Biochimica et Biophysica Acta, 724 (1983) 111–117
Elsevier

BBA 41320

MECHANISM OF THE LIGHT STATE TRANSITION IN PHOTOSYNTHESIS

I. ANALYSIS OF THE KINETICS OF CYTOCHROME *f* OXIDATION IN STATE 1 AND STATE 2 IN THE RED ALGA, *PORPHYRIDIUM CRUENTUM*

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(Received November 29th, 1983)
(Revised manuscript received March 18th, 1983)

Key words: Cytochrome f oxidation; Photosynthesis; Light state transition; Electron transport; Fluorescence; (Porphyridium cruentum)

The kinetics of photooxidation and reduction of cytochrome *f* were examined spectrophotometrically in the red alga *Porphyridium cruentum* in light State 1 and light State 2. Experiments were performed on intact cells that had been chemically fixed and stabilized in the light states. The cytochrome *f* turnover was measured during conditions of linear electron transport driven by both photosystems and during several cyclic reactions mediated by the long-wavelength Photosystem (PS) I. The data show that the rate of photooxidation of cytochrome *f* increased in State 2 when the cells were activated by subsaturating intensities of green light absorbed primarily by the phycobilisome. No differences in kinetics were found between algae in State 1 or State 2 when they were activated by light absorbed primarily by the chlorophyll of PS I. The results confirm that changes in energy distribution between the two photosystems occur as a result of the light state transition and verify that the redistribution of excitation results in the predicted changes in electron transport.

Introduction

It has been established that during oxygenic photosynthesis the distribution of excitation energy between PS I and PS II is regulated [1]. Following extensive studies of fluorescence changes in algae, both Murata [2,3] and Bonaventura and Myers [4] reported that illumination of cells with light absorbed either by PS I or PS II converted the cells to State 1 or State 2, respectively. The transition from State 1 to State 2 results in an adjustment between the excitation energy delivered to the two photosystems such that in State 2 a greater fraction is transferred to PS I. The state transition was shown to be reversible and dark-adapted cells were shown to be intermediate between the two light states.

The energy redistribution in isolated thylakoids was shown to occur in response to changes in concentration of cations [5], and the results of such changes on electron-transport reactions and thylakoid architecture have been extensively documented [1]. More recently, the reversible phosphorylation of an intramembrane pigment-protein complex, the LHC, has been implicated and is most probably a key event in the mechanism for regulating the energy distribution in vivo [6–10]. Although complete details of the mechanism are

Abbreviations: PS, photosystem; LHC, light-harvesting chlorophyll *a*/*b* protein; DCMU, 2-(3,4-dichlorophenyl)-1,1-dimethylurea; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine; DCIP, 2,6-dichlorophenolindophenol.

still forthcoming, the predicted consequences on the rates of electron transport between phosphorylated and dephosphorylated thylakoids have now been established [11,12]. Namely, in phosphorylated membranes that show an increase in the excitation delivered to PS I, the rate of PS I electron flow increases concomitant with a decrease in the rate of PS II [12].

Red algae are oxygenic photoautotrophs that contain phycobilisomes [13] rather than the LHC, which is the major antenna for PS II in the higher algae and green plants. As first demonstrated by Murata [2], red algae undergo energy redistribution via the light state transition and the phenomenon has been recently reinvestigated in detail by Ley and Butler [14,15] in *Porphyridium cruentum*. They confirmed the redistribution of excitation energy during the state transition and showed that the rate constant for the yield of energy transfer from PS II to PS I, $k_T(2 \rightarrow 1)$, in State 2 was twice that in State 1. They also reported that the absorption cross-sections and spectral properties of the two photosystems did not change during the transition [15]. At present there is no information on either the mechanism or physiological consequences of the state transitions in these organisms. It is conceivable that events analogous to those found in higher plants exist in which case the phycobilisome, the thylakoids or both components of the photosynthetic apparatus may be phosphorylated, or an entirely different mechanism may be utilized.

