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CONTROL OF EXCITATION TRANSFER IN PHOTOSYNTHESIS

III. LIGHT-INDUCED DECREASE OF CHLOROPHYLL *a* FLUORESCENCE RELATED TO PHOTOPHOSPHORYLATION SYSTEM IN SPINACH CHLOROPLASTS

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SUMMARY

1. A light-induced reversible decrease in fluorescence yield of chlorophyll *a*, designated as "fluorescence lowering", was discovered in isolated spinach chloroplasts on illumination at high light intensities in the presence of *N*-methylphenazonium methosulfate and 3-(3',4'-dichlorophenyl)-1,1-dimethylurea. The decrease in fluorescence yield amounted to 20–30 %.

2. The "fluorescence lowering" was markedly lessened by addition of uncouplers of photophosphorylation. The concentrations for 50 % suppression of the light-induced effect were 2.0 μM atebrin, 2.5 μM carbonylcyanide *m*-chlorophenylhydrazone and 1 mM methylamine. These findings indicate the involvement of the photophosphorylation system in the "fluorescence lowering".

3. Kinetic analyses showed that the 'fluorescence lowering" commenced at about 0.2 sec and reached an half-level of the "fluorescence lowering" about 2 sec after onset of illumination. The half-decay time of the recovery after cessation of illumination was approx. 5 sec.

4. The time response of the "fluorescence lowering" differed from that of the light-scattering change in the chloroplasts, thus indicating that the "fluorescence lowering" was not caused by the conformational change corresponding to the light-scattering change of the chloroplasts.

5. In experiments at liquid-nitrogen temperature, preillumination before cooling decreased the yields of the three emissions of chlorophyll *a* at 684 nm, 695 nm and 735 nm.

INTRODUCTION

Changes of fluorescence yield of chlorophyll *a* in the chloroplasts have been explained on a basis of a competition of photoreaction II with other processes, including

Abbreviations: DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; PMS, *N*-methylphenazonium methosulfate; CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

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thermal degradation, light re-emission (fluorescence) and transfer of excitation to other pigment molecules[1–7]. On the other hand, several investigators have discovered changes in fluorescence yield which are not explained by this mechanism[8–11]. In the first part of this series of investigation concerning the fluorescence in intact cells of *Porphyridium cruentum*[12], two components of fluorescence change were discriminated in the time-course of fluorescence. It was further shown that the one component which was not explained by the competition of photoreaction II with other processes was attributed to a light-induced change of excitation transfer between chlorophyll *a* molecules in the chloroplasts. By this control of distribution of excitation energy between the two pigment systems, the organism was able to utilize more efficiently the absorbed light energy for driving the electron transport reactions of photosynthesis.

In the second part of the study, which was concerned with the effects of metal ions on the fluorescence yield in isolated chloroplasts[13], Mg^{2+} (and some other divalent metal ions) were shown to control the excitation transfer from the bulk chlorophyll *a* of pigment system II to the bulk chlorophyll *a* of pigment system I.

In the present study, a light-induced decrease in fluorescence yield of chlorophyll *a* was discovered in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) and *N*-methylphenazonium methosulfate (PMS), and it was suggested that the amount of excitation energy available for the photoreactions was controlled by the photophosphorylation system.

METHODS

Spinach chloroplasts were prepared by using the method described previously[13]. The obtained suspension of chloroplasts in a sucrose–Tricine medium (0.4 M sucrose, 0.01 M NaCl and 0.05 M Tricine buffer, pH 7.5) was diluted with the same medium so that the absorbance did not exceed 20% at the absorption peak of the chloroplasts (the concentrations corresponding to less than 1.5 μg chlorophyll per ml). In experiments in which the pH of the medium was varied, the chloroplasts were collected by further centrifugation at 1500 $\times$ *g* (for 5 min), and the precipitate formed was resuspended in a small amount of 0.4 M sucrose solution and diluted with sucrose–Tricine media of desired pH (0.4 M sucrose, 0.01 M NaCl and 0.05 M Tricine buffer).

