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## The rate of P-700 photooxidation under continuous illumination is independent of State 1-State 2 transitions in the green alga *Scenedesmus obliquus*

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Transitions between light-states 1 and 2 (State 1-State 2 transitions) were demonstrated in cells of *Scenedesmus obliquus* as characteristic, slow changes in the yield of variable chlorophyll fluorescence. The transitions were induced by 690 nm light 1 (absorbed predominantly by Photosystem I) and by 650 nm light 2 (absorbed predominantly by Photosystem II). Light-states 1 and 2 were probed further by chlorophyll fluorescence induction kinetic measurements which indicated a decrease of up to 20% in the absorption cross-section of Photosystem II on the transition from State 1 to State 2. No complementary change in the absorption cross-section of Photosystem I could be detected in measurements of the rate of P-700 photooxidation under 640 nm continuous illumination at limiting intensity. A marginal (approx. 3%) average increase in half-time of P-700 photooxidation on transition to State 2 was recorded but is probably within the limits of accuracy of the measurement. It is concluded that excitation energy is diverted away from the reaction centre of Photosystem II in State 2, but that the energy does not become redirected in such a way as to increase the rate of Photosystem I light-absorption in *S. obliquus* in vivo. This in turn suggests that the immediate phenomenon of State 1-State 2 transitions may reflect primarily regulation of excitation energy transfer from the light-harvesting chlorophyll *a/b* complex to the Photosystem II reaction centre. This conclusion is discussed in view of earlier studies on the effect of thylakoid protein phosphorylation in vitro.

### Introduction

Green plants, algae and cyanobacteria possess a regulatory mechanism by which the relative dis-

tribution of excitation energy between the two photosystems becomes adjusted and optimized in response to variations in the light-quality and light-intensity of the environment [1,2]. This response presumably ensures a balanced utilization of photons by the two photochemical reaction centres [3]. Selective excitation of PS I causes a transition to State 1, corresponding to a higher fraction of excitation energy utilized by the reaction centre of PS II. Selective excitation of PS II causes a transition to State 2, that is, an increase in the fraction of excitation energy that is utilized by the reaction centre of PS I. State 1-State 2 transitions, therefore, are thought to represent short-term adaptations to environmental or

Abbreviations: PS, photosystem; Chl, chlorophyll; P-700, reaction centre Chl of PS I; LHC I, light-harvesting Chl-protein complex of PS I; LHC II, light-harvesting Chl *a/b*-protein complex of PS II;  $F_0$ , value of fluorescence when all PS II traps are open;  $F_m$ , value of fluorescence when all PS II traps are closed;  $F_v$ , variable fluorescence ( $F_v = F_m - F_0$ );  $v_f$ , initial rate of fluorescence increase above  $F_0$ ; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

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metabolic changes that would otherwise result in excitation energy being delivered to one or other photosystem in excess of its capacity to convert it into electrochemical potential.

State 1–State 2 transitions are a clear example of a homeostatic process, and they are generally thought to result in the maintenance of a high quantum yield of photosynthesis by means of minimization of total non-photochemical de-excitation. This view depends on the assumption that excitation energy diverted away from PS II in State 2 becomes available instead to PS I, and vice versa. This view has been widely expressed: references [3–6] to review articles are examples only. A different view of the immediate function of state transitions is that they serve merely to control the rate of excitation energy transfer to PS II. This latter view implies that a State 2 transition cannot directly increase overall quantum yield: excitation energy surplus to the photochemical requirements of PS II would not be conserved by redirection to PS I. In this view the State 2 transition would more likely serve as a mechanism to prevent over-excitation of PS II.

In general, the results from steady-state fluorescence spectroscopy have been interpreted to support diversion of excitation energy to PS I at the expense of PS II, while measurements of electron transport rates and kinetics of turnover have been used (with some exceptions) to support the idea that state transitions affect only the absorption cross-section of PS II. Much of this earlier work has been carried out on isolated thylakoids, and has relied on the discovery that phosphorylation of polypeptides of the light-harvesting complex LHC II is the molecular basis of the State 2 transition [7,8].

Thus fluorescence emission spectroscopy of thylakoids at 77 K has consistently shown increased 735 nm (PS I) fluorescence relative to 685 nm (PS II) fluorescence after phosphorylation of LHC II [7,9,10]. In order to permit conclusions to be drawn about redistribution of excitation energy, it is crucial that such studies should distinguish between relative and absolute increases in PS I fluorescence. With this objective, normalization of 77 K fluorescence spectra to emission from exogenous fluorescence standards such as fluorescein [11] and phycoerythrin [12] has been car-

ried out. The results [11,12] indeed suggest that protein phosphorylation in thylakoids gives an absolute increase in PS I fluorescence emission together with an absolute decrease in that from PS II. This conclusion holds for phosphorylated thylakoids from spinach [11] and *Chlorella* [12] as well as for the State 2 transition measured in *Chlorella* cells [12].

