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Mechanism of the light state transition in photosynthesis. III. Kinetics of the state transition in *Porphyridium cruentum*

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The kinetics of the light state transition in the red alga *Porphyridium cruentum* were studied in low intensities of initiating light and in saturating flashes brief enough to elicit single turnovers of the photochemical apparatus. We confirm that the state transition is dose-dependent, but also found that the transition to state 2 was biphasic. The slow phase was correlated with the induction of photosynthesis and was eliminated if the preceding time spent in state 1 was very short. The full transition to state 1 developed following a minimum of 15 turnovers of Photosystem I, and the optimal frequency for the flash sequence was determined to be 2.5 Hz. In contrast, the turnover time required for the transition to state 2 was found to be smaller than 30 ms. The data are consistent with a mechanism we have recently proposed for the state transition in organisms that contain phycobilisomes. The mechanism proposed involves a small conformational change within the thylakoid that is brought about by localized differences in electrochemical potential. A Photosystem-I-generated potential difference of H^+ is prerequisite for the initiation of state 1 and, under certain conditions, a localized electric-field generated by Photosystem II may play a significant role in the transition to state 2.

Introduction

The change in distribution of excitation energy between the two photosystems in response to illumination of photosynthetic cells with light absorbed preferentially by either PS I or PS II was first observed in microalgae. Bonaventura and Myers reported on the green alga *Chlorella pyrenoidosa* [1] and Murata described similar phenomena in the red alga, *Porphyridium cruentum* [2]. Subsequent work has now established that the mechanism for the light state transition in higher plants is a highly complex coordinated series of events including an initial sensing system at the level of plastoquinone that detects imbalance in linear electron transport [3,4], activation of a membrane kinase and phosphorylation of an intramembrane antenna complex, the LHC [5,6]. The phosphorylated LHC is then thought to migrate laterally from the appressed region of the thylakoids to pair with functionally isolated PS I centers located in the unstacked regions [7,8] and thereby increase the absorption cross section of PS I (see Refs. 9 and 10; see also Ref. 11 for review).

In contrast, there is a paucity of information concerning the mechanism of excitation energy redistribution in the cyanobacteria and red algae. The photosynthetic apparatus of these organisms is structurally different from the green algae and higher plants in that they contain phycobilisomes rather than an LHC [12,13], and their thylakoids do not have any stacked regions. In a recent paper [14], we reported that the light state transition in the red alga, *P. cruentum*, and the cyanobacterium *Anacystis nidulans* does not result from, nor is

Abbreviations: PS, Photosystem; LHC, light-harvesting chlorophyll *a*/*b* protein.

accompanied by, a reversible protein phosphorylation analogous to the phosphorylation of the LHC of higher plants, which indicates that the control of energy redistribution in organisms that contain phycobilisomes is substantially different from that in more advanced eucaryotes.

Other important differences between the state transition in phycobilisome-containing organisms and higher plants have been reported that also suggest an entirely different mechanism is employed. In higher plants the transition to state 2 causes an increase in the absorption cross section of PS I [9,10], whereas in *P. cruentum*, the rate constant for excitation energy transfer from PS II to PS I has also been shown to change [15,16]. Previous work from this laboratory [17] on the effect of electron-transport inhibitors and membrane permeability agents on the state transition in vivo in *P. cruentum* established that the transition to state 1 requires PS I cyclic electron transport and coupled proton transport. Satoh and Fork have observed similar behavior in the thermophilic cyanobacterium, *Synechoccocus lividus* [18]. Taken with our recent report that no protein phosphorylation event is implicated [14], these results suggest that a localized ion gradient created by PS I turnover is involved directly in the molecular mechanism triggering the transition to state 1. We suggested a mechanism where the transition to state 1 requires a PS I generated, localized electrochemical potential difference of H^+ that results in a differential in charge between the two photosystems sufficient to cause a small change in thylakoid conformation [14]. As a working hypothesis, we also suggested that the change in conformation is a small change in the spatial relationship between the pigment-protein complexes associated with PS I and PS II in the thylakoid that then modifies the rate of excitation-energy transfer (k_T $2 \rightarrow 1$). If this hypothesis is correct, then the kinetics and stoichiometry of the photochemical activity that initiates the state transition becomes pertinent.

