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MAGNESIUM ION EFFECTS ON CHLOROPLAST PHOTOSYSTEM II FLUORESCENCE
AND PHOTOCHEMISTRY

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Summary

Although there have been several reports that divalent cations, especially Mg^{2+} , can significantly affect chloroplast photoprocesses, the molecular mechanism of the cation interaction is not well understood. We have investigated the interaction of Mg^{2+} with Photosystem II photoprocesses by studying cation effects on chloroplast fluorescence and the Hill reaction. Our results are summarized as follows:

- 1) Mg^{2+} stimulation of background fluorescence (20-30%) saturates at about 0.5 mM Mg^{2+} , while Mg^{2+} stimulation of variable fluorescence (250%) saturates at about 2.5 mM Mg^{2+} .
- 2) Addition of Mg^{2+} to chloroplasts treated with DCMU or dithionite causes a doubling in the amount of total (background + variable) fluorescence.
- 3) Studies on chloroplasts in the presence of DCIP indicate that Mg^{2+} doubles the relative yield of variable fluorescence under light-limiting conditions.
- 4) Mg^{2+} causes large (70-120%) increases in the light-limited rate of the DCIP Hill reaction.

We interpret these results in terms of a model involving two components of chloroplast emission. Our analysis indicates that Mg^{2+} increases the effective absorption cross section (size) of the pigment array associated with Photosystem II photochemistry.

Abbreviations

DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

DCIP, 2,6-dichlorophenolindophenol, oxidized form

Fd, ferredoxin

HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

Pcy, plastocyanin

Introduction

Divalent cations, especially Mg^{2+} , have a significant influence on the primary processes of chloroplast photosynthesis. The addition of Mg^{2+} to chloroplast suspensions 1) stimulates chloroplast fluorescence [1-4], 2) increases the rate of Hill reactions with several electron acceptors [1-3, 5-7], 3) decreases the degree of photo-oxidation of P700 [7-8], and 4) stimulates Emerson Enhancement in broken chloroplasts [8-10]. Other studies have shown that the addition of Mg^{2+} to chloroplasts causes significant changes in chloroplast structure as measured by electron microscopy [11] and light scattering [12]. There is recent evidence that primary photosynthetic processes are also influenced by the addition of monovalent cations [13,14].

Several authors suggest [1,3,4] that many of these Mg^{2+} effects can be explained on the basis of Mg^{2+} -induced blockage of excitation transfer (spillover) from Photosystem II to Photosystem I. In contrast, recent experiments of Jennings and Forti [15] and of Li [16] suggest that Mg^{2+} stimulation of Photosystem II fluorescence and photochemistry may not result from salt-induced changes in the amount of spillover between the photosystems. In this paper we show that Mg^{2+} effects on background fluorescence can be distinguished from Mg^{2+} effects on variable fluorescence. We also show that Mg^{2+} causes similar increases in variable fluorescence and the rate of DCIP reduction. These observations lead us to propose that there are two distinct types of Photosystem II pigment arrays that give rise to chloroplast fluorescence. We explain Mg^{2+} stimulation of variable fluorescence and light-limited DCIP reduction in terms of a Mg^{2+} -induced increase in the size of pigment array which sensitizes these Photosystem II photoprocesses.

Materials and Methods

Fluorescence studies

Isolated broken chloroplasts were prepared from 14 to 21 day old Alaska pea plants (Pisum sativum) grown under the conditions used by Sun and Sauer [5] in their studies on spinach chloroplasts. Pea leaves were picked after 4 to 6 h dark adaptation to provide chloroplasts with reproducible activity. Chloroplasts prepared from dark adapted pea leaves contain less Mg^{2+} than chloroplasts prepared from light adapted leaves [17]. After removal of the stems, the pea leaves were washed with distilled water and ground in 0.45 M sucrose, 0.1 M HEPES, pH 7.6, for 15 to 20 s in a Waring blender equipped with a micro attachment. The leaf homogenate was filtered through 8 layers of Miracloth (Chicopee Mills, New York, N.Y.). The suspension was centrifuged at 200 x g for 1 min to remove cell debris, and the supernatant was centrifuged at 1500 x g for 10 min. The pellet was resuspended in 2 ml of 0.45 M sucrose, 0.05 M HEPES, pH 7.6. For fluorescence measurements this suspension was diluted with the same medium to give a final absorbance (1 cm path) of about 0.3 at 436 nm.