A number of attempts have been made to demonstrate the increased PS I absorption cross-section that is implied by increased PS I (735 nm) fluorescence emission. Horton and Black [13] measured kinetics of cytochrome *f* photooxidation by continuous red light in the presence of DCMU in phosphorylated and unphosphorylated pea thylakoids and found no significant difference, despite a 25–30% decrease in chlorophyll fluorescence on phosphorylation. Haworth and Melis [14] and Deng and Melis [15] found no difference between phosphorylated and control spinach thylakoids with respect to kinetics of P-700 photooxidation, despite appreciably slower PS II kinetics. Moreover, in excitation spectra measurements at 4 K with intact algae, Kramer et al. [16] failed to detect a State 2-induced change in the distribution of Chl *b* excitation between PS II and PS I. In contrast, Telfer and co-workers [17,18] have measured P-700 photooxidation at 820 nm by single-turnover flashes in pea thylakoids, and conclude that phosphorylation increases the absorption cross-section of PS I by 12% [18].

Measurements of PS I electron transport rates [19–21] have also produced at best limited support for the idea of complementary changes in absorption cross-sections of PS II and PS I, with estimates of the increase in PS I absorption cross-section ranging from negligible [19] to 8% as an upper limit even with 650 nm excitation [20], which should produce the maximum effect if relocation of the Chl *b*-containing LHC II is involved. A decrease in PS II electron transport rate of 18% and an increase in PS I electron transport rate of 14% upon phosphorylation of thylakoid proteins was reported by Farchaus et al. [21], who used spinach thylakoids illuminated for electron transport by white light.

The largest estimate of increased PS I absorption cross-section is that of Delepelaire and Wollman [22], who measured flash-induced absorption

changes at 515 nm. These arise from the electrochromic carotenoid bandshift and are a function of the  $\Delta\psi$  (membrane potential) component of the proton motive force. The measurements were carried out on a *Chlamydomonas* mutant that lacks PS II centres, and the observed increase in  $\Delta A_{515}$  upon protein phosphorylation was 50%. Several factors apart from PS I optical cross-section could affect  $\Delta\psi$  in this system. In particular, anaerobic conditions were used to obtain phosphorylation of LHC II, with aerobic conditions giving the unphosphorylated experimental control. In the absence of PS II, cyclic photophosphorylation is known to be inhibited under aerobic conditions by over-oxidation of electron transport components: anaerobic conditions would therefore be expected strongly to promote generation of  $\Delta\psi$  by redox poisoning of the PS I cyclic electron transport chain [23].

Thus, with the possible exception of the study of Delepelaire and Wollman [22], experimental estimates fall short of the predicted increases in absorption cross-section of PS I of at least 16% for broad-band excitation and of 40–70% for Chl *b* excitation [15,20]. Increases of these magnitudes are a clear requirement of the assumption that all LHC II detached from PS II by phosphorylation becomes attached instead to PS I [23–27].

Here we report results of experiments designed to address the question of changes in PS I absorption cross-section while circumventing possible secondary effects (such as photoinhibition) of phosphorylation treatments of isolated thylakoids. We have driven cells of the green alga *Scenedesmus obliquus* into States 1 and 2 using only actinic lights 1 and 2, and have compared the kinetics of the fluorescence rise between light-states in order to confirm their identity. No difference was found between the two light-states with respect to the kinetics of P-700 photooxidation by chlorophyll *b* light at 640 nm. We conclude that the State 2 transition which we observe in vivo underlies a decrease in the absorption cross-section of PS II without an equivalent increase in absorption cross-section of PS I. We propose, therefore, that any increase in quantum yield of photosynthesis on the transition to State 2 [1] is achieved by indirect means. We also consider further implications of this finding for the mechanism of state

transitions and for the consequences of light-harvesting protein phosphorylation in excitation energy dissipation in photosynthetic systems.

## Materials and Methods

*S. obliquus* was grown in Kessler's medium in batches of 300 ml in glass tubes continuously bubbled with 5% CO<sub>2</sub> in N<sub>2</sub> [28]. Cultures were grown for 24 h following a 5–10-fold dilution of the existing culture with fresh medium. Culture vessels were maintained in a water-bath at 30°C and illuminated continuously with six warm-white fluorescence strips at about 15 cm (incident intensity of 180  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ). Cells were harvested typically at a Chl concentration of 10–15  $\mu\text{g} \cdot \text{ml}^{-1}$  by centrifugation at 4000  $\times g$  for 5 min, and resuspended in fresh medium to give a final Chl concentration of 100  $\mu\text{g} \cdot \text{ml}^{-1}$ . Chl was extracted in hot methanol and measured using the absorption coefficients given in Ref. 29.

Light-states 1 and 2 were imposed on cells in continuously stirred aliquots of 20 ml of cell suspension, each aliquot filling an open glass scintillation vial at room temperature. Initially, State 2 was induced by exposure to light from a quartz halogen lamp transmitted through a 650 nm interference filter (Ealing) giving 17  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , and State 1 was induced by superimposing a perpendicular beam from a similar light source, transmitted through a 690 nm interference filter giving 53  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . This protocol was used in the experiment of Fig. 1. The 690 nm interference filter was found to give an excellent light 1, with slightly larger amplitudes for the rapid fluorescence decrease and slow rise than those obtained with a 700 nm filter, and much larger amplitudes than those obtained with a 710 nm filter. 680 nm light also functioned predominantly as light 1 in this system (results not shown). Induction of State 2 in *S. obliquus* cells for measurement of fluorescence yield and optical absorbance changes was obtained with the output of both lamps filtered through 650 nm interference filters. Induction of State 1 was implemented through 690 nm interference filters (Fig. 2). Prior to withdrawal of samples for kinetic studies, States 1 and 2 were induced alternatively in the same

aliquot of cells in order to avoid the possibility of spurious differences between samples, and a full State 2 → State 1 → State 2 cycle (Fig. 1) was carried out initially to confirm physiological activity. State transitions were monitored with a photodiode as a modulated fluorescence signal at 700 nm produced by weak excitation at 580 nm by yellow, pulsed LEDs (Hansatech Modulated Fluorescence System, Hansatech, King's Lynn, Norfolk, U.K.).