Light-induced change of fluorescence yield was measured by two methods, *i.e.* "sector method" and "single-beam excitation method". In the "sector method", the effect of actinic light on the yield of chlorophyll *a* fluorescence was measured, using equipment described previously[12], in which the effect of actinic light could be measured without any contamination of fluorescence and scattered light due to the actinic illumination. Excitation light was obtained from an incandescent lamp (75 W) through an interference filter with transmission peak at 480 nm (12-nm half bandwidth) or 438 nm (10-nm half bandwidth) and blue band-pass filters, V-B46 (Toshiba), two pieces of B-460 (Hoya Glass) and HA-50 (Hoya Glass). Actinic light was obtained from a super-high-pressure mercury lamp (500 W) through blue filters, B-460, HA-50 and an ultraviolet-absorbing filter, U-V39 (Toshiba). Usually, the excitation light (250 ergs·cm^{-2}·sec^{-1}) was much weaker than the actinic light (33 000 ergs·cm^{-2}·sec^{-1}). The fluorescence emitted at right angle to the excitation beam was analyzed with an interference filter having a transmission peak at 684 nm (16-nm half bandwidth).

In the "single-beam excitation method", the kinetics of fluorescence change and

the light-scattering change of chloroplasts on illumination were measured. A strong blue excitation light (usually 30000 ergs·cm^{-2}·sec^{-1}) with a peak at 460 nm was obtained from an incandescent lamp (150 W) operated with a stabilized alternating current at 50 cycles/sec through a blue band-pass filter, V-B46, and an infrared absorbing filter, HA-50. The fluorescence at 685 nm, emitted at right angle to the excitation beam, was analyzed with a Bausch and Lomb grating monochromator (20-nm half bandwidth) equipped with a red cutoff filter, V-R67 (Toshiba), and detected by a photomultiplier, R-136 (Hamamatsu TV). The signal was led to a strip chart servo recorder or a synchroscope. 540-nm light scattered by the chloroplasts at a right angle to the excitation beam was also analyzed with the Bausch and Lomb grating monochromator under the same conditions as in the fluorescence measurements but without attachment of the glass filter.

The measurements of emission spectra at liquid-nitrogen temperature were performed as described previously[14]. Blue excitation light was obtained from a 75-W incandescent lamp through a filter combination of V-B46, B-460 (two pieces) and HA-50. Fluorescence was analyzed with a Bausch and Lomb grating monochromator (set to half bandwidth, 2.5 nm) equipped with a red cutoff filter, V-R65 (Toshiba). The preillumination of the sample before cooling was carried out as follows. A transparent plastic cuvette containing the chloroplast suspension, 0.2 cm thick, was placed in a transparent Dewar flask and illuminated for 1 min with red light (20000 ergs·cm^{-2}·sec^{-1}) obtained from an incandescent lamp with a red cutoff filter, V-R65; then liquid nitrogen was poured into the flask under continued illumination. After cooling for 3 min, the cuvette was rapidly transferred to another Dewar flask equipped in the fluorescence measurement device. For a dark control, the sample was cooled in the same way but in darkness.

RESULTS

Light-induced change of fluorescence yield in the presence of DCMU

In order to exclude the influence of photoreaction II on the yield of chlorophyll *a* fluorescence, an inhibitor, DCMU (20 μM), was always added throughout the present study. 0.5 mM ascorbate (neutralized) was also added to protect PMS from photodestruction. It was discovered that the illumination of spinach chloroplasts in the presence of DCMU decreased the yield of chlorophyll *a* fluorescence, if a cofactor of cyclic photophosphorylation, PMS, had been added to the medium.

