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PHOTOSYNTHETIC REGULATION BY CATIONS IN SPINACH CHLOROPLASTS

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SUMMARY

1. In the presence of $\geqq$ 3 mM divalent cations or $\geqq$ 100 mM monovalent cations, weak 650-nm light, presumably containing an excess of Photosystem II quanta, caused substantial reduction of the electron carrier pool between the two photoacts. In low-salt media, most electron carriers were oxidized during red light.

2. In the presence of low concentrations of carbonylcyanide *m*-chlorophenylhydrazone (CCCP) (0.3 μM) or antimycin A (40 μM) the pool remained oxidized even in the presence of adequate cation concentrations.

3. The non-linear dependence of the O_2-evolution rate upon the concentration of active Photosystem II trapping centers, which implies energy transfer between Photosystem II units, was observed only in the presence of sufficient cation.

4. Enhancement of the rate of methyl viologen reduction in far-red light by red light was found to be a weak function of Mg^{2+} concentration.

5. With ferredoxin–$NADP^+$ as acceptor, enhancement of the rate of O_2 evolution in 650-nm light by far-red light was obtained only with added Mg^{2+}. Furthermore, Mg^{2+} addition markedly stimulated the quantum yields of $NADP^+$ reduction, independent of wavelength, with either water or ascorbate–reduced 2,6-dichlorophenolindophenol (DCIP) as the electron donor. A dual effect of cations in the ferredoxin–$NADP^+$ system is suggested.

6. With methyl viologen or potassium ferricyanide as acceptor and water as donor, Mg^{2+} did not affect the quantum yield in 650-nm light and slightly increased the yield at longer wavelengths. The quantum yield of the ascorbate–reduced DCIP–methyl viologen (Photosystem I) reaction was slightly decreased in 650-nm light and unaffected at long wavelengths. Our interpretation is that cation concentration affects the pigment distribution between Photosystems I and II.

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

INTRODUCTION

The two-light reaction model for photosynthesis requires a balanced input of quanta to both photosystems for optimum efficiency. As recently reviewed by Myers [1], the quantum yield of the overall process seems less wavelength-dependent than expected on the basis of the action spectra of the individual photosystems, suggesting a mechanism which tends to balance the sensitization of the two photosystems. Recent literature has correlated such regulations not only with the wavelength of illumination [2–5], but also with the cation concentration [5–9] and the nature of electron donors and acceptors [8].

Attention has been refocused on an early suggestion [10] that excess excitations of Photosystem II pigments can spillover to Photosystem I. Sun and Sauer [9] proposed a cation-induced type of spillover from Photosystem I to Photosystem II. Bonaventura and Myers [2] and Duysens [4] have suggested regulation by light-induced shifts of pigment from one system to the other. On the other hand, Joliot et al. [11], unable to observe spillover, suggested a low apparent equilibrium constant for the dark reaction chain between the photoacts to explain the flat quantum yield spectrum. This paper reports further observations on the effects of cation concentrations on the distribution of photons in isolated chloroplasts.

MATERIALS AND METHODS

Experiments were done with spinach chloroplasts prepared as described by Schwartz [12]. Chloroplasts were kept on ice in the isolation medium and diluted to appropriate concentrations with indicated mixture just prior to measurements.

Absorption changes of P_{700} were measured with a dual wavelength spectrophotometer as previously described [13]. Chloroplasts containing 25 μg chlorophyll per ml were stirred continuously in a cooled (13 °C) reaction cell having a 3-mm optical path. The basic reaction mixture contained 50 mM Tricine–KOH, pH 7.5, and 0.1 mM methyl viologen with additions as indicated. Actinic light, from a 28-V microscope projection lamp, was passed through 2 cm of water, a heat filter and appropriate color filters. A 725-nm interference filter (half-width 12 nm) was used for far-red actinic light, a 650-nm interference filter (half-width 5 nm) for red actinic light, a Corning cut-off filter (3-67) for bright saturating light and wire screens for neutral density filters. Absorption changes were recorded directly with a Moseley X–Y recorder or first fed onto a signal averager (Fabri-Tek Model 1052) and then recorded.

Enhancement and rates of methyl viologen reduction, where indicated, were measured at room temperature with the modulated polarographic apparatus and technique of Joliot and Joliot [14]. The flowing solution was modified to contain Tricine–KOH, pH 7.5, 10 mM KCl, either 1.0 mM $NADP^+$, or 0.1 mM methyl viologen as electron acceptor. Chloroplasts were diluted in the flow medium to 0.2 mg chlorophyll/ml, enriched with ferredoxin (when using $NADP^+$ as acceptor), placed on the electrode, and allowed to settle in darkness for about 10 min. Sample illumination (modulated and DC beams) was provided as described by Joliot and Joliot [14].

