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# CONTROL OF EXCITATION TRANSFER IN PHOTOSYNTHESIS

## IV. KINETICS OF CHLOROPHYLL *a* FLUORESCENCE IN *PORPHYRA YEZOENSIS*

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SUMMARY

The kinetics of chlorophyll *a* fluorescence were measured at 685 nm in intact cells of *Porphyra yezoensis* during alternate illumination of the organism with two colors of light, one absorbed by phycoerythrin and the other by chlorophyll *a*. Two components of fluorescence change overlapping each other in time were separated; the fast component may be controlled by the rate of Photoreaction II which competes with the fluorescence emission process, and the slow component by the light-induced change in excitation transfer between two pigment systems as suggested in our previous study[6]. The kinetics of the slow change in fluorescence yield were extensively investigated.

Terms, "State I" and "State II" are used to describe the state of excitation transfer. In the State I a lesser amount of excitation energy is delivered in Pigment System I and greater to Pigment System II than in the State II. The conversion of the states is achieved by the selective illumination of pigment systems.

The conversion from the State I toward the State II occurred under Light II (light absorbed by Pigment System II) with a half time of about 10 sec, and it saturated at a light intensity of less than 1000 ergs·cm$^{-2}$·sec$^{-1}$. The reverse conversion occurred under Light I (light absorbed by Pigment System I) with a half time of about 5 sec, and it saturated at about 10000 ergs·cm$^{-2}$·sec$^{-1}$.

Light I and Light II competed with each other in the interconversion of the states.

## INTRODUCTION

Two kinds of light-induced changes in chlorophyll *a* fluorescence have so far been observed in intact cells of photosynthetic plants[1-7]. One kind can be explained by the quenching effect of Photoreaction II (refs. 8–10), or of the primary electron

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; PMS, *N*-methylphenazonium methylsulfate.

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acceptor of Photoreaction II (refs. 2 and 11). The other was not easily explained and suggested another mechanism of fluorescence change. LAVOREL[1] proposed a non-fluorescent and photo-inactive chlorophyll complex. DUYSENS AND SWEERS[2] proposed a quenching by a photochemically inactive form of the primary electron acceptor of Photoreaction II. BANNISTER AND RICE[3] explained the change in fluorescence yield by a term "activation" of Pigment System II, and PAPAGEORGIOU AND GOVINDJEE[4,5] considered a light-induced conformational change of membrane structure.

Measuring the low-temperature fluorescence spectra, we investigated light-induced control of the distribution of excitation energy between the two pigment systems in intact cells of *Porphyridium cruentum*, and concluded that his control mechanism caused the change in fluorescence yield of Pigment System II, which had not been explained by assuming a competition between the fluorescence emission and Photoreaction II (ref. 6).

In this mechanism, upon illumination of algal cells with Light I (light absorbed by Pigment System I), a larger proportion of the absorbed light quanta becomes available to Pigment System II and less to Pigment System I than upon illumination with Light II (light absorbed by Pigment System II). This control of excitation transfer between two pigment systems allows the photosynthetic organisms to utilize efficiently the absorbed light energy for photosynthesis[6].

BONAVENTURA AND MYERS[7], using Chlorella, performed simultaneous measurements of chlorophyll *a* fluorescence and $O_2$ evolution, and found a change in quantum efficiency of Pigment System II upon illumination of the organism. A greater amount of excitation energy was delivered to Pigment System II upon the illumination of the organism with Light I, and less, with Light II. They interpreted their results by assuming a light-induced control of distribution of excitation energy between the two pigment systems, thus reaching the concept of a control mechanism the same as ours, although they obtained no evidence for the amount of excitation energy delivered to Pigment System I.