The object of this report was to investigate the physiological consequences of the changes in energy distribution due to the light state transition in red algae. This was accomplished by studying the kinetics of cytochrome *f* photooxidation spectrophotometrically in cells brought to State 1 or State 2 in vivo and chemically fixed in the light states. The data show that when the cells were activated by light absorbed primarily by the phycobilisome and PS II, the rate of photooxidation of cytochrome *f* was accelerated in State 2. No differences in rate were found between cells in States 1 and 2 when they were activated by light absorbed exclusively by PS I. These results are at variance with those of Horton and Black [16] who did not observe any change in rate of cytochrome *f* photooxidation upon phosphorylation of higher plant thylakoids. The results reported herein verify that the increase in rate of PS I electron transport in State 2 is a direct consequence of a redistribution of excitation energy absorbed by the phycobilisome between the two photosystems.

Methods

P. cruentum (UTEX 161) was obtained from the University of Texas Culture Collection of Algae, Austin, TX, and grown autotrophically on the supplemented seawater medium F of Guillard and Ryther [17] at a light intensity of 25 $\mu E \cdot m^2 \cdot s^{-1}$. The pigment composition of the cells was similar to the L-cells described by Ley and Butler [18].

Samples of *P. cruentum* in 10 mM mannitol, 30 mM potassium phosphate, pH 7.5, were adapted to State 1 or State 2 by illumination of a 10 ml suspension ($A^{1\,cm}_{680\,nm} = 1\text{–}1.5$) in either blue or green light, respectively. Blue light at an intensity of 150 $\mu E \cdot m^2 \cdot s^{-1}$ was isolated from a tungsten halogen source using a Corning glass No. 5.60 plus a 600 nm short-pass filter. Green light at an intensity of 500 $\mu E \cdot m^2 \cdot s^{-1}$ was isolated from the same source using a 500 nm long-pass filter plus a 600 nm short-pass filter. Occasionally, cells were brought to State 1 using far-red illumination ($\lambda \geqslant$ 695 nm) or by illumination of cells poisoned with 10 μM DCMU using green light. The algae were chemically fixed in the light states by incubating the cells with 0.5% glutaraldehyde (grade 1, Sigma Chemical Co., St. Louis, MO) for a further 5 min in the respective light. The fixed cells were then rapidly diluted with 8 vol. of ice-cold 100 mM sucrose, 20 mM Tris-HCl, pH 8.0, centrifuged, washed three times and finally suspended in the same buffer.

Low-temperature fluorescence emission spectra of the cells were resolved at the temperature of liquid nitrogen at a band-pass of 0.9 nm using a Spex Industries Inc. (Metuchen, NJ) 1680 Spectramate double monochromator controlled by a DM1 Datamate processor. Excitation of the samples, contained in 100-μl capillaries, at 560 nm, was provided using a Jobin Yvon monochromator and tungsten halogen source. Emission spectra were corrected for instrument response and smoothed using a running average of 21 data points weighted to the scheme proposed by Savit-

sky and Golay [19]. The spectra were then normalized at 644 nm to compensate for small differences between samples resulting from capillary mismatch and alignment in the dewar.

PS I electron-transfer reactions were measured on algae that had been chemically fixed in the light states by monitoring the kinetics of the photoinduced absorption change at 420 nm using a single-beam spectrophotometer as described previously [20,21]. The change in absorption at 420 nm in red algae has been shown to be primarily due to cytochrome *f* [20,22–24]. Although a small contribution in ΔA results from P-700$^+$, whose maximum absorption change in the blue is centered at 435 nm [25,26], this was not considered a significant artefact in this work because the P-700$^+$ signal is also diagnostic of the turnover of PS I. The activation flashes were subsaturating (20–280 $\mu E \cdot m^2 \cdot s^{-1}$) and of wavelengths absorbed primarily by the phycobiliproteins (500–600 nm). The PS I electron-transport reactions induced as a result of the absorption of such PS II flashes included cyclic and noncyclic reactions as indicated in the figure legends.

