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ENERGY DISTRIBUTION IN THE PHOTOCHEMICAL APPARATUS OF *PORPHYRIDIUM CRUENTUM* IN STATE I AND STATE II

A.C. LEY and W.L. BUTLER

Department of Biology, University of California San Diego, La Jolla, CA 92093 (U.S.A.)

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Summary

Fluorescence of *Porphyridium cruentum* in state I (cells equilibrated in light absorbed predominantly by Photosystem I) and in state II (cells equilibrated in light absorbed appreciably by Photosystem II) was examined to determine how the distribution of excitation energy was altered in the transitions between state I and state II. Low temperature emission spectra of cells frozen in state I and state II confirmed that a larger fraction of the excitation energy is delivered to Photosystem II in state I. Low temperature measurements showed that the yield of energy transfer from Photosystem II to Photosystem I was greater in state II and calculations indicated that the photochemical rate constant for such energy transfer was approximately twice as large in state II. Measurements at low temperature also showed that the cross sections and the spectral properties of the photosystems did not change in the transitions between state I and state II. In agreement with predictions made from the parameters measured at low temperature, the action spectra for oxygen evolution measured at room temperature were found to be the same in state I and state II.

Introduction

When photosynthetic organisms are first irradiated there is an induction period for photosynthesis to reach its final steady-state rate. This time, which is generally in the order of a few minutes for thoroughly dark-adapted organisms, is the time required for the photosynthetic machinery to reach a

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

steady-state rate of turnover. During this period the yield of fluorescence increases rapidly to an early maximum and then decreases slowly to a final steady-state level as the steady-state rate of photosynthesis is approached [1].

According to the terminology which has built up around these phenomena [2], cells which are dark adapted or equilibrated in light absorbed primarily by Photosystem I are said to be in state I while cells at steady-state photosynthesis in light absorbed to an appreciable extent by Photosystem II are said to be in state II. Murata [3], working with *Porphyridium cruentum* and Bonaventura and Myers [4], working with *Chlorella pyrenoidosa* proposed independently that the transition from state I to state II is accompanied by a readjustment in the distribution of excitation energy between Photosystem I and Photosystem II and that the decrease in fluorescence from the initial maximum to the final steady-state level is due to redistribution of excitation energy toward the weakly fluorescent Photosystem I. That proposition was challenged recently by Briantais et al. [5] who concluded, on the basis that the fluorescence emission spectrum of chloroplasts at −196° C was the same regardless of whether the chloroplasts were frozen in state I or in state II, that the distribution of energy between the photosystems did not change in the transitions between state I and state II. The work reported here will show that low temperature fluorescence emission spectra are different in states I and II, that a greater fraction of the excitation energy is delivered to Photosystem I in state II and that this change in energy distribution is due primarily to a change in the rate constant for energy transfer from Photosystem II to Photosystem I. The reason why Briantais et al. reached a different conclusion will also be apparent.

Materials and Methods

Unialgal cultures of *Porphyridium cruentum* were grown in an artificial sea water medium [6] at 19° C in 125 ml Erlenmeyer flasks each containing 50 ml of medium. The cultures were kept on a rotary shaker and were exposed to continuous diffuse laterial illumination (80 $\mu W/cm^2$) from cool-white fluorescent lamps. Cells from such cultures correspond both in pigment content and in the characteristics of their photosynthetic apparatus to cells we have previously described as L-cells [7]. Cultures typically exhibited logarithmic growth for two to three weeks following inoculation. For all experiments described here cells from 7 to 10 day old cultures were collected by centrifugation and were resuspended in fresh growth medium prior to use.

The kinetics of fluorescence yield changes at room temperature were measured on 0.5 ml suspensions of dark-adapted cells ($1 \cdot 10^8$—$2 \cdot 10^8$ cells/ml). Green (560 nm, 800 $\mu W/cm^2$) or blue (440 nm, 300 $\mu W/cm^2$) exciting light was provided by a tungsten-iodide lamp filtered through 7 cm of 2% $CuSO_4$ solution and focused through appropriate blocking and interference filters onto one arm of a three-armed fiber-optics light-pipe assembly [8]. The intensities of actinic irradiations delivered via a light-pipe were measured at the exit end of the light-pipe assembly. The end of the light-pipe assembly where the fiber optics of the three arms were joined together both illuminated the sample and collected light fluoresced from the front surface of the sample. Fluorescence from the sample was directed by the remaining two arms of the light-pipe

assembly onto a photomultiplier (EMI 9558) protected with a red cut-off filter (Toshiba VR-65) and a 692 nm interference filter. The output of the photomultiplier was stored in a small computer (Fabritech 1024) for subsequent analysis.

Fluorescence emission spectra were measured on 0.5 ml suspensions of cells ($5 \cdot 10^6$—$10 \cdot 10^6$ cells/ml) which had been frozen to —196° C during illumination. The samples were irradiated for 5 min with blue (440 nm, 450 $\mu W/cm^2$) or green (560 nm, 1 mW/cm^2) light at room temperature to establish state I or II, respectively, and then were frozen to —196° C by pouring liquid N_2 around the vertical cylindrial cuvette in the Dewar [9] while the irradiation continued. In experiments where a multiple series of irradiations were given, the sample was frozen during continuation of the final irradiation. The end of the light-pipe assembly rested directly on the frozen sample which was immersed in liquid N_2. Excitation at 560, 500 or 435 nm was directed onto the sample through one arm of the light-pipe assembly while fluorescence from the front surface was carried by the other two arms to the entrance slit of a Bausch and Lomb 500 mm monochromator (1.5 nm passband). Light at the exit slit of the monochromator was measured with a GaAs phototube (Hamamatsu R666) and the signal was digitized and stored as a function of wavelength in a small computer. The emission spectra, difference spectra and ratio spectra shown were calculated were calculated by the computer and plotted directly with an *X-Y* recorder.

