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CONTROL OF EXCITATION TRANSFER IN PHOTOSYNTHESIS

II. MAGNESIUM ION-DEPENDENT DISTRIBUTION OF EXCITATION ENERGY BETWEEN TWO PIGMENT SYSTEMS IN SPINACH CHLOROPLASTS

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SUMMARY

1. Effects of metal ions on the yields of chlorophyll *a* fluorescence were investigated in spinach chloroplasts. At room and liquid-nitrogen temperatures, addition of Mg^{2+} increased the yields of two emissions of chlorophyll *a*, having peaks at 684 and 695 nm which were emitted from pigment system II and decreased the yield of another emission of chlorophyll *a* at about 735 nm which was emitted from pigment system I. Mg^{2+} also changed the pattern of the fluorescence induction at room temperature to increase the variable component of fluorescence.

2. Effects of Mg^{2+} on the quantum yields of the two photoreactions were investigated in a region of weak light intensities. Mg^{2+} accelerated the rate of photoreaction II measured by the Hill reaction with 2,6-dichlorophenolindophenol and depressed the rate of photoreaction I measured by the $NADP^+$ reduction in the presence of reduced 2,6-dichlorophenolindophenol and 3-(3',4'-dichlorophenyl)-1,1-dimethylurea.

3. These facts indicate that Mg^{2+} controls the distribution of excitation energy between the two pigment systems and that the control consists mainly in change in rate of excitation transfer from the bulk chlorophyll *a* molecules in pigment system II to those in pigment system I; namely, Mg^{2+} suppresses the spillover of excitation energy from pigment system II to pigment system I.

INTRODUCTION

In our previous study on chlorophyll *a* fluorescence in a red alga, *Porphyridium cruentum*[1], it was suggested that illumination of the organism changes the efficiency of excitation transfer between chlorophyll *a* molecules and that the light-induced change in the excitation transfer controls the distribution of excitation energy between the two pigment systems. Upon illumination of pigment system I, a greater amount of light energy is transferred to pigment system II and a lesser to pigment system I than upon illumination of pigment system II. An inverse change in the excitation transfer occurred upon illumination of pigment system II.

Abbreviations: DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea, CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCIP, 2,6-dichlorophenolindophenol.

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Papageorgiou and Govindjee[2-4] and Bannister and Rice[5], working with Anacystis and Chlorella, also discovered changes in fluorescence yield which were independent of the rate of photoreaction II. However, they attributed the observed changes to mechanisms other than the one we have proposed.

In this study, the changes in fluorescence yields and in quantum yields of the two photoreactions in isolated chloroplasts were investigated to elucidate the control mechanism of excitation transfer. It was discovered that metal ions play an important role in the control of excitation transfer in photosynthesis.

METHODS

Broken spinach chloroplasts were prepared as follows. Three or four fresh spinach leaves were ruptured with a Waring blender at low speed for 3 min in 50 ml of sucrose–phosphate medium (0.4 M sucrose, 0.01 M NaCl, 0.05 M phosphate buffer, pH 7.7), filtered through 8 layers of cheesecloth. The green juice obtained was centrifuged at 1500 × *g* for 5 min. The precipitate was suspended in a sucrose–Tricine medium (0.4 M sucrose, 0.01 M NaCl, 0.05 M Tricine buffer, pH 7.6) and centrifuged at 200 × *g* for 1 min. The supernatant was centrifuged at 1500 × *g* for 5 min, and the precipitate formed was resuspended in the sucrose–Tricine medium and used as test material. In the measurements of fluorescence, the Hill reaction of 2,6-dichlorophenolindophenol (DCIP) and the $NADP^+$ reduction, the suspension was diluted with the sucrose–Tricine medium, so that the light absorption by the sample did not exceed 15 % (fluorescence) and 50 % (the Hill reaction and $NADP^+$ reduction) of the incident light at the absorption peaks of the chloroplasts, the concentrations corresponding to less than 1 and 4 μg chlorophyll per ml, respectively.

