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EFFECTS OF MONOVALENT CATIONS ON LIGHT ENERGY DISTRIBUTION BETWEEN TWO PIGMENT SYSTEMS OF PHOTOSYNTHESIS IN ISOLATED SPINACH CHLOROPLASTS*

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SUMMARY

The effects of monovalent cations on the light energy distribution between two pigment systems of photosynthesis were studied in isolated spinach chloroplasts by measuring chlorophyll *a* fluorescence and photochemical reactions.

The addition of NaCl to the chloroplast suspension produced a 40–80% increase in fluorescence yield measured at 684 nm at room temperature. The fluorescence increase was completed about 5 min after the addition. The effect saturated at 100 mM NaCl. Low-temperature fluorescence spectra showed that NaCl increased the yields of two fluorescence bands of pigment system II at 684 and 695 nm but decreased that of pigment system I at 735 nm. Similar effects on chlorophyll *a* fluorescence at room and at low temperatures were obtained with NaBr, $NaNO_3$, Na_2SO_4, LiCl, KCl, RbCl, CsCl, NH_4Cl and CH_3NH_3Cl.

NaCl suppressed the quantum efficiency of $NADP^+$ reduction supported by the ascorbate–2,6-dichlorophenolindophenol (DCIP) couple as an electron donor system in the presence of 3-(3′,4′-chlorophenyl)-1,1-dimethylurea (DCMU). On the other hand, NaCl only slightly enhanced the quantum yield of photoreaction II measured by the Hill reaction with DCIP.

It is concluded that the monovalent cations tested suppressed the excitation transfer from pigment system II to pigment system I; the effects were the same as those of alkaline earth metals and Mn^{2+} (refs. 1, 2).

INTRODUCTION

In our previous studies[1,2] we investigated the effects of divalent metal ions on the yield of chlorophyll fluorescence and the quantum efficiencies of two photoreactions of photosynthesis in isolated chloroplasts. It was discovered there that some divalent cations increased the light energy available in pigment system II and

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Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3′,4′-chlorophenyl)-1,1-dimethylurea.

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decreased that in pigment system I, and it was concluded that these ions suppressed the excitation transfer from bulk chlorophyll *a* molecules in pigment system II to those in pigment system I.

In the present study, the monovalent cations were found to regulate the light energy distribution between the two pigment systems in the same way as the divalent cations do.

METHODS

Spinach chloroplasts were prepared as follows; three or four leaves of market spinach were ruptured for 5 sec with a Waring blender in about 100 ml of grinding solution (400 mM sucrose, 10 mM NaCl and 50 mM phosphate buffer (pH 7.8)). The green juice was strained through eight layers of cheese cloth and centrifuged at 1000 × *g* for 5 min. The precipitate was suspended in 25 ml of KCl–Tricine solution (300 mM KCl and 50 mM Tricine–KOH buffer (pH 7.8)). The suspension was centrifuged at 1000 × *g* for 5 min. The sediment was again suspended in a small amount of the KCl–Tricine solution and recentrifuged at 200 × *g* for 1 min to remove fragments of cell walls and aggregated chloroplasts. The supernatant was stored in an ice bath until needed.

In the measurements of fluorescence, $NADP^+$ reduction and the Hill reaction with 2,6-dichlorophenolindophenol (DCIP), 0.1 ml of the stored chloroplast suspension was diluted with 2.9 ml of distilled water. The resulting mixture contained 10 mM KCl and 1.7 mM Tricine buffer. Small amounts of concentrated salt solutions were added to the suspension to observe the effects of salts. In the case of CH_3NH_3Cl the pH of the solution was adjusted to 7.8 with NaOH. The concentration of chlorophyll was about 1 μg/ml in the measurements of fluorescence and about 4 μg/ml in the measurements of the Hill reaction and $NADP^+$ reduction.