The light-induced change of fluorescence yield was measured by using the "sector method", with weak excitation light and strong actinic light (Fig. 1). In the absence of PMS, the yield of fluorescence excited by weak excitation light (250 ergs·cm^{-2}·sec^{-1}) was slightly increased (by about 7 %) by illumination of strong actinic light (33000 ergs·cm^{-2}·sec^{-1}). The increase in fluorescence yield in this case is inferred to be caused by the same mechanism which underlies the "weak light effect" in isolated chloroplasts[1,5]; *i.e.* a part of the primary electron acceptor must remain oxidized at the low intensities of excitation light, thus resulting in a slightly lowered fluorescence yield. Under illumination with strong actinic light, the primary electron acceptor will be fully reduced to result in a maximum yield of fluorescence.

The addition of PMS (8 μM) did not significantly change the fluorescence yield under weak excitation light but led to a marked change in time-course of fluorescence

yield caused by the actinic illumination. At onset of actinic illumination, the yield was temporarily increased to be followed by a decrease to attain a steady level, at which the yield was approx. 82 % of that without actinic illumination. On cessation of the actinic illumination, the fluorescence yield temporarily decreased and then increased

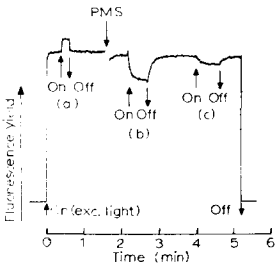


Fig. 1. Effect of actinic light on the time-course of fluorescence yield in spinach chloroplasts measured by the "sector method". 20 μM DCMU and 0.5 mM ascorbate were added. Fluorescence was measured at 684 nm. Excitation light, 480 nm, 250 ergs·cm^{-2}·sec^{-1} (0.10 nEinstein·cm^{-2}·sec^{-1}). Actinic light was obtained from 500-W super-high-pressure mercury lamp and was filtered through glass filters. Intensities of actinic light: a and b, 33 000 ergs·cm^{-2}·sec^{-1}; c, 2600 ergs·cm^{-2}·sec^{-1}. Arrows indicate onset (upward) and cessation (downward) of actinic illumination.

to recover the initial level. These actinic light-induced changes of fluorescence yield were found to be reversible. In the following, the yields of fluorescence with and without the actinic illumination were designated as F_L and F_D, respectively. The degree of light-induced change of fluorescence yield was expressed as $(F_L - F_D)/F_D$.

Two independent changes of fluorescence yield will overlap with each other in the time-course of the actinic light-induced fluorescence change in the presence of PMS. The one is a small rapid increase occurring at the onset of actinic illumination, which corresponds to the rapid decrease upon cessation of actinic illumination. This part of the change will be identical to the small increase caused by actinic illumination in the absence of PMS which is related to the "weak light effect". The other is a large slow decrease which appears upon actinic illumination in the presence of PMS. This part of the change will be designated in the following as "fluorescence lowering". The "fluorescence lowering" is a new discovery concerning the fluorescence changes in the chloroplasts. The magnitude of "fluorescence lowering" was defined as the difference between the values of $(F_L - F_D)/F_D$ in the presence and absence of PMS. Its value was estimated to be 25 % under the experimental conditions as shown in Fig. 1, a and b.

The half-time of the "fluorescence lowering" was estimated to be approx. 3 sec after the onset of actinic illumination (Fig. 1b). (More details concerning the half-time will be described later.) The half recovery time after the cessation of actinic illumination was approx. 5 sec (Fig. 1, b and c).

A similar change of fluorescence yield was also obtained, using 438-nm excitation light which was absorbed mainly by chlorophyll *a*.

The relationship between the intensity of actinic light and the magnitude of light-induced fluorescence change was examined in the presence and absence of PMS (Fig. 2). The magnitude of "fluorescence lowering" is shown by the dashed line in Fig. 2. The increase in fluorescence yield in the absence of PMS (Fig. 2, a), related to the "weak light effect" of chlorophyll *a* fluorescence[1,5], was saturated at a rather low intensity of actinic light (3000 ergs·cm^{-2}·sec^{-1}). On the other hand, the "fluorescence

lowering" required much higher light intensities. The intensity for its saturation was approximately 30000 ergs·cm^{-2}·sec^{-1}.