Results

An investigation was made of the kinetics of PS I in State 1 and State 2 in a variety of PS I reactions energized by excitation absorbed primarily by the pigments in the phycobilisome and PS II. This was accomplished by chemically crosslinking the cells in State 1 and State 2 and then performing electron-transport measurements on the fixed cells. Such fixed cells have been shown by Hallier and Park [27] to be very permeable to metabolic cofactors and to retain a high degree of physiological competence in partial photosynthetic reactions [27,28]. Vernotte et al. [29] also showed previously that *Chlorella* and pea leaves could be chemically fixed in the light states and retain their characteristic fluorescence emission spectra.

Fig. 1 shows low-temperature fluorescence emission spectra of *P. cruentum* brought to State 1 and State 2, before and after fixation with glutaraldehyde. The fluorescence emission spectra of the control cells are very similar to those previously reported for *P. cruentum* in State 1 and State 2 [2,15] and clearly show the change in distribution of energy between the two photosystems. The conversion to State 2 is evidenced by decreases in the intensities of the emissions at 683 and 694 nm due to allophycocyanin B and chlorophyll *a* of PS II, respectively. Butler and Ley [15] have shown by deconvolution of the fluorescence spectra that the long-wavelength emission, 715–730 nm, results from contributions from both the antenna of PS I and the long-wavelength tail of PS II. They determined the quantitative significance of each in State 1 and State 2 and showed that conversion of the cells to State 2 results in an increase in the PS I emission and decrease in the PS II emission at 715–730 nm.

The conditions for fixation were optimized for the electron-transport measurements and were shown to be similar to those reported originally by Hallier and Park [27]. The fluorescence spectra of the fixed cells indicate that some decreases in energy transfer from the phycobilisome to the chlorophylls of PS I and PS II occurred after fixation, but the cells retained their characteristic differences indicative of the two light states. It was not possible to perform the light state transition on cells following glutaraldehyde fixation. As judged by the fluorescence spectra the glutaraldehyde-fixed cells remained unchanged in the light states for 6 h. Longer storage times resulted in further decreases in energy transfer from the phycobilisome to the photosystems, but even after 4 days the characteristic differences between the two states were discernible. All electron-transport measurements on glutaraldehyde-fixed cells were made within 3 h of fixation.

Fig. 2 shows data on the cytochrome *f* absorption change by cells glutaraldehyde-fixed in State 1 and State 2. The fluorescence spectra shown on the right are of the actual samples used in the kinetic experiment. The cytochrome *f* absorption transient is shown for three intensities of green flashes absorbed primarily by the phycobilisome and PS II (A–C) and a far-red flash absorbed almost exclusively by PS I (D). The PS I reaction was linear electron transport; $DCIP_{red} \rightarrow$ methyl viologen. The effect of glutaraldehyde fixation on the rate of photooxidation of cytochrome *f* was not as great as might have been predicted based upon previous reports using isolated chloroplasts. Hardt and Kok [30] showed that the photooxida-

