BBA 45761

CONTROL OF EXCITATION TRANSFER IN PHOTOSYNTHESIS

I. LIGHT-INDUCED CHANGE OF CHLOROPHYLL *a* FLUORESCENCE IN *PORPHYRIDIUM CRUENTUM*

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(Received August 16th, 1968)
(Revised manuscript received November 5th, 1968)

SUMMARY

The emission spectra of chlorophyll *a* in *Porphyridium cruentum* at liquid nitrogen temperature change on illumination of the sample before cooling. Pre-illumination of phycoerythrin decreases the yields of F684 and F695 and increases that of F-l. Pre-illumination of chlorophyll *a* does not significantly change the fluorescence yield. These facts indicate that the pre-illumination of pigment system II changes the efficiency of excitation transfer between chlorophyll *a* molecules. The effects of additional actinic illumination on the yield of F684 at room temperature are also examined. The actinic illumination of chlorophyll *a* has two opposing effects on the fluorescence yield, *i.e.*, decrease and increase of fluorescence yield. The two effects can be distinguished by the difference in times of response towards the onset and end of actinic illumination. The former (fast, decreasing) effect can be explained in connection with the cooperation of the two pigment systems through the photosynthetic electron transport. The latter (slow, increasing) effect is thought to reflect the change in efficiency of excitation transfer, as mentioned above concerning the fluorescence yields at liquid nitrogen temperature. The presence of a light-induced control for the efficient utilization of the absorbed light energy in photosynthesis is suggested.

INTRODUCTION

It has been established from studying the fluorescence of photosynthetic pigments *in vivo* that the light energy absorbed by various photosynthetic pigments is ultimately transferred to chlorophyll *a*[1–3], through which the excitation energy is converted to chemical energy. Attempts to correlate the fluorescence yield with the rate of photochemical reaction have been made[4–10]. The relationship obtained is as follows: A part of the excitation energy transferred to chlorophyll *a* is used to drive the photoreaction, while the rest is dissipated as heat and fluorescence. It is reasonable to assume that the rate constants of the radiative and nonradiative processes do not

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

change, provided the experimental conditions, *e.g.*, temperature and concentrations of static quenchers, remain constant. It follows, therefore, that the fluorescence yield of chlorophyll *a* is proportional to the unused part of the excitation energy, and thus the fluorescence yield is inversely related to the rate of the photoreaction.

It has been assumed throughout these considerations that the transfer efficiency between the photosynthetic pigments in question does not change. There have been some reports of the occurrence of a change in fluorescence yield of chlorophyll *a* in red and blue-green algae during the illumination, suggesting a possible change in efficiency of excitation transfer from phycobilins to chlorophyll *a*[11,12]. However, a change in fluorescence yield of chlorophyll *a* cannot be regarded as definite evidence of a change in excitation transfer, since the fluorescence yield of chlorophyll *a* depends not only on the transfer efficiency but also on the rate of photoreaction II.

Studies on the light-induced oxidation–reduction reactions of the electron carriers in the chloroplasts have led to a generally accepted scheme of electron transport chain in which two photoreactions operate in series[13–15]. There is general agreement that two pigment systems are involved in supplying the light energy to the two photoreactions. In red and blue-green algae, pigment system II consists of the main fraction of phycobilins and a small fraction of chlorophyll *a*, and pigment system I consists of the main fraction of chlorophyll *a* and some phycobilins. It has been inferred from the fact that the fluorescence of chlorophyll *a* is much more efficiently excited by phycobilins than by chlorophyll *a*[1,2], that the fluorescence of chlorophyll *a* observed *in vivo* is emitted by the small portion of chlorophyll *a* in pigment system II, and that the fluorescence yield of chlorophyll *a* is, therefore, inversely related to the rate of photoreaction II. DUYSENS AND SWEERS[16] and BUTLER AND BISHOP[17] found that the fluorescence yield of chlorophyll *a* in several photosynthetic organisms was decreased by additional actinic illumination with light absorbed by pigment system I and was increased by actinic illumination with light absorbed by pigment system II. These results were interpreted in terms of the cooperation of the two pigment systems through the electron transport chain.