Samples (2.5 ml) withdrawn for kinetic studies were mixed rapidly in the spectrophotometer cuvette with DCMU (giving 20  $\mu\text{M}$  final concentration) and DBMIB (giving 20  $\mu\text{M}$ ). Optical absorption changes were measured at 702 and 730 nm as described [30], with continuous actinic light defined by a 640 nm interference filter giving 20  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  at the sample. Fluorescence induction was measured with the same instrument using 640 nm excitation (6  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and 700 nm emission [31]. The kinetics of P-700 photooxidation and of fluorescence induction were limited by the intensity of the actinic (640 nm) excitation (light-limited conditions – this conclusion is confirmed independently by the results in Fig. 6). Both fluorescence and optical absorption kinetics were measured within 10 s of removal of the cells from the actinic light regime, and cells were kept in near-darkness during this interval.

$F_0$  and  $F_m$  values were corrected by subtracting the photomultiplier response to excitation of a suspension from which Chl had been extracted exhaustively with hot methanol prior to resuspension of the pellet to the same final volume as the original cell suspension. This photomultiplier response was found to be consistent, accounted for 35% of the apparent  $F_0$ , and was attributed to overlap in the transmission of the optical filters used in the fluorescence measurement. It was decided that the disadvantages of this correction were offset by the advantage of using Chl *b* actinic excitation.  $F_m/F_0$  ratios of around 3 (e.g., Table II) were obtained routinely by this procedure.

Each absorption and fluorescence transient shown in the sum of four measurements. Each measurement was obtained with a separate 2.5 ml aliquot of cells taken from one batch of cells stably adapted to State 1 or State 2. Samples from each batch were taken for four absorption mea-

surements and four fluorescence measurements in order to guarantee comparability of kinetics of fluorescence and P-700 photooxidation. A further state transition was then carried out on each batch to check the return of the fluorescence values to those obtained in the opposite light-state. This was confirmed in every case, and P-700 photooxidation kinetics were still unchanged (results not shown).

## Results

Fig. 1 shows the observed cycle of fluorescence changes induced by superimposing light 1 (690 nm) on light 2 (650 nm). Such fluorescence changes are characteristic of State 1–State 2 transitions [1]. Addition of light 1 to cells fully in State 2 causes a rapid fluorescence decrease which is attributed to increased photochemical quenching of chlorophyll fluorescence yield as PS II traps open. This opening of PS II traps is a result of oxidation of PS II electron acceptors by increased turnover of PS I. A slow rise in fluorescence intensity follows. This slow rise can be understood to result in part from an increase in the absorption cross-section of PS

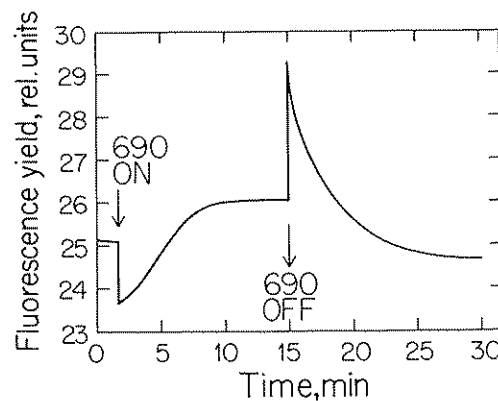


Fig. 1. State 1–State 2 transitions in *S. obliquus* monitored as changes in the yield of fluorescence (given in arbitrary units) at 700 nm and excited by weak modulated light at 590 nm. Continuous actinic light at 650 nm (light 2) was also present throughout, and effects are shown of the addition and extinction of a supplementary beam of light at 690 nm (light 1). Results shown were obtained after 30 min preillumination with actinic light 2, with actinic light 1 also being present for the first 15 min of the preillumination period. Prior to preillumination cells had been stirred in the dark at room temperature for 1–3 h.

II, since PS I makes a negligible contribution to variable fluorescence at room temperature. This increase in absorption cross-section of PS II relative to that of PS I is the State 1 transition and will itself tend to close PS II traps, thereby relaxing photochemical quenching. This relaxation will cause an amplification of the effect of the state transition on fluorescence yield.