Fluorescence excitation spectra were measured at —196° C with the computer-linked, single-beam spectrophotometer as described previously [7,10,11]. Excitation from the scanning monochromator was incident on the top surface of the frozen sample and fluorescence at 693 or 730 nm was measured from the bottom surface with a phototube (EMI 9558) protected by appropriate filters. In some experiments fluorescence excitation spectra were measured on frozen samples at the minimum F_o level. In these cases the monochromator slits were set to 0.28 mm which gave light intensities of 0.1 $\mu W/cm^2$ or less at the sample. These light intensities did not alter the F_o level of fluorescence measurably during the course of a scan. Absorption spectra were also measured with the same spectrophotometer.

Most of the fluorescence measurements were made on cells which had been frozen to —196° C during irradiation and thus were at the maximum F_M level of fluorescence. For some experiments, however, it was necessary to compare cells in state I and state II at the minimum F_o level which occurs when all of the Photosystem II reaction centers are open. For reasons we do not fully understand, we were unable to freeze cells in state II with our usual procedures (i.e. pouring liquid nitrogen around the cuvette) if the cells, after reaching state II, were placed in darkness for a short period to allow the Photosystem II reaction centers to reopen. In order to freeze cells in state II in darkness it was necessary to freeze them much more rapidly. $1 \cdot 10^7$—$5 \cdot 10^7$ cells were collected onto Millipore filters discs (16 mm diameter) by gentle suction filtration. The cells were moistened with a drop of growth medium and illuminated for 5 min at room temperature with the blue or green light sources used to establish state I and state II. The cells were then placed in the dark for 45 s, a time sufficient to completely restore the Photosystem II reaction centers but

too short for any significant changes in state II, and then were frozen rapidly by plunging the filter discs directly into liquid nitrogen. The frozen discs were mounted in darkness in prechilled cuvettes.

Action spectra for oxygen evolution were measured polarigraphically in modulated light using an apparatus described previously [11,12]. A single layer of cells on a bare platinum electrode was illuminated simultaneously with a relatively strong continuous background beam and a weak modulated monochromatic beam of variable wavelength. The background illumination obtained from a 500 W xenon lamp filtered through 2 cm of 3% $CuSO_4$ and appropriate interference filters was either blue (440 nm, 350 $\mu W/cm^2$) or green (560, 300 $\mu W/cm^2$). The modulated light provided by a 150 W xenon lamp and a scanning monochromator (10 nm passband) was chopped at 17 Hz by a rotating sector. The modulated beam was maintained at a constant intensity (5 $\mu W/cm^2$) by a thermopile detector in a servo system which adjusted an optical density wedge at the exit slit of the monochromator. The modulated component of the oxygen evolution was measured with a lock-in amplifier. The amplitude of the modulated signal was found to be linear with the intensity of the modulated beam up to 10 $\mu W/cm^2$ in the presence of the strong background illumination.

Results

Typical fluorescence yield changes which occur during the irradiation of *P. cruentum* with green light (800 $\mu W/cm^2$ at 560 nm) are shown in Fig. 1. The fluorescence increases rapidly during the first few seconds to an initial maximum and then decays more slowly to a final steady-state level which is characteristic of the state produced by light absorbed by Photosystem II. (We

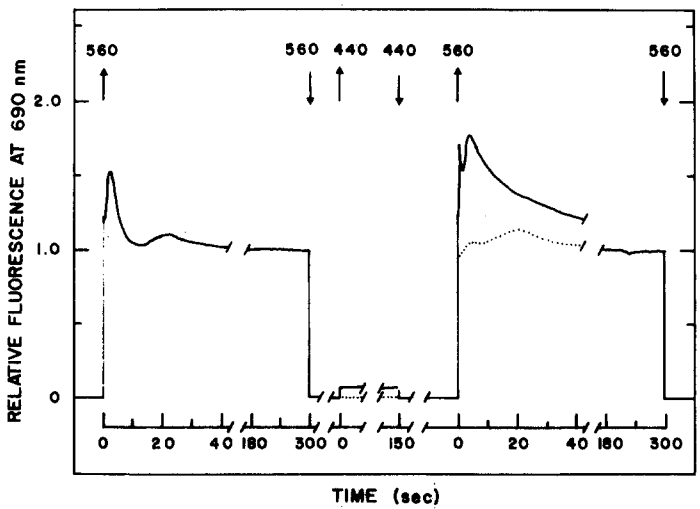


Fig. 1. Time course of fluorescence of *P. cruentum* cells at 690 nm during irradiation with 560 nm light (800 $\mu W/cm^2$) at room temperature. The second irradiation with 560 nm light follows the first after a period of 3 min. Dashed line, 3 min of darkness; solid line, 2.5 min irradiation with 440 nm light (300 $\mu W/cm^2$) in the middle of the 3 min period.

will not consider the initial fast transient spike in the fluorescence curve which often precedes the initial maximum or the secondary maximum which sometimes follows the initial maximum in the approach to state II.) With dark-adapted cells the ratio of the intensity of the fluorescence at the initial maximum to that at the final steady-state level is typically 1.5 to 1.6.