Relative fluorescence intensities were measured using a modification of the method of Park et al. [18]. A Cary 14 spectrophotometer equipped with a linear (% T) slidewire and a Model 1462 Scattered Transmission Accessory, modified for side illumination as described by Sauer and Biggins [19], was operated in the REF mode so that the photomultiplier tube responded only to the modulated signal arising from the sample. Using this technique, first introduced by Duysens and Sweers [20], one can record the effect of an unmodulated actinic beam (side illumination) on the fluorescence signal excited by the low level modulated measuring beam. Blue actinic illumination was provided by a Bausch and Lomb Hi Intensity Monochromator (bandwidth 12 nm) equipped with a 45 watt coil-filament quartz iodine source. The photomultiplier

(EMI 9558 QB) was protected from stray excitation light by a Schott RG 665 filter and an Optical Industries interference filter (bandwidth 10 nm, maximum transmittance 50% at 687 nm). The source for the modulated measuring beam was a Cary High Intensity Light Source operated at 120 volts. The Cary 14 monochromator had a bandwidth of 3.8 nm at 480 nm and 4.5 nm at 430 nm. Light intensities were measured using a Hewlett Packard Radiant Flux Meter #8330A and Probe #8334A or with an El Segundo Solar Cell and Keithley microvoltmeter. Both intensity measuring devices were calibrated against an Eppley thermopile and a standard lamp. Unless otherwise stated, actinic illumination full intensities were $55 \mu\text{W}/\text{cm}^2$ at 480 nm and $38 \mu\text{W}/\text{cm}^2$ at 430 nm. Intensity dependence studies were carried out using Balzers calibrated neutral density filters. The measuring beam intensity at both 480 and 430 nm was determined to be less than $1 \mu\text{W}/\text{cm}^2$.

To obtain a steady baseline for measurement of background fluorescence, previously dark adapted samples were inserted into the sample chamber, where the samples were exposed only to the weak measuring beam and allowed to equilibrate for about 3 min. Unless otherwise stated, all values reported for total and variable fluorescence levels are steady-state values obtained after 90 to 120 s of actinic illumination.

Absorption spectra and quantum yield studies

For quantum yield measurements, chloroplast samples were adjusted to have an absorbance of 3-4 at 436 nm. Absorption spectra and rates of DCIP reduction ($\Delta A_{580}/\text{time}$) were measured with an Aminco-Chance DW-2 spectrophotometer operated in the split beam mode. For absorbance measurements, the photomultiplier was positioned 1 cm from the sample and a square of opal glass was inserted between the photomultiplier and the sample to give uniform scattering. Other details of sample preparation and arrangement of experimental apparatus are as described in reference 21. Absorbed intensities used in quantum yield determinations were computed from measurements of the actinic

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intensity incident on the cuvet and the absorbance of the sample in the direction of actinic illumination. A molar extinction coefficient of 19,800 at 580 nm for DCIP was used in quantum yield calculations [22].

NADP⁺, DCMU, plastocyanin, sucrose and DCIP were obtained as described previously [5]. MgCl₂ was obtained from Mallinckrodt, ferredoxin from Sigma, and HEPES from Calbiochem.

Results

Absorption spectra

The addition of 5 mM MgCl₂ to a chloroplast suspension causes small but significant decreases in both the Soret and red regions of the absorption spectrum (Fig. 1). These absorbance decreases in regions of high optical density most likely result from optical flattening owing to changes in the arrangement of chloroplast membranes. Electron microscope [11] studies and light scattering measurements [12] have shown that addition of Mg²⁺ to chloroplast suspensions causes major alterations in chloroplast membrane organization. The Mg²⁺ induced absorbance changes seen in Fig. 1 correspond to those reported by Gross and Libbey [23]. By contrast, Murata [1] and Briantais *et al.* [3] reported that addition of MgCl₂ does not cause significant changes in the light absorption of chloroplasts.

Fluorescence yield

Illumination of chloroplasts by an actinic source causes an increase in fluorescence efficiency (F_V) over the normal background level (F_B). Variable fluorescence (F_V) and background fluorescence (F_B) are purely experimentally derived quantities which are operationally defined as shown in Fig. 2.

Changes in chloroplast variable fluorescence yield have been attributed to changes in the redox state of the Photosystem II primary acceptor, Q [20,24]. In the presence of the electron acceptor system Pcy/Fd/NADP⁺ we observe

(Fig. 2b) a lower variable fluorescence yield relative to untreated samples (Fig. 2a).¹ This accords with a more oxidized state of Q in the presence of the acceptor system. DCMU, which blocks electron flow from the reduced form of Q (QH) to secondary acceptors [20], produces an increase in total fluorescence yield in DCMU poisoned samples (Fig. 2c) relative to untreated samples.