The dependence of the light-induced change of fluorescence yield on the concentration of PMS added was also examined. As shown in Fig. 3, 5 μM of PMS was sufficient for obtaining the maximum change of fluorescence yield. According to JAGENDORF AND AVRON[15], the cyclic photophosphorylation with PMS in the chloroplasts saturates at 10 μM of PMS. It will be concluded, therefore, that the "fluorescence lowering" and the photophosphorylation exhibit almost the same dependence on the concentration of PMS.

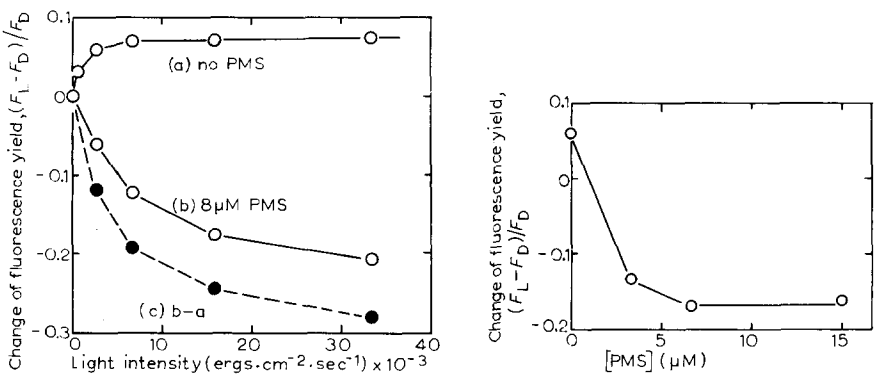


Fig. 2. Relationship between intensity of actinic light and light-induced changes of fluorescence yield. a, no PMS was added; b, 8 μM PMS was added; c, difference between a and b. Other conditions were the same as in Fig. 1, a and b.

Fig. 3. Relationship between concentration of PMS and light-induced change of fluorescence yield. Other conditions were the same as in Fig. 1, b.

Effects of uncouplers, atebrin, carbonylcyanide m-chlorophenylhydrazone (CCCP) and methylamine

The light-induced change in fluorescence yield in the presence of PMS was sensitive towards the presence of uncouplers of photophosphorylation. Atebrin and CCCP at a concentration of 1 μM lessened the light-induced decrease in fluorescence yield, and also partly quenched the fluorescence without actinic illumination. The uncouplers also accelerated the dark recovery after cessation of actinic illumination. In the presence of a much higher concentration of the uncouplers, the actinic illumination caused a small increase in fluorescence yield.

Figs. 4–6 show the relationship between the concentrations of uncouplers (atebrin, CCCP and methylamine) *versus* the fluorescence yields without actinic illumination, F_D, and the actinic light-induced changes in fluorescence yield, $(F_L—F_D)/F_D$. The fluorescence yield without actinic light was decreased by the addition of uncouplers atebrin and CCCP. In the cases of atebrin and CCCP, the sign of actinic light-induced change in fluorescence yield in the presence of PMS, $(F_L—F_D)/F_D$, was negative at low concentrations and positive at high concentrations of these uncouplers. As shown in Figs. 4 and 5, the fluorescence changes in the presence of higher concentrations (*e.g.* 30 μM) of atebrin and CCCP resembled the light-induced changes in the absence of PMS and uncouplers (△ in Figs. 4 and 5), although there was a decrease in F_D. These

findings indicate that these uncouplers markedly lessened the "fluorescence lowering" but little affected the actinic light-induced increase in fluorescence yield related to the "weak light effect" of fluorescence yield. Therefore, the magnitude of "fluorescence