Fluorescence was excited with blue light obtained from an incandescent lamp through a filter combination (V-B46 (Toshiba), HA-50 (Hoya Glass) and B-460 (Hoya Glass) × 2). The incandescent lamp (75 W) was operated with a stabilized direct current. In the experiments at room temperature, the sample was placed in a four-sided transparent cuvette (1 cm × 1 cm × 4 cm), and fluorescence emitted was measured at right angle to the excitation beam by using an interference filter having a transmission peak at 684 nm (16-nm half bandwidth) and a photomultiplier R-136 (Hamamatsu TV) as detector. In the measurements of emission spectra at liquid nitrogen temperature (—196°), the sample was placed in a 0.2-cm-thick plastic cuvette immersed in liquid nitrogen, and the fluorescence emitted for the same side of excitation beam was analyzed with a Bausch and Lomb grating monochromator (2.5-nm half bandwidth) and detected with a photomultiplier, 7102 (RCA). The emission spectra were obtained automatically by driving the monochromator with a synchronous motor at a scanning speed of 1 nm/sec. The emission spectra were not corrected for the spectral sensitivity of the photomultiplier–monochromator combination (for details on the correction, *cf*. Fig. 1 in ref. 6).

In the experiments of the Hill reaction with DCIP and the $NADP^+$ reduction, the reduction of DCIP or $NADP^+$ was measured spectroscopically. The light source of the actinic light was an incandescent lamp. A combination of an interference filter having a transmission peak at 480 nm (12-nm half bandwidth), a blue-band pass filter V-B46 and a far-red absorbing filter HA-50 was used to obtain 480-nm actinic light; another combination of an interference filter having a peak at 695 nm (17-nm half bandwidth) and a red cut-off filter V-R67 (Toshiba), for the 695-nm actinic light. The intensity of actinic light was changed with the use of neutral density filters, N-50, N-25 and N-10 (Hoya Glass). The absolute intensity of light was measured with a calibrated thermopile (Kipp and Zonen). The reduction of DCIP or NADP$^+$ was measured by the absorbance changes at 600 nm or 340 nm, respectively. Monochromatic light of 600 or 340 nm, obtained from a Hitachi EPU-2A spectrophotometer, was incident on the sample contained in a four-sided transparent cuvette (1 cm × 1 cm × 4 cm). A Bausch and Lomb grating monochromator was placed behind the cuvette, in order to isolate the measuring light and eliminate the scattered actinic light and the fluorescence emitted by the sample. A V-R60 glass filter for the Hill reaction of DCIP or V-V40 for the NADP$^+$ reduction (both filters, products of Toshiba) was placed between the cuvette and the monochromator.

The absorption spectrum of the chloroplasts was measured with a Shimadzu MPS-50 spectrophotometer.

RESULTS

According to our previous study[1], the yield of chlorophyll *a* fluorescence depends (inversely) on the rate of photoreaction II and the efficiency of excitation transfer between pigments. In the present study, therefore, an inhibitor of the photosynthetic electron transport, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU), was always added in fluorescence measurements at room temperature to discriminate the fluorescence changes due to changes in excitation transfer from those due to changes in rate of photoreaction II.

Under this condition, the addition of oxidation–reduction reagents, ferrocyanide (2 mM), ascorbate (2 mM) and ferricyanide (0.5 mM), did not alter the fluorescence yield, thus showing that the oxidation–reduction conditions had no significant effect on the excitation transfer between pigments.

On the other hand, addition of some divalent metal ions, such as Mg^{2+}, Ca^{2+} and Mn^{2+}, increased the yield of chlorophyll *a* fluorescence at room temperature. Mg^{2+} had marked effects on the fluorescence yield. As shown in Fig. 1, the addition of DCMU at the steady level of fluorescence slightly increased the fluorescence yield. A further addition of $MgCl_2$ (2 mM) enhanced the fluorescence yield by about 40 %