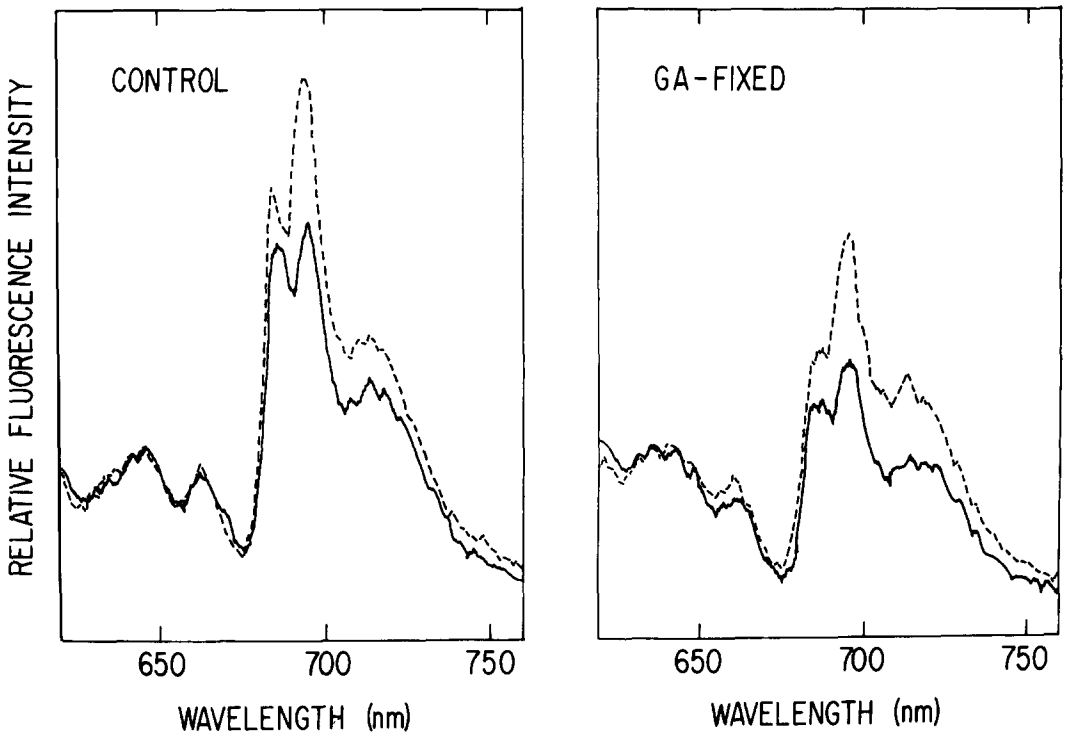

Fig. 1. 77 K fluorescence emission spectra of *P. cruentum* in State 1 (- - - - - -) and State 2 (———) before and after fixation with glutaraldehyde. Excitation at 560 nm.