State II persists for some time in the dark following an irradiation. The dotted curve in Fig. 1 shows fluorescence changes during a second irradiation with 560 nm light following a dark period of 3 min. The small initial maximum indicates a rather small return to the dark-adapted state. The half time to regenerate the dark-adapted state is 5 to 10 min. However, if the second irradiation is preceded by a 2.5 min irradiation with 300 $\mu W/cm^2$ of blue light (440 nm), which has very little action for photosynthesis in these cells, the condition for the high initial maximum is regenerated even beyond the original dark-adapted state (see solid curve in Fig. 1). This state, referred to as state I because it results from light absorbed by Photosystem I, shows a ratio of the intensities at the initial maximum to the final level of about 1.8 and a longer induction period during a subsequent irradiation to reach state II. State I decays to the original dark-adapted state with a half-time of about 3 min. Thus, the dark-adapted state is intermediate between state I and state II but is considerably closer to state I than to state II. Most of these characteristics of states I and II have been described previously [2—4]. Our purpose here is to determine how the photochemical properties of the cells change during the transition between those states.

Cells were placed in state I or state II by irradiation for 5 min with blue or green light at room temperature and that condition was frozen in by freezing the cells to —196° C during continued irradiation. Emission spectra of the cells at —196° C in states I and II are shown in Fig. 2 with fluorescence excitation at 560, 500 and 435 nm. 560 nm light is absorbed predominantly by the phycobilisomes; 435 nm light is absorbed predominantly by chlorophyll and 500 nm light is absorbed by both sets of the pigments. The emission bands at 642, 662, and 683 nm are due to phycocyanin, allophycocyanin and allophycocyanin B of the phycobilisomes, respectively, the bands at 694 and 755 nm are due to the antenna chlorophyll of Photosystem II and the band at 716 nm is due to the antenna chlorophyll of Photosystem I [11,13].

In each of the cases in Fig. 2, the state I minus state II difference spectrum (plotted just above the emission spectra) shows a greater yield of fluorescence from Photosystem II chlorophyll (694 nm) and allophycocyanin B (683 nm) in state I but no difference in the yields of fluorescence from phycocyanin or allophycocyanin. The fluorescence yield changes of allophycocyanin B are known to follow those of Photosystem II chlorophyll because of the tight energy coupling. The data in Fig. 2 show clearly that the fluorescence yield of the Photosystem II pigments is greater in state I than in state II.

Yields of energy transfer from Photosystem II to Photosystem I were determined by methods developed previously [13] for cells frozen in states I and II. In brief, measurements were made of the excitation spectrum for 730 nm fluorescence and of the absorption spectrum for a dilute suspension of cells (see Figs. 3B and 3E). The ratio spectra F_{730}/A in Figs. 3A and 3D indicate the wavelength dependence of the relative yield of the 730 nm fluorescence. If the

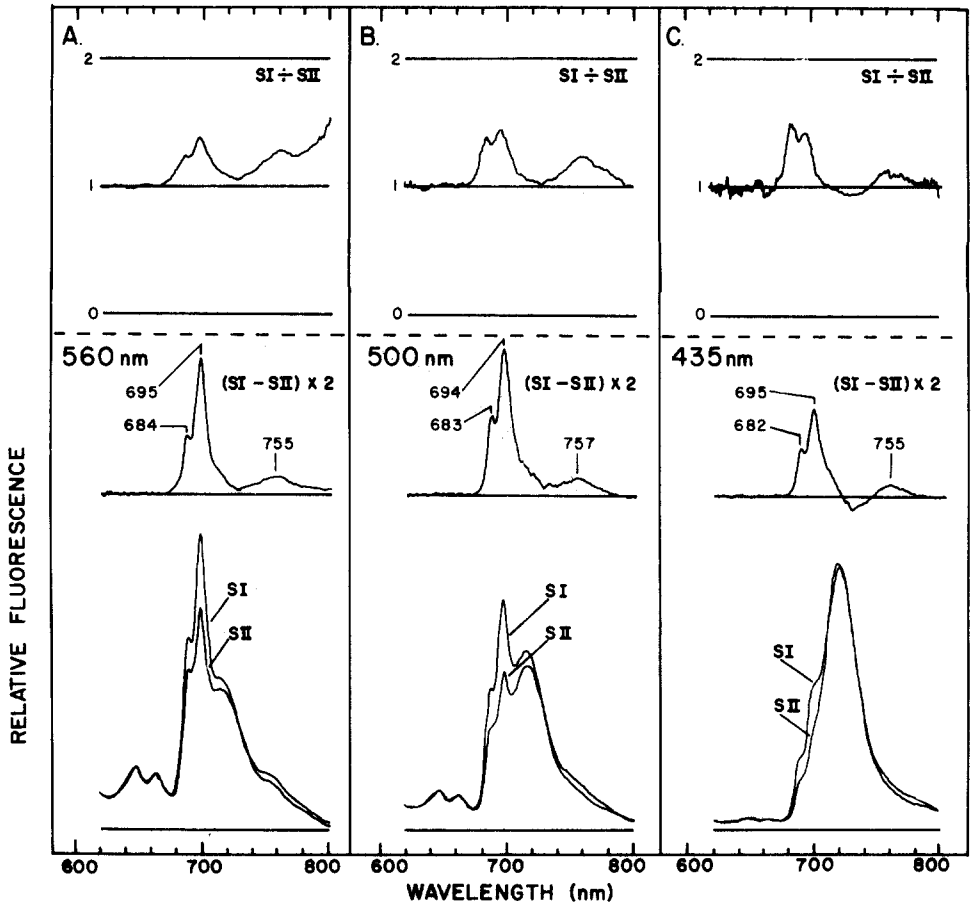


Fig. 2. Low temperature emission spectra of *P. cruentum* cells frozen to —196°C in state I (SI) during irradiation with blue light or frozen in state II (SII) during irradiation with green light. A, excitation at 560 nm; B, 500 nm; C, 435 nm. Difference spectra state I — state II and ratio spectra state I/state II are plotted above the emission spectra.