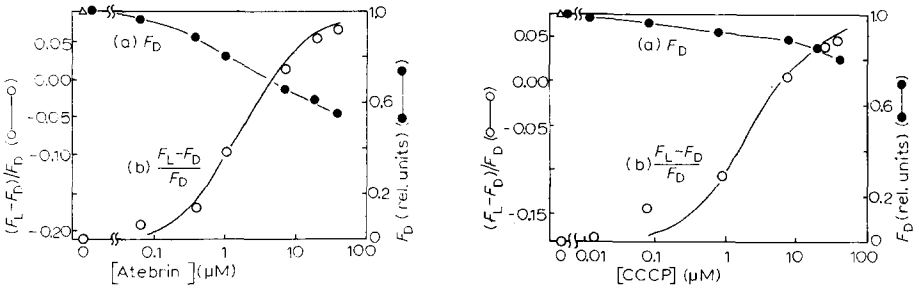


Fig. 4. Effects of atebrin at various concentrations on the fluorescence yield without actinic light and the actinic light-induced change of fluorescence yield. a, fluorescence yield without actinic light, F_D (●); b, actinic light-induced changes of fluorescence yield in the presence (○) and absence (△) of PMS. Other conditions were the same as in Fig. 1, b.

Fig. 5. Effects of CCCP at various concentrations on the fluorescence yield without actinic light and the actinic light-induced change of fluorescence yield. a, fluorescence yield without actinic light, F_D (●); b, actinic light-induced changes of fluorescence yield in the presence (○) and absence (△) of PMS. Other conditions were the same as in Fig. 1, b.

lowering" will be estimated by the suppression in value for $(F_L—F_D)/F_D$ at each concentration of the uncouplers, the value in the absence of PMS and uncouplers being taken as the control. The curves for $(F_L—F_D)/F_D$ in Figs. 4 and 5 were drawn according to the first-order sigmoid. The concentrations for 50 % suppression were 2.0 μM atebrin and 2.5 μM CCCP under the experimental conditions shown in Figs. 4 and 5. These concentrations are in the same order as those for the inhibition of photophosphorylation in the isolated chloroplasts[16–18]. In contrast to these uncouplers with which the actinic illumination caused the increase in fluorescence yield at high concentrations, another well-known uncoupler of photophosphorylation, methylamine[19], behaved in a different way (Fig. 6). The light-induced change in fluorescence yield was

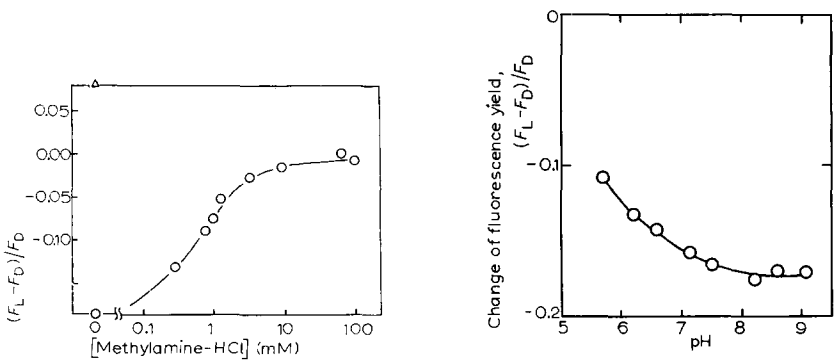


Fig. 6. Effects of methylamine–HCl at various concentrations on the actinic light-induced change of fluorescence yield in the presence (○) and absence (△) of PMS. Other conditions were the same as in Fig. 1, b.

Fig. 7. Relationship between the light-induced change of fluorescence yield and pH of the medium. Other conditions were the same as in Fig. 1, b.

eliminated at a high concentration (100 mM) of methylamine. The sensitivity towards methylamine was, however, fairly significant, 50 % of the suppression occurring at a concentration of methylamine as low as 1 mM.

pH dependence

The pH dependence of the "fluorescence lowering" was measured between pH 6 and 9. Fig. 7 shows the magnitude of "fluorescence lowering" at various pH's. The optimum pH was 7.5–9.0. The pH dependence of photophosphorylation shows the optimum at the same pH region[20]. However, its curve is much steeper than that for the "fluorescence lowering". At the optimum pH for the light-induced light-scattering change, *i.e.* pH 6.0 (refs. 21 and 22), the "fluorescence lowering" was about 2/3 that of its maximum obtained at pH 7.5–9.0.