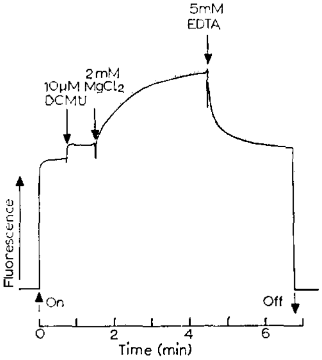


Fig. 1. Effects of $MgCl_2$ and EDTA on the yield of chlorophyll *a* fluorescence at room temperature in spinach chloroplasts. Fluorescence was measured at 684 nm.

within several minutes. A similar effect was obtained on addition of $MgSO_4$ instead of $MgCl_2$. Thus, the presence of Mg^{2+} appeared to be essential for the observed effect. The effect of Mg^{2+} was eliminated by addition of EDTA, which is known to chelate divalent metal ions (Fig. 1). A similar eliminating effect was observed on addition of ATP (3 mM) instead of EDTA.

The addition of $CaCl_2$ (3 mM) and $MnCl_2$ (3 mM) also increased the fluorescence yield. As compared with the case of $MgSO_4$ at the same concentration, the extent of the fluorescence increase was almost the same with $MnCl_2$ and $CaCl_2$.

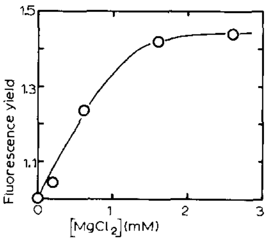
No significant change in light absorption of the chloroplasts was observed on addition of Mg^{2+}.

Effects of carbonylcyanide *m*-chlorophenylhydrazone (CCCP), phlorizin and NH_4Cl on the Mg^{2+}-induced change in fluorescence yield were examined. According to BANNISTER[7], CCCP at a concentration of 10 μM decreases the fluorescence yield in Chlorella cells. In the present study on spinach chloroplasts, CCCP decreased the fluorescence yield at concentrations higher than 50 μM but did not at concentrations lower than 5 μM. Phlorizin also did not change the fluorescence yield at a concentration of 1 mM. NH_4Cl had no effect on the fluorescence yield at concentrations lower than 5 mM.

CCCP (3 μM), phlorizin (1 mM) and NH_4Cl (4 mM), at least at concentrations which did not by themselves affect the fluorescence yield, showed no effect on the Mg^{2+}-induced change of the fluorescence yield.

The relationship between the concentrations of $MgCl_2$ and the increase in fluorescence yield was also investigated (Fig. 2). A concentration of 1.5 mM was sufficient to obtain a maximum increase in fluorescence yield. The maximum increase amounted to 44 % in the case of Fig. 2. The extent of the increase, however, depended on the sample. In some cases an increase as high as 80 % was obtained. When aged spinach leaves were used as the starting material, the increase tended to be smaller or sometimes no increase was observed at all.

The time-courses of chlorophyll *a* fluorescence during the induction period were compared in the presence and absence of $MgCl_2$ and EDTA. Fig. 3 shows oscilloscope traces of the fluorescence intensity in the induction period. $MgCl_2$ significantly increased the steady level of fluorescence, F_s, but had only a slight effect on the initial rise component of fluorescence, F_0. The addition of EDTA to the sample containing $MgCl_2$ restored the initial induction pattern. Although $MgCl_2$ increased F_s by about



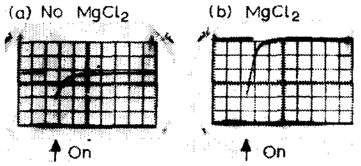


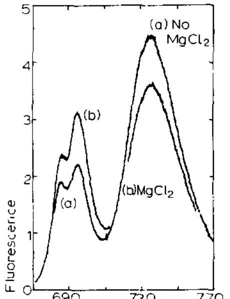
Fig. 2. Dependence of fluorescence yield at room temperature on concentration of $MgCl_2$. Fluorescence was measured at 684 nm in the presence of DCMU (10 μM) and expressed in relative units.

Fig. 3. Effect of $MgCl_2$ on the induction of chlorophyll *a* fluorescence at room temperature. Fluorescence was measured at 684 nm in the presence of DCMU (10 μM). One division, 0.1 sec. (a) $MgCl_2$ was not added. (b) 2 mM $MgCl_2$ was added 5 min before measurement.