720 nm fluorescence were a pure Photosystem I emission, we would expect that the maximum yield of fluorescence would occur at 690 nm which is absorbed solely by Photosystem I chlorophyll and that shorter wavelengths, which excite Photosystem I at least in part via energy transfer from Photosystem II, should show lower yields of fluorescence. On that basis, the ratio spectra F_{730}/A present something of an anomaly in that the yield of the 730 nm fluorescence is greater in the 560 nm region than it is at 690 nm but that anomaly is readily resolved by an inspection of the emission spectrum excited at 560 nm. It is apparent that the 730 nm fluorescence excited by 560 nm light is not a pure Photosystem I emission but also includes a significant contribution from the long wavelength tail of the Photosystem II emission. Thus, in order to proceed with the analysis, we need to determine the fraction of the 560 nm-excited 730 nm fluorescence which is due solely to Photosystem I.

The 560-excited emission spectrum can be resolved into its Photosystem I and Photosystem II component parts as was done previously [13]. The low

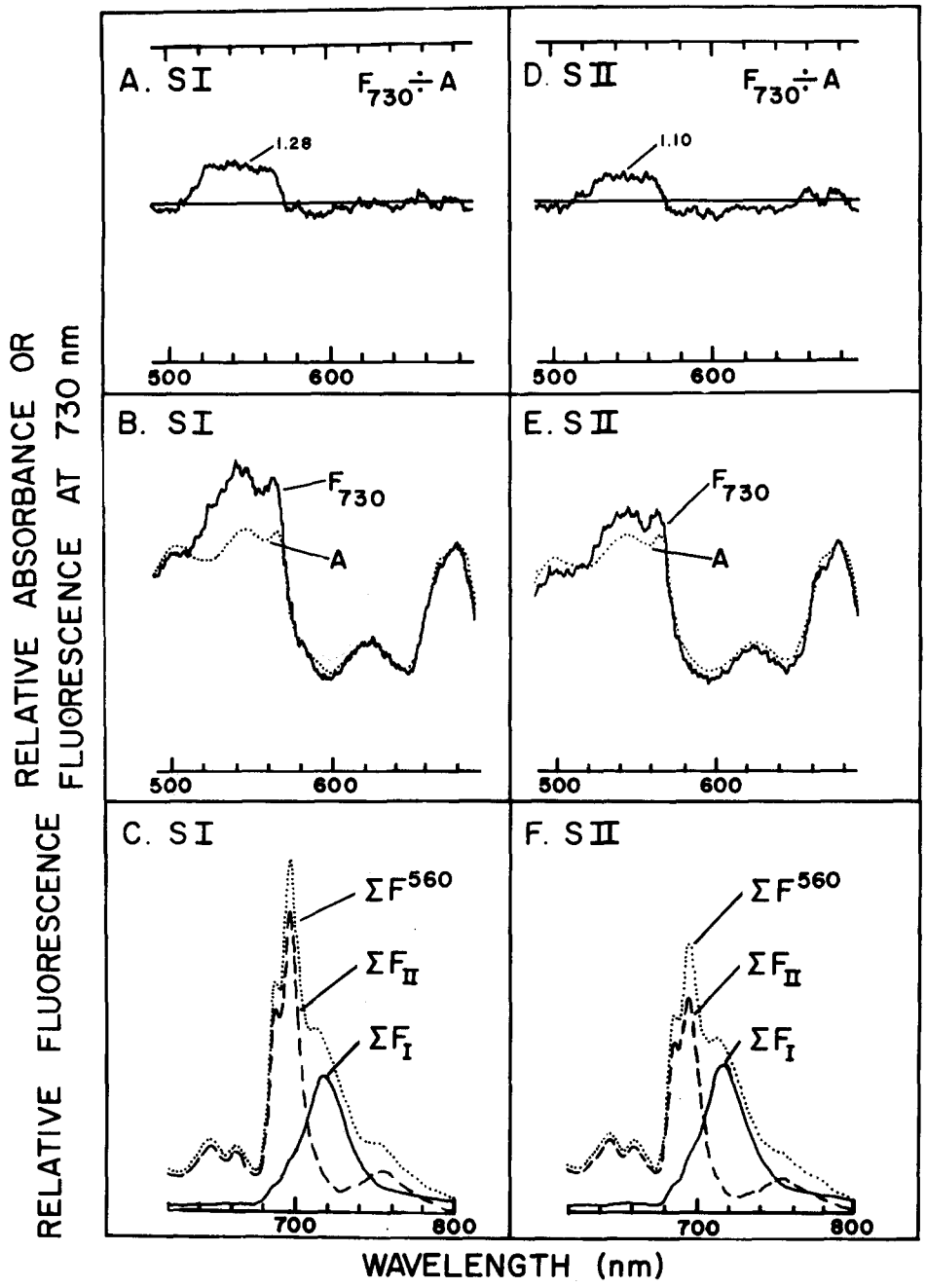


Fig. 3. Wavelength dependence of the relative yield of the 730 nm fluorescence of *P. cruentum* cells at −196°C and deconvolution of the emission spectrum into Photosystem I and Photosystem II components. A and D, ratio spectra F_{730}/A; B and E, fluorescence excitation spectra F_{730}, corrected for a constant quantum flux of excitation, and absorption spectra A. C and F, deconvolution of the fluorescence emission spectrum excited at 560 nm, ΣF^{560}, into its Photosystem I and Photosystem II components, ΣF_I and ΣF_{II}. Cells in state I (SI) or state II (SII) as indicated.