Fig. 3 shows the effect of $MgCl_2$ on the levels of variable, background and total fluorescence of chloroplasts without added electron acceptors. Addition of $MgCl_2$ causes an increase of 30% in background fluorescence (Fig. 3a) saturating at about 0.5 mM. This Mg^{2+} concentration dependence is similar to that for the inhibition of the 515 nm absorbance change reported by Gross and Libbey [23]. By contrast, the Mg^{2+} -induced increase in variable fluorescence is about 250% and saturates at 2.5 mM $MgCl_2$ (Fig. 3b). The Mg^{2+} concentration dependence of variable fluorescence agrees with the data of Rurainski and Hoch [25]. Recently Wydryzynski *et al.* [26] have also reported the existence of different Mg^{2+} concentration dependences for background and variable fluorescence. Using a different measurement technique, Homann [2], Mohanty *et al.* [4], and Briantais *et al.* [3] also reported that Mg^{2+} stimulates larger increases in variable fluorescence than in background fluorescence. Since the total modulated emission (background + variable) is 50-70% variable and the Mg^{2+} effect is tenfold greater on the variable component, the Mg^{2+} concentration dependence of total emission (Fig. 3c) is similar to that observed for variable emission alone (Fig. 3b). $MgSO_4$ produced similar effects. The aliphatic amine spermine is capable of replacing Mg^{2+} in these fluorescence stimulation experiments. The addition of EDTA reverses the Mg^{2+} stimulation of fluorescence.

¹ The small decrease (10%) in the background fluorescence level between an untreated sample (no added acceptor) and the sample containing Pcy/Fd/NADP⁺ probably indicates that in untreated chloroplasts the measuring beam causes some change in the redox state of Q.

Chloroplasts with added electron acceptors also show much greater stimulation of variable fluorescence than of background fluorescence (Table I); however, the Mg^{2+} -induced changes in background, variable and total fluorescence are smaller than those for untreated chloroplasts. The data of Table I support earlier reports that Mg^{2+} is capable of doubling the total fluorescence yield of DCMU [2,4] and dithionite [2] treated chloroplasts. The data of Fig. 4 are in agreement with the report of Murata [1] that Mg^{2+} stimulation of total fluorescence in DCMU poisoned samples saturates at a Mg^{2+} concentration of 2.5 mM.

In the presence of the electron acceptor DCIP, F_V is directly proportional to I_{act} in chloroplast samples with or without Mg^{2+} (Fig. 5). Added Mg^{2+} doubles the slope of the F_V versus I_{act} plot. This result indicates that Mg^{2+} causes a twofold increase in the variable fluorescence quantum yield. Fig. 5 also shows that the F_V versus I_{act} plots do not extrapolate through the origin, for reasons as yet unknown.

Photosystem II photochemistry: $H_2O \rightarrow DCIP$

Fig. 6 shows the effect of Mg^{2+} and actinic intensity on the rate of the DCIP Hill reaction. For samples with and without Mg^{2+} , the rate of DCIP reduction is proportional to the actinic intensity; hence we are working in a photon-limited region of the saturation curve. In this experiment we also see that Mg^{2+} doubles the light-limited rate of DCIP reduction. In agreement with others [1,8], we find some variation in the degree of Mg^{2+} stimulation of rate from one sample to another. The average Mg^{2+} -induced increase in the rate of DCIP reduction was $76 \pm 7\%$ for 18 pairs of dark adapted samples. As explained in the Discussion, the Mg^{2+} -induced increase in the light limited rate of this Photosystem II reaction is of the same magnitude as the Mg^{2+} -induced increase in the slope of the F_V versus I_{act} plot in the linear intensity region (Fig. 5). Our pea chloroplasts show a stimulation

by Mg^{2+} of light-limited DCIP reduction significantly greater than that reported by Murata [1] for spinach chloroplasts at the same actinic wavelength (480 nm). Using 640 nm illumination and spinach chloroplasts, Briantais et al. [3] found that Mg^{2+} causes a 40% increase in the light limited rate of the DCIP Hill reaction. For the $H_2O \rightarrow$ DCIP data shown in Fig. 6 we have computed an approximate quantum yield of 0.3 equivalents/einstein for the sample without Mg^{2+} and 0.6 for the sample with Mg^{2+} . Quantum yield values ranging from 0.26 [1] to 1.0 [5] have been reported in previous studies of the $H_2O \rightarrow$ DCIP reaction in spinach chloroplasts.

The Hill reaction with ferricyanide is another measure of Photosystem II activity. Ben-Hayyim and Avron [27], using lettuce chloroplasts, reported that Mg^{2+} increased the light limited rate of the $H_2O \rightarrow$ ferricyanide reaction by about 75%. A 5-fold Mg^{2+} stimulation of the light limited rate of this reaction in oat chloroplasts has also been reported recently by Li [16]. In contrast, other laboratories using spinach [8] and lettuce [28] chloroplasts have reported that Mg^{2+} does not alter the rate of this reaction. The reason for the discrepancy among these results is not clear.

Discussion

Fluorescence studies

Variable and background fluorescence are experimentally derived quantities which are operationally defined as shown in Fig. 2. In a discussion of theoretical aspects of chloroplast fluorescence it is useful to consider the concepts of active and inactive fluorescence. Inactive fluorescence is sensitized by pigments which are photochemically inactive; i.e., they are not connected to the Photosystem II reaction center (RC II). Active fluorescence is sensitized by pigments which are connected to RC II and hence are also active in sensitizing Photosystem II photochemistry. (These definitions for active and inactive fluorescence are similar to those used by Clayton [29]

for live and dead fluorescence.)