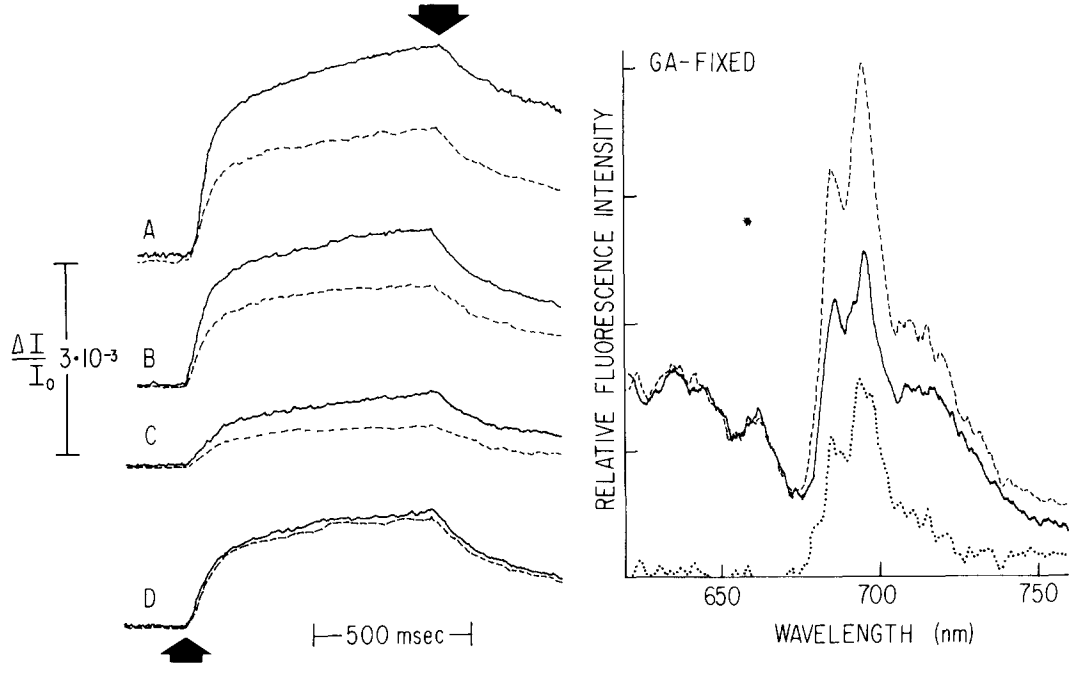

Fig. 2. 77 K fluorescence emission spectra of *P. cruentum* cells glutaraldehyde fixed in State 1 (- - - - - -) and State 2 (———). The difference, State 1 minus State 2 is shown below (· · · · · ·). On the left are photoinduced absorption changes due to cytochrome *f* of the same samples of fixed cells in response to broad-band green activation flashes (500–600 nm) at three intensities (A, 280; B, 150 and C, 20 $\mu E \cdot m^2 \cdot s^{-1}$) and D, far-red activation ($\lambda \geqslant 695$ nm, 40 $\mu E \cdot m^2 \cdot s^{-1}$). The cells were supplied with 10 μM DCMU, 100 μM methyl viologen, 10 mM sodium ascorbate and 3 mM DCIP.

tion of cytochrome *f* decreases by 70% following glutaraldehyde fixation wheras only a 10% decrease in the rate was routinely observed under the conditions adopted in this work with *P. cruentum* (data not shown). The kinetic data show that the photooxidation of cytochrome *f* was considerably accelerated in State 2 [31]. As expected, the dark decay kinetics were similar due to the chemical reduction of cytochrome *f* by $DCIP_{red}$-ascorbate. There was no difference in the rate of photooxidation between State 1 or State 2 cells when they were activated by a far-red flash absorbed exclusively by PS I (traces D). These data show that changes in energy distribution in State 2 result in the increase in electron-transport rate of PS I.

Similar experiments using cells fixed in State 1 and State 2 were performed where the cytochrome turnover was measured in response to several PS I cyclic reactions mediated by DCIP, *N*-methylphenazonium methosulfate and TMPD. As was the case in Fig. 2, no differences were observed in the decay kinetics between cells fixed in State 1 and State 2 but significant changes were found in the kinetics of photooxidation and the steady-state signal amplitudes. Fig. 3 shows data for cytochrome *f* photooxidation during the DCIP cyclic reaction as a function of PS II flash intensity. Similar data were found for the *N*-methylphenazonium methosulfate- and TMPD-mediated reactions but the kinetics were slightly different. In all reactions the steady-state amplitude of the signal and, therefore, the rate of photooxidation of cytochrome *f* was greater in cells fixed in State 2 [31]. The data in Fig. 3 show that at subsaturating intensities the relative quantum efficiency of PS I is greater in State 2. Although it was not possible to conduct experiments at light saturation the data also suggest that the steady-state amplitudes may be different at saturation. This may be a result of the glutaraldehyde fixation and requires further study. However, as was shown in Fig. 2 no differences in cytochrome photooxidation were noted between cells in State 1 or State 2 upon direct activation of PS I by a far-red flash ($\lambda \geq 695$ nm). This confirms that the kinetic differences found between cells in the light states upon activation by green flashes absorbed primarily by the phycobilisome reflect changes in distribution of the phycobilisome-absorbed excitation at the level of PS I electron transfer.

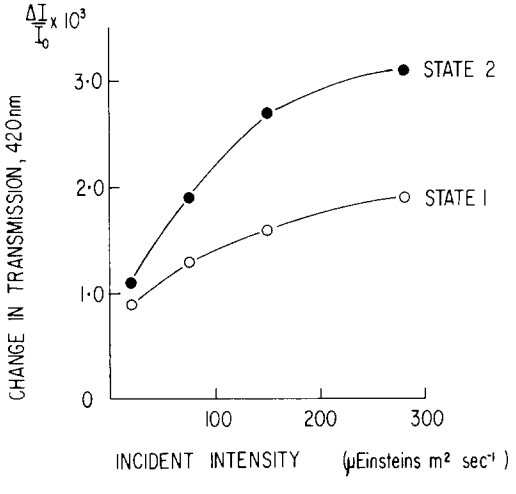

Fig. 3. Relationship between the amplitude of the absorption change due to cytochrome *f* in the DCIP cyclic reaction by *P. cruentum* cells fixed in State 1 (○) and State 2 (●) and intensity of the activation light (500–600 nm). The cells were supplied with 3 mM DCIP, 10 mM sodium ascorbate and 10 μM DCMU. Other conditions as in Fig. 2.