temperature emission spectrum of cells in state II excited at 435 nm can be taken as a virtually pure Photosystem I emission spectrum. We have determined that the ratio F_{716}/F_{693} for a pure Photosystem II emission spectrum is approx. 0.14 [7]. Thus, the deconvolution requires that the proper amount of the pure Photosystem I emission spectrum ΣF_I be subtracted from the 560 nm-excited emission spectrum ΣF^{560} so that the resulting difference spectrum ΣF_{II} has a ratio F_{716}/F_{693} of 0.14. Figs. 3C and 3F show such deconvolutions for cells in SI and SII. The relative contributions of Photosystem I and Photosystem II to the 730 nm fluorescence were then calculated by integrating the product of the deconvoluted emission spectra times the spectral transmission curve of the 730 nm filter used in the measurement over the spectral passband of the filter (see Ref. 7). These calculations indicated that 76% of the 730 nm fluorescence was due to Photosystem I in state I and that 85% was due to Photosystem I in state II. These values allow us to correct the ratio F_{730}/A at 560 nm to include only the Photosystem I component of the 730 nm fluorescence. The product of the values in the first two columns in Table I gives the corrected value for the ratio of the yields of the Photosystem I fluorescence at 730 nm excited at 560 and 690 nm (given in the third column) and this corrected value of the ratio is taken as the maximum yield of energy transfer from Photosystem II to Photosystem I [7].

The excitation spectra of the 730 nm fluorescence were measured at the maximal F_M level where the Photosystem II reaction centers are all closed (recall that the cells were frozen to −196° C during irradiation) so that the yield of energy transfer will be the maximal $\varphi_{T(II\rightarrow I)(M)}$ value. The values of $\varphi_{T(II\rightarrow I)(M)}$ in Table I are not significantly different for cells in state I or state II; both are close to unity. However, it is more meaningful to consider values of $\varphi_{T(II\rightarrow I)(o)}$ which are obtained when the Photosystem II reaction centers are all open since that is the condition for optional photosynthesis. The ratio of $\varphi_{T(II\rightarrow I)(M)}/\varphi_{T(II\rightarrow I)(o)}$ will be the same as the ratio of F_M/F_o for Photosystem II fluorescence so that the value of $\varphi_{T(II\rightarrow I)(o)}$ can be determined from the value of $\varphi_{T(II\rightarrow I)(M)}$ and the ratio $F_{693(M)}/F_{693(o)}$. The ratio determination requires that cells be frozen in states I and II in the dark with the Photosystem II reaction centers fully open in order to measure the increase of fluorescence at 693 nm which occurs when the Photosystem II centers are closed photochemically. Cells can be frozen in state II quite readily if the freezing occurs in the light but some difficulty was experienced in trying to freeze cells in state II in the dark after a short dark period to allow the regeneration

TABLE I

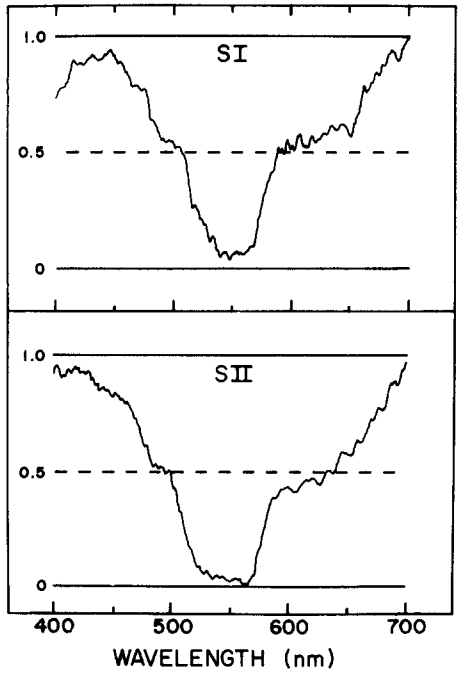
COMPARISON OF FLUORESCENCE AND ENERGY TRANSFER PROPERTIES OF CELLS OF *P. CRUENTUM* AT −196°C IN STATE I AND STATE II

	$\frac{\varphi_{F730}^{560}}{\varphi_{F730}^{690}}$	$\frac{F_{I(730)}}{F_{730}}$	φ_T (II → I) (M)	$\frac{F_{693(M)}}{F_{693(o)}}$	φ_T (II →) (o)
State I	1.28	0.76	0.97	2.04	0.48
State II	1.10	0.85	0.94	1.54	0.61

of open Photosystem II reaction centers. Similar problems were reported previously with green leaves [14]. Even though state II is reasonably stable in the dark at room temperature, it appears to escape quite rapidly to state I during the freezing process. This escape from state II which occurs when the temperature is lowered is the reason why Briantais et al. [5] concluded that there were no differences in energy distribution between state I and state II; they assumed that they had frozen chloroplasts to −196° C in state II while, in fact, the chloroplasts were frozen in state I. The escape from state II may be even more rapid in chloroplasts than in whole cells. However, state II can be trapped in spinach chloroplasts at −196° C if the chloroplasts are frozen during the actinic irradiation (Kitajima, M. and Butler, W.L., unpublished data). With our usual freezing procedures we would invariably obtain *P. cruentum* cells in state I when we tried to freeze the cells in state II in the dark. To overcome these problems samples were prepared by filtering cells of *P. cruentum* onto Millipore filter discs to provide very thin samples which could be frozen rapidly by plunging them directly into liquid nitrogen. With these rapid freezing procedures we were able to freeze in state II after a short dark period (45 s was sufficient for all of the Photosystem II centers to open) so that the ratio of $F_{693(M)}/F_{693(o)}$ could be determined for cells in both state I and state II (Table I). The values of $\varphi_{T(II\rightarrow I)(o)}$ were then calculated by dividing the values of $\varphi_{T(II\rightarrow I)(M)}$ by the ratios of $F_{693(M)}/F_{693(o)}$ (Table I). It is apparent that $\varphi_{T(II\rightarrow I)(o)}$ is significantly larger in state II (0.61) than in state I (0.48). It will be shown in the discussion (vide infra) that the values of $\varphi_{T(II\rightarrow I)}$ in Table I are consistent with a doubling of the rate constant for energy transfer, $k_{T(II\rightarrow I)}$, in the transition between state I and state II.

