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ENERGY TRANSFER FROM PHOTOSYSTEM II TO PHOTOSYSTEM I IN *PORPHYRIDIUM CRUENTUM*

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SUMMARY

Rates of photooxidation of *P*-700 by green (560 nm) or blue (438 nm) light were measured in whole cells of *Porphyridium cruentum* which had been frozen to −196 °C under conditions in which the Photosystem II reaction centers were either all open (dark adapted cells) or all closed (preilluminated cells). The rate of photooxidation of *P*-700 at −196 °C by green actinic light was approx. 80 % faster in the preilluminated cells than in the dark-adapted cells. With blue actinic light, the rates of *P*-700 photooxidation in the dark-adapted and preilluminated cells were not significantly different. These results are in excellent agreement with predictions based on our previous estimates of energy distribution in the photosynthetic apparatus of *Porphyridium cruentum* including the yield of energy transfer from Photosystem II to Photosystem I determined from low temperature fluorescence measurements.

INTRODUCTION

Quantitative estimates of the partitioning of excitation energy between Photosystem I and Photosystem II of *Porphyridium cruentum* were made previously from an analysis of low temperature fluorescence measurements [1, 2]. The distribution of energy was specified in terms of three parameters; α, the fraction of absorbed quanta delivered initially to Photosystem I, β, the fraction going to Photosystem II and $\varphi_{T(II\to I)}$, the yield of energy transfer from Photosystem II to Photosystem I. The latter parameter is variable ranging from a minimum value, $\varphi_{T(II\to I)(o)}$, when the Photosystem II reaction centers are all open, to a maximum value, $\varphi_{T(II\to I)(M)}$, when the Photosystem II centers are closed. The low temperature fluorescence measurements [2] indicated that the excitation energy from blue light, absorbed predominantly by the chlorophyll of the red algae, is distributed almost entirely to Photosystem I ($\alpha \approx 0.95$, $\beta \approx 0.05$) while the energy from green light, absorbed predominantly by phycoerythrin, appears to be distributed initially almost entirely to Photosystem II ($\alpha \approx 0.05$, $\beta \approx 0.95$). However, energy distributed initially to Photosystem II can be transferred subsequently to Photosystem I according to the value of $\varphi_{T(II\to I)}$. $\varphi_{T(II\to I)(M)}$ was estimated from measurements of fluorescence excitation and absorption of *Porphyri-*

dium cells at −196 °C to be about 0.95 and $\varphi_{T(II\rightarrow I)(o)}$ to be about 0.50 [1], independent of the wavelength of excitation. The action spectrum for oxygen evolution measured at room temperature [2] was consistent with the wavelength dependence of β and the value of $\varphi_{T(II\rightarrow I)(o)}$. The photochemical apparatus of this red alga was envisaged to consist of relatively large Photosystem I units and very small Photosystem II units with the phycobilisomes connected directly to the small Photosystem II units [2]. The highly specific transfer of energy from the phycobilisomes to Photosystem II appeared to be mediated through allophycocyanin B [3].

In previous studies with chloroplasts [4] and bean leaves [5] the increase in the efficiency of energy transfer from Photosystem II to Photosystem I which occurs when the Photosystem II reaction centers close was manifest in the rate of photooxidation of *P*-700 at −196 °C. Similar experiments can be carried out with *Porphyridium* cells which allow us to test predictions based on our values of α, β and $\varphi_{T(II\rightarrow I)}$. In green light we would predict that the fraction of absorbed energy delivered to Photosystem I, $\alpha+\beta\varphi_{T(II\rightarrow I)}$, should increase from 0.52 to 0.95, a ratio of 1.8, as the Photosystem II reaction centers close. In blue light, the fraction delivered to Photosystem I should increase from 0.975 to 0.997, a ratio of only 1.02. The purpose of the present work is to test these predictions by measuring the rate of photooxidation of *P*-700 in cells of *P. cruentum* at −196 °C with green actinic light and with blue actinic light when the Photosystem-II reaction centers are either all open or all closed.

MATERIALS AND METHODS

Whole cells of *P. cruentum*, grown as described previously [2], were collected by centrifugation, resuspended in growth medium, and kept on ice in complete darkness until use. Stock suspensions consisted of algae suspended in growth medium at a chlorophyll concentration of $5 \cdot 10^{-3}$ μg/ml in the presence of 2 mM NH_4OH and 20 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). 0.5-ml aliquots of the stock suspension were used in all experiments.