The purpose of this study was to investigate the detailed kinetics of the state transition in red algae paying particular attention to the effect of single turnover flashes of light. Evidence will be presented to show that the full transition to state 1 occurred following a minimum of approx. 15 turnovers of PS I presented at a frequency of 2.5 Hz. The conversion to state 2 was observed to occur with a rapid initial phase, the extent of which was dependent on intensity, followed by a slow phase. The slow phase was correlated with photosynthetic induction kinetics and could be eliminated if the preceding time spent in state 1 was very short. The conversion to state 2 initiated by saturating single turnover flashes followed the slow phase if the flashes were presented at a frequency of 30 Hz or less, indicating that the rapid photochemical component has a turnover faster than 30 ms. Our data are consistent with, and provide further details of the general mechanism we have proposed for the state transition in phycobilisome-containing organisms [14]. In the accompanying report we provide additional evidence for this mechanism based on the picosecond fluorescence decay kinetics of the phycobilin pigments and chlorophyll-*a* of both *P. cruentum* and *A. nidulans* [19].

Methods

P. cruentum (UTEX 161) was grown autotrophically on the enriched seawater medium F/2 of Guillard and Ryther [20] at a light intensity of 25 $\mu E \cdot m^{-2} \cdot s^{-1}$. Exponential phase cells were harvested by centrifugation, washed and resuspended in *F/2* to a final concentration of approx. 10 μg Chl-*a* per ml. Cells were brought to state 1 and state 2 by illumination in blue (light 1) and green (light 2) light, respectively, and the extent of the state transition was determined by analysis of their 77 K fluorescence emission spectra. All techniques were as previously described [21] and further details are provided in the legends to the figures.

For the rapid kinetic experiments, the exposure time to light 1 or light 2 needed to initiate the state transition was controlled via mechanical shutters, or by using either a linear zenon flash lamp or a frequency doubled Nd-Yag laser as light sources. The flash lamp (EG&G FX-33C-2) was operated at 1 kV with a discharge capacitance variable from 4 to 52 μF resulting in a pulse width at half height adjustable from 15 to 100 μs. Results obtained with the flash lamp were independent of the pulse width or intensity over this range, and all data shown are for 15 μs pulses. The frequency-doubled

(532 nm) Nd-Yag laser (Raytheon SS313) had a pulse duration of 10 ns and averaged intensity of 750 $\mu E \cdot m^{-2} \cdot s^{-1}$ when operated at 20 Hz. Reactions were terminated by rapid freezing of the samples in liquid nitrogen and the rate of freezing was shown to be nonlimiting in the experiments.

Results

Transition to state 1

The transition of *P. cruentum* from steady state 2 to state 1 was investigated as a function of the intensity and time of exposure to light 1. In Fig. 1, the extent of the transition to state 1 as induced by a short exposure of cells to light 1 was determined from the relative increase in the 77 K fluorescence emission at 695 nm due to PS II (see Fig. 3 for fluorescence emission spectra). The half-time of the transition decreased from 3.8 to 1.3 s as the intensity of light 1 was increased from 7 to 20 $\mu E \cdot m^{-2} \cdot s^{-1}$. In agreement with Ried and Reinhardt [22], the transition is dose-dependent at these intensities and under our conditions half transition to state 1 was induced by 25 $\mu E \cdot m^{-2}$ of light 1.