In the fluorescence measurement at room temperature, monochromatic excitation light was obtained by filtering light from an incandescent lamp through interference filters (Balzers) in combination with two pieces of blue bandpass filters (Corning 9782). The sample was placed in a four-sided transparent cuvette (1 cm × 1 cm × 4 cm), and the fluorescence of chloroplasts at 684 nm was measured at right angles to the excitation beam by using an interference filter with a transmission peak at 684 nm (Balzers). The intensity of fluorescence was detected with a photomultiplier, EMI 9558 B. The photomultiplier signal was amplified and recorded on a strip chart servo recorder, or oscilloscope traces of the signal were photographed.

Low-temperature fluorescence spectra were measured by a method described elsewhere[3]. Excitation light came from a Xenon lamp through a Bausch and Lomb "High-intensity" grating monochromator set at 475 nm with half bandwidth of 20 nm. The emission spectra were automatically corrected for variation in transmission of the analyzing monochromator and sensitivity of detecting photomultiplier with wavelength. The half bandwidth of the analyzing monochromator was 3.5 nm. The thickness of the cuvette was about 0.02 cm. Small amounts of concentrated salt solutions were added 5 min before cooling the sample.

Absorption spectra were measured with a spectrophotometer developed by French and Lawrence[4].

In the experiments on the Hill reaction with DCIP and $NADP^+$ reduction, the reduction of these compounds was measured spectroscopically at 591 and at 340 nm, respectively. The measuring beam was obtained by using appropriate interference filters in conjunction with an incandescent lamp powered by a battery. Another interference filter with a transmission peak at the same wavelength as the measuring beam was placed between the sample and a photomultiplier (RCA 1 P22) that measured the small change in transmission of the sample. A blue bandpass filter (Corning 9780) or an ultraviolet-transmitting filter (Corning 5860) was also inserted there in addition to the interference filter to prevent the scattered actinic light from reaching the photomultiplier. Actinic light was obtained from a 2000-W high-pressure mercury lamp used with a monochromator having a half bandwidth of 1.5 nm, a 4-cm water layer, a red cut-off filter (Corning 2403) and a heat-reflecting filter (Balzers Calflex).

RESULTS

The fluorescence yield of chlorophyll *a* in isolated spinach chloroplasts varied with changing concentrations of monovalent cations in the chloroplast suspension, as reported by HOMANN[5]. The effects of NaCl and $MgCl_2$ upon the fluorescence yield at room temperature are shown in Fig. 1. The 0.1-ml aliquot of the chloroplast suspension containing 300 mM KCl and 50 mM Tricine buffer (pH 7.8) was rapidly mixed with 2.9 ml distilled water in the cuvette. The resulting suspension contained 10 mM KCl and 1.7 mM Tricine buffer. The fluorescence yield rapidly rose to the maximum, then declined to a steady level within 30 sec. This decline was not observed when the chloroplast suspension was diluted with the solution of the same concentration (300 mM KCl and 50 mM Tricine buffer) instead of distilled water. These findings indicate that the reduction of KCl concentration in the chloroplast suspension caused the decline of fluorescence yield after the attainment of the maximum. 3-(3',4'-chlorophenyl)-1,1-Dimethylurea (DCMU) was added to avoid the quenching effect of photoreaction II. The fact that there was only a slight increase in fluorescence yield after the addition suggests that the light intensity was strong