Quantum yields (relative) and saturation levels were measured with a Clark-type O_2 electrode covered with a 0.5-mil Type A Teflon FEP membrane. Reactions were run in a lucite vessel having a reaction chamber of 1.17 ml capacity, magnetic

stirring and thermoregulation at 20 °C. Light from a 500-W projection lamp was filtered through 6 cm water, heat filter and narrow-band (5 nm) interference filters (Thin Films).

RESULTS

Influence of cations on photoreduction of intermediate electron carriers

Fig. 1 shows the effects of illumination and cation concentration on the state of reduction of the pool of electron carriers between the two photoacts. As previously reported [13], when chloroplasts are illuminated with bright saturating light, the intermediates (about 12 electron equivalents) associated with the reducing side of Photosystem II become reduced while P_{700} and two other components on the oxidizing side of Photosystem I (about 3 electron equivalents) become oxidized [13]. After the strong light is switched off, P_{700} is rapidly reduced (a few ms). This initial reduction is not recorded due to the relatively slow response time of the instrument ($\leqq$ 1 s). P_{700} remains reduced until the electron carrier pool which was photo-reduced during the bright light becomes photo-oxidized due to the action of the far-red beam (Curve A). The presence or absence of mono- or divalent cations does not affect the amount of reductant accumulated in strong light as evidenced by the time course of the subsequent photo-oxidation in far-red light viewed via P_{700}. Divalent cations, however, have a pronounced effect on the degree of reduction of the intermediate electron carrier pool attained in weak 650-nm light which presumably contains an excess of Photosystem II quanta [15, 16]. As shown in Curve B (Fig. 1), if Mg^{2+} is present during illumination with weak 650-nm light (long enough to attain a steady-state) a substantial fraction of the intermediate pool becomes reduced. Curve C shows that after a comparable period of 650-nm illumination, in the absence of cations, a relatively rapid photo-oxidation of P_{700} occurs which suggests that in this case, very few electron equivalents accumulate in the pool.

Several other features should be mentioned about the experiments illustrated

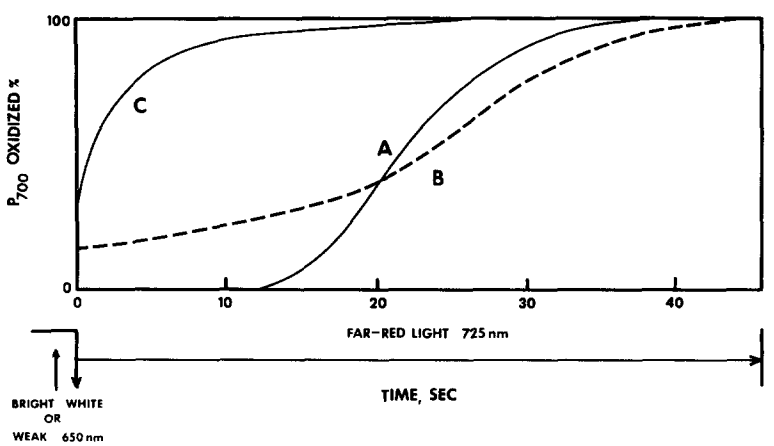

Fig. 1. Time-course of P_{700} oxidation in far-red light following pre-illumination with 5-s bright saturating light (Curve A) or 2-min weak red light (Curves B and C). Chloroplasts suspended in basic reaction mixture (Curve C) or mixture with 3 mM $MgCl_2$ (Curves A and B).

in Fig. 1. Irrespective of the type of pre-illumination, the time required to photo-oxidize the pool was inversely proportional to the intensity of the 725-nm beam. This intensity × time relation proves that the P_{700} oxidation transients reflect fixed amounts of reductant accumulated during the illumination and that the kinetics of photo-oxidation are not dark-limited. Secondly, it appears that in the steady-state P_{700} is only about 85% reduced during illumination with weak 650-mn light. Thirdly, the kinetics of the pool depletion via P_{700} photo-oxidation in far-red light are different following pre-illumination with strong white light and with weak 650-mn light. This suggests that the apparent equilibration of P_{700} with other electron transport compounds is light dependent. The apparent equilibrium constant is high after strong light and low after weak Photosystem II light, an observation which was already made some time ago by P. Joliot (personal communication).

In parallel with the spectroscopic observations of P_{700}, we performed polarographic experiments monitoring the rate of viologen reduction with the modulated rate electrode. The reaction mixture was the same as that used in the spectrophotometric experiments except that 10 mM KCl was always present to maintain electrical conductance. The results were entirely similar. This shows that the observed behavior of P_{700} is not due to scattering or other spurious phenomena.