Terms "State I" and "State II" will now be used to describe the control mechanism of excitation transfer between the two pigment systems. In the State 1 a greater amount of excitation is available to Pigment System II, and a smaller amount is available to Pigment System I, than in the State II. Consequently, Light I is more, and Light II is less, efficiently utilized to drive the photosynthetic electron transport in the State I than in the State II. The States I and II correspond to "light 1 state" and "light 2 state", respectively, of BONAVENTURA AND MYERS[7] in respect to the energy distribution between the two pigment systems. The low-temperature fluorescence spectra in our previous study[6] indicated that the intact cells of *P. cruentum* are in the State I when the cells were kept in the dark or illuminated with Light I, and they are in the State II when the cells were illuminated with light II. In the presence of a photosynthetic inhibitor, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU), the cells were in the State I in the dark and under illumination with Light II as well as with Light I.

Our previous study[12,13] on the low-temperature fluorescence spectra in isolated spinach chloroplasts showed that ions of the alkaline earth metals and manganese increased the amount of excitation energy delivered to Pigment System II and decreased that to Pigment System I. The measurements of activities of the $NADP^+$ reduction in the presence of DCMU, 2,6-dichlorophenolindophenol (DCIP) and as-

corbate, and the Hill reaction with DCIP in the same material also supported the idea that $Mg^{2+}$ enhanced the quantum yield of Photoreaction II and suppressed that of Photoreaction I (ref. 12). Kinetic analyses of the fluorescence induction showed that $Mg^{2+}$ depressed the excitation transfer from Pigment System II to I (spillover of excitation)[12]. These findings suggested that the metal ions play an important role in the light-induced control of excitation transfer.

On the other hand, it has been reported that the metal ions are transported through the chloroplast membrane upon illumination[14,15]. According to NOBEL[16], some kinds of metal ions such as $K^+$ and $Mg^{2+}$ were extruded from chloroplasts upon illumination of the leaves of *Pisum sativum*, though, unfortunately, the effects of selective illumination of the two pigment systems upon this ion transport have not yet been studied. In any case, the light-induced transport of metal ions is probably related to the light-induced control of excitation transfer between the two pigment systems[6], *i.e.*, the interconversion between the State I and the State II.

In the present study, kinetics of chlorophyll *a* fluorescence were studied in a red alga, *Porphyra yezoensis*, to investigate the light-induced interconversion of the State I and the State II.

## METHODS

The marine alga, *P. yezoensis*, used in these experiments was supplied by the courtesy of Yamamoto Nori Research Laboratory and was grown in synthetic sea water under fluorescent lamp light. The absorption of a thallus measured with the Shimadzu-MPS spectrophotometer was approx. 0.4 at absorption peaks near 435, 500, 570 and 675 nm.

One layer of a thallus of Porphyra which was attached to a thin glass plate was placed along the diagonal of the 1 cm × 1 cm × 4 cm four-sided transparent cuvette containing the synthetic sea water.

Green excitation light absorbed mainly by phycoerythrin was obtained from a 150-W incandescent lamp powered by a stabilized source by filtering the light through a combination of glass filters, G-520 (Hoya Glass), two pieces of B-460 (Hoya Glass) and 9780 (Corning), which transmitted wavelengths between 470 and 600 nm and had a peak at 525 nm. The chlorophyll *a* fluorescence at 685 nm emitted from the same side of thallus facing the exciting light was isolated with a Bausch and Lomb grating monochromator set at 20 nm half bandwidth and equipped with a glass cutoff filter, V-R67 (Toshiba) that transmitted wavelengths longer than 670 nm. Blue preillumination light absorbed mainly by chlorophyll *a* was obtained from the 150-W incandescent lamp or from a 500-W high-pressure mercury lamp (Ushio Electric Co.) used in conjunction with a blue band-pass filter, V-V44 (Toshiba), or with interference filters. The filter V-V44 transmitted wavelengths between 370 and 480 nm and had a transmission peak at 440 nm. The preillumination light was incident on the sample at a small angle to the excitation light. The intensities of exciting and preillumination light were varied with neutral density filters, N-10, N-25, N-50 (Hoya Glass). Fluorescence was detected with a photomultiplier, 7102 (RCA) cooled with solid $CO_2$. The photomultiplier signal was amplified and recorded with a strip chart servo recorder (Riken Denshi). The time response of the recording system was 0.3 sec. Light intensities were measured with a calibrated thermopile equipped with a

filter, IRO-1A (Toshiba). The filter absorbs infrared light. The light intensities were corrected for the absorption of the filter at the visible region.