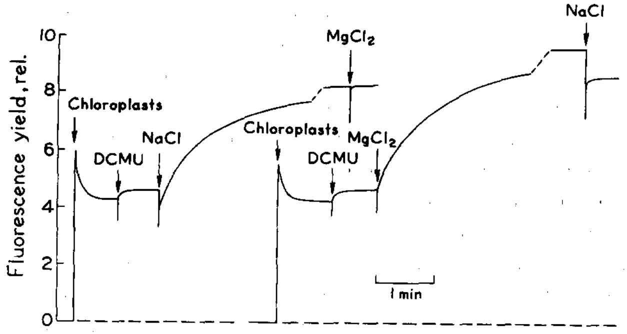


Fig. 1. Time-courses of fluorescence yield at room temperature in isolated spinach chloroplasts. 0.1 ml chloroplast suspension containing 300 mM KCl, 50 mM Tricine buffer (pH 7.8) was added to the cuvette containing 2.9 ml distilled water. Consequently chloroplasts were transferred from 300 to 10 mM KCl solution. DCMU (20 μl) was added, final concentration being 7 μM. Upon the additions of NaCl and $MgCl_2$ 0.1 ml of solution (3000 mM NaCl or 60 mM $MgCl_2$) was added to the sample. Mixing of the solution at the time of addition was complete so that no further mixing produced any change. Excitation light, 478 nm, 2100 ergs·cm^{-2}·sec^{-1}. Fluorescence was measured at 684 nm.

enough to reduce most of the primary electron acceptor (Q, according to DUYSENS AND SWEERS[6]) in the absence of DCMU.

Addition of 0.1 ml distilled water (not shown in Fig. 1) served to check the effect of a small volume change of the suspension, and produced a negligibly small change in fluorescence yield.

Addition of 0.1 ml of 3 M NaCl produced about 100 mM final NaCl concentration in the chloroplast suspension. There was a small fast decrease in fluorescence yield followed by a large slow increase. As suggested later, the small fast decrease may be produced by the change of light absorption due to the reduction of the chloroplast size with the addition of NaCl. The slow fluorescence increase continued for about 5 min before attaining its maximum level. The final level was about 1.6 times as high as that before the addition. However, the relative increase in fluorescence yield after the addition varied with the chloroplast preparations. It ranged from 40 to 80%.

Turning off the excitation light for a while during the time-course gave no modification in the change in fluorescence yield. Thus, it is inferred that illumination does not produce any influence on the fluorescence change with NaCl.

The addition of $MgCl_2$ (2 mM) gave almost the same time-course of fluorescence yield in respect to the large and slow change. However, the small fast decrease upon the addition was missing.

Experiments were performed in which $MgCl_2$ (2 mM) was subsequently added to a suspension of chloroplasts containing 100 mM NaCl and showing the maximum fluorescence level. The addition did not further induce a fluorescence increase. In another experiment with a chloroplast suspension that contained 2 mM $MgCl_2$, the addition of NaCl produced only a small and fast decrease in fluorescence yield. These findings indicate that the effects of $MgCl_2$ and NaCl were the same with respect to the large slow change in fluorescence yield, and that the small fast change is specific to the addition of NaCl.

The slow increase in fluorescence yield saturates at 100 mM NaCl (see Fig. 4), while the effect of divalent cations saturates at 2–5 mM (ref. 2).

Fig. 2 shows absorption spectra of the chloroplasts with and without 100 mM NaCl. NaCl decreased the absorption of the chloroplasts most significantly around the

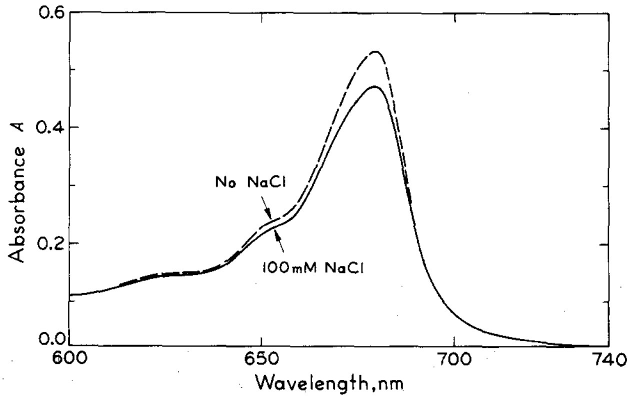


Fig. 2. Absorption spectra of spinach chloroplasts at room temperature with and without NaCl

absorption peak at 675 nm and less in the regions where the absorption was small. There was no evidence that the salt caused changes of the chlorophyll absorption forms. On the other hand, 2 mM $MgCl_2$ had no distinct effects on the absorption spectrum of the chloroplasts.