60 %, the value for $\int_0^s (F_s - F(t))\mathrm{d}t/F_s$ was augmented by 10 % by the addition of Mg^{2+} (see Table I in DISCUSSION).

On the other hand, $MgCl_2$ significantly changed the emission spectrum of the chloroplasts at liquid-nitrogen temperature (Fig. 4). It increased the yields of F684 and F695 (emissions having peaks at 684 nm and 695 nm, respectively[6]), while the yield of F-1 (an emission having a peak at 735 nm[6]) was decreased by the addition. In the experiments shown in Fig. 4, blue light (having a transmission peak at 470 nm) absorbed mainly by chlorophyll *b* was used as the excitation light. In other experiments, using 438-nm light or 630-nm light absorbed mainly by chlorophyll *a*, similar results were obtained concerning the mode of changes in yields of the three emissions.

Effects of Mg^{2+} on the quantum yields of photoreactions I and II were investi-



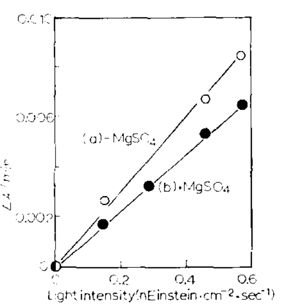
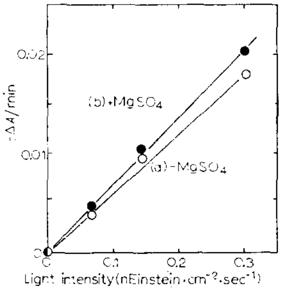


Fig. 4. Emission spectra of spinach chloroplasts at liquid-nitrogen temperature in the presence and absence of $MgCl_2$. a, $MgCl_2$ was not added; b, 4 mM $MgCl_2$ was added 5 min before cooling.

Fig. 5. Effect of $MgSO_4$ on the photoreduction of $NADP^+$ in the presence of DCMU and reduced form of DCIP. 10 μM DCMU, 1 mM $NADP^+$, 0.08 mM DCIP and 1 mM ascorbate and sufficient amount of spinach ferredoxin were added. Reduction of $NADP^+$ was continuously measured at 340 nm. 480-nm light was used as actinic light. The chloroplast suspension absorbed 50 % of incident light at 480 nm. The ratio of the fluorescence yields in the presence and absence of $MgSO_4$ was 1.55 at room temperature. a, $MgSO_4$ was not added; b, 3 mM $MgSO_4$ was added.



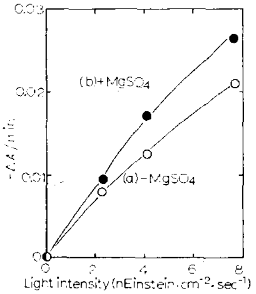


Fig. 6. Effect of $MgSO_4$ on Hill reaction of DCIP with 480-nm light. 0.033 mM DCIP was added. Reduction of DCIP was continuously measured at 600 nm. 480-nm light was used as actinic light. The chloroplast suspension absorbed 44 % of incident light at 480 nm. The ratio of fluorescence yields in presence and absence of $MgSO_4$ was 1.65 at room temperature. a, $MgSO_4$ was not added; b, 3 mM $MgSO_4$ was added.

Fig. 7. Effect of $MgSO_4$ on Hill reaction of DCIP with 695-nm light. 0.017 mM DCIP was added. Reduction of DCIP was measured at 600 nm. 695-nm light was used as actinic light. The chloroplast suspension absorbed 5 % of incident light at 695 nm. The ratio of fluorescence yields in presence and absence of $MgSO_4$ was 1.33 at room temperature. a, $MgSO_4$ was not added; b, 3 mM $MgSO_4$ was added.

gated by measuring, respectively, the photoreduction of $NADP^+$ in the presence of DCMU and the reduced form of DCIP and the Hill reaction with DCIP. The reactions were carried out at low light intensities, so that the reaction rates were limited by the light intensity. As shown in Figs. 5–7, the rates of the reactions were in fact almost proportional to the light intensity, indicating that the rates of reactions were limited by the amounts of excitation energy available for the reactions.