Kinetics

The fast kinetics of the light-induced fluorescence change was measured by the "single-beam excitation method" with a strong excitation light (30000 ergs·cm^{-2}·sec^{-1}). As shown in Fig. 8a, the fluorescence yield rapidly rose to a maximum (P) and

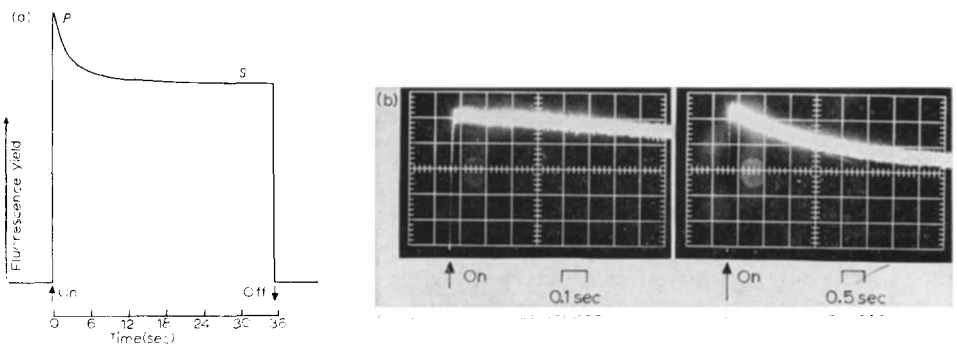


Fig. 8. Time-course of fluorescence yield in spinach chloroplasts measured by the "single-beam excitation method". Excitation light, blue light obtained from 150-W incandescent lamp through blue band-pass filters (30000 ergs·cm^{-2}·sec^{-1}). Fluorescence was measured at 685 nm (20-nm half bandwidth). 20 μM DCMU, 10 μM PMS and 0.5 mM ascorbate were added. Time scales in (b): 1 division corresponds to 0.5 sec in the right and 0.1 sec in the left. P and S in (a) represent the maximum and steady level of fluorescence yield.

then decreased to reach a final steady level (S) within 20 sec. The fluorescence yield at P will correspond to the maximum fluorescence yield obtained in the "sector method" experiments without addition of PMS and under strong actinic light (Fig. 1, a), since, at the initial period immediately after the onset of illumination at which the "fluorescence lowering" has not yet appeared, the primary electron acceptor of photoreaction II must be in a fully reduced state. The time required for the "fluorescence lowering" to attain its half level ($\tau_{1/2}$) was approx. 2 sec after onset of excitation.

The early stage of kinetics of the fluorescence induction was measured by the same method using a synchroscope. As shown in Fig. 8b, the fluorescence yield reached a maximum (P) within 0.01 sec after onset of illumination, remained at the same level for 0.2 sec and then started to decrease.

Experiments with varied intensities of excitation light were performed, using the

"single-beam excitation method". The magnitudes of the "fluorescence lowering", expressed as $(P—S)/P$, and its half-time, $\tau_{1/2}$, were observed in the presence and absence of 4 μM CCCP (Fig. 9). In the absence of CCCP and at high intensities of excitation light (higher than 30000 ergs·cm^{-2}·sec^{-1}), both $(P—S)/P$ and $\tau_{1/2}$ showed the maximum and the minimum values, 0.28 and 2.2 sec, respectively. As shown in Fig. 9, CCCP decreased $(P—S)/P$ and increased $\tau_{1/2}$ markedly especially at lower light intensities (lower than 10000 ergs·cm^{-2}·sec^{-1}). With stronger excitation light (47000 ergs·cm^{-2}·sec^{-1}), CCCP had less marked effects both on $(P—S)/P$ and $\tau_{1/2}$.