After 12 min in excess light 1 the cells became stably adapted to state 1 (Fig. 1). When light 1 is extinguished, PS I turnover decreases, and there follows a rapid fluorescence increase as PS II traps become closed. The rapid increase is followed by a slow decrease in fluorescence as the absorption cross-section of PS II decreases relative to that of PS I. The slow fluorescence decrease therefore reports on the State 2 transition, but (as with the State 1 transition) it also contains a contribution from a secondary effect on photochemical quenching: the effect of the State 2 transition on fluorescence will be amplified by its opening of PS II traps. The State 1-State 2 transition shown in Fig. 1 is measured purely by changes in variable fluorescence emission. The results therefore show effects of relative changes in absorption cross-section of PS II and PS I combined with secondary effects on PS II photochemistry: there does not appear to be any way of distinguishing between relative and absolute changes in absorption cross-section of PS I purely by these means. The cycle shown in Fig. 1 begins with cells previously adapted to State 2 with excess light 2. The dark state appeared to be an intermediate state close but not identical to State 2 (results not shown). Although factors other than photochemical quenching and absorption cross-section may affect fluorescence intensity [32] and may thus contribute to the changes, there is no obvious way in which to explain results such as those of Fig. 1 other than by state transition.

Having established the ability of the cells to carry our state transitions (Fig. 1), light 1 (690 nm) and light 2 (650 nm) were used individually to drive the same cells as far as possible into State 1 and State 2. Fig. 2 (upper trace) shows a State 2 transition upon replacement of light 1 by light 2. Cells initially in State 1 showed a rapid increase in fluorescence emission on addition of light 2, followed by a slow fluorescence decrease that was

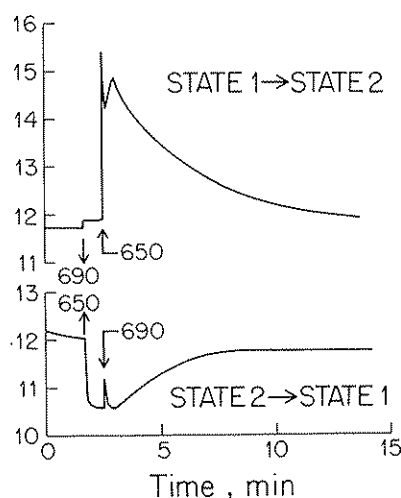


Fig. 2. The State 2 transition (upper trace) and State 1 transition (lower trace) in *S. obliquus* monitored as in Fig. 1, but with either 650 nm light 2 or 690 nm light 1 provided, each as a monochromatic actinic light regime. Secondary effects also contribute to changes in fluorescence yield, as described in the text.

interrupted by a brief, secondary fluorescence rise. This 'Kautsky' phenomenon is normally characteristic of plants illuminated after a period of dark adaptation and indicates multiple effects on fluorescence yield of photochemical and  $\Delta pH$ -quenching [32], not just of redistribution of excitation energy between PS I and PS II [33]. Since the same cells were clearly capable of state transitions (Fig. 1), it is reasonable to assume that a State 2 transition also contributed to the overall fluorescence decrease, and that the cells in the terminal, low-fluorescence state were fully adapted to State 2. This is confirmed by the subsequent effect (Fig. 2, lower trace) of replacement of light 2 by light 1. After light 2 was extinguished, fluorescence dropped to a value lower than that observed for cells in State 1. This new fluorescence value, obtained with the weak excitation beam alone, represent a direct measure of the lower  $F_0$  level and reflects the lower absorption cross-section of PS II in State 2 (at least 10% in this case). Addition of light 1 (690 nm) then caused a fast transient fluorescence increase, which is expected since light 1 is also absorbed to some extent by PS II and will therefore serve initially to close a proportion of PS II traps. When increased PS I turnover opens PS

II traps then the transient fluorescence increase is reversed. The subsequent, slow fluorescence rise is again likely to have a number of components, but the overall rise to a new, higher terminal fluorescence value can be attributed in part to the cells having become fully adapted to State 1.

In our view it is essential to monitor State 1–State 2 transitions by means such as those shown in Figs. 1 and 2 before any conclusions can be drawn about their characteristics in the system under investigation. An independent check on light-state transitions is often missing from studies of this kind. Light-dependent changes in themselves do not necessarily report on state transitions, particularly in view of the fact that respiratory electron flow can reduce plastoquinone and make the dark-adapted state approximate to State 2 [34,35]. Neither do effects of lights 1 or 2, defined purely by wavelength, necessarily report on state transitions: one can envisage physiological and metabolic circumstances under which regeneration of the terminal electron acceptor of non-cyclic electron transport will be blocked, thereby making light absorbed primarily by PS I ineffective in increasing PS I turnover and therefore, functionally, light 2. In order to explain the data presented in Fig. 1 the operation of light-state transitions is a necessary and sufficient assumption. Since the objective of these experiments was to establish unambiguously the identity of the light-states prior to kinetic analysis, no attempt was made to quantify the possible contribution of  $\Delta\text{pH}$ -quenching that may also be a necessary (though not sufficient) assumption for a complete explanation of Fig. 2. In support of our assertion that state transitions are a sufficient explanation of the data of Fig. 1, it is worth pointing out that essentially the same fluorescence changes have been observed in pea thylakoids in the presence of the uncouplers nigericin and valinomycin, where  $\Delta\text{pH}$ -effects cannot occur [4].