Comparison of the effect of monovalent and divalent cation concentrations on the degree of reduction of intermediate pools in weak 650-nm light is shown in Fig. 2. These results, obtained with different chloroplast preparations, were normalized with respect to the area (reducing equivalents) obtained following bright light (dashed line). The degree of reduction obtained in steady-state weak light is most sensitive to the presence of divalent cations, e.g. Mg^{2+} or Ca^{2+}. Approximately 30–40-fold higher concentrations of monovalent cations (Na^+ or K^+) are required to obtain a comparable degree of reduction in weak light. The number of reducing

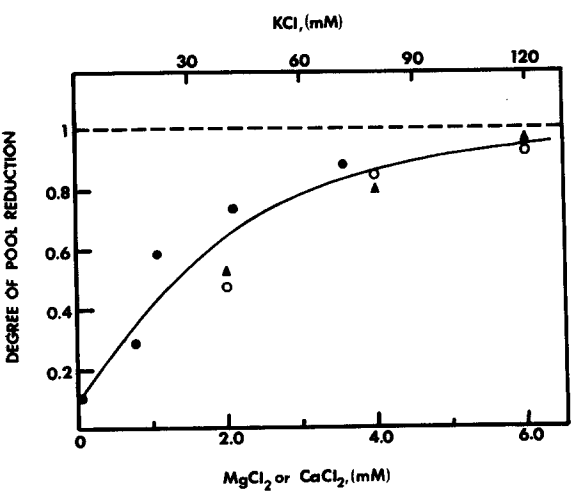

Fig. 2. Influence of cation concentration on the reduction of intermediate electron carriers (pool) in weak red light as measured via P_{700}. Areas bounded by P_{700} oxidation curves (see Fig. 1) were determined for chloroplasts suspended in varying concentrations of $MgCl_2$ (solid dots), $CaCl_2$ (triangles) or KCl (open dots).

equivalents accumulated in weak light approaches that obtained in bright saturating light. This does not necessarily imply, however, that the Q and A pools are completely reduced as thought to be the case in strong light [13]. In weak 650-nm light but not in strong light, the carriers on the oxidizing side of Photosystem I are also partially reduced, e.g. P_{700} about 85%.

The concentrations of monovalent and divalent cations found effective in restoring the photoreduction of intermediate carriers in weak Photosystem II light (Fig. 2) compare well with those reported to affect a variety of other chloroplast phenomena, e.g. fluorescence yield, quantum yields and enhancement [6–9, 17, 18]. We feel that these cation-dependent events are more than casually related.

Influence of cations on enhancement

The experiments in Figs 1 and 2 illustrate that in the presence of sufficient cation, the intermediate electron carriers become substantially reduced in weak 650-nm light. In this condition, Photosystem II apparently receives an excess of quanta relative to Photosystem I. O_2 enhancement studies with the modulated electrode support this conclusion. As reported earlier [11] using ferredoxin–$NADP^+$ as acceptor the modulated rate of O_2 evolution in modulated 650-nm light can be enhanced by an unmodulated far-red beam. The effect proved to be cation-dependent. With low cation concentrations (10 mM KCl), no enhancement was observed. Sun and Sauer [9] and Sinclair [16] have reported a similar dependence of enhancement on cations with isolated chloroplasts. The simplest explanation for the absence of enhancement and pool reduction would be a loss of efficiency of Photosystem II in low cation media.

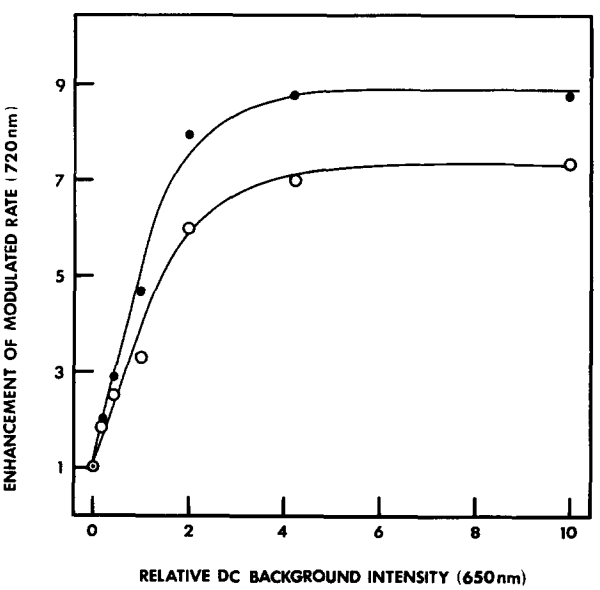

Fig. 3. Effect of $MgCl_2$ on enhancement of the rate of methyl viologen reduction in modulated 720-nm light by a non-modulated 650-nm beam. Flowing solution contained basic reaction mixture plus 5 mM NH_4Cl with (solid dots) or without (open dots) 6 mM $MgCl_2$. The modulated rates of methyl viologen reduction in weak 720-nm light were normalized in the two experiments for zero background intensity. Positive polarization: +0.65 V. Modulation frequency 46 cycles/s.