We then proceeded to investigate the state

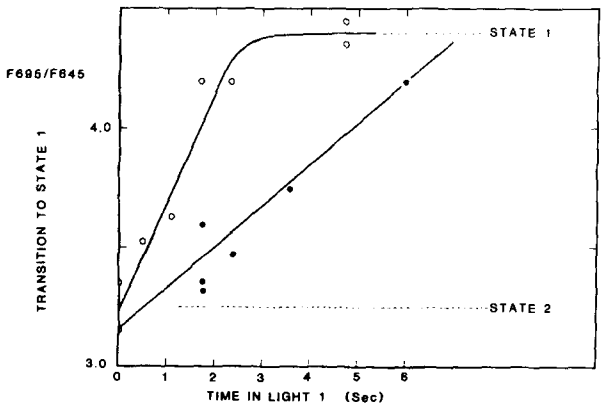

Fig. 1. Dose dependence for the transition from state 2 to state 1 at two light intensities. The extent of the transition to state 1 expressed as the relative increase in the 77 K fluorescence emission at 695 nm (F_{695}/F_{645}) versus the time of exposure to light 1 immediately following an initial 2 min exposure to light 2 at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. The upper curve (O) is for various exposure times to 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ of light 1 and the lower (●) for exposure to 7 $\mu E \cdot m^{-2} \cdot s^{-1}$ of light 1. The dashed lines show the limits of the F_{695}/F_{645} ratio at 77 K for cells exposed only to 2 min of light 2 at 200 $\mu E \cdot m^2 \cdot s^{-1}$ (state 2) or 2 min of light 1 at 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ (state 1).

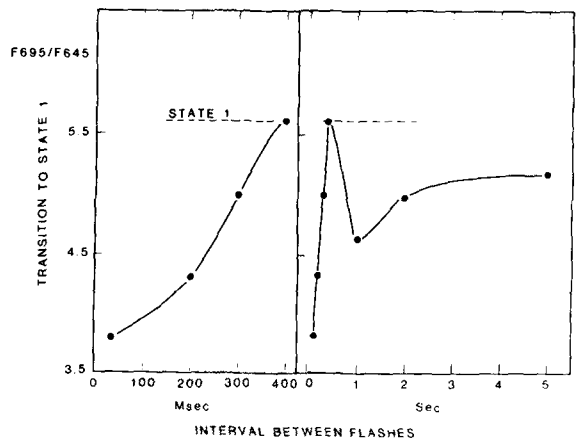

Fig. 2. Effect of dark interval between flashes on the extent of transition from state 2 to state 1 induced by a train of fifteen 15 μs flashes of light 1. The extent of transition to state 1 is expressed as the relative increase in the fluorescence emission at 695 nm (F_{695}/F_{645}). The dashed line shows the limit of this ratio after exposure of cells for 2 min with light 1 at 38 $\mu E \cdot m^{-2} \cdot s^{-1}$.

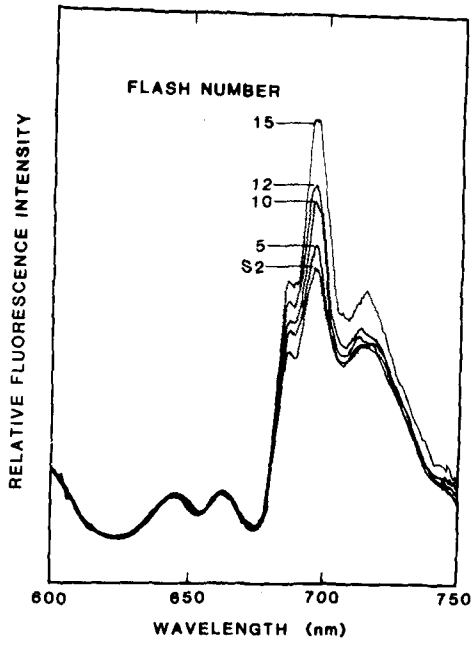

Fig. 3. 77 K fluorescence emission spectra. Trace labelled S2 resulted from exposure to 2 min of light 2 at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. Upper traces are for cells initially exposed to 2 min of light 2, and subsequently exposed to five, ten, twelve or fifteen 15 μs flashes of light 1 with a dark interval between flashes of 400 ms.