As shown in Fig. 5, Mg^{2+} depressed the $NADP^+$ reduction with 480-nm actinic light. This corresponds to the Mg^{2+}-induced decrease of F-I emitted from pigment system I. The Hill reaction of DCIP with 480-nm actinic light was slightly promoted by the addition of Mg^{2+} (Fig. 6), while the same reaction with 695-nm actinic light was markedly enhanced by the addition (Fig. 7).

From the data of the experiments shown in Figs. 5–7, the quantum yields of the two reactions (number of electrons transported through the photoreaction per absorbed quanta) were computed to be 0.21 ($-Mg^{2+}$) and 0.15 ($+Mg^{2+}$) for the $NADP^+$ reduction with 480-nm light at a light intensity of 0.30 nEinstein $\cdot cm^{-2} \cdot sec^{-1}$; and 0.26 ($-Mg^{2+}$) and 0.29 ($+Mg^{2+}$) for the Hill reaction of DCIP with 480-nm light at an intensity of 0.15 nEinstein $\cdot cm^{-2} \cdot sec^{-1}$; and 0.08 ($-Mg^{2+}$) and 0.11 ($+Mg^{2+}$) for the Hill reaction of DCIP with 695-nm light at an intensity of 2.2 nEinsteins $\cdot cm^{-2} \cdot sec^{-1}$.

DISCUSSION

In our previous study on the chlorophyll *a* fluorescence in *P. cruentum*[1], it was discovered that the illumination of the organism caused changes in yields of chlorophyll *a* emissions; the sign of the changes in F684 and F695 emitted from pigment system II was inverse to that of the change in F-I emitted from pigment system I. In this study the same relationship was obtained concerning the changes in the three emissions on addition of Mg^{2+}, as well as the recovery on their removal by addition of EDTA. It will be concluded, therefore, that Mg^{2+} and some other divalent metal ions play an important role in the light-induced control of excitation transfer between chlorophyll *a* molecules.

It was reported that Mg^{2+} were released from spinach chloroplasts under illumination[8,9]. If we assume that the same ion transport and the same effects of Mg^{2+}, as discovered in spinach chloroplasts, also occur in *P. cruentum*, our previous findings with this alga that the illumination of pigment system II decreases the yields in F684 and F695 and increases that in F-I will be explained as a result of a decrease in concentration of Mg^{2+} in the chloroplasts caused by the illumination.

In the previous study on Porphyridium[1], two possible mechanisms were considered concerning the change of excitation transfer to account for the competition of fluorescence yields between pigment systems I and II. Also in the present case of Mg^{2+}-induced changes in fluorescence yields in spinach chloroplasts, the same two mechanisms have to be considered. In one mechanism, the control of excitation transfer occurs at the steps of excitation transfer from the light-absorbing pigments to fluorescent ones in pigment systems I and II. In the other mechanism, it occurs at the step of excitation transfer from the bulk chlorophyll *a* of pigment system II to the bulk chlorophyll *a* of pigment system I, *i.e.* at the step of spillover. The following analyses of fluorescence induction of F684 at room temperature proved the latter mechanism to be likely.

According to the theory concerning the relationship between the fluorescence induction and photoreaction II proposed independently by our group[10,11] and by MALKIN AND KOK[12] and MALKIN[13], the parameters of fluorescence induction in the isolated chloroplasts at a sufficiently strong intensity of excitation light are expressed as follows:

$$F_s = cKI_a \frac{k_f}{k_h + k_f + k_t} \tag{1}$$

$$\frac{F_s - F_0}{F_s} = \frac{k_r}{k_h + k_f + k_t + k_r} \tag{2}$$

$$[X_1] = \frac{KI_a \int_0^s (F_s - F(t))\mathrm{d}t}{F_s} \tag{3}$$

where, F_0, $F(t)$ and F_s represent the fluorescence intensities at onset of illumination, at time t and at the final steady state. K is the fraction of light quanta, transferred to and directly absorbed by the fluorescent chlorophyll *a* molecules in pigment system II, to the total light quanta absorbed. I_a is the total amount of light quanta absorbed by the chloroplasts; c is a constant; k_h, k_f, k_t and k_r represent, respectively, the rate constants of thermal degradation of excitation, fluorescence emission, transfer of excitation to bulk chlorophyll *a* in pigment system I and photoreaction II.