## RESULTS

The fluorescence spectrum of intact cells of *Porphyra yezoensis* excited by light absorbed by phycoerythrin showed bands of phycobilins at 580, 645 and 655 nm and of chlorophyll *a* at 685 and 720 nm (ref. 17). The action spectrum for chlorophyll *a* fluorescence at 685 nm indicated that this fluorescence was emitted from Pigment System II (ref. 17).

### *Time-course of chlorophyll a fluorescence after switching illumination from Light I to Light II*

Fig. 1a shows a time-course of the chlorophyll *a* fluorescence in intact cells of *P. yezoensis*. After a long dark period the fluorescence showed an induction phenomenon upon the illumination with 525-nm light (6000 ergs·cm$^{-2}$·sec$^{-1}$) absorbed mainly by phycoerythrin (Light II). Two peaks appeared during this induction period. With much stronger excitation light, the fluorescence displayed more complex time-courses.

After the illumination with 525-nm light for about 200 sec, the fluorescence yield reached a steady level, S. Then, the illumination was changed to 440-nm light with approximately the same intensity (7500 ergs·cm$^{-2}$·sec$^{-1}$) which was mainly absorbed by chlorophyll *a* (Light I). After the illumination with Light I for about 30 sec, the illumination was again switched to Light II. The fluorescence yield showed a fast rise from the initial level, O, to a peak, P, which was about 1.5 times as high as at the steady level and gradually declined to the steady level, S (Fig. 1a). In Fig. 1b, where the time scale was four times expanded as compared in Fig. 1a, the increase of fluorescence from O to P could be easily seen.

The period between O and P was shorter with a higher intensity of excitation light. It was 1.8 sec at an intensity of 6000 ergs·cm$^{-2}$·sec$^{-1}$, and 4.0 sec at 2100 ergs·cm$^{-2}$·sec$^{-1}$ (see also Fig. 5a).

In order to examine what pigment was active in increasing the fluorescence

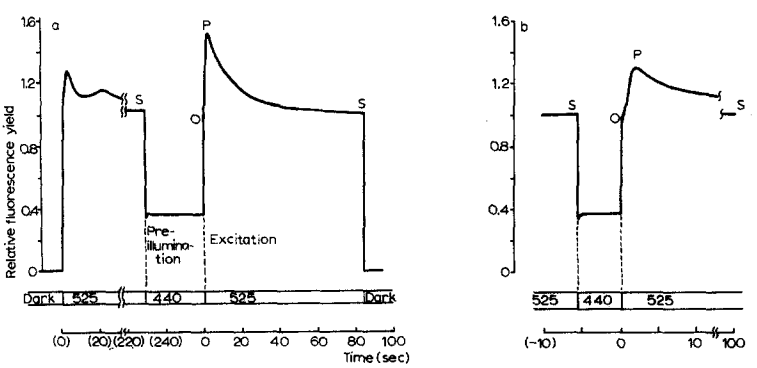



Fig. 1. Time-course of chlorophyll *a* fluorescence measured at 685 nm in *P. yezoensis*. Excitation light; 525 nm, 6000 ergs·cm$^{-2}$·sec$^{-1}$. Preillumination; 440 nm, 7500 ergs·cm$^{-2}$·sec$^{-1}$ obtained from an incandescent lamp. O, P and S represent the initial, peak and steady levels of the fluorescence yield.

yield after switching the light from preillumination to excitation, several colors of monochromatic light obtained through interference filters were used for the preillumination. 438- and 678-nm light which were absorbed by chlorophyll *a* were remarkably effective, but 568-nm light which was absorbed by phycoerythrin had no effects. While the preillumination with 618-nm light absorbed mainly by phycocyanin showed a slight effect in increasing the fluorescence yield after the switching, the effectiveness was much less marked than with 438- or 678-nm light. Thus, it is concluded that preillumination of Pigment System I results in the increased fluorescence yield after switching to 525-nm light.