However, interpretation is complicated by the fact that in the O_2 enhancement measurements, ferredoxin–$NADP^+$ was used as the terminal electron acceptor. As discussed below, in low salt media we routinely obtained anomolous low quantum yields with the ferredoxin–$NADP^+$ systems compared to other electron acceptors, e.g. methyl viologen or potassium ferricyanide. Consequently, to check whether in low salt media Photosystem II operates or is sensitized suboptimally, we performed the inverse enhancement experiment using methyl viologen as the acceptor. The modulated rate of viologen reduction induced by modulated 720-nm light was measured with various intensities of unmodulated 650-nm background light. NH_4Cl (5 mM), added to uncouple phosphorylation, did not affect the results. As shown in Fig. 3, both with and without added Mg^{2+}, the steady-state rate (modulated) of viologen reduction induced by 720-nm light was increased by an unmodulated 650-nm beam. Fig. 3

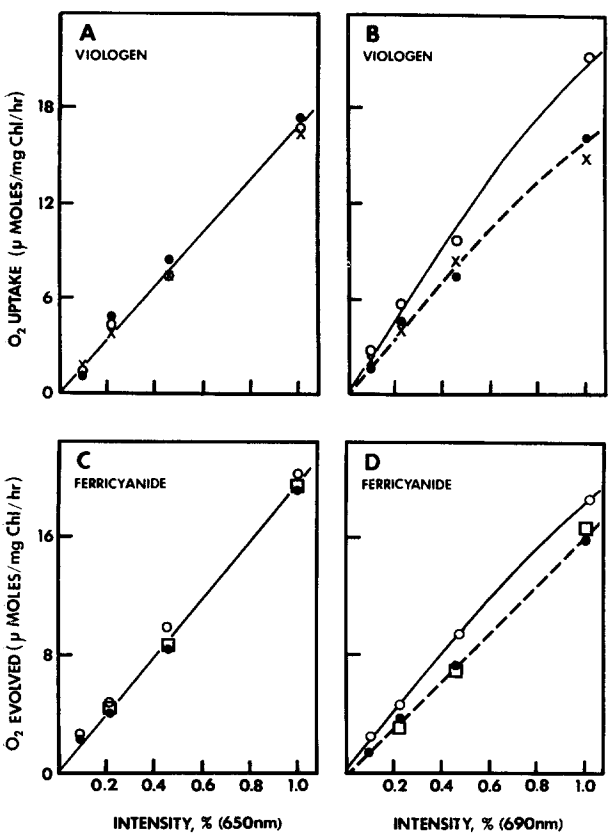

Fig. 4. (A) Effect of $MgCl_2$ and antimycin A on rates of methyl viologen reduction measured polarographically by net O_2 uptake. Basic reaction mixture plus 0.1 mM KCN, 30 mM methylamine, 25 μg total chlorophyll per ml. (○), 3 mM $MgCl_2$; (●), no $MgCl_2$; (×), 3 mM $MgCl_2$ plus $4 \cdot 10^{-5}$ M antimycin A. Actinic 650-nm light. (B) As in A except actinic 690-nm light. (C) Effect of $MgCl_2$ and CCCP on rates of O_2 evolution. Reaction medium contained 0.1 mM potassium ferricyanide, 50 mM Tricine buffer (pH 7.5), 30 mM methylamine and 25 μg total chlorophyll per ml. (○), 3 mM $MgCl_2$; (●), no $MgCl_2$; (□), 3 mM $MgCl_2$ and 0.3 μM CCCP. Actinic 650-nm light. (D) As in C except actinic 690-nm light.

shows the relative rates normalized for the rates without background light. The enhanceability of the modulated 720-nm rate by 650-nm light proved slightly greater (20%) when Mg^{2+} was added to the medium, a difference observed irrespective of the 650-nm background intensity. Using modulated 710-nm or 700-nm light instead of 720-nm gave similar results except that the maximum enhancement obtained was lower.

The results suggest that the yield of Photosystem II in weak 650-nm light is slightly decreased by lack of cation.