$[X_1]$ represents the amounts of the electron pool between photoreaction II and the step inhibited by DCMU.

In our analyses of fluorescence induction at —196°, it was concluded that C_{f684} and C_{f695} (pigments emitting F684 and F695) represent, respectively, the bulk chlorophyll *a* and the energy sink which is connected to the photoreaction in pigment system II[14]. Therefore, the yield in F695 should directly reflect the utilization of excitation energy in photoreaction II according to the relationship defined by Eqns. 1–3. At room temperature, at which the thermal energy is sufficient to cover the energy deficit for the return transfer of excitation from C_{f695} to C_{f684}, the rate of return transfer is much higher than the rates of radiative and nonradiative degradation in C_{f695}. Therefore, an induction which reflects directly the utilization of excitation energy in photoreaction II also occurs with respect to F684. For this reason, Eqns. 1–3 can be applied to the induction of F684 at room temperature.

There are two possibilities to be considered as the cause of the observed effects of Mg^{2+}. According to the mechanism by which the excitation transfer is controlled at the steps of transfer from the light-absorbing pigments to the respective fluorescent molecules of chlorophyll *a*, an increase in K will result in an increase in fluorescence yield as observed in the above experiments. According to the other mechanism, in which the transfer is controlled at the step of excitation transfer from the bulk chlorophyll *a* of pigment system II to the bulk chlorophyll *a* of pigment system I, it is a decrease in k_t that causes similar changes of fluorescence yield. In these arguments other rate constants, k_h, k_f and k_r, are assumed to be unchanged by the addition of Mg^{2+}. It will also be reasonable to assume that the amount of the electron pool $[X_1]$ is not altered by the addition of Mg^{2+}.

The values for the parameters of fluorescence induction computed from the data of fluorescence induction at room temperature (experiments shown in Fig. 3) are summarized in Table I.

In this set of experiments, Mg^{2+} enhanced F_s by about 60 %, which, according to Eqn. 1, can be interpreted in terms of an increase in K and/or decrease in k_t.

Mg^{2+}, on the other hand, increased $(F_s - F_0)/F_s$ by about 50 %. This fact will indicate that there was a decrease in k_t, since $(F_s - F_0)/F_s$ is a quantity which does not depend on K as shown in Eqn. 2.

A small increase in value of $\int_0^s (F_s - F(t))dt/F_s$ (about 10 %), according to Eqn. 3, will indicate that Mg^{2+} also slightly decreased the value for K by about 10 %; if $[X_1]$, the pool size of electron associated with photoreaction II, was not changed by the addition.

According to Eqn. 1, the increase in F_s caused by the addition of Mg^{2+} is not contradictive with the significant decrease in value for k_t and the slight decrease in value for K.

Summarizing these considerations, it may be concluded that the main change induced by the addition of magnesium ions consists in a decrease in k_t, namely a decrease in rate constant of the excitation transfer from the bulk chlorophyll *a* in pigment system II to the bulk chlorophyll *a* in pigment system I. A subsidiary change may also occur with respect to the value for K, although this seems to be rather insignificant in its extent. Thus, a scheme as shown in Fig. 8 will be presented in the control of excitation transfer between pigments in the isolated spinach chloroplasts.

Low-temperature studies on the action spectra of the three fluorescence emissions in various photosynthetic organisms and chloroplasts[6,15,16] and the emission spectra

TABLE I

PARAMETERS FOR INDUCTION OF CHLOROPHYLL *a* FLUORESCENCE IN SPINACH CHLOROPLASTS AT ROOM TEMPERATURE

The data are taken from the experiments shown in Fig. 3.