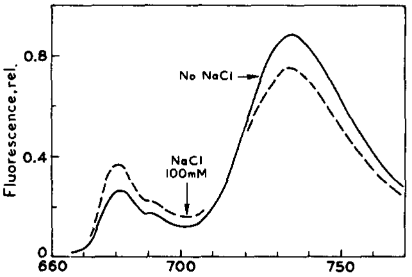
DUYSENS[7] discovered the flattening effect of light absorption spectrum of particles in suspension. According to the theory, the light absorption becomes smaller when the pigment molecules are in particles suspended in a solution compared to being homogeneously dispersed in the same solution. The suppression of absorption is greater at wavelengths where the absorption is stronger and when the projected area of the particles is smaller.

The chlorophyll molecules are located in the chloroplasts, and therefore their absorption must be more or less influenced by the flattening effects. The increase in osmotic pressure upon the addition of NaCl may reduce the size of the chloroplasts. resulting in a decrease in projected area. This may lead to a more marked flattening effect, and produce the most marked suppression of light absorption at the peak.

The decrease in absorption at 475 nm upon the addition of NaCl was about 10% (not shown in Fig. 2). This decrease corresponds to the small fast decrease in fluorescence yield with the addition of NaCl to the chloroplast suspension. This is supported by the fact that the change was not produced by $MgCl_2$ (at 50 times less concentration) but was by NaCl even after $MgCl_2$ addition.

Fluorescence emission spectra were measured at liquid N_2 temperature in the presence and absence of NaCl. Fig. 3 shows that NaCl decreased the yield of the fluorescence band at 735 nm that is emitted from pigment system I (ref. 8), while it increased the yield of fluorescence bands at 684 and 695 nm that are emitted from pigment system II (ref. 8). These effects of NaCl are the same as those of some divalent metal ions except that with these ions much lower concentrations are sufficient[1,2].

Induction of chlorophyll *a* fluorescence in the chloroplasts was measured at room temperature in the presence and absence of NaCl (100 mM). It is known that there are two fluorescence components in the induction period; one is a time-dependent



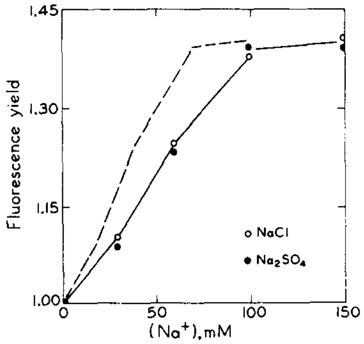


Fig. 3. Fluorescence spectra at liquid N_2 temperature with and without NaCl (100 mM). NaCl was added 5 min before cooling the sample. Excitation light, 475 nm.

Fig. 4. Relationship between concentration of Na^+ and fluorescence yield in NaCl and Na_2SO_4. Excitation light, 554 nm, 3000 ergs·cm^{-2}·sec^{-1}. ○—○, NaCl; ●, Na_2SO_4. Dashed line, expected for Na_2SO_4 from the solid curve of NaCl provided that effects of salts depended on ionic strength. See text for details.

increase component (variable fluorescence) and the other is not (constant fluorescence)[9]. NaCl increased the variable fluorescence by 80% and the constant fluorescence by 10%. This effect of NaCl specific for the variable fluorescence is similar to that of $MgCl_2$ investigated in the previous study[1].