A quantitative evaluation of these data is not possible since the absolute rates of Photosystem I and Photosystem II under the various conditions are unknown. However, the experiments do demonstrate that the absence of Mg^{2+} does not greatly affect the normal push–pull effects predicted by the standard two-light electron transport reaction model. It should be noted that this type of enhancement is obtained in conditions where CO_2 fixation and photophosphorylation processes are eliminated.

Influence of cations on relative quantum yields for System I and System II

To aid interpretation of the above-discussed observations, which mainly pertain to the ratio between the two photosystems rather than absolute rates, we studied the effect of cations on the (relative) quantum yields of the various systems. Figs 4A and 4C (open and solid dots), show that Mg^{2+} concentration had no effect on the relative quantum yields in 650-nm light using either methyl viologen or ferricyanide as the terminal electron acceptor. Since 650-nm light presumably contains a relative excess of Photosystem II quanta, an inhibition or stimulation of Photosystem II activity should be more readily detectable at longer wavelengths where this system is rate-limiting. Low salt proved to result in a slight decrease ($\leqq$ 20–25%) of the relative quantum yields (solid vs open dots) at 690 nm (Figs 4B and 4D) or at 710 nm (not shown).

On first sight, these quantum yield measurements are consistent with the idea

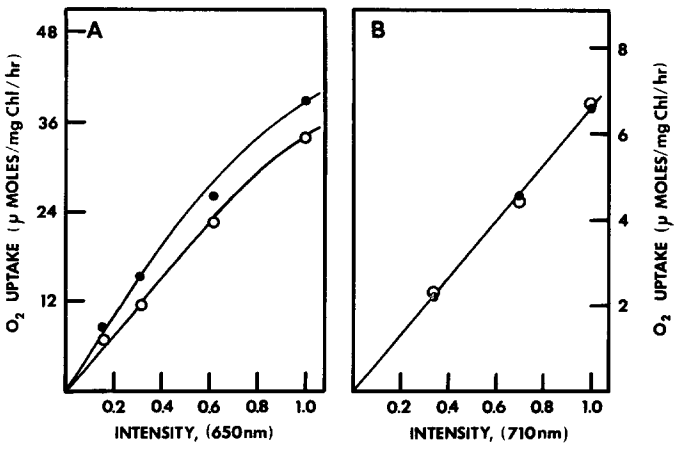

Fig. 5. Effect of $MgCl_2$ on rates of methyl viologen reduction in DCMU-inhibited chloroplasts. (A) Basic reaction medium containing 0.01 mM DCMU; 0.1 mM KCN; 2.0 mM sodium ascorbate; 0.08 mM DCIP and 6.6 μg total chlorophyll per ml. Solid dots, no $MgCl_2$; open dots, 3 mM $MgCl_2$. Actinic 650-nm light. (B) As in A except 38 μg total chlorophyll per ml and actinic 710-nm light.

that low cation concentrations slightly inhibit Photosystem II. The experiments shown in Fig. 5, however, indicate that this simple model of cation action is incorrect. In these experiments, we determined the effect of Mg^{2+} on the relative quantum yield of Photosystem I by measuring the rate of methyl viologen reduction (O_2 uptake) in the presence of 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) and ascorbate–reduced 2,6-dichlorophenolindophenol (DCIP). Using 650-nm light (Fig. 5A), Mg^{2+} addition resulted in a decrease in quantum yields (20%), while no effect was observed with long wavelengths, e.g. 710 nm (Fig. 5B). Identical results were obtained using ascorbate–reduced diaminodurene as the electron donor system.

Effects of cations on $NADP^+$ reduction

While with viologen and ferricyanide as acceptors, the effects of Mg^{2+} upon the quantum yields were consistent but relatively small, with ferredoxin–$NADP^+$ more dramatic and probably more complex effects were seen. Mg^{2+} addition consistently resulted in a marked increase (40–100%) of the relative quantum yields measured via O_2 evolution or spectrophotometrically at 340 nm. Such increases of the yield were seen for both the open system ($H_2O \rightarrow NADP^+$) and the isolated Photosystem I reaction (ascorbate–reduced DCIP $\rightarrow NADP^+$) irrespective of wavelength between 650 and 700 nm.