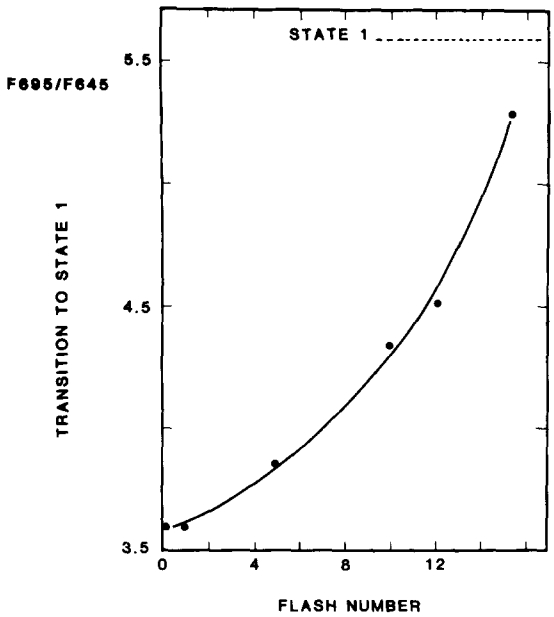

Fig. 4. The transition to state 1 expressed as the relative increase in the fluorescence emission at 695 μm (F_{695}/F_{645}) versus the number of 15 μs flashes of light 1 given after an initial 2 min exposure to 130 $\mu E \cdot m^{-2} \cdot s^{-1}$. Dark interval between flashes was 400 ms. The dashed line shows the limit of the transition to state 1 achieved with 2 min exposure to light 1 at 38 $\mu E \cdot m^{-2} \cdot s^{-1}$.

transition as induced by saturating single turnover flashes and observed that the transition to state 1 required a sequence of between ten and twenty flashes presented at a frequency of 2.5 Hz. To illustrate these requirements Fig. 2 shows the effect of varying the dark interval between flashes on the extent of the transition to state 1 induced by a sequence of 15 flashes. The maximum transition to state 1 occurred with a 400 ms dark interval between flashes. A decrease or increase in the dark interval between flashes inhibited the transition to state 1 resulting from the flash sequence indicating that the transition was not dose-dependent when induced by single-turnover flashes. The inhibition that occurred at dark intervals both shorter and longer than 400 ms shows that there was a cumulative effect of the individual flashes in the sequence that was strongly dependent on flash frequency. The inability of a sequence of 15 flashes to cause a complete transition to state 1 when spaced at intervals shorter than 400 ms implies a turnover time for the mechanism triggering the transition on the order of hundreds of milliseconds. Furthermore, the inability of the cells to convert to state 1 when the flash sequence was presented with dark intervals longer than 400 ms, indicates that the contribution of each individual flash to the cumulative effect of the sequence was not stable in the dark and had a decay time of several seconds. The subsequent slow rise toward state 1 seen at longer intervals between flashes (2–5 s) reflects the slow dark conversion to a point between state 2 and state 1 normally observed when cells are maintained in the dark. This observation is in general agreement with that of Ley and Butler [15] who reported that the half-time to convert the dark condition in *P. cruentum* is 3 min.

Fig. 3 shows the effect of flash number in the transition of cells to state 1. The figure shows 77 K fluorescence spectra of samples following five, ten, twelve and fifteen flashes of light 1 presented with an optimal dark interval of 400 ms. Fig. 4 shows the same data graphically and it can be seen that the conversion behaves with positive cooperativity.

Transition to state 2

The transition to state 2 from steady state 1 was investigated as a function of light intensity and time of exposure to light 2. Fig. 5 shows the extent of the transition to state 2 for two light intensities, and it can be seen that the conversion is biphasic. Both phases were dependent on intensity and, in a separate experiment where the conversion was monitored as a function of 1 s flashes of varying intensity, we found half conversion to state 2 required 50 $\mu E \cdot m^{-2}$ and 90% conversion required 2000 $\mu E \cdot m^{-2}$ (data not shown).