If the modulated measuring beam used in the experimental apparatus is sufficiently weak and does not alter the fraction of functional reaction centers (open traps), then background and inactive fluorescence are equivalent, as are variable and active fluorescence. For chloroplasts without DCMU or dithionite, these conditions are often approximately satisfied; hence, in such studies we can approximate background fluorescence by inactive fluorescence and variable fluorescence by active fluorescence. For DCMU and dithionite poisoned samples we must make indirect estimates of the fraction of active fluorescence.

Mg^{2+} stimulation of active (variable) fluorescence saturates at a higher Mg^{2+} concentration than does Mg^{2+} stimulation of inactive (background) fluorescence (Fig. 3). This result indicates that the Mg^{2+} stimulation of active and inactive fluorescence have different origins. One possible interpretation is that there are separate sites of active and inactive fluorescence (two site model). Active and inactive fluorescence are then emitted by different groups of pigments, and the Mg^{2+} effects are independent of one another. An alternative model which involves a single fluorescing site is discussed in reference 21.

Two site model. According to the two site model (Fig. 7), the Photosystem II chlorophylls are arranged in two independent emitting arrays. Pigments in the active array sensitize both Photosystem II photochemistry and active fluorescence. Consequently active fluorescence competes with photochemistry and is sensitive to conditions which alter the ability of the Photosystem II reaction center to carry out electron transfer reactions. Since the pigments in the inactive array are unable to transfer excitation to the photochemical trap, inactive fluorescence is unaffected by conditions which alter the efficiency of Photosystem II photochemistry.

Nature of the active fluorescence site. In our analysis of chloroplast fluorescence we treat active fluorescence as a property of the Photosystem II active antenna array including the reaction center. In this view (the fluorescent array model) a reaction center chlorophyll which cannot carry out photochemistry (a closed trap) cannot act as energy sink and is no longer distinguishable from other molecules in the active pigment array. The fluorescent array model for the site of active fluorescence is supported by reports from several laboratories [3,30,31] of sigmoidal fluorescence induction curves for normal chloroplast preparations. According to Lavorel and Joliot [30], sigmoidal induction curves are compatible only with a fluorescent array model. Mohanty et al. [4] and Briantais et al. [3] have implicitly used the assumption of an active fluorescent array in their discussions of chloroplast fluorescence.

Effect of actinic intensity. Using the modulated detection technique discussed in the Methods section, we measure only the fluorescence excited by the modulated measuring beam (I_m) and not the fluorescence excited by unmodulated actinic source (I_{act}). Increases in I_{act} decrease the fraction of open traps and consequently increase the efficiency of fluorescence excited by I_m . We can summarize the processes involved in active fluorescence and Photosystem II photochemistry as follows:

Active Fluorescence - Two Site Model

<u>Processes</u>		<u>Rate</u>
Excitation	$Chl_A + h\nu \rightarrow Chl_A^*$	$\sigma_A I_m$

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<u>Processes</u>		<u>Rate</u>
Trapping & Photochemistry	$\text{Chl}_A^* + \text{P}_{680}\text{Q} \xrightarrow{k_T} \text{Chl}_A + \text{P}_{680}\text{Q}^*$ $\text{P}_{680}\text{Q}^* \downarrow \text{P}_{680}^+\text{Q}^-$	$k_T (\text{P}_{680}\text{Q}) (\text{Chl}_A^*)$ $+$
Fluorescence	$\text{Chl}_A^* \xrightarrow{k_f} \text{Chl}_A + h\nu'$	$k_f (\text{Chl}_A^*)$
Thermal decay & spillover to Photosystem II	$\text{Chl}_A^* \xrightarrow{k_{ha}} \text{Chl}_A + \text{"heat"}$	$k_{ha} (\text{Chl}_A^*)$

where Chl_A = ground state pigment in active array

Chl_A^* = excited singlet state pigment in active array

P_{680}Q = ground state of open reaction center

P_{680}Q^* = excited state of reaction center

$\text{P}_{680}^+\text{Q}^-$ = products of Photosystem II primary photochemistry

k_T = rate constant for energy transfer from active pigment to reaction center

k_{ha} = rate constant for de-excitation of active pigments by thermal losses and spillover to Photosystem I

[†] We assume that once excitation reaches an open reaction center there is essentially unit probability that the excitation will be utilized for electron transfer.

k_f = intrinsic rate constant for fluorescence

σ_A = effective absorption cross section (size) of Photosystem II
active pigment array

I_m = intensity of modulated measuring beam

F_A = intensity of active fluorescence (at a fixed value of I_m this
quantity is a measure of relative fluorescence yield or efficiency)

