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CONTROL OF EXCITATION TRANSFER IN PHOTOSYNTHESIS

V. CORRELATION OF MEMBRANE STRUCTURE TO REGULATION OF EXCITATION TRANSFER BETWEEN TWO PIGMENT SYSTEMS IN ISOLATED SPINACH CHLOROPLASTS*

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SUMMARY

1. Changes in fluorescence yield of chlorophyll *a* in isolated chloroplasts have been interpreted by means of regulation of excitation transfer between two pigment systems of photosynthesis[5-7]. In order to investigate the relationship between the membrane structure of chloroplasts and the regulation of excitation transfer, changes of light scattering and chlorophyll *a* fluorescence of isolated spinach chloroplasts were measured upon addition of cations, Mg^{2+} and Na^{+}. The cations increased the intensities of both light scattering and fluorescence yield. The changes showed similar time courses and concentration dependences. These facts suggest that modification of membrane structure produced by the cations suppresses the excitation transfer between the two pigment systems.

2. In another case of structural change which is induced by light in the presence of *N*-methylphenazonium methosulfate, there was little correlation between light-scattering and fluorescence changes.

3. Changes in fluorescence yield induced by the addition of Mg^{2+} were measured in disintegrated chloroplasts and fractionated particles. The effects of Mg^{2+} on fluorescence were observed only in preparations of grana stacks, but not in preparations of stroma lamellae. These findings suggest that the excitation transfer is regulated between the two pigment systems located in the grana thylacoid membranes.

INTRODUCTION

In photosynthesis of green plants, two photoreactions cooperate in the electron transport system, to which two pigment systems supply light energy[1]. For an efficient reaction of photosynthesis, the light quanta have to be equally distributed in the

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

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two pigment systems. Otherwise, if one of the two absorbs much more quanta than the other, the latter system becomes the rate-limiting step of the overall reaction and the former system loses most of its absorbed light energy. A control mechanism has been found with which plants improve the efficiency of photosynthesis when the incident light is absorbed preferentially by one of the two systems[2-4].

Upon illumination of algal cells with light absorbed preferentially by Pigment System I, Photoreaction II becomes the rate-limiting step. After the illumination for 10–20 sec the distribution of light quanta changes to supply more light quanta into Pigment System II and less into Pigment System I than before. Thus, the overall reaction of photosynthesis becomes faster. Upon illumination with light absorbed preferentially by Pigment System II, the reverse change in the distribution takes place.

In the experiments in isolated spinach chloroplasts, some cations were found to produce effects similar to the preferential illumination of Pigment System I in intact algal cells[5-7]. In the presence of cations (2–5 mM for the divalent and 100 mM for the monovalent), more light quanta go to Pigment System II and less to Pigment System I than in their absence; the cations suppressed the fluorescence yield and the quantum yield of System I, and enhanced those of System II. However, in System II, the increase in fluorescence yield attained 50–90 % (refs. 5–7), while the quantum yield of Photoreaction II measured by the Hill reaction with 2,6-dichlorophenolindophenol was only slightly (5–15 %) stimulated. These discrepant effects of cations on the chlorophyll fluorescence and the quantum yield of Pigment System II led us to conclude that the cations suppressed the excitation transfer from Pigment System II to I at the step of spillover[5,7].

The efficiency of excitation transfer between pigment molecules depends on the distance between them and on their mutual orientation. In the chloroplasts, chlorophyll molecules are embedded in the thylacoid membrane. These findings led us to investigate the relationship between the membrane structure of the chloroplasts and the regulation of excitation transfer between the two pigment systems.

Light-scattering changes were measured as an indicator for the structural change of chloroplast membrane, and changes in fluorescence yield at 684 nm were measured at room temperature in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) to estimate the degree of suppression of excitation transfer from Pigment System II to I.

Changes in fluorescence yield upon addition of $MgCl_2$ were measured also in disintegrated chloroplasts as well as in preparations of grana and stroma lamellae to obtain further information about the correlation of the chloroplast structure with the regulation of excitation transfer.

METHODS

Isolated spinach chloroplasts were used as test materials. Leaves of market spinach were ruptured for 10 sec with a Waring blender in grinding solution containing 400 mM sucrose, 10 mM NaCl and 50 mM phosphate buffer (pH 7.8). The green juice was filtered through 8 layers of cheese cloth and centrifuged at 1000 × *g* for 5 min. The precipitate thus obtained was suspended in a low-salt solution containing 400 mM sucrose, 10 mM NaCl and 10 mM Tricine–KOH buffer (pH 7.8). The suspension was

centrifuged at 1000 × *g* for 5 min. The sediment was again suspended in the same solution and recentrifuged at 200 × *g* for 1 min to remove aggregated chloroplasts and other large particles. The supernatant was stored at 0° until used.