The fluorescence of chlorophyll *a* at low temperatures has also been extensively studied[18–24]. There are three emissions of chlorophyll *a*, F684, F695 and F-l, showing peaks at 684 nm, 695 nm and 715–740 nm, respectively. The excitation spectra for the emissions showed that F684 and F695 are emitted from pigments in pigment system II, and in most organisms F-l is emitted from pigment system I[20–23]. The kinetic analysis of the induction of fluorescence at liquid nitrogen temperature indicated that F684 is emitted from the bulk chlorophyll *a* and F695 from the energy sink directly connected with photoreaction II[24].

In the present study, we will report the changes in fluorescence yield upon pre-illumination or additional actinic illumination and present evidence for the changes in transfer efficiency of excitation energy between chlorophyll *a* molecules in the chloroplasts.

METHODS

Porphyridium cruentum was cultured at 25° under incandescent lamp light (4000 lux) in KOCH's medium[25]. Concentration of the sample was controlled so that the absorbance did not exceed 20 % at the absorption peaks.

Emission spectra at liquid nitrogen temperature were measured as described previously[20]. Immediately after pre-illumination at room temperature, the sample, in a transparent plastic cuvette (0.2 cm thick), was rapidly chilled by dipping in liquid nitrogen in the dark; the temperature was thus lowered to —196° within 1.5 min, as measured with a thermocouple. The monochromatic light for the pre-illumination or excitation was obtained from a xenon lamp with a combination of interference filter and band pass filters. The fluorescence emitted from the sample was analyzed by a Bausch and Lomb grating monochromator (bandwidth, 2.5 nm) equipped with a red cutoff filter. Fluorescence intensity was measured with a photomultiplier, 7102 (RCA). Emission spectra were not corrected for the spectral sensitivity of the equipment (see Fig. 1 in ref. 20).

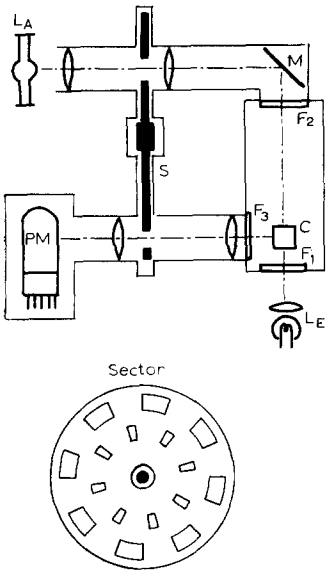


Fig. 1. Schematic diagram of apparatus for measuring the effects of additional actinic illumination on fluorescence yield. C, cuvette; F_1, F_2 and F_3, filters or filter combinations for excitation light, actinic light and fluorescence, respectively; S, sector; PM, photomultiplier; L_E and L_A, lamps of excitation light and actinic light, respectively; M, mirror.

The effect of actinic illumination on the fluorescence yield at room temperature was measured, using an apparatus in which the actinic illumination and the fluorescence measurement could be performed alternately without any overlapping (Fig. 1). A cuvette containing the algal sample was illuminated by a continuous beam of excitation light and an intermittent beam of additional actinic light passing through the holes cut in a circle on a rotating sector. By the use of another set of holes cut on the same sector, the fluorescence was measured during the dark period with respect to the actinic illumination, so that there was no danger of any contamination of the fluorescence and the scattered light caused by actinic illumination. On the sector there were two circles of 8 holes, one for the beam of actinic light and the other for the fluorescence. The sector was turned at 20 cycles/sec. The duration of 1 cycle of actinic illumination and fluorescence measurement was 6.25 msec: 1.39 msec for actinic illumination, 2.78 msec for fluorescence measurement, leaving two 1.04-msec dark

intervals between the periods of actinic illumination and fluorescence measurement. The excitation light was obtained from a tungsten lamp with a combination of an interference filter and band pass filters. The actinic light was obtained from a super-high-pressure mercury lamp or a xenon lamp with glass filter(s) or an interference filter. A combination of an interference filter and a red cutoff filter was placed between the sample and the detector to exclude the scattered light. The fluorescence intensity was detected with a photomultiplier, R-136 (Hamamatsu TV). The signal from the photomultiplier, square wave in form, was smoothed by an *RC*-combination and was amplified and recorded on a strip chart servo recorder.