The intensity (relative yield) of active fluorescence is given by

$$F_A = k_f I_m \sigma_A / [k_T(P_{680}Q) + k_{ha} + k_f] \quad (1)$$

Equation 1 expresses active fluorescence in terms of molecular excitation and de-excitation parameters. Therefore, we can relate the Mg^{2+} -induced increases in active fluorescence to one or more specific molecular excitation and relaxation processes. Increases in I_{act} serve to close traps, resulting in decreases in the magnitude of the term $(P_{680}Q)$ in Eqn 1. When active (variable) fluorescence increases linearly with actinic intensity for samples with and without Mg^{2+} , as in Fig. 5, we can conclude that $(P_{680}Q)$ is proportional to $1/I_{act}$ and $k_T(P_{680}Q) \gg (k_f + k_{ha})$. Therefore Mg^{2+} -stimulated fluorescence increases must arise from either a decrease in $k_T(P_{680}Q)$ or an increase in σ_A . The data of Table I and reference 2 show that DCMU- or dithionite-treated chloroplasts, which cannot carry out photochemistry [$k_T(P_{680}Q) \cong 0$], still show a doubling of total fluorescence upon addition of Mg^{2+} . Thus, it is unlikely that Mg^{2+} -stimulated increases in active fluorescence arise from a decrease in the term $k_T(P_{680}Q)$ in Eqn 1. Furthermore, chloroplasts with and without acceptors, which therefore contain different fractions of open and closed traps [different values of $k_T(P_{680}Q)$], show similar large Mg^{2+} -induced active

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fluorescence increases (Table I). Making the usual assumption that k_f is constant [1,3], the most reasonable origin in this model for a Mg^{2+} -induced increase in active fluorescence is an increase in σ_A . An increase in σ_A indicates an increase in the effective size (absorption cross section) of the Photosystem II active pigment array. A Mg^{2+} -stimulated increase in spillover from Photosystem I to Photosystem II, previously proposed by Sun and Sauer [9], would represent such an increase in σ_A . Mg^{2+} activation or connection of previously inactive or disconnected chlorophyll would also produce an increase in σ_A . As discussed above, our analysis of Fig. 5 indicates that k_{ha} , the rate constant for thermal losses and spillover to Photosystem I, must be much less than $k_T(P_{680}Q)$. This is significant, because it means that a Mg^{2+} -induced decrease in k_{ha} cannot account for the Mg^{2+} -induced doubling of active fluorescence shown in Fig. 5. Previously, Murata [1] and others [3,4] have proposed that Mg^{2+} -stimulated fluorescence increases arise from Mg^{2+} -induced blockage of spillover from Photosystem II to Photosystem I. On the basis of our analysis of the data of Fig. 5 and Table I, we feel this proposal is no longer tenable.

Inactive fluorescence. In terms of our two-site model, the processes involved in inactive fluorescence are:¹

<u>Process</u>		<u>Rate</u>
Excitation	$Chl_{In} + h\nu \rightarrow Chl_{In}^*$	$\sigma_{In} I_m$

¹The process, $Chl_A^* + Chl_{In} \rightarrow Chl_A + Chl_{In}^*$, which represents sensitization of inactive fluorescence by energy transfer from the active array, predicts that inactive fluorescence should be sensitive to the state of the trap. Since inactive fluorescence is defined to be independent of the state of the trap, we have not included this process in our analysis of inactive fluorescence.

<u>Process</u>		<u>Rate</u>
Thermal decay	$\text{Chl}_{\text{In}}^* \xrightarrow{k_{\text{hi}}} \text{Chl}_{\text{In}} + \text{"heat"}$	$k_{\text{hi}}(\text{Chl}_{\text{In}}^*)$
Fluorescence	$\text{Chl}_{\text{In}}^* \xrightarrow{k_{\text{f}}} \text{Chl}_{\text{In}} + h\nu'$	$k_{\text{f}}(\text{Chl}_{\text{In}}^*)$

The expression for the intensity (relative yield) of inactive fluorescence is:

$$F_{\text{In}} = k_{\text{f}} \sigma_{\text{In}} I_{\text{m}} / (k_{\text{hi}} + k_{\text{f}}) \quad (2)$$

where Chl_{In} = ground state pigment in inactive array

Chl_{In}^* = excited singlet state of pigment in inactive array

σ_{In} = effective absorption cross section (size) of inactive pigment array

k_{hi} = rate constant for thermal decay of excited inactive pigment

f_{In} = intensity of inactive fluorescence (relative yield of inactive fluorescence at fixed value of I_{m})

I_{m} , k_{f} , Chl_{A} and Chl_{A}^* are defined as previously.