Preilluminated samples were exposed to bright white light (70 mW/cm^2) for 45 s and returned to darkness for 2 min at room temperature before being rapidly frozen to liquid nitrogen temperature in our vertical cuvette and Dewar system [6]. Dark-adapted samples were kept in complete darkness prior to freezing. The frozen samples were irradiated during measurement by a series of flashes of green (560 nm) or blue (438 nm) light. Green light at an intensity of 80 μW/cm^2 was provided by the combination of a 75 W tungsten lamp with three Califlex heat reflecting filters and a Corning 559 nm interference filter. Blue light at an intensity of 100 μW/cm^2 was provided by the combination of a 75 W xenon lamp with three Califlex heat reflecting filters, a Corning 5031 glass filter and a Balzers 437 nm interference filter. 33-ms flashes of light at a rate of one flash every 6 s were generated by placing a solid rotating wheel with a single small opening in the optical path between the light source and the sample.

Photooxidation of *P*-700 by the incident light flashes was monitored continuously as the change in transmission of the sample measured by a weak 703 nm beam from a monochromator (2.4 nm passband) similar to the methods described previously [4, 5]. The light flashes and the measuring beam were delivered directly to the front surface of the frozen sample by a dual-armed fiber-optics light pipe. The light transmitted by the sample was detected by an EMI 9558C photomultiplier protected by the

combination of a Toshiba VR65 glass filter, a Corning 9830 glass filter and a Corning 705 nm interference filter. The output of the photomultiplier was recorded against time on an X-Y recorder.

RESULTS

The combination of DCMU and hydroxylamine has the same influence on cells of *P. cruentum* [7] that it has on spinach chloroplasts [8, 9]. Irradiation of the cells at room temperature in the presence of these inhibitors causes the Photosystem II reaction centers to close and to remain closed during a subsequent dark period as noted by the maintenance of a high fluorescence yield in the dark. During that dark period the *P*-700 photooxidized during the irradiation is restored to the fully reduced state by the dark metabolism. Thus, cells can be frozen to −196 °C in a state in which the Photosystem II reaction centers are fully closed and the *P*-700 is fully reduced by giving the cells a short saturating irradiation in the presence of DCMU and hydroxylamine a short time (1–2 min) before freezing. In contrast, if the cells are frozen to −196 °C in a fully dark-adapted state (no preillumination) the Photosystem II reaction centers are fully open. Irradiation of the preilluminated cells at −196 °C causes no fluorescence yield changes because the fluorescence yield of the sample is already at the maximal F_M level but normal light-induced fluorescence yield changes are observed with the dark-adapted cells at −196 °C. However, the extent of the light-induced absorbance change at 703 nm due to the photooxidation of *P*-700 is the same for both types of samples.

The photooxidation of *P*-700 was measured in cells of *P. cruentum* at −196 °C in a repetitive series of flashes of blue or green light (a 0.33 s flash every 6 s). The flashes produced small accumulating increments of photooxidized *P*-700 which were assayed between the flashes by the weak measuring beam at 703 nm. Fig. 1 shows the results of a typical experiment with 438 nm actinic light. The transmission of the frozen sample at 703 nm increases due to the photooxidation of *P*-700 by the repetitive flashes from an initial stable dark level, to a final maximum level. Following the end of the flash regime, there is a slow decrease in transmission due to a multiphasic dark reduction of the oxidized *P*-700. In separate experiments we have found that 30–60 % of the *P*-700 photooxidized at −196 °C can be rereduced in the dark at −196 °C and that this reversible part remains reversible in subsequent irradiations. Similar examples of the partial reversibility of the photooxidation of *P*-700 at low temperatures have been reported for isolated spinach chloroplasts [10–12], blue-green algae [11, 13, 14], and the blue-green-colored red alga *Porphyridium aerugineum* [12].

As was found in higher plants [4, 5], the photooxidation of *P*-700 of *P. cruentum* cells at low temperature appears to follow second-order reaction kinetics. The data shown in Fig. 2A were obtained from several experiments in which green (560 nm) flashes of light were used to photooxidize *P*-700. The rate of photooxidation of *P*-700 in the preilluminated algae (k_p) was found to be 80 % faster than the rate in algae which had been kept in complete darkness prior to exposure to the flash regime (k_d), i.e. the ratio k_p/k_d was 1.8 ± 0.2.

When blue (438 nm) flashes of light were used to photooxidize *P*-700 (Fig. 2B), there was very little difference in the rates of photooxidation between preilluminated and dark-adapted algea. For the data shown in Fig. 2B, k_p is actually slightly smaller than