RESULTS

The emission spectra at liquid nitrogen temperature in *Porphyridium cruentum* were compared with and without illumination prior to cooling the sample (Fig. 2). The emission spectra were measured after a sufficiently long period of illumination of the cooled sample to eliminate the influence of the induction of fluorescence occurring even at such a low temperature[24]. As described previously[20], the emission spectra caused by excitation of phycoerythrin consisted of three emissions, F684, F695 and F-l, showing maxima at 685 nm, 695 nm and 712 nm, respectively, and two emissions of phycobilins at 648 nm and 664 nm (Fig. 2). On illumination, before cooling, with 567-nm light (10 nEinsteins·cm^{-2}·sec^{-1}, 60 sec) absorbed by phycoerythrin, the yields of F684 and F695 at liquid nitrogen temperature decreased, while the fluorescence yields of phycobilins remained unchanged. On pre-illumination with 694-nm light (16 nEinsteins·cm^{-2}·sec^{-1}, 60 sec) absorbed by chlorophyll *a*, the yields were little changed. As shown in Fig. 2, the lower intensity of F-l as compared with the other two emissions of chlorophyll *a* made it difficult to decide whether F-l increased or decreased on pre-illumination. Further, on excitation of chlorophyll *a* with 433-nm light (Fig. 3), the three emission bands of chlorophyll *a* were observed at 685 nm,

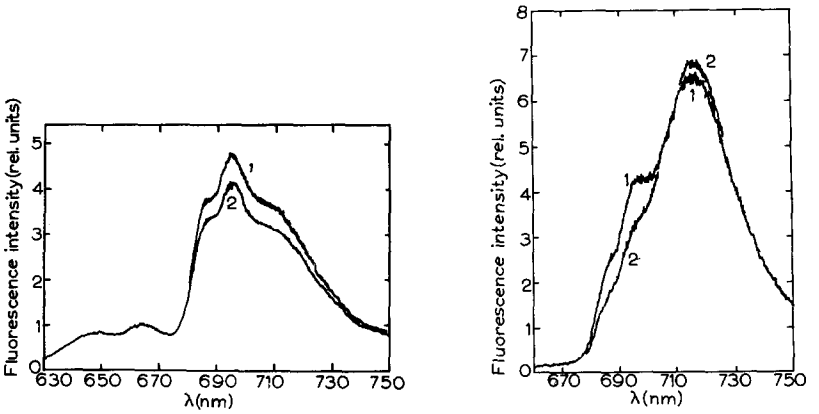


Fig. 2. Effects of pre-illumination of Porphyridium at room temperature on the emission spectrum at liquid nitrogen temperature. Excitation light, 553 nm (8 nm half-bandwidth). 1, No pre-illumination; 2, pre-illumination with 567-nm light (10 nEinsteins·cm^{-2}·sec^{-1}, 60 sec).

Fig. 3. Effects of pre-illumination at room temperature on the emission spectrum at liquid nitrogen temperature. Excitation light, 433 nm (13 nm half-bandwidth). 1, No pre-illuminatioon; 2, pre-illumination with 567-nm light (13 nEinsteins·cm^{-2}·sec^{-1}, 30 sec).

695 nm and 715 nm. In this case, however, F-1 was much stronger than the other two emissions. On illumination of phycoerythrin with 567-nm light (13 nEinsteins·cm^{-2}·sec^{-1}, 30 sec) before cooling, the yields of F684 and F695 decreased, while that of F-1 showed a slight increase. Also in this case, the pre-illumination of chlorophyll *a* with 694-nm light (17 nEinsteins·cm^{-2}·sec^{-1}, 30 sec) did not significantly affect the yields of the three emissions.