In the treatments of sonic oscillation and French-pressure extrusion, the chloroplasts were subjected to the treatments with and without addition of 5 mM $MgCl_2$. The sonic oscillator (MSE, 100 W) was operated at full intensity for 1 min. French-pressure treatment was performed 3 times at a pressure of 500 kg/cm^2. To fractionate the press-treated particles they were centrifuged on the sucrose density gradient according to the method by MICHEL AND MICHEL-WOLWERTZ[8].

Changes in fluorescence yield and light scattering were measured simultaneously for comparison. Blue excitation light covering wavelengths from 390 to 580 nm was obtained by using an incandescent lamp, and two blue filters, Corning 9782, and an infrared-reflecting filter, Calflex C .Its intensity was 8000 ergs/cm^2/sec. Light scattered by the chloroplasts in the cuvette at right angles to the excitation beam was isolated through an interference filter with a transmission peak at 534 nm (Balzer). Fluorescence emitted from the sample was measured by using another interference filter with a transmission peak at 684 nm (Balzer) at right angles to the excitation beam but at the position opposite to the measurement of light scattering. Scattered light and fluorescence were detected with photomultipliers, RCA 1P22 and EMI 9558B, respectively. The signals of the photomultipliers were amplified separately and recorded on strip-chart recorders. In measurement of the fluorescence yield in the disintegrated chloroplasts, 480-nm monochromatic light was used instead of the broad band light for the excitation of fluorescence. An interference filter (Balzer) with a transmission peak at 480 nm was placed in the excitation beam in addition to the above-mentioned blue filters. The intensity of this excitation light was 800 ergs/cm^2/sec.

RESULTS

Time courses of chlorophyll *a* fluorescence and light scattering were measured simultaneously upon the addition of $MgCl_2$ (Fig. 1a). A small amount of chloroplasts suspended in the low-salt medium was rapidly diluted with the same medium contained in the cuvette. DCMU, which was added after the fluorescence yield reached the steady level, produced only a slight change in fluorescence yield, indicating that the intensity of excitation light was so high as to reduce all of the primary electron acceptor even in the absence of DCMU. Addition of $MgCl_2$ induced a slow increase in fluorescence yield as shown in a previous study[5]. The increase took about 5 min to attain the maximum steady level, which was 60 % higher than before the addition. The light scattering showed a similar time course on the addition of $MgCl_2$. It took about 5 min to reach the steady level. Two phases (fast and slow) seemed to appear in the time course of the increase, but the fast kinetics of the light-scattering change could not be followed, because a large artifact signal produced by the mixing procedure overlapped the real signal of light scattering. A later addition of EDTA, a chelating agent for divalent metal cations, eliminated the effects of $MgCl_2$ upon the fluorescence yield and the light scattering.

Fig. 1b shows concentration dependences of fluorescence yield and light scattering for $MgCl_2$. Both changes attained the saturation level at 2 mM of $MgCl_2$. The time

courses and concentration dependences exhibited rather good parallelism between changes in fluorescence yield and light scattering upon the addition of $MgCl_2$.

Monovalent cations were discovered to increase the fluorescence yield in a way similar to that of the divalent cations[7]. The addition of 100 mM NaCl induced a small rapid decrease and then a large slow increase in fluorescence yield.

The rapid decrease is always observed when high concentrations of salts of monovalent cations are added to the chloroplast suspension, and is probably due to a change in degree of the flattening effects of chloroplasts[7]. The large slow increase must be caused by the suppression of excitation transfer between two pigment systems. The light scattering showed a fast increase and then a slow increase upon the addition as in the case of $MgCl_2$ (Fig. 2). A later addition of EDTA to this sample did not produce the changes in fluorescence yield nor in light scattering. Thus, also in this case,