Discussion

The data presented show that the light state transition in *P. cruentum* was accompanied by changes in rate of photosynthetic electron transport. Specifically, the rate of photooxidation of cytochrome *f*, a PS I-catalyzed reaction, increased in State 2 during turnover in linear and cyclic electron-transfer reactions. The differences in cytochrome kinetics between cells in State 1 and State 2 were substantial when the activation light was of wavelengths primarily absorbed by the phycobilisome and PS II. However, when the cells were activated by light absorbed predominantly by the chlorophylls of PS I, no such differences were observed. Horton and Black [16] have also investigated the kinetics of cytochrome *f* in the thylakoids of higher plants following membrane phosphorylation. From a comparison of the low-temperature emission spectra available [8,10], dephosphorylated and phosphorylated thylakoids should be physiologically equivalent to cells in State 1 and State 2 [2,15], respectively. Although Horton and Black [16] showed a decrease in the

photoreduction of cytochrome *f* in phosphorylated thylakoids, they did not observe a concomitant increase in rate of photooxidation using 650 nm illumination. They concluded that membrane phosphorylation did not alter the distribution of excitation energy and suggested that it decreased the fraction of energy arriving in PS II by an effect on the LHC. It should be noted, however, that major differences in the mechanism of energy distribution most likely exist between the higher plants and the phycobilisome-containing organisms. The transition to State 2 in higher plants has been shown to result in increases in absorption cross-section following lateral migration of the phosphorylated LHC into the stroma lamellae [32,33]. This is in contrast to red algae where it has been shown that a change in the rate constant for energy transfer between the two photosystems, $k_T(2 \rightarrow 1)$, occurs rather than a change in cross-section [15].

The observed increase in PS I activity in State 2 in this work using a red alga is in agreement with the recent work of Farchaus et al. [12] using isolated plant thylakoids. They measured substrate conversions by both PS I and PS II electron-transport reactions in the same preparation of thylakoids and demonstrated a 15–20% increase in the electron-transport rate of PS I and a corresponding decrease in rate of PS II upon membrane phosphorylation. The differences in rates were apparent when the thylakoids were assayed using limiting intensities of white light but small differences were noted even at light saturation. Although it was not possible to perform measurements at light saturation in the present study because illumination of more defined chromaticity was required, the intensity relationship shown in Fig. 3 suggests that the light-saturated rate of cells in State 1 might be lower than those in State 2 and requires further clarification.

As noted in Results the rate of photooxidation of cytochrome *f* in *P. cruentum* was only reduced approx. 10% following fixation by glutaraldehyde. This was considerably less than the 70% inhibition observed by Hardt and Kok [30] who reported in detail on the effect of glutaraldehyde fixation of isolated thylakoids. They concluded that the site of glutaraldehyde inhibition was most likely plastocyanin, which is intermediate between cytochrome *f* and P-700, the reaction center of PS I. The reason for the discrepancy could be attributable to the milder fixation conditions employed in this work, or the fact that whole cells were treated rather than isolated membranes. Less likely would be the absence of plastocyanin in these cells or possibly its inaccessibility to glutaraldehyde fixation. The fact that cells can be stabilized in the light states using a bifunctional cross-linking reagent such as glutaraldehyde, and that the state transition cannot be observed in cells following chemical fixation, supports the previously advanced hypothesis [3,4] that the state transition is accompanied by a structural change. The structural change most likely involves functional proteins of the thylakoid complexes or the phycobilisome.

In summary this report presents data that demonstrates that the light state transition in a *P. cruentum*, a phycobilisome-containing organism, is accompanied by changes in rate of electron transfer as a consequence of the redistribution of excitation energy between the two photosystems.

Acknowledgements

The research was supported by the Science and Educational Administration of the U.S. Department of Agriculture under Grant No. 59-2442-1-1-767-0 from the Competitive Research Grants office and Biomedical Research Support Grant No. S07RR07085-16 from the National Institutes of Health.

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