These effects were only observed on illumination before cooling; the illumination of the chilled sample, even at high intensities, caused no change in fluorescence yields except for the transient changes due to the induction of chlorophyll *a* fluorescence. After the induction period, the fluorescence yields did not change if the sample was left at this temperature. The effect of pre-illumination at room temperature was also examined in the presence of an inhibitor, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) (20μM). Under these conditions, the emission spectrum was not influenced by pre-illumination of phycoerythrin or chlorophyll *a*.

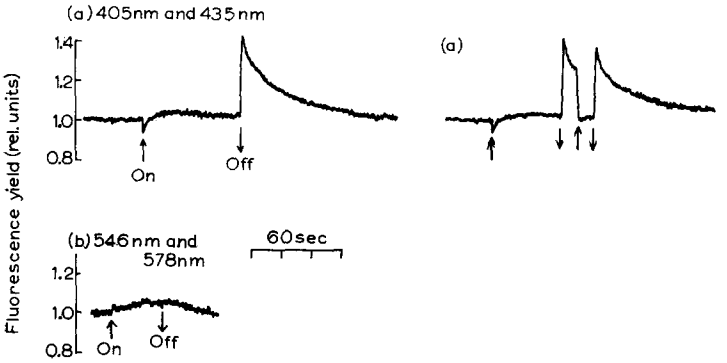


Fig. 4. Effects of actinic illumination on fluorescence yield of chlorophyll *a* at room temperature in Porphyridium. Fluorescence was measured at 684 nm (16 nm half-bandwidth). Excitation light was obtained from tungsten lamp with a combination of an interference filter and glass band pass filters; 553 nm, 0.88 nEinstein·cm^{-2}·sec^{-1}. Actinic light was obtained from Hg-lamp with filters, (a) 405 nm and 435 nm, approx. 0.67 nEinstein·cm^{-2}·sec^{-1}, and (b) 546 nm and 578 nm, approx. 1.4 nEinsteins·cm^{-2}·sec^{-1} (average value).

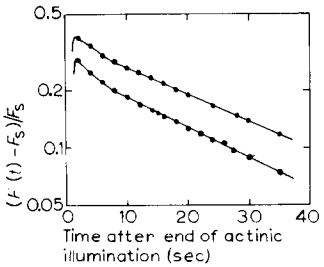
The effect of actinic illumination on the fluorescence yields of chlorophyll *a* at room temperature was somewhat different from that of the pre-illumination on fluorescence yields at the low temperature. Fig. 4 shows the effects of actinic light absorbed by chlorophyll *a* and phycoerythrin. When the steady state of fluorescence had been attained after a sufficiently long illumination with excitation light absorbed by phycoerythrin, the sample was further illuminated with the additional actinic light for several seconds. After the onset of actinic light absorbed by chlorophyll *a*, the fluorescence showed a temporary decrease, then recovered slowly to reach a second steady level. Whether the second steady level of fluorescence was higher or lower than the initial steady level depended on the sample and the intensities of the actinic and excitation light. After the end of actinic illumination, the fluorescence yield rapidly increased to attain a maximum, and then gradually decreased to recover the initial steady level observed before onset of actinic illumination. The half-decay time of the temporarily increased portion of fluorescence was 16 sec under the experimental conditions of Fig. 4a. If the actinic light was turned on soon after attainment of the

maximum level, the second level of fluorescence was recovered. As shown in Fig. 4b, the actinic illumination absorbed by phycoerythrin slightly increased the fluorescence yield. However, there was no rapid transient change at the onset of actinic illumination nor any rise in fluorescence yield at the end of actinic illumination.

The kinetics of the fluorescence changes were studied with the actinic light absorbed by chlorophyll *a*. Fig. 5 shows the relationship between the time after the end of actinic illumination and the logarithms of the fluorescence increment above the initial steady level. Two distinct phases will be noticed in the course of the decay.

The experiments with varied intensities of excitation light showed that the first fast phase was more marked with stronger excitation light and disappeared when the excitation light was weak. The second slow phase was exponential, and the decay constant was almost independent of the elevated level of fluorescence.