The time-course of fluorescence yield in Fig. 1 is essentially the same as that discovered in our previous study (Fig. 4a of ref. 6) in *P. cruentum*, although a slightly different measurement system was used. We suggested there that the rapid increase in fluorescence yield after the cessation of light absorbed by Pigment System I was caused by the co-operation of the two photochemical reactions through the electron transport chain as proposed by DUYSENS AND SWEERS[2]. The slowly decreasing component after the attainment of the peak was attributed to the light-induced change in efficiency of excitation transfer between chlorophyll *a* molecules, which resulted in the control of energy distribution between two pigment systems.

Also in the case of Porphyra, the time-course of fluorescence after switching the light from 440 to 525 nm is explained by the superposition of two kinds of fluorescence changes. The fast increase in fluorescence yield from O to P corresponds mainly to the change in rate of Photoreaction II. The fluorescence yield is low when the electron carriers situated between the two photoreactions are in the oxidized state[2], and the electron carriers become reduced upon illumination with Light II, resulting in the increase in fluorescence yield from O to P. The slow decline after the attainment of the peak is mainly caused by the change in excitation transfer, *i.e.* the conversion from the State I toward the State II.

The fact that the fluorescence yield at the first peak in the induction of fluorescence after a long dark period is situated between the steady level, S, and the peak, P, indicates that the dark state of excitation transfer falls between the States I and II.

Fig. 1 also shows that the conversion from the State I toward the State II under 525-nm light was completed within 2 min and had a half time 10 sec under the experimental conditions in Fig. 1.

Most of the following experiments were performed using the same illumination procedure as in Fig. 1 but by varying the experimental conditions such as the intensity of preillumination and excitation light and the period of preillumination. The switching from excitation to preillumination took place after the fluorescence yield reached the steady level.

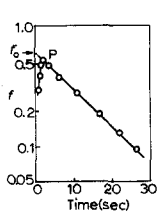
The decline of fluorescence yield from P to S displayed an exponential decay in most cases. Fig. 2 is a semilogarithmic plot of the time-course for the increased component of fluorescence after switching illumination light from 440 to 525 nm. It shows an exponential decay after the attainment of P. The relative increase in fluorescence yield, $f$, is expressed as follows:

$$f = \frac{F(t) - F_s}{F_s} \qquad (1)$$

where $F(t)$ and $F_s$ represent the fluorescence yields at time $t$ after switching the light

and at the steady state, respectively. $f_0$ is the value for $f$ at the intersection of the ordinate and the extension line of the exponentially decaying part. As shown in Fig. 2, $f_0$ attained 0.6 under the experimental conditions in Fig. 1a.

In the following, the relative increase in fluorescence, $f$, will be used as a parameter to express the degree of conversion between the two states; $f$ is zero in the State II and $f$ is about 0.6 in the State I.



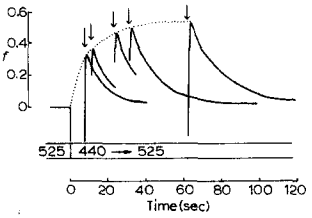




Fig. 2. Semilogarithmic plot of the increased component of chlorophyll *a* fluorescence, $f$, after switching the light from 440 to 525 nm. $f$ is presented by the ratio of the yield of increased component and the steady-state yield; $f = (F(t)-F_s)/F_s$; $F(t)$ and $F_s$ represent the fluorescence yields at time $t$ after the switching and the steady state, S, respectively. $f_0$ is the value at the intersection of the ordinate and the extension line of the exponentially decaying component. The data was taken from Fig. 1a.