The effects of other sodium salts such as NaBr, $NaNO_3$ and Na_2SO_4 on fluorescence yields were examined at room and liquid N_2 temperatures. These salts had effects similar to NaCl on chlorophyll *a* fluorescence in the chloroplasts. Their addition decreased the fluorescence yield of pigment system I but increased the yield of pigment system II. The time-courses of fluorescence increase after the addition and the maximum levels of fluorescence increase were the same as in the case of NaCl. The same concentration dependences were observed in NaCl, NaBr and $NaNO_3$. The increase saturated at 100 mM. In the case of Na_2SO_4 the fluorescence yield reached its maximum level at 50 mM.

Fig. 4 shows the relationship between the concentration of Na^+ and the fluorescence yield in NaCl and Na_2SO_4. In both cases, the fluorescence yield reached the maximum level at 100 mM of Na^+. These concentration curves give an answer to a question whether the effects of the salts upon the light energy distribution depend on the concentration of Na^+ or on the ionic strength of the medium of chloroplast suspension. If Na^+ controls the distribution, NaCl and Na_2SO_4 should show the same concentration dependences on the basis of Na^+ concentration. If the effects were due to the ionic strength, then Na_2SO_4 should be 1.5 times as effective as NaCl on the same concentration base. The dashed line in Fig. 4 is the expected for Na_2SO_4 in the latter case. It is clear from Fig. 4 that the concentration of Na^+ determines the degree of fluorescence yield increase.

Chloride salts of other monovalent cations including Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+ and $CH_3NH_3^+$ were compared. Table I shows the effects of their chloride salts as well as of $MgCl_2$ upon the fluorescence yield at room temperature. The increase in fluorescence yield was almost the same in all the salts tested. The concentration dependence curves showed the effects to saturate around 100 mM with each monovalent cation. Low-temperature fluorescence spectra showed that all the monovalent

TABLE I

EFFECTS OF SALTS ON FLUORESCENCE YIELD OF CHLOROPHYLL *a* IN SPINACH CHLOROPLASTS AT ROOM TEMPERATURE

Experimental conditions were the same as in Fig. 1. Fluorescence yield was measured 5 min after the addition of salts.

Salt	*Concentration (mM)*	*Relative fluorescence yield*
No addition	—	1.00
LiCl	100	1.69
NaCl	100	1.63
KCl	100	1.64
RbCl	100	1.60
CsCl	100	1.59
NH_4Cl	100	1.57
CH_3NH_3Cl	100	1.50
$MgCl_2$	2	1.75

cations enhanced the fluorescence yield of pigment system II, but suppressed that of pigment system I. Therefore, it is concluded that all the monovalent cations tested have the same effects upon the chloroplasts in changing the light energy distribution between the two pigment systems.

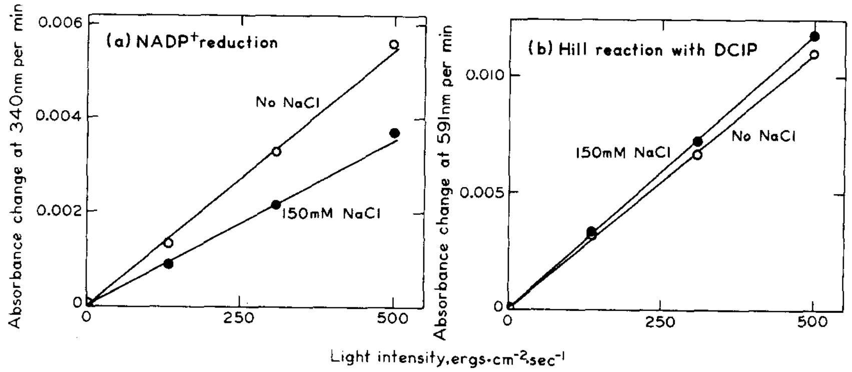


Fig. 5. (a) Effects of NaCl on NADP$^+$ reduction supported by ascorbate and DCIP in the presence of DCMU. NADP$^+$ reduction was measured by absorption change at 340 nm. Reaction medium contained 0.5 mM NADP$^+$, 0.5 mM sodium ascorbate, 0.03 mM DCIP, 0.02 mM DCMU, 10 mM KCl, 1.7 mM Tricine buffer (pH 7.8) and sufficient amount of ferredoxin. ○, no NaCl; ●, 150 mM NaCl. Actinic light was 650-nm monochromatic light. (b) Effects of NaCl on the Hill reaction with DCIP. Reduction of DCIP was measured by the absorption change at 591 nm. Reaction medium contained 0.017 mM DCIP, 10 mM KCl and 1.7 mM Tricine buffer (pH 7.8). ○, no NaCl; ●, 150 mM NaCl. Actinic light was 650-nm monochromatic light.