The dependence of the increase in fluorescence after the end of actinic illumination, described above, on the wavelength of the actinic light was examined. 400-nm,



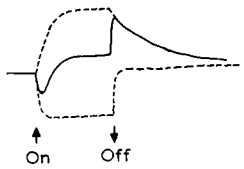


Fig. 5. Time course of decay of temporarily increased portion of chlorophyll *a* fluorescence after the end of actinic illumination of chlorophyll *a*. $F(t)$ and F_s represent the fluorescence yields at time t and at the steady state. Data are taken from Fig. 4a.

Fig. 6. Schematic illustration showing two opposing effects of additional actinic illumination of chlorophyll *a* upon fluorescence yield of chlorophyll *a* at room temperature.

438-nm or 678-nm light, absorbed preferentially by chlorophyll *a*, was effective, whereas 568-nm or 618-nm light, absorbed preferentially by phycobilins, was ineffective. These facts lead one to conclude that pigment system I participates in the observed increase in fluorescence yield at the end of actinic illumination.

The fluorescence at 650 nm, which was emitted by phycobilins, was not influenced by the actinic illumination.

DISCUSSION

At the end of the induction period reached after a sufficiently long illumination at liquid nitrogen temperature, there is no electron transport in the chloroplasts[24]. Under these conditions, the observed fluorescence yields of chlorophyll *a* must directly reflect the transfer efficiency of excitation energy from the light-collecting pigments to the fluorescent species of chlorophyll *a*. The pre-illumination of phycoerythrin caused changes in the yields of F684 and F695; these changes were observed equally on excitation both with light absorbed by chlorophyll *a* and with light absorbed by phycoerythrin. The fluorescence yields of phycobilins were not changed by the pre-illumination. These facts indicate that the change in transfer efficiency observed must

have occurred, not during the transfer between the phycobilins and chlorophyll *a* or between phycobilins, but during the transfer between the chlorophyll *a* species.

On the other hand, the fluorescence yield at room temperature depends on both the efficiency of excitation transfer between pigments and the rate of photoreaction II. The complex time course of the fluorescence yield of chlorophyll *a* observed on actinic illumination with light absorbed by chlorophyll *a* suggests that the actinic illumination has two opposing effects on the fluorescence yields (Fig. 6). One is a rapid decrease of the yield at the onset of illumination which is rapidly recovered at the end of actinic illumination; the other is a slow increase at the onset and a slow decrease at the end of actinic illumination. The superposition of these two effects accounts for the rather complicated pattern of the fluorescence changes observed (Fig. 6).

The rapid responses mentioned above are the ones which have been observed by DUYSENS AND SWEERS[16] and also by BUTLER AND BISHOP[17]. These findings are explained by the interaction of the two pigment systems *via* the electron transport chain including two photoreactions: a light-induced acceleration of photoreaction I, which in turn induces the acceleration of photoreaction II by increasing the oxidized form of its primary electron acceptor, results in a decrease in fluorescence yield of chlorophyll *a* in pigment system II[16].

On the other hand, the slow response cannot be explained in terms of the relationship between the fluorescence yield and the rate of photochemical reaction. Illumination of pigment system I, which accelerates the rate of photoreaction II, in fact caused an increase in fluorescence designated here as the slow response. The direction of such a change is the opposite of that expected from the interaction of the light-induced electron transport. The most probable mechanism for the cause of the slow response will be a change in mode of excitation transfer between chlorophyll *a* species, as suggested in the above discussion for the changes in fluorescence yield at liquid nitrogen temperature.