According to this model, a Mg^{2+} stimulated increase in inactive fluorescence could arise from a Mg^{2+} -induced increase in k_{f} or σ_{In} , or from a Mg^{2+} -induced decrease in k_{hi} . Again assuming that k_{f} is a true constant of the system which is unaltered by the presence of Mg^{2+} [1,3], we can still account for a Mg^{2+} stimulated increase in inactive fluorescence by either an increase in σ_{In} , corresponding to an increase in the effective size of the inactive pigment array, or a decrease in the rate constant for non-radiative decay, k_{hi} . Since little is known about the detailed molecular properties of the inactive chlorophyll, it is not yet possible to determine which of these two

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terms is responsible for the observed Mg^{2+} -induced increase in inactive fluorescence.

The only significant experimental evidence that argues against our proposal that Mg^{2+} increases the absorption cross section of the Photosystem II active pigment array is found in the work of Briantais et al. [3]. For preilluminated chloroplast samples, both the (total) fluorescence intensity (F) and the fluorescence lifetime (τ) were measured with a phase fluorometer. Briantais et al. [3] concluded that the same colinear relationship between τ and F is obtained in the presence or absence of Mg^{2+} (reference 3, Fig. 1). On this basis they concluded that Mg^{2+} causes a decrease in the rate of Photosystem II $\rightarrow$ Photosystem I spillover rather than an increase in the fraction of absorbed excitation which reaches the Photosystem II reaction center. Implicit in their use of the phase fluorometric technique is the assumption that the decay of fluorescence is a single exponential. As discussed by Müller et al. [32] and Pearlstein [33], one should employ a chopping frequency of about 10^9 Hz to determine the validity of this assumption. With their 7.25 MHz chopping frequency, Briantais et al. [3] could not know whether their fluorescence decay was truly exponential. A recent report indicates that the fluorescence decay of chloroplasts is non-exponential [34]. We feel that a truly meaningful τ versus F plot requires direct measurement of fluorescence decay curves.

Photosystem II photochemistry. The DCIP Hill reaction is a measure of Photosystem II photochemistry [5,35]. Using the model for active fluorescence and Photosystem II photochemistry presented above, we obtain:¹

$$V_{II} = k_T(P_{680}Q)\sigma_A I_{act} / [k_T(P_{680}Q) + k_{ha} + k_f] \quad (3)$$

where V_{II} = rate of trapping (and photochemistry); other quantities as defined previously. The direct proportionality between DCIP and actinic intensity seen in Fig. 6 indicates that the quantity $k_T(P_{680}Q) / [k_T(P_{680}Q) + k_{ha} + k_f]$ must be independent of I_{act} . As discussed above, changes in I_{act} cause changes in $(P_{680}Q)$. We would expect to observe $k_T(P_{680}Q) / [k_T(P_{680}Q) + k_{ha} + k_f]$ to be independent of I_{act} when either $k_T(P_{680}Q) \gg (k_{ha} + k_f)$ or when $k_T(P_{680}Q) \ll (k_{ha} + k_f)$. The relatively high quantum yield (0.3 to 0.6) which we have obtained for the DCIP Hill reaction indicates that we are in a situation where $k_T(P_{680}Q) \gg (k_{ha} + k_f)$. Under these conditions $k_T(P_{680}Q) / [k_T(P_{680}Q) + k_{ha} + k_f] \cong 1$ and $V_{II} \cong \sigma_A I_{act}$. The Mg^{2+} -induced doubling of the light-limited rate of DCIP reduction (Fig. 6) apparently corresponds to a Mg^{2+} -induced doubling of σ_A . As discussed above, increases in σ_A can result from increased spillover from Photosystem I to Photosystem II or from the connection of previously disconnected chlorophyll to Photosystem II. Because our data require that $k_T(P_{680}Q) \gg k_{ha}$, we conclude that a Mg^{2+} -induced blockage of Photosystem II $\rightarrow$ Photosystem I spillover cannot account for the

¹ Trebst suggested recently that DCIP can also function as a Photosystem I electron acceptor [36]. The site of DCIP reduction appears to be dependent on the state of the chloroplasts after isolation [35]. If DCIP accepts at both Photosystem II and Photosystem I sites, then the above analysis must be modified to include only DCIP reduced at the Photosystem II site.

Mg^{2+} -induced doubling of the light-limited rate of DCIP reduction seen in Fig. 6. The similarities in the magnitude of the Mg^{2+} -induced increase in active fluorescence (Fig. 5) and Photosystem II photochemistry (Fig. 6) argue that both of these effects result from a Mg^{2+} -induced increase in σ_A .

It would be of great interest to know if this Mg^{2+} -induced increase in the size of the Photosystem II antenna array occurs at the expense of the Photosystem I antenna array. We were unable to resolve this question because of conflicting results obtained with different Photosystem I electron acceptors in our laboratory [21] and elsewhere [8,28].