Fig. 3 shows fluorescence induction transients obtained in the presence of DCMU from cells in States 1 and 2. From these results the State 2 transition in *Scenedesmus* is seen to cause decreased  $F_0$  (13%),  $F_m$  (18%) and  $F_v$  (21%). The largely unchanged  $F_v/F_m$  ratio (4% decrease on transition to State 2) is consistent with the interpretation that a State 2 transition lowers the ab-

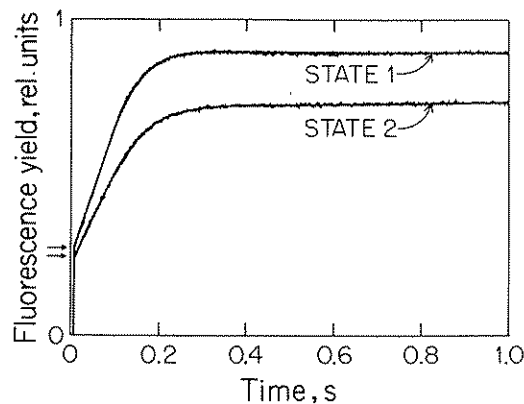


Fig. 3. Fluorescence induction transients in cells of *S. obliquus* in State 1 and State 2 and in the presence of DCMU (20  $\mu\text{M}$ ). Fluorescence emission (arbitrary units) at 700 nm was excited at 640 nm. State 1 and State 2 were induced by actinic light 1 and light 2 as shown in Fig. 2.

sorption cross-section of PS II rather than increasing energy transfer to PS I by 'spillover' [5]. These results (Fig. 3) are therefore in agreement with those obtained upon protein phosphorylation in pea chloroplasts [36] and upon state transitions in the green alga *Chlorella* [37] and the cyanobacterium *Synechococcus* 6301 [38]. A decreased sigmoidicity of the fluorescence rise in State 2 was seen in semilogarithmic plots of the data of Fig. 3 (results not shown), in further agreement with results obtained with other systems [37–40]. This decreased sigmoidicity indicates a decrease in cooperativity of PS II units. Fig. 3 therefore provides further validation of our experimental protocol for induction of light-states.

Fig. 4 shows the kinetics of P-700 photooxidation and subsequent dark recovery (re-reduction) in the presence of DCMU and DBMIB in cells adapted to States 1 and 2. A maximum decrease in  $\Delta A_{700}$  was obtained within 2 seconds of actinic illumination, and a slower increase in  $\Delta A_{700}$  followed when the actinic light was switched off. This dark re-reduction of P-700 is probably caused by respiratory electron flow or by cyclic electron flow around PS I, and although DBMIB minimized the rate of electron donation to P-700<sup>+</sup>, it did not inhibit it completely. The presence of DBMIB blocks the function of the Rieske Fe-S centre. However, at 20  $\mu\text{M}$  concentration, DBMIB may also shuttle electrons from PQH<sub>2</sub> directly to

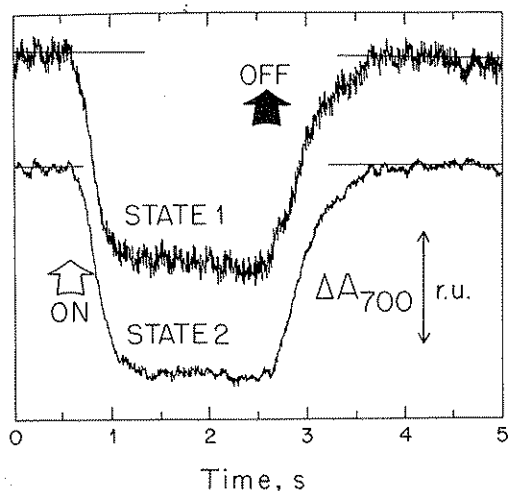


Fig. 4. Photooxidation and dark recovery (re-reduction) of P-700 in cells of *S. obliquus* in State 1 and State 2 and in the presence of DCMU ( $20 \mu\text{M}$ ) and DBMIB ( $20 \mu\text{M}$ ). 'ON' and 'OFF' refer to onset and offset of continuous actinic light at  $640 \text{ nm}$  and at  $20 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .  $\Delta A_{700}$  is given in arbitrary units. State 1 and State 2 were induced by actinic light 1 and light 2 as shown in Fig. 2.

P-700<sup>+</sup> [41]. Whatever the source of electrons for the dark re-reduction of P-700, the important feature of Fig. 4 for the purposes of this investigation is the independence from state transitions of both the rate and amplitude of P-700 photooxidation and of dark recovery. We determined that the rate of P-700 recovery in the dark was independent on the state in which the cells were preconditioned. Thus, the P-700 recovery process could not have disguised any differences in the rate of P-700 photooxidation under State 1 and State 2 conditions.