We have come to the conclusion that these unique cation effects observed with the ferredoxin–$NADP^+$ acceptor system are a consequence of a secondary influence on the coupling of $NADP^+$ reduction to electron transport. We found, for example, that to increase the rate of the donor system (ascorbate–reduced DCIP $\rightarrow NADP^+$), Mg^{2+} could be replaced by a relatively low concentration of monovalent cations (30 mM). Furthermore, in the presence of low monovalent cation concentrations, the ferredoxin–$NADP^+$ system responded to Mg^{2+} as did the methyl viologen system. For instance, in weak 650-nm light, the addition of 3 mM $MgCl_2$ to the medium now resulted in a slight decrease (about 20%) of the relative quantum yield. It should be noted that these dual cation effects were observed using salt-free ferredoxin prepared in this laboratory. A commercial preparation of ferredoxin (Sigma Co.) did not show the secondary (monovalent) cation effect. A relatively large volume of this preparation had to be added, which possibly contained sufficient salt.These two different effects of salt probably underlie the conflicting reports in the literature [6, 8, 9, 16, 17, 19] regarding the effect of Mg^{2+} on the rates of $NADP^+$ reduction and enhancement of O_2 evolution.

Effect of cations on System II photon transfer

Joliot et al. [11] have previously shown that the rate of O_2 (v_{O_2}) evolution is non-linear with respect to the concentration of open Photosystem II reaction centers (E). Their results indicated a finite probability (a) of photon transfer between Photosystem II units, the relation between v_{O_2} and E being:

$$v_{O_2} = E \frac{1}{1-a(1-E)} \tag{1}$$

With chloroplasts and *Chlorella* they found a to vary between 0.5 and 0.6, which suggested that photon transfer between Photosystem II units was an invariable characteristic of photosynthetic material. Their experiments with chloroplasts were made

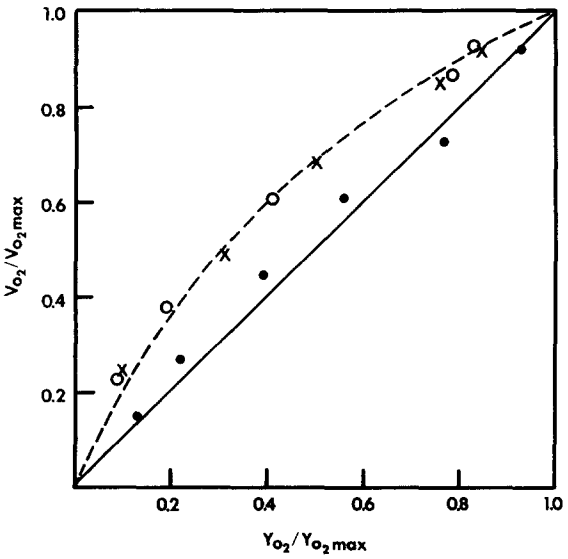

Fig. 6. Effect of $MgCl_2$ on the relative rate ($v_{O_2}/v_{O_{2max}}$) vs relative flash yield ($Y_{O_2}/Y_{O_{2max}}$) of O_2 evolution. Reaction mixture contained 50 mM Tricine buffer (pH 7.4), 10 mM KCl, no acceptor and 3 mM $MgCl_2$ (×), 3 mM $MgCl_2$ and 100 mM KCl (○), no further additions (●). Negative polarization: −0.6 V. Weak 700-nm detecting beam modulated at 40 cycles/s. A continuous 650-nm beam of varying intensity was used to vary the relative rates and flash yields. Dashed curve for transfer between units assuming probability $a = 0.55$ (see text). Solid line for no transfer.

using relatively high concentrations of cations. We repeated Joliot's Photosystem II transfer experiments using various concentrations of cations. As shown in Fig. 6, we obtained results similar to those of Joliot et al., when Mg^{2+} was present (open dots and crosses). The dotted curve shows the theoretical relationship according to Eqn 1, assuming $a = 0.55$. With low cation concentrations (10 mM KCl), however, an approximately linear relationship between v_{O_2} and E is observed (solid dots). Again, as in the other phenomena, the transition from a linear v_{O_2} vs E relationship to a non-linear relationship did not specifically require divalent cations, it occurred with either 3 mM Mg^{2+} or $\geqq$ 100 mM K^+. We thus conclude that the transfer of excitation energy between Photosystem II units (a) is dependent upon the presence of cations in the medium.