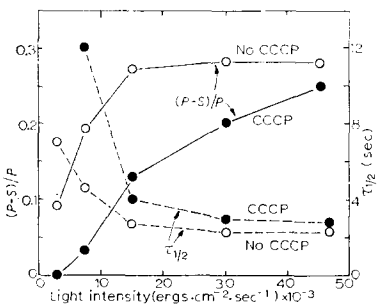
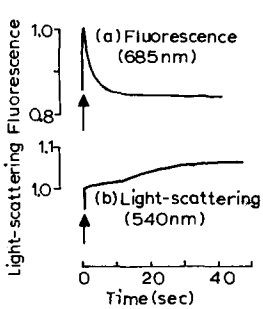


Fig. 9. Relationship between the light intensity and the change of fluorescence yield, $(P—S)/P$, and the half-time of the change, $\tau_{1/2}$, in the presence and absence of CCCP. Conditions were the same as in Fig. 8.

Comparsion of time-courses of fluorescence and light-scattering changes

The time-courses of fluorescence and light-scattering changes were compared under the same experimental conditions, using the "single-beam excitation method" (Fig. 10). The response of the light-scattering change was much slower than the fluorescence one. In addition, the light scattering of the chloroplasts at 540 nm was increased by about 7 %, while the "fluorescence lowering" amounted to 15 %.



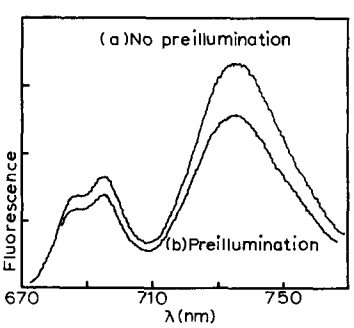


Fig. 10. Comparison of time-courses of changes in fluorescence yield and light-scattering at 540 nm under the same conditions. Experimental conditions were the same as in Fig. 8.

Fig. 11. Low-temperature fluorescence spectra (−196°) of spinach chloroplasts with and without preillumination before cooling. 20 μM DCMU, 10 μM PMS and 0.5 mM ascorbate were added. Excitation light was a blue light obtained from an incandescent lamp through blue band-pass filters. Fluorescence was measured with 2.5-nm half bandwidth. a, no preillumination; b, preillumination with red light (20000 ergs·cm^{-2}·sec^{-1}).

Emission spectra at —196°

Emission spectra were measured at liquid-nitrogen temperature with and without preillumination before cooling (Fig. 11). The preillumination decreased the yields of the emissions, F684, F695 and F-l (fluorescence emitted at 684 nm, 695 nm and 735 nm[14]), the effect being most significant with respect to F-l. These effects of preillumination were in contrast to those of Mg^{2+}, with which there was a decrease in F-l accompanied by increases in F684 and F695 (ref. 13).

DISCUSSION

The "fluorescence lowering" was observed in the presence of PMS at low concentrations. Since an inhibitor of electron transport, DCMU, was always present throughout the experiments in this study, the change in rate of photoreaction II cannot be a cause for the "fluorescence lowering" observed. A small change in fluorescence yield ascribable to such competition of photoreaction II with other processes of excitation degradation was observed upon actinic illumination of the chloroplasts in the absence of PMS (Fig. 1, a). However, the sign of this type of fluorescence change was always opposite to that of the "fluorescence lowering". A possibility that PMS accelerated photoreaction II by making a DCMU-insensitive bypass, or a short circuit, of electron flow will also be excluded. If there were such a bypass or short circuit, the effect of PMS should have been more significant under low than high light intensities. The experimental results showed that the "fluorescence lowering" was much more marked under illumination at high intensities.