We can also determine the relative cross sections of Photosystem I and Photosystem II, α and β (where $\alpha + \beta = 1.0$), for any wavelength of excitation [11]. Cells which had been brought to state I or II by irradiation with 440 or 560 nm light at room temperature were frozen rapidly to −196° C after a 45-s dark period. Excitation spectra were measured for fluorescence at 693 and 730 nm before any actinic irradiation (F_o), after actinic irradiation with far-red light which closed the Photosystem I reaction centers (i.e., photooxidized *P*-700) but had no influence on the Photosystem II centers (F_o') and after a saturating irradiation with white light which closed all of the Photosystem II centers (F_M). Photooxidation of *P*-700 caused the yield of the 730 nm fluorescence to increase 15 to 20% but had no effect on the 693 nm fluorescence. The wavelength distribution of α was then calculated by the computer from the excitation spectra for the 693 and 730 nm fluorescence measured at the F_o' and F_M levels and the value of $\varphi_{T(II\rightarrow I)(M)}$ according to equations presented previously [7,11]. We conclude from the similarity between the spectra of α in state I and state II presented in Fig. 4 that the cross sections of Photosystem I and Photosystem II do not change during transitions between states I and II.

The above results on the spectral distribution of α lead to certain predictions for the action spectra of oxygen evolution measured in state I and state II. To make these measurements the photochemical state of the cells was established with a constant background irradiation of 300 $\mu W/cm^2$ at 560 nm (for state II) or 350 $\mu W/cm^2$ at 440 nm (for state I) and the rate of oxygen evolution was measured with a weak monochromatic modulated beam. Action



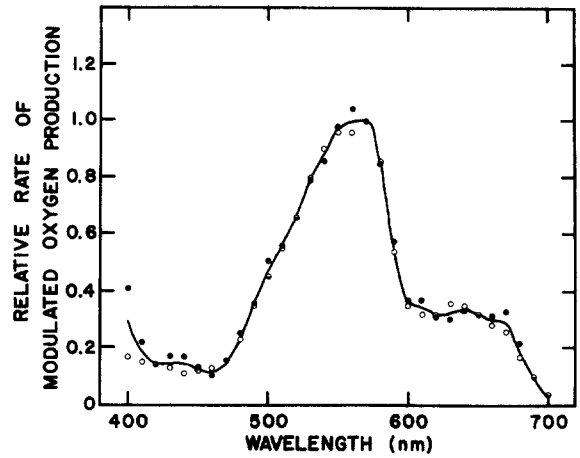


Fig. 4. Wavelength dependence of α for *P. cruentum* cells in state I (SI) and state II (SII) at −196°C.

Fig. 5. Relative rate of modulated oxygen evolution as a function of wavelength with a constant quantum flux of excitation. Measurements were made with a very weak (3—5 μW/cm^2) modulated monochromatic beam superimposed on a strong constant beam of 440 nm light (350 μW/cm^2) to hold the cells in state I (open circles) or 560 nm light (300 μW/cm^2) to hold the cells in state II (closed circles).

spectra for the modulated oxygen evolution corrected for equal incident quantum flux and normalized are presented in Fig. 5 for cells held in state I and state II. The two spectra which are, in essence, action spectra for Photosystem II show no significant differences. Energy distribution in the photochemical apparatus can be described in terms of two parameters, α, which is a function of wavelength and $\varphi_{T(II\to I)}$, which is independent of wavelength. The observation that $\varphi_{T(II\to I)(o)}$ changes between states I and II, while α does not, leads to the prediction that the action spectrum for Photosystem II (which follows the wavelength dependence of β) should be the same in both states. That prediction is born out in the results of Fig. 5. Furthermore, we note that the prediction, which was based on measurements made at low temperature, was tested by measurements made at room temperature under physiological conditions.

Earlier work [15] established that DCMU blocks the transition from state I to state II but not the reverse transition from state II to state I. Fig. 6 shows the results of a similar study in which low temperature emission spectra at the F_M level were used as the assay for the two states. Fig. 6A shows the normal state I and state II emission spectra as well as the state I-state II difference spectrum for cells which had been irradiated for 5 min with blue light (state I) and green light (state II) before being frozen to −196°C while the