Effect of uncouplers on cation-induced effects

The peculiar kinetics of P_{700} oxidation in far-red light following pre-illumination with weak 650-nm light (Curve B, Fig. 1) suggested that Mg^{2+} may affect a dark reaction step in the intermediate electron carrier sequence, perhaps related to energy-coupling processes. We tested the effect of various phosphorylation uncouplers and antibiotics, which selectively influence membrane permeability. Of the various agents and combinations tried (CCCP; antimycin A; nigericin, valinomycin, valinomycin plus dinitrophenol; methylamine; gramicidin D), only carbonylcyanide *m*-chlorophenylhydrazone (CCCP) and antimycin A were effective in annihilating the cation-dependent accumulation of reducing equivalents in weak 650-nm light. As shown in

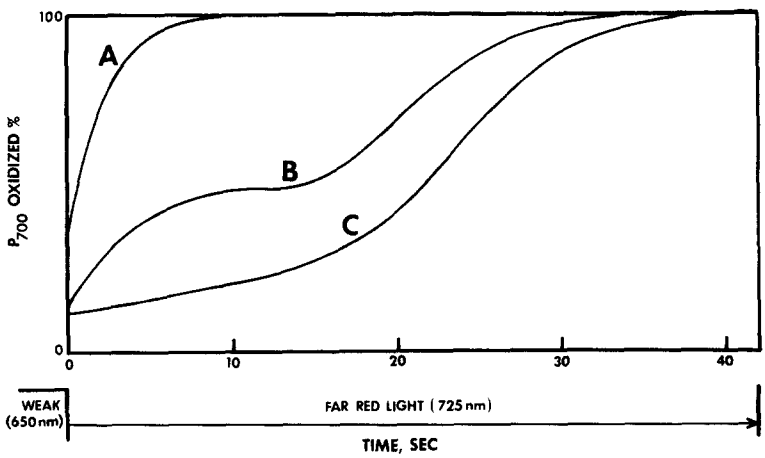

Fig. 7. Influence of varying concentrations of CCCP on the time-course of P_{700} oxidation in far-red light following pre-illumination with 2 min red light. Basic reaction medium plus 3 mM $MgCl_2$ and 0.3 μM CCCP (Curve A); 0.2 μM CCCP (Curve B); no CCCP (Curve C).

Fig. 7, CCCP at a relatively low concentration ($\geqq$ 0.3 μM), essentially eliminated the influence of Mg^{2+} on the photo-reduction of intermediate carriers in weak 650-nm light. Antimycin A yielded similar results at a concentration of $\geqq$ 40 μM. The corresponding effect of CCCP and antimycin A on relative quantum yields in Mg^{2+} containing suspensions is shown in Fig. 4. CCCP (0.3 μM) or antimycin A (40 μM) did not influence the yields in 650-nm illumination (Figs 4A and 4C) while at longer wavelengths the yields were decreased to the level observed without added Mg^{2+} (Figs 4B and 4D). These results indicate that the cation-induced changes are selectively eliminated by these two agents.

DISCUSSION

The experiments of Fig. 6 show that the apparent transfer of excitation energy between Photosystem II units is dependent upon the concentration of cations supplied in the external medium. While at higher concentrations, transfer occurs with a probability of about 0.55, at low concentrations this transfer is minimal. Consequently, because of the low apparent equilibrium constant (K) for the overall reaction chain between the photosystems [11], one would expect low salt to decrease the quantum efficiency especially at wavelengths which sensitize Photosystem II in excess (see later). How cations affect the probability of transfer between Photosystem II units is unknown. One possibility is that the distance between units and consequently the probability of energy transfer, varies dependent upon cation concentrations. Dramatic cation-induced alteration in chloroplast membrane structure are well-known [20–23].

In light of the fact that cations affect the probability of energy transfer between Photosystem II units, a likely assumption would be that they also influence excitation transfer between Photosystem II and Photosystem I (spillover). Two models have been proposed which assume spillover and regulation of it by cation concentration. Murata [6, 7] suggested that there is significant spillover from Photosystem II to

TABLE I

SUMMARY OF THE OBSERVED EFFECTS OF MAGNESIUM DEFICIENCY (Φ = RELATIVE QUANTUM YIELD)

1. Redox state of intermediate electron carriers in weak 650-nm light	oxidized
2. System II (O_2 evolution) enhancement	eliminated
3. System I (viologen reduction) enhancement	slight decrease
4. Excitation transfer between System II units	eliminated
5. Φ ($H_2O \rightarrow$ viologen) 650 nm	none
6. Φ ($H_2O \rightarrow$ viologen) 690, 710 nm	decrease
7. Φ (ascorbate + reduced DCIP $\rightarrow$ viologen) 650 nm	increase
8. Φ (ascorbate + reduced DCIP $\rightarrow$ viologen) 710 nm	none