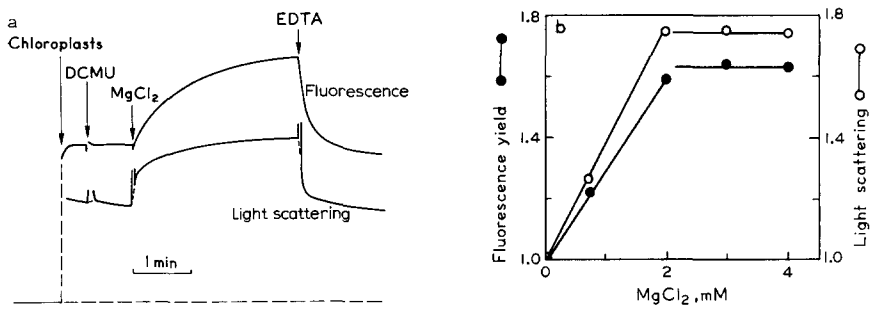

Fig. 1. Effects of $MgCl_2$ and EDTA upon chlorophyll *a* fluorescence yield and light scattering of spinach chloroplasts. Fluorescence was measured at 684 nm with 10-nm band width. Light scattering was measured at 534 nm at a right angle to the excitation light. Excitation blue light had a broad band covering 390–580 nm. Concentrations in the chloroplast suspension: 1.4 μM chlorophyll, 5 μM DCMU, 400 mM sucrose, 10 mM NaCl and 10 mM Tricine–KOH buffer (pH 7.8). a. Time courses of fluorescence and light scattering. Final concentrations of $MgCl_2$ and EDTA were 3 and 5 mM, respectively. b. Concentration dependences of fluorescence yield and light scattering for $MgCl_2$. Levels of the changes were taken 5 min after the addition of $MgCl_2$.

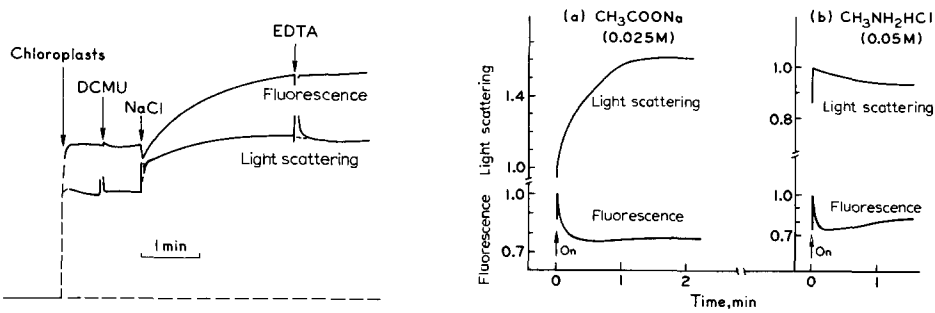

Fig. 2. Effects of NaCl and EDTA upon chlorophyll *a* fluorescence yield and light scattering. Measuring system was the same as in Fig. 1 except for the addition of 100 mM NaCl instead of $MgCl_2$.

Fig. 3. Comparison of time courses of light-induced fluorescence and light-scattering changes in the presence of PMS. Reaction mixture contained 2.1 μM chlorophyll, 10 mM NaCl, 10 mM Tricine–KOH buffer (pH 7.9), 10 μM DCMU and 10 μM PMS. a. 25 mM sodium acetate added. b. 50 mM methylamine hydrochloride added.

the changes in fluorescence yield and light scattering exhibited similar behaviours to the addition of NaCl.

It is known that a light-scattering change is induced by illumination of chloroplasts in the presence of an electron carriers such as *N*-methylphenazonium methosulfate (PMS)[9–11]. The direction of change depends on the solute of the suspension medium. Fig. 3 shows changes in fluorescence yield and light scattering. Light scattering was increased by 60 % during the 1-min illumination period in the presence of 25 mM sodium acetate. On the other hand, the fluorescence yield decreased with a higher speed than the increase in light scattering, and then slowly increased slightly. In the presence of methylamine, the light scattering showed a slow decrease, while the fluorescence yield decreased rapidly upon the onset of illumination and then slowly increased a little. The fluorescence yield showed almost the same time courses in both cases, while the light scattering changed to the opposite directions.

It is concluded, therefore, that the changes in fluorescence yield and light scattering do not correspond to each other in the case of light-induced conformational changes.

The effects of Mg^{2+} upon the fluorescence yield were studied in chloroplast fragments prepared by treatments with French-pressure extrusion and sonic oscillation. The chloroplast suspension containing 400 mM sucrose, 10 mM NaCl and 10 mM Tricine–KOH buffer (pH 7.8), with and without 5 mM $MgCl_2$ was subjected to these treatments. The suspension was then diluted 50 times with the above-mentioned medium excluding $MgCl_2$. The concentration of $MgCl_2$ in the diluted suspension was 0 or 0.1 mM, at which concentration $MgCl_2$ has practically no effect on the fluorescence yield[5]. $MgCl_2$ was added to this sample for the investigation of the effects of $MgCl_2$ on the fluorescence yield.