Fig. 3. Effect of varying the preillumination period on the time-course of fluorescence after switching the light from 440 to 525 nm. The arrows represent the time when 440-nm light was switched to 525-nm light. Excitation light, 525 nm, 6000 ergs·cm$^{-2}$·sec$^{-1}$. Preillumination light, 440 nm, 7500 ergs·cm$^{-2}$·sec$^{-1}$, obtained from an incandescent lamp.

*Kinetics of interconversion between State I and State II*

Fig. 3 shows the time-course of fluorescence with various periods of preillumination with 440-nm light. The degree of the increase in fluorescence yield represented by $f_p$ (the relative increase in fluorescence yield, $f$, at the peak) was augmented by increasing the period of preillumination. Although $f_0$ is a better parameter to express the degree of conversion between the two states at the time of switching than $f_p$, the values for $f_p$ were used in this case. The decay kinetics after P, however, were little affected by varying the preillumination period. The half time $\tau_{1/2}$ was approx. 10 sec after switching the light from 440 to 525 nm.

The dotted line in Fig. 3 indicates the increase in the value for $f_p$, during illumination of Pigment System I. The effect of 440-nm light on increasing the fluorescence yield at P saturated at about 60 sec of illumination, at which time the value for $f_p$ attained 0.53. The half value for $f_p$ was reached after illumination with 440-nm light for 6 sec. Thus, the results in Fig. 3 indicate that the conversion from the State II toward the State I occurs with a half time approx. 5 sec under Light I, and the reverse conversion occurs with half time approx. 10 sec under Light II.

The dark conversion after the attainment from the State I to the dark state was measured by inserting a dark period between the preillumination and the excitation. Fig. 4 shows the time-courses of fluorescence yield with various dark periods before excitation. The thick line indicates the time-course of fluorescence after 30 sec preillumination without any dark time. It shows the largest value for $f_p$ and a monophasic decay of the increased component of fluorescence. The thin lines indicate the time-courses after inserting various dark periods between the end of the 30-sec preillumination and the onset of excitation, as indicated by the arrows. In this case a second peak

of fluorescence yield was observed during the decay from the peak to the steady-state level.

The dashed line in Fig. 4 is an approximation of the decay of the State I toward its dark level. The half time of this decay was approx. 20 sec. If one compares the dark decay (dashed line) to the time-course of fluorescence without inserting a dark interval (thick solid line), it will be realized that Light II accelerated the conversion

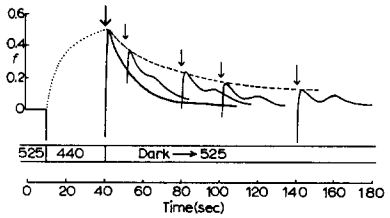



Fig. 4. Effect of inserting a dark period between the cessation of preillumination and the onset of excitation light on the time-course of fluorescence. The arrows indicate the onset of excitation light. Excitation light, 525 nm, 6000 ergs·cm$^{-2}$·sec$^{-1}$. Preillumination light, 440 nm, 7500 ergs·cm$^{-2}$·sec$^{-1}$, obtained from an incandescent lamp. The dotted line was redrawn from Fig. 3, and represents the increase of $f_p$ during the preillumination with 440-nm light.