The effects of NaCl on two photoreactions were investigated by measuring NADP$^+$ reduction supported by ascorbate and DCIP in the presence of DCMU for photoreaction I and the Hill reaction with DCIP for photoreaction II. The intensities of actinic light were so low that the primary photoreactions were the rate-limiting steps in both reactions. Under this condition, the quantum efficiency of the reaction corresponds to the light energy available to each photoreaction. Fig. 5 shows the light-intensity dependences of NADP$^+$ reduction in the presence of DCIP, ascorbate, ferredoxin and DCMU and the Hill reaction with DCIP. The linear relationships between the light intensity and the reaction rates show that the primary photoreactions were the rate-limiting steps. The addition of NaCl suppressed the quantum efficiency of NADP$^+$ reduction by about 35% (Fig. 5a). On the other hand, it stimulated the quantum efficiency of the Hill reaction with DCIP by 5% (Fig. 5b). It is inferred here that Na$^+$ suppresses the quantum efficiency of photoreaction I and slightly enhances that of photoreaction II.

DISCUSSION

It was shown in the low-temperature fluorescence spectra that NaCl decreased the fluorescence yield of pigment system I. The quantum efficiency of photoreaction I measured by NADP$^+$ reduction with ascorbate and DCIP was suppressed by the addition of NaCl. These results are consistent and indicate that Na$^+$ decreases the number of light quanta in pigment system I.

On the other hand, the fluorescence yield of pigment system II measured at room temperature was increased by 40–80%, while the quantum efficiency of photoreaction II measured by the Hill reaction with DCIP was only slightly stimulated. These different effects on the fluorescence yield and quantum efficiency of system II will be explained as follows: In the measurement of fluorescence yield photoreaction II does not quench the excited state because in the presence of DCMU Q is reduced in the light. The excitation energy is dissipated through heat production, fluorescence emission and transfer to other pigment molecules. The experimental results show that Na^+ increased the fluorescence yield of pigment system II under this condition. In the measurement of the Hill reaction Q is in the oxidized form, because the reaction was measured in the presence of electron acceptor under weak light intensities (lower 500 ergs$\cdot$cm$^{-2}\cdot$sec^{-1}). The primary photochemical reaction or Q captures the excitation energy so fast as to predominate over other degradation processes such as heat production, light emission and transfer of excitation to other pigment molecules, and most of the excitation energy in pigment system II must be trapped by photoreaction II. The obtained data show that photoreaction II is only a little stimulated by the addition of Na^+.

In summary, when Q is in the oxidized form and the quenching effect of photoreaction II is at the maximum, Na^+ does not enhance the quantum efficiency of system II. When Q is in the reduced form and there is no quenching by photoreaction II, Na^+ increases the fluorescence yield of pigment system II. These findings lead us to consider a mechanism shown in Fig. 6.

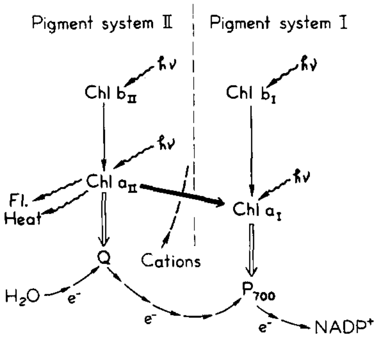


Fig. 6. Scheme for excitation transfer between pigments in spinach chloroplasts. Arrows indicate the excitation transfer steps. Chl a_I and Chl a_{II} are chlorophyll *a* in pigment systems I and II, respectively. Chl b_I and Chl b_{II} are chlorophyll *b* in pigment systems I and II, respectively. The thick closed arrow is the step which is suppressed by Na^+ and other monovalent cations. The thick open arrows are photoreactions.