In the chloroplasts treated with the French-pressure extrusion in the absence of $MgCl_2$, the addition of $MgCl_2$ induced only a slight increase in the fluorescence

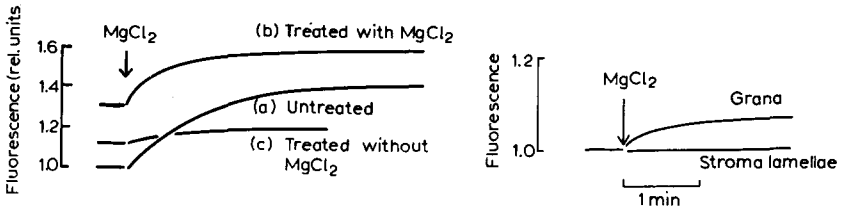

Fig. 4. Effects of $MgCl_2$ upon chlorophyll *a* fluorescence yield in disintegrated chloroplasts. Chloroplasts suspended in 400 mM sucrose, 10 mM NaCl and 10 mM Tricine–KOH buffer (pH 7.8) in the presence and absence of 5 mM $MgCl_2$ were treated with French-pressure extrusion. The suspension of disintegrated chloroplasts was diluted 50 times with the above-mentioned solution, and 10 μM DCMU was added. Addition of 3 mM $MgCl_2$ to this suspension was used to see the effects of Mg^{2+}. a. Untreated chloroplasts. b. Treated in the presence of 5 mM $MgCl_2$. c. Treated in the absence of $MgCl_2$.

Fig. 5. Effects of $MgCl_2$ upon the yield of chlorophyll *a* fluorescence in fractionated particles after French-pressure treatment. Chloroplasts suspended in 150 mM KCl and 50 mM Tricine–KOH buffer (pH 7.8) were treated 3 times with French pressure and then fractionated by centrifugation on a sucrose density gradient. Stroma lamellae (small particles) coming from the top band had a high chlorophyll *a* to chlorophyll *b* ratio, about 7, and only System I activity. Grana stacks (large particles) coming from the bottom band had a low chlorophyll *a* to chlorophyll *b* ratio, about 2.5, and a high System II and a low System I activities. Experimental conditions were the same as in Fig. 4, except that fluorescence yields before addition of $MgCl_2$ were normalized in both preparations.

yield. In the chloroplasts treated under the same condition but in the presence of 5 mM $MgCl_2$, the fluorescence yield was increased upon the addition of $MgCl_2$ (Fig. 4). After the treatment with the sonic oscillation, a similar situation was found with respect to the presence of $MgCl_2$ in the medium at the time of treatment.

The same results were obtained in the disintegrated chloroplasts, when 5 mM $MgCl_2$ was replaced by 100 mM NaCl in the suspending medium at the treatment time.

The treatment with French pressure cell and centrifugation on sucrose density gradient produce two fractions of disintegrated chloroplasts; one is a preparation of stroma lamellae having only System I activity, and the other is a preparation of grana stacks having System II and weak System I activities[8,12–14]. These fragments prepared according to the procedure of MICHEL AND MICHEL-WOLWERTZ[8] were 50 times diluted, and then the fluorescence yield was measured. The fluorescence yield increased upon the addition of $MgCl_2$ in the preparation of grana stacks, but there was no response in the preparation of stroma lamellae (Fig. 5).

DISCUSSION

The changes in fluorescence yield and light scattering showed similar time courses and concentration dependences upon the addition of cations. These facts suggest that a close relationship must exist between the regulation of excitation transfer between the two pigment systems and the membrane structure of the chloroplasts.

The chloroplasts, when excited, emit chlorophyll *a* fluorescence having a peak at 684 nm at room temperature[15]. Since this fluorescence originates mainly from bulk chlorophyll *a* of Pigment System II, we can estimate the degree of suppression of excitation transfer from Pigment System II to I by measuring the fluorescence yield. Some treatments of chloroplasts are known to produce a modification of their membrane structure; such as the addition of salts[16–19] and illumination in the presence of suitable electron carriers[9–11,20–24]. These facts were studied by measuring the light scattering and packed volume as well as electron microscopy of the chloroplasts. In most cases, shrinkage of the chloroplasts corresponds to the increase in intensity of light scattering.