DUYSENS AND SWEERS[16] have also reported changes in fluorescence yield in red algae which cannot be explained in terms of changes in rate of electron transport; they found that on switching the light absorbed by chlorophyll *a* to the light absorbed by phycobilins, the fluorescence yield slowly decreased after attaining a maximum by a rapid increase. The slow decrease of fluorescence yield observed in the present study on removal of additional illumination of pigment system I seems to represent the same change as that observed by DUYSENS AND SWEERS[16]. They explained the phenomenon by the quenching effect of a hypothetical form, Q', of the reaction center of pigment system II which they assumed to be formed by a dark reaction from the reduced form, QH, of the reaction center. LAVOREL[26] observed, in the induction of fluorescence in Chlorella, a slow decrease of the fluorescence yield after attainment of the maximum of fluorescence, and interpreted the phenomenon in a similar way as did DUYSENS AND SWEERS[16]. The slow changes of the fluorescence yield at room temperature may well be explained by the hypothesis proposed by DUYSENS AND SWEERS, although it is rather difficult to understand exactly what the photoinactive quenching form, Q', represents. The results obtained at liquid nitrogen temperature, however, cannot be accounted for by such a mechanism. It is incomprehensible to assume that a supposed increase of a quencher, Q', should increase the yield of any component of fluorescence. The experimental results obtained indicated that on excitation of chlorophyll *a*, the decrease of the yields of F684 and F695 with the illumination before cooling the sample

was accompanied by an increase in yields of F-1. It is reasonable to assume that a common mechanism should underlie the slow change of F684 at room temperature and the changes of fluorescence yields at liquid nitrogen temperature. Therefore, it will be concluded that the slow response of fluorescence change is caused by the mechanism based on the light-induced change of excitation transfer efficiency.

At both room and liquid nitrogen temperatures, it may be generally stated that the yields of F684 and F695 are lower in the samples illuminated by light absorbed by pigment system II than in the samples prepared in the dark or illuminated by light absorbed by pigment system I. In the experiments at room temperature (Fig. 4), the samples were subject to continuous illumination with excitation light (553 nm) of a rather high intensity, and therefore the yields of F684 and F695 must have been suppressed before the onset of actinic illumination. This may explain why there was no further suppression of the fluorescence yield on additional actinic illumination of pigment system II, while an increase in fluorescence yields occurred on actinic illumination of pigment system I, to counteract the decreasing effect of the excitation light.

There are two possible schemes for explaining the decrease in yields of F684 and F695 on pre-illumination of pigment system II. One is the decrease in transfer efficiency at some step(s) of the sequence of excitation transfer from light-collecting pigments to C_{f684} (chlorophyll *a* emitting F684). The other is the increase in rate of transfer from C_{f684} to some molecules other than C_{f695} (chlorophyll *a* emitting F695). The yield of F-1 caused by excitation of chlorophyll *a* at liquid nitrogen temperature was not decreased but rather increased after pre-illumination of phycoerythrin. According to the former mechanism this would indicate that pre-illumination of phycoerythrin caused an increase in efficiency of direct transfer of excitation energy to C_{f-1} (chlorophyll *a* emitting F-1); according to the latter mechanism, the same fact is interpreted to mean that the transfer rate from C_{f684} to C_{f-1} increases (*i.e.*, an acceleration of spillover) (Fig. 7).

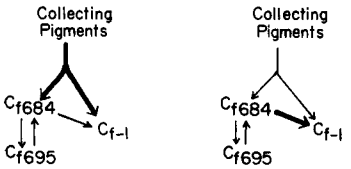


Fig. 7. Two possible schemes for light-induced changes in efficiency of excitation transfer between pigments in Porphyridium. Arrows represent transfer of excitation energy. Thick arrows represent the site at which the regulation of excitation transfer occurs.

On pre-illumination of chlorophyll *a* in a blue-green alga Schizothrix at room temperature, VREDENBERG AND DUYSENS[27] have also observed an increase in yield of chlorophyll *a* fluorescence having an emission peak near 685 nm. These investigators attributed the increment to the fluorescence emitted from pigment system I. However, it will, at least in part, be interpreted in the light-induced increase of fluorescence yield of pigment system II based on the change of excitation transfer efficiency.