We discovered that the kinetics of conversion to state 2 was strongly dependent upon the preceding time of exposure of the cells in state 1 in contrast to the corresponding conversion to state 1 which was independent of the time in state 2. The data illustrated in Fig. 5 were typical of cells that had been maintained in state 1 for 2 min or longer (steady-state 1). When a systematic decrease in the time in state 1 was given prior to the state 2 conversion we noted that the slow phase became less predominant. If the time in state 1 was only on the order of a few seconds, then the state 2 conversion occurred very rapidly (less than 1 s) and was dose-dependent as anticipated. We sug-

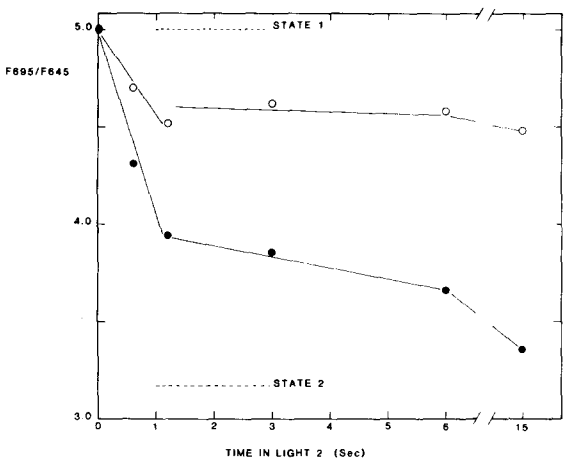

Fig. 5. Dose dependence for the transition from state 1 to state 2 at two light intensities. The extent of the transition to state 2 is expressed as the relative decrease in the 77 K fluorescence emission at 695 nm (F_{695}/F_{646}) versus the time of exposure to light 2 immediately following an initial exposure to light 1 at 20 $\mu E \cdot m^{-2} \cdot s^{-1}$. The upper curve (○) is for various exposure times to 55 $\mu E \cdot m^{-2} \cdot s^{-1}$ of light 2 and the lower curve (●) for exposure to 325 $\mu E \cdot m^{-2} \cdot s^{-1}$ of light 2. The dashed lines show the limits of the F_{695}/F_{645} ratio at 77 K for cells exposed only to 2 min of light 1 at 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ (state 1) or 2 min of light 2 at 325 $\mu E \cdot m^{-2} \cdot s^{-1}$ (state 2).

gest that the slow phase be associated with the kinetics of photosynthetic induction which would be required for establishment of linear electron transport and carbon dioxide assimilation in state 2 following a prolonged period of the cells in state 1.

Attempts were made to study the state 2 conversion using single-turnover flashes from the zenon flash lamp and the Nd-Yag laser. Experiments using both sources indicated that much higher flash frequencies were required than was found to be necessary for the conversion to state 1, and instrumental limitations precluded an exhaustive study of the frequency dependence. However, using the laser which delivered saturating flashes at a maximum frequency of 30 Hz, we determined that half conversion to state 2 required approx. 1 min, indicating that the turnover time of the photochemistry necessary to observe the fast component of the transition to state 2 must be shorter than 30 ms.

Discussion

The data presented confirm the earlier observations of Ried and Reinhardt [22] that the transition of red algae to state 1 and state 2 exhibits a photochemical dose dependence when the cells were illuminated with excitation of relatively low incident intensities. Under our conditions we found that 25 $\mu E \cdot m^{-2}$ was required for the transition to state 1, a value similar to that noted in the original report by Murata [2]. The conversion to state 2 was found to be complex, and the kinetics were dependent upon the immediate history of the cells prior to the conversion. At relatively low intensities of light 2 the conversion was observed to be biphasic, the slow phase being correlated with long exposure times in state 1. We suggest that long exposure to state 1 would result in deactivation of the regulatory enzymes involved in CO_2 assimilation [23] and, to some degree, also result in a metabolic condition that would necessitate a full photosynthetic induction [24] once linear electron transport is initiated in response to light 2. We did observe that the conversion to state 2 was very rapid if the previous time in state 1 was reduced to a few seconds. Under such conditions the rapid phase was dose-dependent and the slow phase was less predominant.