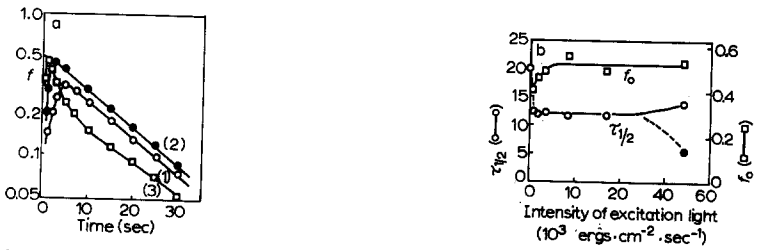



Fig. 5. a. Semilogarithmic plot of time-course of relative increase in fluorescence yield $f$ after the switching of illumination from 440- to 525-nm light with various intensities. Preillumination condition; 440-nm light, 33000 ergs·cm$^{-2}$·sec$^{-1}$, 30 sec. Excitation light; 525-nm light with intensities, (1), 850; (2), 8500; and (3), 34000 ergs·cm$^{-2}$·sec$^{-1}$. b. The half time $\tau_{1/2}$ and $f_0$ of the fluorescence decay after the peak at various intensities of excitation light. ○—○, half time $\tau_{1/2}$; ●, half time of the fast decay component appearing at 34000 ergs·cm$^{-2}$·sec$^{-1}$; □—□, $f_0$.

from the State I toward the State II and that the dark state came between the States I and II.

The fluorescence kinetics were measured after switching the illumination from 440- tot 525-nm light with a wide range of intensities of excitation light. Fig. 5a shows semilogarithmic plots of the time-course of relative increase in fluorescence yield, $f$, at the excitation light intensities of (1) 850, (2) 8500, (3) 34000 ergs·cm$^{-2}$·sec$^{-1}$. The plots with other intensities, 1700 and 3300 ergs·cm$^{-2}$·sec$^{-1}$, fell between Curves 1 and 2. The time-course with excitation light of 17000 ergs·cm$^{-2}$·sec$^{-1}$ also came between Curves 1 and 2 except during the early period after the switching, *i.e.* the fluorescence yield reached the peak earlier.

The value for $f_p$ was smaller with weaker excitation light. For example, 0.31 at 850 ergs·cm$^{-2}$·sec$^{-1}$ and 0.47 at 8500 ergs·cm$^{-2}$·sec$^{-1}$. It can also be seen that the fluorescence yield reached the peak earlier with the stronger excitation light.

While the increased component of fluorescence decayed exponentially with almost the same decay constant at the light intensities tested between 850 and 17000 ergs·cm$^{-2}$·sec$^{-1}$, with stronger excitation light, 34000 ergs·cm$^{-2}$·sec$^{-1}$ (Curve 3 in Fig. 5a), there appeared another fast decaying component after the attainment of the peak.

Fig. 5b shows the relationship between the intensity of excitation light, the values for $f_0$, and the half time, $\tau_{1/2}$. The value for $\tau_{1/2}$ in the dark (0 erg·cm$^{-2}$·sec$^{-1}$) was obtained from the experiment as shown in Fig. 4. At the intensity 34000 ergs·cm$^{-2}$·sec$^{-1}$, the slow and fast components are presented as open and closed circles, respectively.

The $\tau_{1/2}$ displayed a constant value of 12 sec over a wide range of intensities of excitation light from 850 to 17000 ergs·cm$^{-2}$·sec$^{-1}$. The value for $f_0$ was also almost constant throughout the intensities of excitation light tested, although it displayed a slight decline at low intensities of excitation light (less than 2000 ergs·cm$^{-2}$·sec$^{-1}$). It can be inferred from the results in Fig. 5 that the conversion from the State I to the State II under Light II occurs at the maximum rate approx. 10 sec in light intensities higher than 1000 ergs·cm$^{-2}$·sec$^{-1}$.