Fig. 5 shows the results of a separate experiment carried out with the same protocol as that of Fig. 4. In Fig. 5, however, the horizontal axis has an expanded scale in order to permit a more accurate estimate of the half-time of P-700 photooxidation. Only a very small difference in half-time between the two light states is seen: 261 ms in State 2 and 252 ms in State 1. This result (Fig. 5) is representative of several experiments that we have carried out, and is experimental result number 5 in the summary presented in Table I. Table I shows that only one experiment of five gave a higher rate of P-700 photooxidation in State 2, an increase in rate of about 2%. The remaining ex-

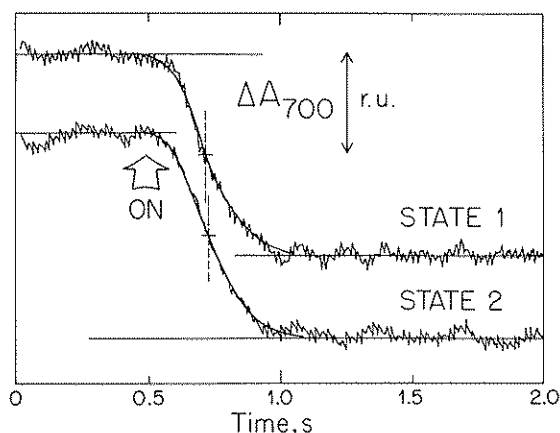


Fig. 5. Photooxidation kinetics of P-700 as in Fig. 4, but from a separate experiment and presented on an expanded time-scale to facilitate comparison of half-times.

periments resulted in lower rates of P-700 photooxidation in State 2. In all cases, however, differences in half-time of P-700 photooxidation are probably too small to be significant and may fall within the order of accuracy of the measurement. Absolute differences in half-time between experiments with different cell cultures are attributed to differences in light scattering in the cuvette and in final cell density. This contention was supported by the observation that qualitatively similar varia-

TABLE I  
HALF-TIME OF P-700 PHOTOOXIDATION IN CELLS OF *S. OBLIQUUS* IN STATE 1 AND STATE 2

Half-times of P-700 photooxidation were obtained in the presence of DCMU and DBMIB with cells preconditioned in light-state 1 and light-state 2, as shown in Fig. 5. Each experiment was carried out on a different cell culture and each half-time was obtained from the average of four separate measurements.

Experiment	Half-time of P-700 photooxidation ( $t_{1/2} \Delta A_{700}$ , ms)		Change (%)
	State 1	State 2	
1	287	304	+6
2	378	370	-2
3	315	331	+5
4	222	243	+9
5	252	261	+4

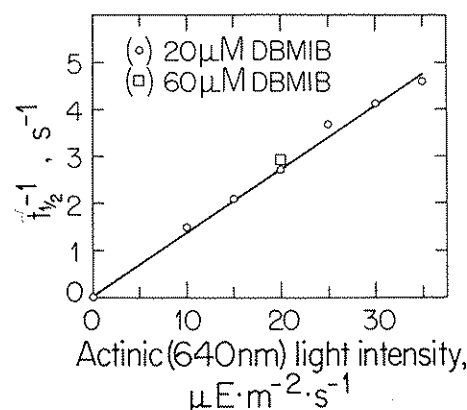


Fig. 6. Rate of P-700 photooxidation as a function of actinic light-intensity, showing that  $20 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  is a limiting light-intensity under the conditions of Figs. 4 and 5. It is also seen that the rate of P-700 photooxidation at that light intensity is unaffected by increased DBMIB concentration. The rate of P-700 photooxidation is given as the reciprocal half-time of  $\Delta A_{700}$ .

tions between batches of cells were observed in the fluorescence induction kinetics of cell suspensions (not shown). For each half-time of P-700 photooxidation a full transient such as that in Fig. 4 was obtained, and no differences in dark recovery between the light-states were observed. The uncoupler gramicidin also had no effect on the kinetics of the P-700 photooxidation and dark recovery in either State 1 or State 2 (results not shown). There were no apparent absorption changes at 730 nm, an isosbestic point for P-700, in either State 1 or State 2 (results not shown), which eliminates the possibility that light-artifacts could have overlapped the apparent changes in absorption at 700 nm.

TABLE II

SUMMARY OF CHANGES IN FLUORESCENCE INDUCTION DATA AND HALF-TIMES OF P-700 PHOTOOXIDATION IN CELLS OF *S. OBLIQUUS*

Fluorescence induction data were obtained as in Fig. 3 and half-times of P-700 photooxidation as in Fig. 5. Fluorescence values are given as a percent of  $F_0$  in State 1.  $v_f$  is the initial rate of fluorescence increase.  $t_{1/2}$  in State 1 (100%) is 291 ms. Each value is the mean of five experiments  $\pm$  standard deviation.

	$F_0$	$F_m$	$F_v$	$F_v/F_m$	$v_f$	$t_{1/2}$ $\Delta A_{700}$
State 1	100	306 $\pm$ 8	206 $\pm$ 8	0.673 $\pm$ 0.044	100	100
State 2	89 $\pm$ 2	262 $\pm$ 9	172 $\pm$ 7	0.656 $\pm$ 0.049	78 $\pm$ 6	103 $\pm$ 4
Change (%)	-11 $\pm$ 2.0	-14.4 $\pm$ 3.0	-16.5 $\pm$ 4.0	-2.5 $\pm$ 7.0	-22 $\pm$ 6	+3 $\pm$ 4

Fig. 6 shows that the rate of P-700 photooxidation is a linear function of intensity of actinic illumination under these conditions, with the experimental actinic intensity of  $20 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  being clearly light-limiting. The constant half-time of P-700 photooxidation reported in Table I cannot therefore have resulted from kinetic limitations on electron transport rate, and is most simply interpreted as a constant absorption cross-section of PS I. Increasing the DBMIB concentration from 20 to  $60 \mu\text{M}$  was also without effect on the rate of P-700 photooxidation (Fig. 6), which eliminates the possibility that electron donation by DBMIB was a rate-limiting step.