The facts that the addition of PMS did not change the fluorescence yield under weak light indicate that PMS, at low concentrations used in this study, does not by itself act as a static quencher of excited state of chlorophyll *a*. A static quenching by some transient form of PMS which may be produced under strong illumination is also unlikely, since the "fluorescence lowering" was found to be strongly suppressed by various types of uncouplers of photophosphorylation, which do not block the photoreactions nor the electron transport reactions.

The facts that the "fluorescence lowering" requires the presence of PMS and high intensities of illumination and begins at 0.2 sec after the onset of illumination and recovers with a half-decay time of 5 sec indicate that the "fluorescence lowering" is related to an intermediate state formed by the electron transport reactions mediated by PMS. Its suppression by various types of uncouplers suggests the involvement of a high energy state (or intermediate) of photophosphorylation in the "fluorescence lowering", as are the cases in other light-induced changes of chloroplasts, such as light-scattering change[23–26], volume change[27] and ion transport[27]; It will be assumed, therefore, that a high energy state of photophosphorylation may influence in some way the state of chlorophyll *a* molecules in the chloroplasts, and, in turn, modify the fate of excited state to result in a decrease in fluorescence yield.

PAPAGEORGIOU AND GOVINDJEE[10,11] stated that there occurred, in intact cells of algae, a fluorescence induction which was not explained by the competition between fluorescence emission and photoreaction II. They inferred that the fluorescence induction was related to the energy-dependent structural changes of the chloroplasts. The change in state of chlorophyll *a* in the chloroplasts which may underlie the "fluo-

rescence lowering" under investigation in the present study, however, must be different from the conformational changes responsible for the light-scattering change of chloroplasts, since the time responses of these two kinds of changes differed from each other as shown in Fig. 10. The similar effects of atebrin and CCCP towards the "fluorescence lowering" in contrast to their different behaviors towards the high-energy-dependent light-scattering change[26] will also support the above view.

In the experiments at −196°, the preillumination before cooling of the chloroplasts in the presence of PMS decreased the fluorescence yields of pigment system II (F684 and F695[14]) and that of pigment system I (F-l[14]). The decrease in yield of F-l was more significant than those of F684 and F695. These facts indicate that the excitation energy available to photoreactions I and II is depressed by the strong illumination of the chloroplasts in the presence of PMS. We have shown that a change in efficiency of excitation transfer from pigment system II to I is induced by the addition of divalent metal ions[13]. This, however, cannot be regarded as a cause for the "fluorescence lowering", since the pattern of fluorescence spectrum at −196° was affected in different ways by the addition of magnesium ions and by illumination at high light intensities in the presence of PMS.

Most plausible mechanisms thus far reached are the following: (1) a high energy state accelerates thermal degradation of the excited state of chlorophyll *a*, or (2) it accelerates the excitation transfer to pigment molecules which fluoresce weakly or not at all at room and liquid-nitrogen temperatures.

The mechanism underlying the "fluorescence lowering" will be another control of utilization of excitation energy, through which the excitation energy available to both photoreactions I and II is decreased. Presently, it is still unknown whether the "fluorescence lowering" has any biological role. It may be that the mechanism perhaps reduces the inactivation of the chloroplasts under too strong light.

In our previous study on the fluorescence of isolated spinach chloroplasts[28], PMS did not significantly decrease the fluorescence yield. This will be due to the low intensity of excitation light used in the previous study. ARNON *et al.*[29] reported that the fluorescence yield in isolated chloroplasts was significantly lowered by addition of PMS. They inferred that the fluorescence yield was lowered in a competitive way by the cyclic electron transport related to the photophosphorylation. Their conclusion, however, cannot be adopted. In their experiments, no inhibitor of electron transport was added and the concentration of PMS used was much higher than in this study. Therefore, the decrease of fluorescence yield observed must have been a result of 3-fold action of PMS, an acceleration of photoreaction II due to the Hill reaction of PMS, static quenching of excited state by PMS and the "fluorescence lowering" stated above.

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