Conclusion

The most significant conclusion to be drawn from our studies on the effect of Mg^{2+} on Photosystem II photochemistry and fluorescence is that the size of the active pigment array is not constant, but can be increased by the addition of Mg^{2+} . Murata [12] showed that salt-induced changes in chloroplast structure (light scattering) and total chloroplast fluorescence have a very similar dependence on Mg^{2+} concentration. Electron microscope measurements [11,37] also indicate that Mg^{2+} causes significant changes in the organization of chloroplast membranes. The model calculations of Seely [38] indicate that changes in the orientation of only a small number of molecules in a pigment array which contains both Photosystem I and Photosystem II can significantly alter the distribution of excitation between the two photosystems. In view of the known ability of Mg^{2+} to affect chloroplast membrane structure [11,12,37], we propose that Mg^{2+} also alters the arrangement of pigments in the thylakoid so as to increase the size of the Photosystem II antenna.

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TABLE I

EFFECT OF ELECTRON TRANSPORT COFACTORS ON Mg^{2+} STIMULATED FLUORESCENCE INCREASES

Experimental conditions: excitation wavelength, 480 nm; excitation intensity, $55 \mu W/cm^2$; $MgCl_2$, 5 mM; Fd, 34 $\mu g/ml$; $NADP^+$, 0.5 mM; dithionite and Pcy, saturating amount; DCMU, 50 μM ; DCIP, 3 μM ; other conditions as described in Methods section. F_B , F_V , F_T as defined in Fig. 2.

(F^+/F^-) is defined as follows: $(F^+/F^-) = F^{+Mg^{2+}}/F^{-Mg^{2+}}$

Chloroplasts	$(F^+/F^-)_B$	$(F^+/F^-)_V$	$(F^+/F^-)_T$
Untreated	1.29 ± 0.08	3.70 ± 0.25	2.24 ± 0.17
+ Fd/ $NADP^+$	1.16 ± 0.05	3.05 ± 0.19	1.84 ± 0.05
+ Pcy/Fd/ $NADP^+$	1.20 ± 0.04	2.95 ± 0.16	1.46 ± 0.07
+ DCIP	1.14 ± 0.03	3.09 ± 0.19	1.64 ± 0.05
+ DCMU	---	---	2.11 ± 0.11
+ dithionite	---	---	2.10 ± 0.15

Figure Legends

Figure 1. Effect of MgCl_2 on chloroplast absorption spectra. Spectral bandpass, 3.0 nm. ---, 5 mM Mg^{2+} ; —, no Mg^{2+} . To correct for the volume change due to the addition of MgCl_2 , an equivalent volume of buffer was added to the sample which lacked Mg^{2+} . Samples were allowed to equilibrate in the dark for five minutes after the addition of Mg^{2+} (or buffer). Other details as described in the text.

Figure 2. Fluorescence yield of chloroplasts. Excitation wavelength, 480 nm; excitation intensity, $55 \mu\text{W}\cdot\text{cm}^{-2}$; instrument time constant, 300 ms; fresh dark-adapted samples used in (a), (b) and (c); other experimental details as described in Methods section. Start of actinic illumination, +. Variable fluorescence (F_V) is computed as the difference between the total fluorescence (F_T) and the background fluorescence (F_B). (a) Untreated chloroplasts. (b) Chloroplasts containing Pcy/Fd/ NADP^+ . Conditions as in (a) except that sample also contained the following additions: Pcy, saturating amount; Fd, 40 $\mu\text{g}/\text{ml}$; NADP^+ , 0.6 mM. (c) DCMU-treated chloroplasts. Conditions as in (a) except that sample also contained 0.01 mM DCMU.

Figure 3. Effect of Mg^{2+} concentration on background, variable and total fluorescence levels in untreated chloroplasts. Other conditions as in Figure 2(a). F_V computed as described for Figure 2. Correction for volume change due to addition of Mg^{2+} and equilibration procedure as described for Fig. 1. Fresh dark-adapted samples were used at each Mg^{2+} concentration. (a) F_B ; (b) F_V ; (c) F_T .

Figure 4. Effect of Mg^{2+} concentration on total fluorescence level of DCMU-treated chloroplasts. Sample preparation as described in Fig. 3 except that samples contained 0.01 mM DCMU.

Figure 5. Effect of Mg^{2+} on intensity dependence of variable (live) fluorescence in chloroplasts containing DCIP. DCIP concentration, 0.02 mM; I_{act} value of 100% represents an actinic intensity of $40 \mu W-cm^{-2}$. Neutral density filters were used to obtain F_V values for a given sample at different actinic intensities. Volume correction and equilibration procedure as in Fig. 1. Fresh dark-adapted samples used in (a) and (b). (a) 5 mM $MgCl_2$; (b) no added $MgCl_2$; other experimental conditions as in Fig. 2(a). F_V is computed as in Fig. 2.

Figure 6. Effect of Mg^{2+} and actinic intensity on the rate of DCIP reduction. Actinic wavelength, 480 nm; actinic intensity ($I_{rel} = 100$), $60 \mu W-cm^{-2}$ DCIP concentration, 0.02 mM. Volume correction and equilibration procedures as described in Fig. 1. Fresh dark-adapted samples used in (a) and (b). (a) no added Mg^{2+} ; (b) 7.5 mM Mg^{2+} . I_{rel} values were varied with calibrated neutral density filters. + indicates start of actinic illumination at relative intensity indicated in the figure. Other experimental procedures as described in Methods section.