The experiments reported in this article using flashes brief enough to elicit single turnover of the reaction centers and electron-transfer carriers provides much greater insight into the mechanism of the state transition. We found that conversion of cells to state 1 required a sequence of approx. 15 flashes and maximal transition to state 1 was not attained if the interval between flashes was shorter or longer than 400 ms. The transition to state 1, is therefore, not dose-dependent when induced by single-turnover flashes. The failure of cells to convert to state 1 when the dark interval was shorter than 400 ms suggests that the turnover time for the mechanism is on the order of several hundred milliseconds. This observation is in accordance with our earlier reports on the in vivo turnover of PS I cyclic electron transport in *P. cruentum* where we showed that the post-illumination reduction of cytochrome *f* [25] and P-700 [26] occurred with a half-time of 150 ms in cells inhibited with DCMU or activated with light of wavelengths longer than

680 nm. The inability of *P. cruentum* to convert to state 1 when the interval between flashes was longer than 400 ms indicates that the flash product was not stable in the dark.

The conversion to state 2 was found to be biphasic and the experiments performed with single-turnover flashes revealed that the frequency dependence was different to that required to attain state 1. Activation using the laser, which delivered saturating light 2 (532 nm) flashes, was characteristic of the slow phase of the conversion even at the highest possible repetition rate of 30 Hz. This suggests that the turnover rate of the mechanism responsible for the rapid component was faster than 30 ms, and because of instrumental limitations it was not possible to determine the minimum number of flashes and optimal frequency required to achieve state 2.

These kinetic data are consistent with the model we have proposed for the mechanism of the state transition in phycobilisome-containing organisms [14] and provide further details. We postulated that the transition to state 1 is driven by coupled PS I cyclic electron transport which results in a localized electrochemical potential difference of H^+. This would lead to a difference in charge within the thylakoid sufficient to invoke a small conformational change between the two photosystems. We now show that only fifteen turnovers of PS I are required for the complete transition if the dark interval between the activation flashes is approx. 400 ms. This optimal frequency of 2.5 Hz is in line with both the turnover time of the in vivo cyclic electron-transport pathway in *P. cruentum* [25,26] and the conditions determined by Graan and Ort [27] for accumulation of H^+ as a result of synchronous turnover flash activation of thylakoids. Their data indicates that our sequence of 15 flashes would result in accumulation of at least 15 H^+ per PS I unit. At a frequency of 2.5 Hz we would point out that the dark intervals between flashes are sufficiently long to ensure relaxation of any electrical field [28].

The photochemical transition to state 2 by light 2 was also very rapid if photosynthetic induction effects were eliminated. Although we were unable to determine precisely the optimal conditions for the transition to state 2 using single-turnover flashes, we did show that the transition can be completed within 1 s, and the turnover time of the photochemistry was shorter than 30 ms. Presumably, activation of linear electron transport by light 2 is sufficient to reverse the partial redistribution of charge that maintains the conformation of state 1. We suggest that under certain conditions the photochemical transition to state 2 will require photosynthetic induction and, most probably, full activation of the regulatory enzymes in the Calvin cycle [23]. It is not clear whether the association of the transition to state 2 with induction is mechanistically at the level of enzyme activation, or simply related to a limitation of PS II activity. As the rapid component of the transition to state 2 has a turnover time shorter than 30 ms, a local influence of PS II activity on the mechanism of transition to state 2 seems likely. On this time-scale (30 ms), the electric field generated by single-turnover flashes will still be significant [28]. The possible contribution of localized electric-potential differences generated by PS II in the transition to state 2 remains to be elucidated.

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