In this scheme, cations suppress the excitation transfer from chlorophyll *a* of pigment system II to that of pigment system I. The rate constant of the heat-producing process is estimated to be about $1 \cdot 10^9$ sec^{-1} from the life-time of chlorophyll *a* fluorescence in isolated chloroplasts[10]. The rate constant of fluorescence emission is estimated as about $1 \cdot 10^8$ sec^{-1} (ref. 11) and can be neglected in the estimation of the fate of excitation energy. The rate of photoreaction II must be much faster than the heat-producing process, because almost all the light quanta are probably captured by the photoreaction when all of the Q is in the oxidized state[12]. It is estimated therefore that when all of Q is oxidized, the excitation energy is trapped by photoreaction

II with an apparent rate constant which is equal to or faster than $1 \cdot 10^{10}$ sec^{-1}. When Q is reduced and photoreaction II does not quench the excitation as in the presence of DCMU, the fluorescence yield is markedly increased by the cations. This fact indicates that the rate of excitation transfer from pigment system II to I is comparable to that of heat-producing process. The rate constant is estimated to be about $1 \cdot 10^9$ sec^{-1}. The apparent rate constant of photoreaction II with fully oxidized Q is still much faster than the sum of the rate constants of heat-producing process and excitation transfer from pigment system II to I. Under this condition, therefore, photoreaction II is not so strongly influenced with the change in rate of excitation transfer between the pigments systems. Thus, the different effects of NaCl on the fluorescence yield of pigment system II and the quantum efficiency of photoreaction II can be explained by assuming the scheme in Fig. 6 and the above mentioned rate constants.

In the above consideration we assumed that Na^+ suppresses the excitation transfer from pigment system II to pigment system I, instead of stimulating the transfer from light collecting pigments, such as chlorophyll *b* or carotenoids, to chlorophyll *a*. The possibility of the latter case is excluded because if Na^+ increased the transfer efficiency from the light collecting pigments to chlorophyll *a* of pigment system II (Chl a_{II}), the Hill reaction with DCIP should be stimulated with the addition of Na^+ in parallel to the increase in fluorescence yield.

Another action of salts upon isolated chloroplasts is to induce a change in membrane structure of chloroplasts[13–15]. IZAWA AND GOOD[15] as well as ANDERSON AND VERNON[16] showed, by electron microscopy, that in the chloroplasts suspended in high-salt medium there were clear grana stacks that became diffuse or disappeared in low-salt medium. They also pointed out that salts of divalent cations are more effective in retaining the grana stacks than are salts of monovalent cations. The experimental results in the present study indicate that the excitation transfer from pigment system II to I is less efficient when the grana stacks are formed in the high-salt medium than when there are only diffuse lamellae as in the low-salt medium. Since the efficiency of excitation transfer between two pigment molecules is inversely proportional to the sixth power (resonance transfer model) or the third power (exciton model) of the distance between them, the less efficient transfer suggests the longer distance between the two pigment systems in the high-salt medium than in the low-salt medium.

Several methods have been developed for a physical separation of the pigment systems. In all the treatments employed, such as by detergent, needle valve extrusion and sonic oscillation treatments, the chloroplasts have to be suspended in high-salt medium to obtain a good separation of the systems[16–18]. In recent work of OHKI *et al.*[19], cations but not anions are essential in this regard, and the concentration required is about 50 mM for monovalent cations and is about 3–5 mM for divalent cations. These findings suggest that the two pigment systems are separate from each other in the presence of salts, and that the concentration dependences for monovalent and divalent cations for the physical separation of the pigment systems are similar to those of suppression of excitation transfer between them. Since the two pigment systems are considered to be situated in the thylacoid membranes, it is inferred that cations produce some structural change at the inside of the thylacoid membrane, in addition to the formation of grana stacking. That results in a pulling apart of the two pigment systems and facilitates their physical separation and a suppression of excitation transfer between them.