Table II presents a summary of all fluorescence and  $\Delta A_{700}$  kinetic data obtained in this investigation.  $F_0$ ,  $F_m$  and  $F_v$  are lower in State 2 than in State 1, while the  $F_v/F_m$  ratio is not significantly changed. The initial rate of fluorescence increase,  $v_f$ , is lower in State 2 than State 1, as predicted by the model. The fluorescence data taken together indicate a consistently lower absorption cross-section of PS II in State 2 relative to State 1, a minimal estimate of the magnitude of this decrease being 11%, that is, the decrease in  $F_0$ . Since  $F_0$  arises from the antennae of both PS II and PS I units, the effect of a State 2 transition on the absorption cross-section of PS II is probably greater than 11%.

The initial rate of fluorescence rise,  $v_f$ , is taken as a conservative estimate of the rate of closing of PS II traps and therefore of the rate of excitation energy transfer to the reaction centre of PS II. It should be noted that a decrease in sigmoidicity in fluorescence induction a priori will give an in-

crease in apparent  $v_f$ . In this study, the State 2 transition caused a decreased sigmoidicity in fluorescence induction and, at the same time, a 22% lower  $v_f$  (Table II). Hence, it may be concluded that a 22% lower  $v_f$  upon the State 2 transition actually underestimates the decrease in absorption cross-section of PS II.

If 20% of excitation energy is redirected from PS II in the State 2 transition and if all this excitation energy arrives instead at the reaction centre of PS I, then a complementary increase in the rate of P-700 photooxidation of 20% (a 20% decrease in half-time) at the very least should have been observed, the amplitude of the  $\Delta A_{700}$  being constant. No significant change in P-700 photooxidation kinetics was observed (Table II). Instead, the mean of five experimental results gave an increase in half-time, that is, a decrease in rate of P-700 photooxidation in State 2, of 3%, a value smaller than the standard deviation (4%) and therefore unlikely to be of significance. In summary (Table II), a consistent decrease in absorption cross-section of PS II and in excitation energy transfer to the reaction centre of PS II produced no measurable, complementary change in the kinetics of PS I photochemistry.

## Discussion

Evidence is available from a wide variety of experimental approaches [5,6,23–27,34] to support the proposal [7,8] that phosphorylation of LHC II is the basis of the State 2 transition in green algae and higher plant chloroplasts, and there is also compelling evidence for lateral movement of phospho-LHC II from appressed regions of thylakoid membrane, rich in PS II, to unappressed regions, rich in PS I [24–27,34]. Despite the considerable attractions of the idea of complementary changes in absorption cross-section between PS II and PS I, there is still no agreed experimental basis for excitation energy transfer from LHC II to P-700 under physiological conditions, or for regulation of such an energy transfer pathway.

The assumption of complementary changes in absorption cross-section of PS II and PS I has been made implicitly in a number of studies, notably those where measurements have been made of room-temperature fluorescence (e.g., Ref.

42) or of oxygen yield by photoacoustic spectroscopy (e.g., Ref. 43). In each case analysis of data proceeds on the assumption that a measured decrease in light absorbed by PS II,  $\beta$ , is accompanied by a corresponding increase in light absorbed by PS I,  $\alpha$ , such that  $\alpha + \beta = 1$  regardless of light-state.

Several studies of lifetimes and amplitudes of fluorescence decay components have led to the conclusion that PS II absorption cross-section is lowered upon phosphorylation of LHC II in thylakoids [44,45]. Such studies have generally shown no effect of phosphorylation on the fast decay component attributed to PS I [45,46]. Studies on thylakoids by Berens et al. [45] and on *Chlorella* by Holzwarth [46] have suggested instead that the major effect of the State 2 transition is on PS II heterogeneity. Thus the State 2 transition is thought either to convert  $\alpha$ -centres into  $\beta$ -centres [45], as proposed originally by Kyle et al. [47], or to cause complementary changes in the absorption cross-section of PS II $_{\alpha}$  and PS II $_{\beta}$ , as proposed by Holzwarth [46]. Apparently contrasting fluorescence lifetime data have recently been obtained by Hodges et al., however [48]. The Holzwarth model [46,49] also predicts increased chlorophyll *b* excitation of the slow, exponential component of fluorescence induction in State 2. To our knowledge this has not been investigated.

A further problem arising from the results presented here is how one may account for the agreed increase in steady-state 77 K fluorescence emission at about 735 nm [7–12]. This now remains the only uncontested finding that can be taken as evidence for increased excitation energy transfer to PS I. One solution might be to postulate an increased exciton density in a PS I-associated pigment bed such as to cause large increases in fluorescence emission without proportional increases in the rate of exciton transfer to the PS I reaction centre. This could be feasible thermodynamically if the relevant LHC I pigment bed carried excitons primarily at a lower energy than that required for photochemical trapping. Control by cation concentration of LHC II  $\rightarrow$  LHC I excitation energy transfer has recently been demonstrated for an isolated pigment-protein complex containing LHC II and PS I, though even here it is not yet known whether increased 735 nm fluores-

cence emission correlates with increased PS I photochemistry [50].