What mechanism is actually operating in the observed control of the excitation transfer remains to be investigated. However, it may be that the oxidation–reduction reactions in the electron transport chain play a role in this phenomenon. This is suggested by the following facts: (1) the illumination before cooling of phycobilins

suppressed the yields of F684 and F695 at liquid nitrogen temperature, while that of chlorophyll *a* did not; (2) in the presence of an inhibitor, DCMU, which depresses the electron transport at a step close to photoreaction II, the pre-illumination of phycobilins was no longer effective in changing the fluorescence yields at liquid nitrogen temperature; and (3) the actinic illumination at liquid nitrogen temperature did not cause any change in fluorescence yields.

The physical basis of such changes in transfer efficiency is still unknown. Some conformational change of the lamella structure caused by the illumination, which must modify the distances between and the mutual orientations of the chlorophyll *a* molecules, might cause alterations of the rate of excitation energy transfer.

In the Emerson enhancement, oxygen production, driven by the light absorbed by one of the pigment system, is accelerated by the complementary illumination of the other system[28]. FRENCH[29] inferred that some hypothetical product formed by illumination of chlorophyll *a* caused the enhancement of oxygen evolution and calculated that the half-decay time of the product was about 18 sec in *Porphyridium cruentum*. The enhancement of oxygen evolution by pre-illumination of pigment system I can be interpreted in terms of the electron pool between the two photoreactions, which is oxidized by photoreaction I and reduced by photoreaction II. However, the same effect can also be explained by assuming an increased efficiency of utilization of excitation energy in photoreaction II caused by pre-illumination of pigment system I. The good agreement of the half-decay time (16 sec) of the light-induced portion of fluorescence at room temperature with the half-life time of the product obtained by FRENCH seems to support such a view. We consider that a light-induced control of excitation transfer can, at least in part, be a possible cause of the Emerson enhancement.

It will be suggested that the light-induced control of excitation transfer enables the chloroplasts to utilize light energy efficiently in photosynthesis. Upon illumination of pigment system II, a greater amount of absorbed light energy is transferred to chlorophyll *a* in pigment system I and a lesser amount of light energy is transferred to chlorophyll *a* in pigment system II than occurs upon illumination of pigment system I. Such a change of excitation transfer reduces the difference between the amounts of excitation energy available for photoreactions I and II. With weak light, the rate of total electron transport is limited by one of the two photoreactions with the slower reaction rate. Thus, by controlling excitation transfer between chlorophyll *a* species, the alga should efficiently utilize the absorbed light energy in photosynthesis.

ACKNOWLEDGEMENTS

The author wishes to express his thanks to Prof. A. TAKAMIYA for helpful suggestions and support during the work. The work was supported in part by a grant from the Ministry of Education. Financial aid from the Mishima Kaiun Memorial Foundation is also acknowledged.

ADDENDUM

The fundamental idea on which the above discussion is based is that the photochemical reaction itself causes the quenching process of chlorophyll *a* fluorescence.

Another mechanism, however, has also been proposed by some other investigators. DUYSENS AND SWEERS[16] proposed a mechanism in which the chlorophyll *a* fluorescence is quenched by the oxidized form of the reaction center, Q, and Q is converted to QH by the photoreaction II and reversed by photoreaction I, and the reduced form, QH, has no quenching action. However, it is difficult to interpret the change of the fluorescence yield by such static quenching of the singlet excited state of chlorophyll *a* by Q. In static quenching, the excitation energy should be dissipated as thermal energy. The fluorescence yield should be proportional to the fraction of excitation energy which is not quenched by Q and is consequently available for the photochemical reaction. MURATA, NISHIMURA AND TAKAMIYA[5] found that the fluorescence yield at the beginning of the induction period is about 1/3 that at the final steady state. This means, according to the theory of static quenching, that about 2/3 of the excitation energy was lost as heat when Q was initially in the fully oxidized state. Such an extensive loss seems unlikely if we consider that the quantum yield of the Hill reaction with ferricyanide or dichlorophenolindophenol is reported to attain values as high as 0.5–0.7 (refs. 9, 30); at the low intensities of light generally used for the determination of quantum yield, the quenching effect must be nearly at its maximum, since almost all reaction centers are in the oxidized form, Q.

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