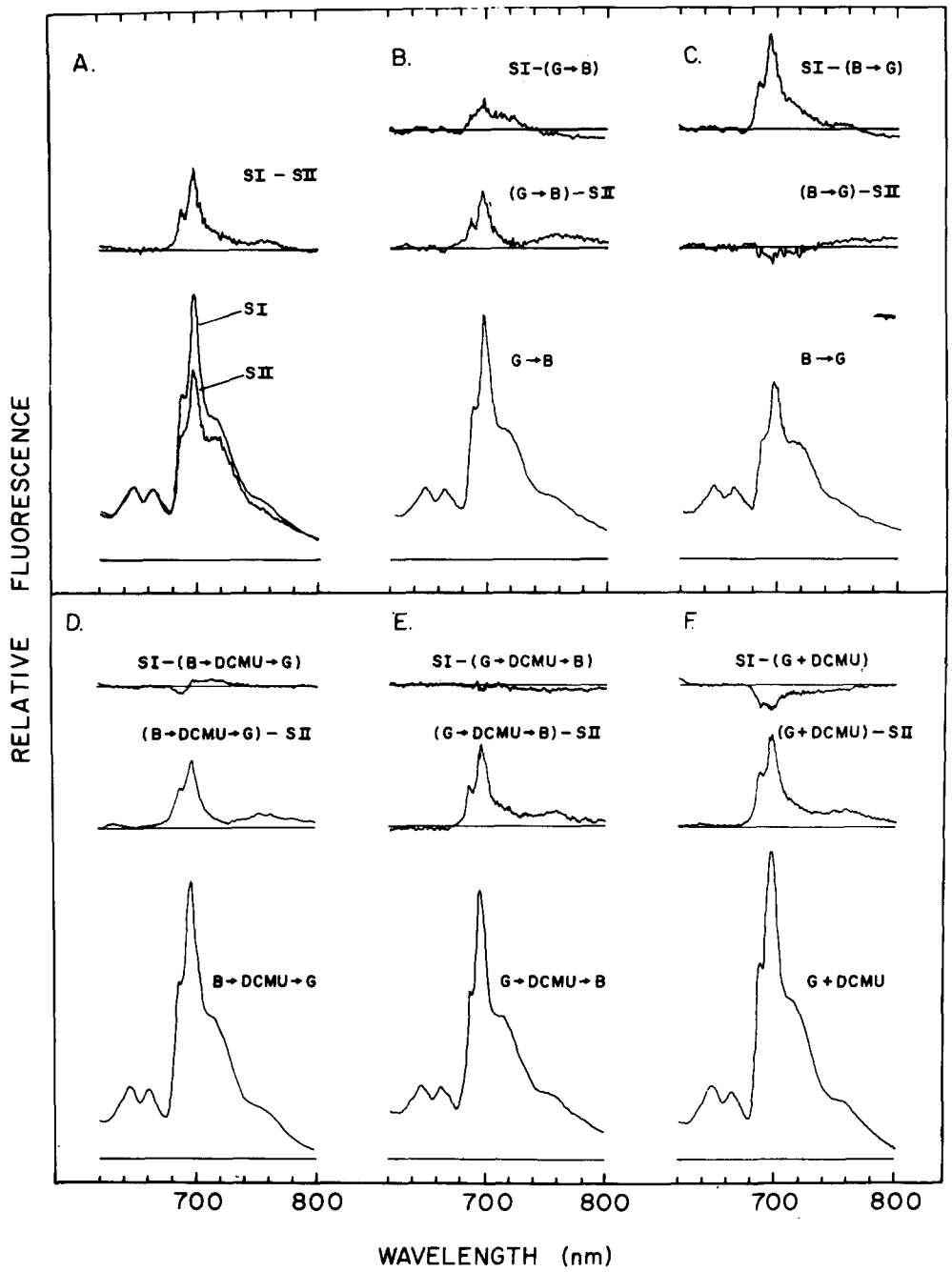


Fig. 6. Effects of DCMU on transitions between states I (SI) and II (SII) in *P. cruentum* cells. A, emission spectra of cells irradiated 5 min with blue light and frozen to —196°C in the blue light, state I, and of cells irradiated 5 min with green light and frozen to —196°C in the green light, state II, and the difference spectrum state I — state II. B, emission spectrum of cells irradiated 5 min with green light, then 5 min with blue light and frozen to —196°C in the blue light, G → B, and difference spectra of these cells versus cells in state I and state II. C, emission spectrum of cells irradiated 5 min with blue light, then 5 min with green light and frozen to —196°C in the green light, B → G, and difference spectra versus cells in state I and state II. D, same as C but with 5 μM DCMU added between the blue and green inrradiation. E, same as B but with 5 μM DCMU added between the green and blue irradiation. F, emission spectrum of cells irradiated 5 min with green light with 5 μM DCMU added during the irradiation after 4 min. The cells were then frozen to —196°C in the green light.

irradiation continued. The two states are fully reversible at room temperature in that cells which were irradiated for 5 min with green light and then for 5 min with blue light before being frozen in blue light were in state I due to the final irradiation with blue light (Fig. 6B) while cells which were irradiated for 5 min with blue and then 5 min with green before being frozen in green light were in state II (Fig. 6C). However, if DCMU was added after the initial blue irradiation but before the green, the cells remained in state I (Fig. 6D). DCMU prevented the transition from state I to state II. On the other hand, if DCMU was added after an initial green but before a subsequent blue, the cells were also in state I (Fig. 6E). DCMU did not prevent the state II to state I transition mediated by blue light. Furthermore, if DCMU was added during a green irradiation one minute before the cells were frozen to −196° C in the green light, the cells reverted from state II to state I during the final minute of irradiation (Fig. 6F). In the presence of DCMU even green light acts as Photosystem I light. Adding DCMU during a blue irradiation had no effect on the final state (data not shown). Thus, it is clear that DCMU blocks the Photosystem II mediated state I to state II transition but has no effect on the Photosystem I mediated state II to state I transition.

Discussion

Our original investigation of the low temperature fluorescence properties of *P. cruentum* in the context of the tripartite model indicated that the photochemical apparatus was comprised of relatively large Photosystem I units which contained approximately 95% of the chlorophyll and small Photosystem II units which contained the remaining 5% of the chlorophyll and that the large phycobilisomes transferred their excitation energy almost exclusively to the small Photosystem II units [11]. In addition, the yield of energy transfer from Photosystem II to Photosystem I was found to be quite high ranging from values of about 0.50 when the Photosystem II reaction centers were open to 0.95 when the centers were closed [13]. Recently, it was shown in a study of chromatic adaptation in *P. cruentum* [7] that these photochemical properties are characteristic of cells grown in light absorbed primarily by the phycobilisomes while cells grown in light absorbed primarily by chlorophyll make much larger Photosystem II units which contain approx. 40% of the chlorophyll and which transfer energy less efficiently to Photosystem I. The cells used in the present study were of the original type which showed very small Photosystem II units with high probabilities for energy transfer to Photosystem I. Those characteristics are confirmed by the large values of α (approaching unity) at 435 nm and the low values of α (approaching zero) in the 560 nm region (see Fig. 4) and by the high yields for energy transfer from Photosystem II to Photosystem I (Table I).

