and in Synechococcus 6301

in state 2 (c) and in state 1 (d), the absolute yield of P-700 photooxidation is increased in state 2.





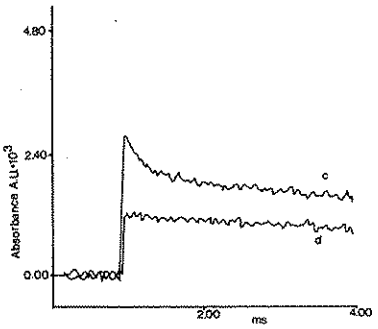


FIG.2

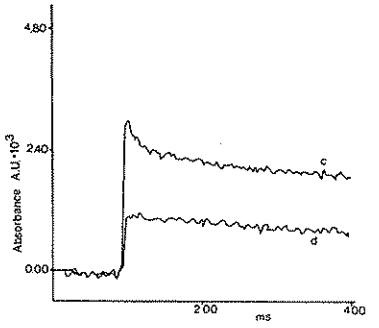


FIG.3

These results suggest that there is an increase in the absorption cross-section of PS I for phycobilisome-absorbed light. The absorption decay after the 337 nm and 532 nm flashes is the same for both species. Thus, the absorption decay is monophasic during state 1 ($t_{1/2}$= 8-10 ms) but multiphasic kinetics in state 2 ($t_{1/2}$= 100-150 us and $t_{1/2}$ = 8-10 ms).

Using 337 nm and 532 nm laser flashes an increase in the absolute yield of $P\text{-}700^{+}$ in state 2 in both species was observed. 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 [4].

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 1 the P-700 re-reduction kinetics are biphasic while in state 1 they are monophasic, with no fast phase (figs 2,3). DBMIB (5 uM) also removes the fast phase in state 2 (data not shown). This difference in kinetics could be due to the redox state of an electron carrier which is located at the donor site of PS I: reduction of this carrier in state 2 causes the biphasic kinetics, while oxidation of this carrier causes the monophasic kinetics. Haehnel et al. [11] also observed biphasic kinetics of P-700 re-reduction in intact chloroplasts upon pre-illumination at 655 nm and monophasic upon far-red pre-illumination. Finally we conclude that state transitions in cyanobacteria are regulated by the redox state of plastoquinone [7]. The reduction of this electron carrier may trigger activation of a protein kinase causing the phosphorylation of light harvesting-polypeptides [12-13]. 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 phycobisome and reattachment of the phycobilisome to PS I.

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