The excitation transfer between pigment molecules depends upon the distance between them and upon their mutual orientation[25]. The excitation transfer from Pigment System II to I must depend on similar factors. It is generally accepted that the units of the two pigment systems lie on the thylacoid membranes. The suppression of excitation transfer between the two pigment systems on the addition of cations suggests that the cations induce conformational change of the thylacoid membrane pulling apart the units of the two pigment systems. It is expected that such conformational changes of membrane structure taking place upon the addition of cations are detected by the change in light scattering of the chloroplasts.

The close parallelism between the changes in the fluorescence yield and the intensity of light scattering strongly supports the above-mentioned explanation. These experimental results indicate that the distance between the units of the two pigment systems is greater in the chloroplasts shrunken in the presence of cations. This fact, in turn, may suggest that a microscopic structural change at the inside of the thylacoid membrane, but not the whole shape of the chloroplasts, is important in the regulation of excitation transfer between the two pigment systems.

It should be noticed that there is a similar situation in the physical separation of the two pigment systems in respect to the requirement of cations. In the study by OHKI, KUNIEDA AND TAKAMIYA[26], divalent cations at a concentration of 3–5 mM or monovalent cations at a concentration of 50 mM had to exist in the medium of chloroplast suspension for an efficient separation of the pigment systems when the chloroplasts were treated with detergent or French-pressure extrusion. An electron-microscopic study of the chloroplasts showed that in the presence of Mg^{2+} or Na^{+} the chloroplasts shrank and the grana stacking appeared. Upon the additon of 100 mM NH_4Cl or ammonium acetate, on the other hand, the chloroplasts swelled extensively, while the pigment systems were well separated with the detergent treatment. Thus, there was no relationship between the bulk structure of the chloroplasts and the physical separation of the pigment systems, but the separation depends only on the presence of cations, the same situation as in the case of the regulation of excitation transfer between the two pigment systems.

In the case of the light-induced conformational change in the presence of PMS, there was no parallelism between the changes in fluorescence yield and light scattering. The fast fluorescence decrease after the onset of strong illumination light was called "fluorescence lowering" in which the chlorophyll *a* fluorescence of Pigment System II is quenched by mechanism related to an accumulation of high energy state of phosphorylation[27,28]. If this effect is subtracted from the observed fluorescence change, a time course is obtained in which the fluorescence yield stays at almost constant levels. The light scattering, on the other hand, is increased by 60 % in the presence of sodium acetate and decreased by 10 % in the presence of methylamine hydrochloride. It is known that the chloroplasts under these conditions shrink in the presence of ammonium acetate and swell in the presence of methylamine hydrochloride. These facts, therefore, suggest again that the bulk change in the whole chloroplast structure has nothing to do in the regulation of excitation transfer between the two pigment systems.

It was discovered in this study that the effect of $MgCl_2$ in increasing fluorescence yield was found in the preparation of grana stacks but not in the preparation of stroma lamellae. The grana contained Pigment Systems I and II, and the stroma lamellae contained only Pigment System I (ref. 13). The fact that the change in fluorescence yield is observed only in the preparation of grana stacks suggests that the excitation transfer between Pigment Systems I and II that are located in the grana is regulated by Mg^{2+}.

In the resonance excitation transfer (in case of weak interaction between pigment molecules), the critical distance between chlorophyll *a* molecules for 50 % transfer can be calculated according to FÖRSTER[25]. He obtained a value, 80 Å, by using 30 nsec for a natural life-time. However, it is 2 nsec in the chloroplasts[29]. This life-time and values used by FÖRSTER[25] produces 50 Å as the critical distance between the chlorophyll *a* molecules for the excitation transfer. It can be noted that the sizes of grana and stroma lamellae are both about 5000 Å; therefore, the excitation transfer from Pigment System II in the grana to Pigment System I in the stroma does not exist or is negligibly small.

In the physical separation of two pigment systems with detergent as well as mechanical treatment, salts were found to be required for an efficient separation[8,19,26]. The treatment of chloroplasts with French pressure or sonic oscillation in the presence

of the cations in the medium produces large particles (grana stacks) containing System II and weak System I activities and small particles (stroma lamellae) having System I activity, which can be separated by a differential centrifugation or a centrifugation on sucrose density gradient[8,30]. The same treatment but in the absence of cations produces only small particles having both System I and II activities. In order to explain no or little effects of $MgCl_2$ on the fluorescence yield in the particles disintegrated in the absence of cations, it is plausible to assume that the linkage between Pigment Systems I and II in grana is broken after the treatments, forming small System I particles and small System II particles that are not separated by centrifugation.

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