Measurements of the wavelength distribution of α did not indicate any significant changes in the cross sections of the photosynthesis in the transition between state I and state II. However, the transition from state I to state II was found to be accompanied by a decrease in the fluorescence yield of Photosystem II, an increase in the minimum yield of energy transfer from Photosystem II to Photosystem I, $\varphi_{T(II\to I)(o)}$, and a decrease in the ratio of F_M/F_o

for Photosystem II fluorescence. In order to relate those observations to changes in fundamental photochemical parameters we will adopt the simplified bipartite formulation of the tripartite model [16]. According to that formulation:

$$\frac{F_M}{F_o} = \frac{1}{1 - \psi_{TII}\psi_{tII}}$$

where ψ_{TII} is the probability that excitation energy in a Photosystem II unit will be trapped by the reaction center chlorophyll and ψ_{tII} is the probability that the energy trapped by the chlorophyll of a closed Photosystem II reaction center will be returned to the antenna chlorophyll of that unit. We will assume for simplicity that there are no fluorescent or nonradiative decay losses at the reaction center chlorophyll so that $\psi_{tII} = 1.0$. The sum of the probabilities of all of the deexcitation processes in the antenna chlorophyll of Photosystem II must add up to unity; i.e., $\psi_{FII} + \psi_{DII} + \psi_{TII} + \psi_{T(II\to I)} = 1.0$ where the subscripts indicate fluorescence, nonradiative decay, trapping by the reaction center chlorophyll and energy transfer to Photosystem I, respectively. Thus,

$$\frac{F_M}{F_o} = \frac{1}{1 - \psi_{TII}} = \frac{\psi_{FII} + \psi_{DII} + \psi_{TII} + \psi_{T(II\to I)}}{\psi_{FII} + \psi_{DII} + \psi_{T(II\to I)}}$$

and since $\psi_{XII} = k_{XII}/\Sigma\, k_{II}$:

$$\frac{F_M}{F_o} = \frac{k_{FII} + k_{DII} + k_{TII} + k_{T(II\to I)}}{k_{FII} + k_{DII} + k_{T(II\to I)}}$$

If we assign relative values of $(k_{FII} + k_{DII}) : k_{TII} : k_{T(II\to I)}$ of 5 : 100 : 90 for cells in state I we calculate theoretical values which are in reasonably good agreement with the experimental data obtained in state I : $F_M/F_o = 2.05$ (vs the measured value of 2.04 in Table I), $\varphi_{T(II\to I)(o)} = 0.46$ (vs. 0.48) and $\varphi_{T(II\to I)(M)} = 0.95$ (vs. 0.97). Most of the experimental data for the cells in state II can be accomodated quite well if we assume only that the relative value of $k_{T(II\to I)}$ increases from 90 to 180 in the transition from state I to state II. In that case in state II, $F_M/F_o = 1.54$ (vs. the measured value of 1.54), $\varphi_{T(II\to I)(o)} = 0.63$ (vs. 0.61) and $\varphi_{T(II\to I)(M)} = 0.97$ (vs. 0.94). However, the state I/state II ratio of the 693 nm fluorescence at the F_M level may not be predicted adequately by assuming that only $k_{T(II\to I)}$ changes. The measured value of that ratio was 1.5 ± 1.0 while the value predicted from the relative values of rate constants is 1.95. The measured value could be too low because of overlap from the 680 and 716 nm bands in the measurement at 693 nm but such effects are probably not sufficient to account for the discrepancy. It may be that one of the other rate constants in the system such as k_{TII} also changes during the transition from state I to state II. All of the experimental data can be predicted quite closely by assuming changes in various pairs of rate constants but in each case one member of the pair must be $k_{T(II\to I)}$. We can state unequivocally from our data that the transition from state I to state II is accompanied by a major increase (60—100%) in $k_{T(II\to I)}$ but we cannot assert that this is the only photochemial parameter to change. We assume that the

increase in $k_{T(II \rightarrow I)}$ reflects a closer physical association between Photosystem I and Photosystem II units.

It has been suggested that the changes of energy distribution which occur during transitions between states I and II are similar to those which are produced in chloroplasts by the presence and absence of divalent cations. In such correlations cells in state II are assumed to be analogous to chloroplasts suspended in the absence of divalent cations in that both conditions induce an increase in the distribution of excitation energy to Photosystem I. Mechanistically, it could be envisaged that outward movement of magnesium which occurs in response to the inward pumping of H^+ into the thylakoids results in a membrane conformational change which increases energy transfer from Photosystem II to Photosystem I in state II and that these same changes occur when isolated chloroplasts are depleted of divalent cations. However, the experiments with DCMU indicate that such correlations are probably oversimplifications. In the presence of DCMU, we would expect that Photosystem I could produce ion gradients by cyclic electron transport that were smaller but in the same direction as those produced by noncyclic electron transport in the absence of DCMU. If states I and II were solely a question of ion gradients or the concomitant energization of thylakoid membranes we would not expect strong antagonistic effects between Photosystem I and Photosystem II. At best the effect of Photosystem I light would be similar to darkness in causing the conversion of state II to state I but we would not expect Photosystem I to actively stimulate that conversion. The fact that Photosystem I activity does stimulate the transition from state II to state I indicates additional factors must be involved. The results with DCMU suggest (in agreement with the earlier work and suggestions of Duysens [15]) that some component or components between Photosystem II and Photosystem I must be reduced in order for state II to occur and that state I prevails when those components are oxidized. However, some cooperative effects between ion gradients and the redox state of components in the electron transport system between Photosystem II and Photosystem I may play an important role in these phenomena.

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