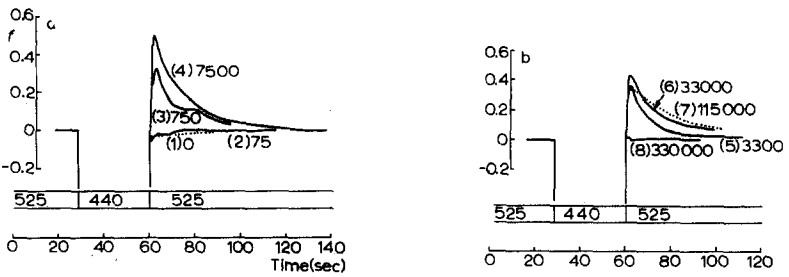



Fig. 6. Effects of varying the intensity of preillumination light on the time-course after switching the wavelength. Intensities of preillumination light are given in units of ergs·cm$^{-2}$·sec$^{-1}$. a. Intensity of excitation light was 6000 ergs·cm$^{-2}$·sec$^{-1}$. Preillumination light was obtained from an incandescent lamp. b. Intensity of excitation light was 8500 ergs·cm$^{-2}$·sec$^{-1}$. Preillumination light was obtained from a high-pressure mercury lamp.

To investigate the conversion process from the State II toward the State I with Light I, experiments were undertaken with various intensities of preillumination light. Fig. 6 shows the time-courses of fluorescence with various intensities of preillumination light. The dashed line in Fig. 6a represents the time-course with a dark time instead of preillumination light. With weak (75 ergs·cm$^{-2}$·sec$^{-1}$) and strong (330000 ergs·cm$^{-2}$·sec$^{-1}$) preillumination light, the State II was not converted toward the State I. The most significant effect was observed with intensities of 5000–30000 ergs·cm$^{-2}$·sec$^{-1}$. At an intensity of 750 ergs·cm$^{-2}$·sec$^{-1}$ of preillumination light, the time-course of fluorescence displayed a second peak during the decay from the peak to the steady level. With an intensity of 115000 ergs·cm$^{-2}$·sec$^{-1}$ of preillumination light, a slower decay was observed after the peak.

*Interaction of Light I and Light II in the conversion of State I and State II*

In order to investigate the interaction of Light I and Light II, the time-courses of fluorescence were compared with and without superimposing Light II (525 nm) on

the preillumination light (440 nm, Light I). Fig. 7 shows the semilogarithmic plots of time-courses of fluorescence with and without superimposing Light II. Light II superimposed on the preillumination light suppressed the conversion from the State II to the State I, indicated by the lowered value for $f_0$. The $f_0$'s were 3.4 and 4.7 with

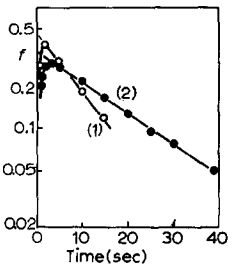



Fig. 7. Effects of superimposing 525-nm light on the preillumination light on the time-course of fluorescence after the switching the preillumination light to excitation light. Excitation light; 525 nm, 8500 ergs·cm$^{-2}$·sec$^{-1}$. Preillumination condition; (1), 440 nm, 34000 ergs·cm$^{-2}$·sec$^{-1}$, 20 sec; (2), 525-nm light, 8500 ergs·cm$^{-2}$·sec$^{-1}$ was superimposed on the 440-nm light during the preillumination.

and without superimposing light, respectively. The superimposition, however, had another effect. It delayed the decay of fluorescence yield after the peak as shown in the slower decline. This indicates that the superimposition of Light II on preillumination with Light I suppresses the conversion from the State I to State II under Light II after the switching. Consequently, the fluorescence yield after 10 sec was higher with the preillumination superimposed by Light II.

## DISCUSSION

After switching from Light I to Light II, the yield of chlorophyll *a* fluorescence showed a characteristic feature in the time-course (Fig. 1). The yield rose rapidly from the initial level (O) to the peak (P) within a few seconds and then gradually declined to the steady level (S) with half time with about 10 sec or more. The time-course is regarded as consisting of two components of time-dependent changes in fluorescence yield overlapping each other; the fast rise component (from the initial level to reach its steady level), and the slowly declining component decaying exponentially.