BRODY *et al.*[20] reported the effects of salts on the fluorescence and absorption spectra in Euglena chloroplast fragments. They observed a similar change in low-temperature fluorescence spectrum as in the present study in spinach chloroplasts. Addition of NaCl increased the fluorescence yield at 685 nm and decreased that at 730 nm. Furthermore, they found an increase of absorption band at 705 nm. We did not observe in spinach chloroplasts such an absorption change as due to a transformation of chlorophyll forms, although there was a change in absorption spectrum which was produced by a more marked flattening effect by the addition of salts. The reason of this discrepancy between their and our results is not so far obvious. However, it is possible that the Euglena and spinach chloroplasts respond to the salts in different ways. They concluded that a transformation from monomer to aggregate of chlorophyll *a* diminished the excitation transfer between them. However, this is not adopted in the case of spinach chloroplasts, because there is no evidence for the interconversion of chlorophyll forms (Fig. 2).

We observed, in our previous study, the effect of Mg^{2+} on light energy distribution with the chloroplasts suspended in a 50 mM Tricine buffer solution containing 400 mM sucrose and 10 mM NaCl. When 50 mM phosphate buffer was used instead of Tricine buffer, there is no effect of Mg^{2+} on fluorescence yield[5]. These findings can be explained as follows: 50 mM phosphate buffer solution at pH 7.8 contains Na^+ and K^+; the sum of their concentrations is about 95 mM. The effects of monovalent cations in increasing the fluorescence yield saturate around this concentration. Therefore, further addition of Mg^{2+} is not expected to induce the change in light energy distribution.

In the study of HOMANN[5], the effects of Mg^{2+} in increasing the fluorescence yield at room teperature was less distinguishable in Tris–HCl buffer than in Tricine–NaOH buffer at the same concentrations (50 mM). Tris is partly associated with H^+ at neutral pH to produce hydrated amine ion, a monovalent cation, and must act in the same way on the chloroplasts investigated in this study.

AVRON AND BEN-HAYYIM[21] also observed changes of quantum yields of the two photoreactions by the salt contained in the medium of the chloroplast suspension. They reported an opposite effect of monovalent and divalent cations upon the light quanta distribution between the two pigment systems; the distribution of light energy favored photosystem I in the presence of KCl or NaCl while it favored photosystem II in the presence of $MgCl_2$ or $CaCl_2$. This is inconsistent with our present study that shows the same effects of monovalent and divalent cations. They did not show a control experiment without cations, but added the monovalent cations at 40 mM to the reaction mixture. It was seen in Fig. 4 that this concentration produces only one-third the maximum fluorescence change that might have occurred. It appears that they did not obtain the maximum effect of the monovalent cations in changing the light distribution. On the other hand, they used the divalent cations at 27 mM, which is about 10 times higher than the concentration necessary for the saturation of their effects. Therefore, they reached the incorrect conclusion of opposite effects of monovalent and divalent cations.

HEATH AND HIND[22] reported that NaCl induced a small change of the low-temperature fluorescence spectrum of spinach chloroplasts. There were 5 mM $MgSO_4$

in their medium which is sufficient to produce the maximum effects of cations upon the light energy distribution. Then, they measured the effect of 10 mM NaCl, a 10 times lower concentration than necessary to get the maximum effects of Na^+ in changing the light energy distribution. In their experiment NaCl must produce another effect, perhaps, as they suggested, an effect of Cl^- on the electron transport between the O_2-producing reaction and photoreaction II.

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