Figure 7. Two site model for chloroplast fluorescence and Photosystem II photochemistry. Details explained in the Discussion section.

References

1. Murata, N. (1969) *Biochim. Biophys. Acta* 189, 171-181.
2. Homann, P. (1969) *Plant Physiol.* 44, 932-936.
3. Briantais, J. M., Vernotte, C. and Moya, I. (1973) *Biochim. Biophys. Acta* 325, 530-538.
4. Mohanty, P., Braun, B. and Govindjee (1973) *Biochim. Biophys. Acta* 459-476.
5. Sun, A.S.K. and Sauer, K. (1971) *Biochim. Biophys. Acta* 234, 399-414.
6. Avron, M. and Ben-Hayyim, G. (1969) *Prog. in Photosynthesis Research*, Vol. III, ed. by H. Metzner, H. Laupp, Tübingen 1185-1196.
7. Rurainski, H. J., Randles, J. and Hoch, G. E. (1971) *FEBS Letters* 13, 98-100.
8. Marsho, T. V. and Kok, B. (1974) *Biochim. Biophys. Acta* 333, 353-365.
9. Sun, A.S.K. and Sauer, K. (1972) *Biochim. Biophys. Acta* 256, 409-427.
10. Sinclair, J. (1972) *Plant Physiol.* 50, 778-783.
11. Murakami, S. and Packer, L. (1971) *Arch. Biochem. Biophys.* 146, 337-347.
12. Murata, N. (1971) *Biochim. Biophys. Acta* 245, 365-372.
13. Murata, N. (1971) *Biochim. Biophys. Acta* 226, 422-432.
14. Gross, E. L. and Hess, S. C. (1973) *Arch. Biochem. Biophys.* 159, 832-836.
15. Jennings, R. C. and Forti, G. (1974) *Biochim. Biophys. Acta* 347, 299-310.
16. Li, Y. S. (1975) *Biochim. Biophys. Acta* 376, 180-188.
17. Nobel, P. S. and Lin, D. C. (1971) *Arch. Biochem. Biophys.* 145, 622-632.
18. Park, R. B., Steinback, K. E. and Sane, P. V. (1971) *Biochim. Biophys. Acta* 253, 204-207.
19. Sauer, K. and Biggins, J. (1965) *Biochim. Biophys. Acta* 102, 55-72.
20. Duysens, L.N.M. and Sweers, H. (1963) *Studies on Microalgae and Photosynthetic Bacteria*, ed. by Japanese Soc. of Plant Physiologists, Univ. of Tokyo Press, Tokyo 535-372.

21. Henkin, B. M. (1975) Ph.D. Thesis, Univ. of California, Berkeley, ERDA-LBL-Report-3880.
22. Sauer, K. and Park, R. B. (1965) *Biochemistry* 4, 2791-2798.
23. Gross, E. L. and Libbey, J. W. (1972) *Arch. Biochem. Biophys.* 153, 457-467.
24. Yamashita, T. and Butler, W. L. (1968) *Plant Physiol.* 43, 1978-1986.
25. Rurainski, H. and Hoch, G. E. (1972) Proc. 2nd Intl. Conf. on Photosynthesis Research, Vol. I, ed. by M. Avron and G. Forti, Dr. W. Junk, The Hague 133-143.
26. Wydrzynski, T., Gross, E. L. and Govindjee (1975) *Biochim. Biophys. Acta* 376, 151-161.
27. Ben-Hayyim, G. and Avron, M. (1971) *Photochem. Photobiol.* 14, 389-396.
28. Harnischfeger, G. and Shavit, N. (1974) *FEBS Letters* 45, 286-289.
29. Clayton, R. K. (1969) *Biophys. J.* 9, 60-76.
30. Lavorel, J. and Joliot, P. (1972) *Biophys. J.* 12, 815-831.
31. Delosme, R. (1967) *Biochim. Biophys. Acta* 143, 108-128.
32. Müller, A., Lumry, R. and Walker, M. (1969) *Photochem. Photobiol.* 9, 113-126.
33. Pearlstein, R. M. (1966) Ph.D. Thesis, Univ. of Maryland.
34. Yu, W., Ho, P. P., Alfano, R. R. and Seibert, M. (1975) *Biochim. Biophys. Acta* 387, 159-164.
35. Trebst, A. (1972) *Methods in Enzymology* 24, 146-165.
36. Trebst, A. (1974) *Ann. Rev. Plant Physiol.* 25, 423-458.
37. Wang, A-Y. J. and Packer, L. (1973) *Biochim. Biophys. Acta* 305, 488-492.
38. Seely, G. R. (1973) *J. Theor. Biol.* 40, 189-199.

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