Additions	F_s (*rel. units*)	F_0 (*rel. units*)	$\frac{F_s - F_0}{F_s}$	$\frac{\int_0^s (F_s - F(t))dt}{F_s}$ (*sec*)
No addition	97	53	0.45	0.040
$MgCl_2$ (2 mM)	153	51	0.67	0.045
$MgCl_2$ (2 mM) + EDTA (5 mM)	96	51	0.47	0.039

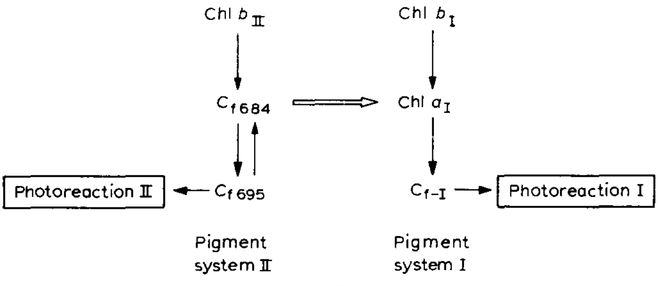


Fig. 8. Scheme for excitation transfer between pigments in chloroplasts. Thick arrow represents the site of control at which the efficiency of excitation transfer is depressed by the addition of Mg^{2+} (see also ref. 14).

of detergent-treated subparticles of spinach chloroplasts[17, 18] have indicated that F684 and F695 are emitted from pigment system II and F-l from pigment system I. However, in the case of *Anacystis nidulans*, F-l was found to be more efficiently excited by illumination of pigment system II than by pigment system I[19,20], a finding which makes at least for this particular case, the attribution of F-l to pigment system I rather questionable.

Of special interest in this connection is the relationship between the Mg^{2+}-induced changes in quantum yields of the two photoreactions and the changes in fluorescence yields of the two pigment systems. As the yields of F684 and F695 were increased and that of F-l was decreased by the addition of magnesium ions, the quantum yield of photoreaction II measured by the Hill reaction of DCIP with 695-nm actinic light was accelerated, while the quantum yield of photoreaction I measured by the $NADP^+$ reduction with 480-nm actinic light was depressed. This parallelism between the changes of fluorescence yields and quantum yields of the two photoreactions will be another and decisive evidence for the attribution of F-l to pigment system I. This makes another ground for concluding that, at least in spinach chloroplasts, F-l is emitted from pigment system I.

In contrast to the Hill reaction of DCIP under 695-nm actinic light, the same reaction under 480-nm actinic light was not so significantly enhanced by the addition of Mg^{2+} as expected from the increase in fluorescence yield. This will be explained if one assumes that DCIP is reduced at two sites in the electron transport chain, *i.e.* one at the reducing side of photoreaction I (type I) and another situated between photoreactions I and II (type II). The sum of these two types of the DCIP reduction will correspond to the actually observed reduction of DCIP. The illumination of the spinach chloroplasts with 695-nm actinic light excites pigment system I more strongly than pigment system II. Photoreaction II, therefore, makes the limiting step in both types of DCIP reduction, and thus, the addition of Mg^{2+}, which increases the excitation energy available to photoreaction II, results in an acceleration of DCIP reduction in parallel with the Mg^{2+}-induced increase in fluorescence yield. On the other hand, on illumination with 480-nm light, pigment system II is more strongly excited than pigment system I; and therefore the DCIP reduction of type I is limited by the step of photoreaction I, while the DCIP reduction of type II is still limited by the step of photoreaction II. In this circumstance, the addition of Mg^{2+}, which increases the excitation energy available to photoreaction II and decreases the excitation energy available to photoreaction I, accelerates the DCIP reduction of type II and suppresses the DCIP reduction of type I. Consequently, under 480-nm actinic light, the Hill reaction of DCIP will not be so significantly enhanced by the addition of Mg^{2+} as calculated from the induced increase of fluorescence yield.