Regulation of the rate of excitation energy transfer from phospho-LHC II to PS II is not a strict requirement for an explanation of the phenomenon of state transitions in terms of the  $\alpha$ -changes described by Myers [3]. The amount of excitation energy converted photochemically by PS I as a proportion of the total excitation energy converted by both photosystems would increase during the State 2 transition merely as a consequence of decreased excitation energy transfer to PS II. Such a mechanism could be sufficient to explain all the effects of state transitions on room-temperature fluorescence yield, whether obtained *in vitro* in thylakoids [4,51] or *in vivo* as shown, for example, in Fig. 1. Increased excitation energy transfer to PS I does, however, seem to be required in order to explain the increase in quantum yield of oxygen evolution during a State 2 transition [1]. However, an increased efficiency of photosynthesis might also be observed *in vivo* if a lower absorption cross-section of PS II had some indirect effect on the total photosynthetic process. This, for example, could be manifested in the form of a proper redox poising of the electron-transport intermediates between PS I and PS II, which would otherwise tend to become over-reduced. This would enhance the yield of ATP formation. An increased quantum yield of ATP synthesis by photophosphorylation would result in increased turnover of the Calvin cycle, an increased rate of NADPH oxidation, and hence an increased efficiency of PS I as a result of an increase in PS I electron acceptor availability [52]. On the other hand, one might argue that cyclic photophosphorylation itself would be more efficient if excitation energy could be redistributed to PS I at the expense of PS II [23,53].

Another possible indirect route for effects of altered PS II absorption cross-section on quantum yield might involve a variable  $H^+/e^-$  stoichiometry for electron transport through the cytochrome *b/f* complex [54]. Such indirect mechanisms for increased quantum yield during the State 2 transition could occur only under *in vivo* conditions, and there is in fact little evidence from isolated, uncoupled thylakoids on the question of quantum yield. Measurements of state transitions *in vitro*

tend to concentrate exclusively on fluorescence changes. An exception to this is the work of Sinclair and Cousineau [55] showing no LHC II phosphorylation-dependent increase in rate of  $O_2$  evolution by spinach thylakoids, despite an observed ATP-dependent decrease in enhancement by light 1 of  $O_2$  evolution due to modulated light 2.

The rate of light absorption by PS I can regulate LHC II phosphorylation by determining the redox poising of plastoquinone [7]. An implication of our findings is that PS I is not in turn affected directly by LHC II phosphorylation. This would mean that the direct effects of phosphorylation are confined to control of absorption cross-section and cooperativity of PS II. Similar regulation of excitation energy transfer might then reasonably be expected to occur in photosynthetic bacteria that contain only one photosystem. Regulation of cooperativity among photosystems has been proposed as a function for phosphorylation of light-harvesting polypeptides in the purple bacterium *Rhodobacter sphaeroides* [56–58]. Allen and Holmes [57] have put forward a general model for control of photosynthetic unit function by which protein phosphorylation could regulate excitation energy transfer in purple bacteria and cyanobacteria, despite the absence from these organisms of the lateral heterogeneity in thylakoid membrane organisation that is often thought to be a requirement for regulatory effects of phosphorylation of light-harvesting proteins.

If excitation energy directed away from PS II does not contribute to PS I photochemistry, where does it go instead? One possibility is that it is converted ultimately to heat in an energy-dissipating pathway. This could take the form of its diversion to a photochemical reaction centre with a high probability of an electron transfer back-reaction, essentially a photochemical futile cycle. Switching to an energy-dissipating cyclic pathway around PS II has been proposed as a function for LHC II phosphorylation by Horton and Lee [59]. If PS II <sub>$\beta$</sub>  could be demonstrated to provide an energy-dissipating pathway via a cyclic electron flow, then such a model would be fully compatible with that proposed by Holzwarth [46,49].

Spillover of excitation energy from PS II <sub>$\beta$</sub>  to PS I is also permitted by Holzwarth's model [46], and

could account for some of the electron transport results obtained *in vitro*, especially where cation concentrations are low. This spillover could be increased after membrane fractionation, where phospho-LHC II, PS II<sub>β</sub> and PS I are all found in a stromal fraction. Horton and co-workers have obtained electron transport data consistent with a 20–30% contribution of phospho-LHC II to increased PS I turnover in isolated stroma lamellae [60]. Again, it is important to note that much smaller percentage increases in PS I turnover are observed upon phosphorylation in intact thylakoids [19–21].

An alternative possibility for energy dissipation might be that light absorbed by phosphorylated light-harvesting systems is converted directly to heat via non-radiative decay. This might also be compatible with the data of Wendler and Holzwarth [49] if the fluorescence decay component attributed to PS II<sub>β</sub> arose instead from phospho-LHC II, though in this event it would be difficult to account for the dependence of the fluorescence amplitude of this component on the photochemical state of the PS II reaction centre.

Whatever the mechanism of energy dissipation, it is clear that a State 2 transition that had no direct effect on PS I photochemistry *in vivo* could nevertheless serve primarily as a defence against the destructive effects of over-excitation of PS II, with any energetic gains at low light intensities being a result of additional, indirect factors. This in turn would call for a reappraisal of the physiological role of state transitions, suggesting that they serve as adaptive responses to changes in light intensity rather than spectral composition.

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