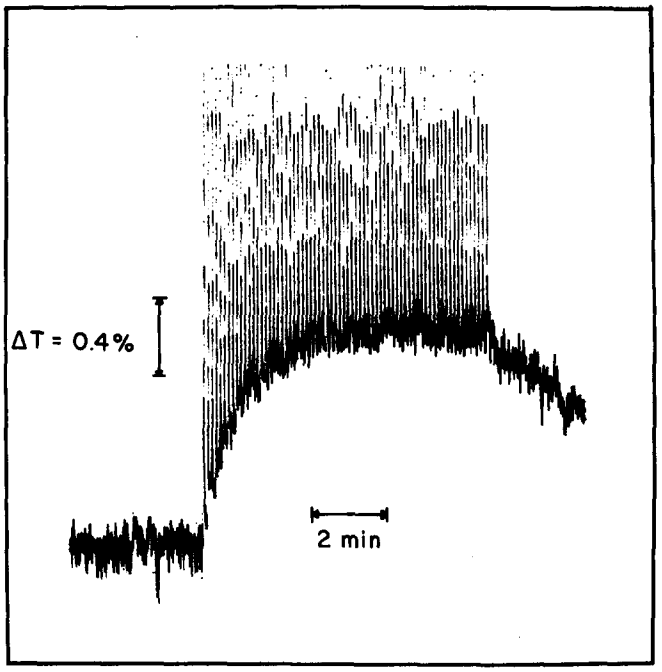

Fig. 1. Kinetics of the photooxidation of *P*-700 in *P. cruentum* at −196 °C. The transmission of the frozen sample was monitored at 703 nm during simultaneous irradiation from repetitive 438 nm flashes at a rate of one flash every 6 s. The trace goes off scale during the flash but recovers to give a valid reading between flashes. The algae were suspended at a chlorophyll concentration of $5 \cdot 10^{-3}$ μg/ml in the presence of 2 mM hydroxylamine and 20 μM DCMU.

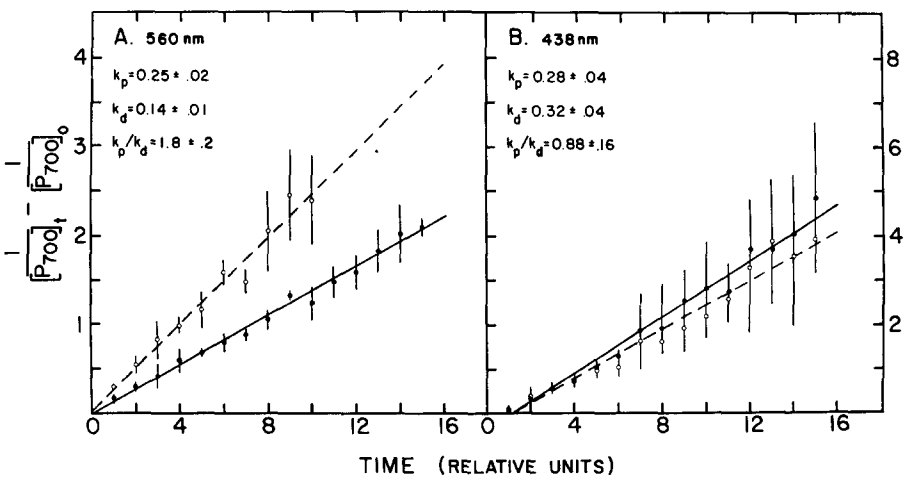

Fig. 2. Second-order plots of *P*-700 photooxidation at −196 °C in green and blue flashing lights. Solid lines and closed circles indicate data for dark-adapted algae. Dashed lines and open circles indicate data for preilluminated algae. Each point represents the arithmetic mean of four measurements and the error bars represent one standard deviation about that mean. The lines are linear regressions on the data. k_p is the slope of the regression line for the data from preilluminated algae and k_d is the slope for dark-adapted algae. The time scale is the number of flashes from the start of the flash regime. (A) 560 nm flashes were used to oxidize *P*-700. (B) 438 nm flashes were used.

k_d, but there is large variability in that data (probably due in part to fluctuations in the intensity of the xenon lamp used to provide the blue light). An analysis of variance [15] on the regressions shown in Fig. 2B indicate that the differences in slope between the preilluminated and dark-adapted samples are not significant ($0.50 < p < 0.75$) whereas a similar analysis on the data shown in Fig. 2A indicates a significant difference ($0.01 < p < 0.025$). Because of the large variability encountered in the blue-light experiments, several sets of such experiments were performed. Values for k_p/k_d ranged from a minimum of 0.88 ± 0.16 to a maximum of 1.37 ± 0.19. The average value of k_p/k_d of all such experiments was determined to be 1.10 ± 0.23.

CONCLUSION

Our experimental results on the degree to which closing the Photosystem II reaction centers stimulates the rate of photooxidation of *P*-700 in green light and in blue light agree closely with the values predicted in the Introduction. The question of whether energy transfer from Photosystem II to Photosystem I (often termed "spillover" in the literature) even occurs has been a source of controversy. We take the results reported here to be strong support for the concept that such energy transfer plays an important role in determining and controlling energy distribution in the photochemical apparatus of these algae. We also take the close agreement between the measurements and our predictions to be a further confirmation of our general model for the photosynthetic apparatus of plants and the ability of that model to accommodate systems as diverse as those representative of red algae and higher plants.

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