In a previous study of fluorescence induction at liquid-nitrogen temperature[14], we concluded the occurrence of spillover of excitation energy from pigment system II to I in spinach chloroplasts and some red algae. On the other hand, JOLIOT AND KOK[21] inferred that the spillover, if present, does not exceed 30% of the total absorbed excitation energy in Chlorella. The finding of the present study that the Mg^{2+} cause fluorescence changes ascribable to the changes in excitation transfer from pigment system II to I furnishes another evidence for the spillover mechanism in spinach chloroplasts.

It has been known that the rate of excitation transfer between weakly coupled pigment molecules, as chlorophyll *a* in the chloroplasts, depends on the mutual orientations and the distances between the molecules[23]. The results obtained, that the addition of Mg^{2+} caused the change in excitation transfer between chlorophyll *a* molecules, suggest that Mg^{2+} may modify the interaction of chlorophyll *a* molecules in the chloroplasts. On the other hand, it has been shown in the studies of light-scattering and volume changes in the chloroplasts that the isolated chloroplasts shrink with the addition of Mg^{2+} (ref. 24). The electron microscopic studies have also indicated that the ions drastically change the membrane structure of chloroplasts[25]. Since the chlorophyll *a* molecules are localized in the matrix of the chloroplast membrane, the Mg^{2+} must change the mutual orientations and the distances between chlorophyll *a* molecules in the chloroplasts, thus changing the efficiency of excitation transfer between them.

The findings that the most remarkable change in efficiency of excitation transfer occurred at the step from bulk chlorophyll *a* of pigment system II to that of pigment system I will be explained as follows. The efficiency of excitation transfer between two pigment molecules, Φ_t, is presented as follows:

$$\Phi_t = \frac{k_t}{k_t + k_f + k_h} \qquad (4)$$

The notations are the same as in Eqns. 1–3. If it is assumed that k_t is a variable number and k_f and k_h are invariable, Φ_t depends upon k_t only when the value for k_t is comparable with, or less than, the value for the sum of k_f and k_h. The value for $(k_f + k_h)$, the reciprocal of the actual life time of fluorescence, has been estimated to be approx. $5 \cdot 10^8$ sec^{-1} (ref. 26). In the excitation transfer between bulk chlorophyll *a* molecules in each pigment system, the value for k_t (estimated to be $1 \cdot 10^{11}$–$1 \cdot 10^{12}$ sec^{-1} (refs. 27–29) is much larger than that for $(k_f + k_h)$. In this case, a small change in k_t has little effect on the value for Φ_t. On the other hand, in the excitation transfer from bulk chlorophyll *a* in pigment system II to that of pigment system I, the value for k_t must be comparable with that of $(k_f + k_h)$; if k_t were much larger than $(k_f + k_h)$, the fluorescence emission from pigment system II should be too weak to be observable. In this case, Φ_t will be effectively influenced with the change of k_t. These will be the reasons why the most remarkable change in efficiency of excitation transfer with the addition of Mg^{2+} occurred at the step of transfer from bulk chlorophyll *a* of pigment system II to that of pigment system I.

BANNISTER AND RICE[5] and PAPAGEORGIO AND GOVINDJEE[2–4], in their studies of the induction of fluorescence in some algae, also observed light-induced changes in fluorescence yield which are independent of the electron transport through photoreaction II. BANNISTER AND RICE[5] attributed the observed changes in fluorescence yield to a light-induced "activation" of pigment system II unit from a nonfluorescent and photochemically inactive form to fluorescent and photochemically active form through a process mediated by photoreaction I. The mechanism of BANNISTER AND RICE[5], however, does not apply to the Mg^{2+}-induced changes of fluorescence yield, since the increases in yields of F684 and F695 accompanied by the observed decrease in yield of F-I cannot be explained in terms of such "activation" of pigment system II.

PAPAGEORGIOU AND GOVINDJEE[2–4] suggested that the light-induced conformational change of the lamellae coupled with the phosphorylation system is a cause for the changes in fluorescence yield. Although such a connection may be possible between the structure and function of the chloroplasts, the observed effects of Mg^{2+} in decreasing the yield of F-1 and increasing those of F684 and F695 are in clear contrast with the effects of a high energy state (or intermediate) of photophosphorylation on the fluorescence yields which will be seen in the next study[22].

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