The former fast rise component corresponds to the fluorescence change due to the reduction of electron carriers between the two photoreactions from the more oxidized state under Light I to the more reduced state under Light II; under the more reduced state of the electron carriers the fluorescence yield is higher. This change in fluorescence yield is expected to reach its steady state within a shorter time when the stronger excitation light is used. This was obtained in the experiment of Fig. 5a. There the fluorescence excited by the higher intensities of Light II reached P more rapidly while the decline of fluorescence after P was almost independent of the intensity of excitation light except with extremely strong excitation light. The latter (slowly decaying) component appears to be caused by the light-induced control of excitation transfer and corresponds to the change in the excitation energy available

to Pigment System II, as previously studied in *P. cruentum*[6]. The superposition of the two components of fluorescence change will form a time-course which was observed in the induction of chlorophyll *a* fluorescence after switching from Light I to Light II.

The value of $f$ for the exponentially decaying component extrapolated to the time of switching ($f_0$) was 0.6 under the optimum conditions of the intensities of preillumination and excitation light. The chlorophyll *a* fluorescence measured at 685 nm was contaminated by fluorescence emitted from phycobilins (see Fig. 5 in ref. 17), which did not vary during the short time of illumination used in this study. Therefore, if the value for $f_0$ were obtained for only chlorophyll *a* fluorescence (F684) by subtracting the fluorescence of phycobilins, the value for $f_0$ would attain a value larger than 0.6. BONAVENTURA AND MYERS[7], in their study in Chlorella, obtained the value for $f_0$ about 0.5 (calculated from Fig. 13 in ref. 7).

Although the value for $f_0$ is a parameter for the expression of the interconversion of the States I and II, the meaning of the value for $f_0$ is not simple. During the conversion from the State I to the State II after switching the illumination from Light I to Light II, the number of light quanta delivered in Pigment System I increases and that in Pigment System II decreases. This results in a shift of oxidation–reduction level of the electron carriers situated between the two photoreactions toward oxidation, which induces an increase of fluorescence quenching by Photoreaction II and causes a decrease in fluorescence yield. Therefore, the change in distribution of excitation energy between the two pigment systems has two effects in decreasing the slow component of fluorescence; through the decrease in light quanta delivered in Pigment System II, as well as through the increase in the quenching effect of Photoreaction II. Thus, $f_0$ (the value for the relative increase in fluorescence yield of the slowly delaying part extrapolated to the time of switching from Light I and II) must have displayed a larger value than the relative increase in light energy delivered in Pigment System II.

Recently, VREDENBERG[18] studied time-courses of chlorophyll *a* fluorescence in Porphyra in a method similar to ours. His interpretation of the slow fluorescence change does not seem adequate because he used the model proposed by DUYSENS AND SWEERS[2], in which Q′, a quenching and photo-inactive form of the primary electron acceptor, was postulated. However, the results obtained in his study in respect to the effects of uncouplers on the fluorescence change indicate that nigericin and carbonyl cyanide *m*-chlorophenylhydrazone accelerate the conversion from the State II toward the State I in the dark. This suggests that ion exchange through the chloroplast membrane plays an important role in the light-induced control of excitation transfer.

The light-induced decrease in fluorescence yields of both Pigment Systems I and II was previously discovered in the presence of DCMU and *N*-methylphenazonium methylsulfate (PMS) in isolated spinach chloroplasts and the phenomenon was designated as "fluorescence lowering"[19]. The high energy state of the intermediate of photophosphorylation was suggested to play an important role in the fluorescence lowering. Fluorescence lowering is observed also in intact cells of algae in the presence of DCMU and PMS (K. SUGAHARA, personal communication).

Although a similar change in fluorescence yield may be operating in the absence of PMS in the intact cells of Porphyra, its contribution seems small. In the above discussion, therefore, the slow change in fluorescence yield which could not be explained

by the competition between fluorescence emission and Photoreaction II was attributed to the change in distribution of excitation energy between the two pigment systems.

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