Photosystem I with low but not with high cation concentrations. Sun and Sauer [9] assumed that (1) excitation transfer occurs in the reverse direction (Photosystem I to Photosystem II) in the presence of cations and not in their absence; and (2) the intrinsic absorption (minus cation) of Photosystems II and I is equal at all wavelengths between 620 nm and 680 nm. Neither model appears to fully accommodate our data summarized in Table I. While observations 1 through 3 are compatible with either of the above models, neither explains the effects on quantum yields. The model proposed by Sun and Sauer does not predict identical yields at 650 nm for the open system. An inbalance caused by a Mg^{2+} enhanced spillover of photons from Photosystem I to Photosystem II would result in lower yields relative to the optimum (low cation) condition. If anything, the gain of Photosystem II transfer (with Mg^{2+}) would accentuate this change. Murata's model predicts that, in an open system, cations lower the O_2 yield at 650 nm and do not change the yield at long wavelengths. Considering our observation of a cation requirement for Photosystem II transfer and assuming a low K value (see above), Murata's model can still accommodate the constant quantum yield at 650 nm, and a slight increase at 690 nm, but not the increase at 710 nm observed by us. In 710-nm light, all Photosystem II centers are open and spillover would be minimal, yet we observed a Mg^{2+}-induced increase.

Our data are interpreted more readily by the model developed from studies with *Chlorella* by Bonaventura and Myers [2] and Duysens [4]. This model assumes absence of active spillover between the photosystems, but instead, that α and $1-\alpha$, the fraction of quanta delivered to Photosystem II and I, respectively, at a particular wavelength, is not fixed. We can assume that in isolated chloroplasts, α and $1-\alpha$ are altered by changes in cation concentration, with cations inducing the State I condition and lack of cations the State II condition according to Bonaventura and Myers notation. We infer that with high cation concentrations, the chloroplasts are fixed into State I in which relatively more quanta are directed to Photosystem II. This would result in a more reduced state in weak 650-nm light, more Photosystem I enhancement, a higher quantum yield for an open system with wavelengths where Photosystem II is limiting ($\leqq$ 690 nm). Furthermore, for a donor system, we expect lower yields in 650-nm light, and negligible effects in far-red light, excitation of Photosystem II being minimal at these wavelengths. On the other hand, chloroplasts fixed in State II

(minus cation) would show the converse results, characteristic of decreased Photosystem II excitation. Again, the similar quantum yields for the $H_2O \rightarrow$ viologen system observed at 650 nm, plus and minus cation must be fortuitous. We must assume that in State II an increase of Photosystem I excitation is balanced by the loss of transfer between Photosystem II units. It should also be noted that the large Mg^{2+}-induced changes in fluorescence yield, observed by others [6, 18, 24], is not simply interpretable in terms of this model. The pronounced fluorescence changes could be explained with the added assumption that Mg^{2+} affects radiationless de-excitation losses.

Qualitatively, the discussed phenomena represent an important aspect of the light associated processes in photosynthesis, e.g. the physical arrangement of the two photosystems in the lamellar structure and their interactions. Quantitatively, the effects are small and difficult to analyze. Bonaventura and Myers [2] and Duysens [4] conclude that $\leqq 10\%$ of the total pigment is involved in the readjustment. Our data suggest slightly larger changes (about 20%).

A few additional remarks should be made concerning the chloroplast pigment shifts suggested in this paper relative to those presumed to occur in whole cells. Our observations concern exclusively the reversible responses to the concentration of externally supplied cations. In chloroplasts we have, as yet, not observed similar changes in state induced by light as are found in whole cells [2–4]. Even though the same effects are seen in both systems, their causes may not be related. A recent report [25] indicated that (very high) salt concentrations affected whole cell photosynthesis in a direction opposite to that seen in chloroplasts.

While we found in chloroplasts that the transfer of excitation energy between Photosystem II units depends upon salt concentration, Delrieu [5] found that in *Chlorella* this probability was the same in State I and State II. In addition, she concluded that in algae the State I–State II transition rests upon changes of the apparent equilibrium constant (K) of the interconnecting reaction chain, rather than upon changes in pigment distribution. This proposal was recently disputed by Wang and Myers [26]. We have also considered a change of K to explain the cation effect in chloroplasts. This hypothesis, however, fails to account for the decrease in quantum yield of the donor system upon addition of Mg^{2+} (No. 7, Table I).

Some circumstantial evidence suggests a correlation between the light-induced change in state in whole cells and the cation-induced change in isolated chloroplasts. Several investigators [27, 28], using whole algae, found that low concentrations of FCCP, CCCP and atabrin, markedly reduced the amplitude of the slow fluorescence change, which presumably reflects a change of the energy distribution between the two photosystems. This effect of CCCP parallels the selective removal of cation-induced phenomena we observed. Although these results suggest that both light- and cation-induced changes are related to energy coupling processes, the relationship is unclear. It should be noted that unlike the above uncoupling agents, methylamine does not alter the Mg^{2+}-induced changes, and in high concentration actually mimics its action.

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