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INTERSYSTEM EXCITON TRANSFER IN ISOLATED CHLOROPLASTS

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SUMMARY

The following arguments in favor of exciton transfer between the two photosystems are presented:

(1) $MgCl_2$ (1–10 mM range) decreases the intersystem transfer but does not modify the partition of absorbed photons between the photosystems. $MgCl_2$ addition causes a simultaneous increase of excitation life time (τ) and of fluorescence intensity (F). The same linear relationship is obtained with or without added Mg^{2+}

(2) The deactivation of Photosystem II by the Photosystem II to Photosystem I transfer increases with the level of reduced Photosystem II traps. When all Photosystem II traps are closed, half of Photosystem II excitons are deactivated by transfer to Photosystem I

(3) From the relative values of the 685-nm fluorescence yield and System II electron transport rate in limiting light, measured with and without $MgCl_2$, the values of rate constants of Photosystem II deactivation were calculated.

(4) The intersystem transfer determines a 715-nm variable fluorescence, which is lowered by $MgCl_2$ addition. When this transfer is decreased by $MgCl_2$ the efficiency of the transfer between Photosystem II-connected units is enhanced, and a more sigmoidal fluorescence rise is obtained

A double-layer model of the thylakoid membrane where each photosystem is restricted to one leaflet is proposed to explain the decrease of the intersystem transfer after adding cations. It is suggested that $MgCl_2$ decreases the thickness of the Photosystem I polar region, increasing the distance between the pigments of the two photosystems.

INTRODUCTION

The addition of cations to chloroplast suspensions induces changes of the partition of absorbed energy between the two photosystems[1–5]. These changes could result either from changing the partition of the sensitizers related to each photosystem (Hypothesis I) or by changing an intersystem exciton transfer (Hypothesis II, sug-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F, fluorescence intensity, τ, excitation lifetime

gested by Murata[1-3]). After considering the extensive analysis done by Malkin[6] and more recently by Sun and Sauer[7,8] on "spill-over" we concluded that additional experiments were needed in order to choose between these two hypotheses. The results reported here, measuring fluorescence and electron transport in the presence or absence of $MgCl_2$, argue in favor of Hypothesis II; *i.e.* there is a Photosystem II → Photosystem I exciton transfer which is inhibited by Mg^{2+}.

EXPERIMENTAL

Chloroplasts used in these experiments were isolated from lettuce leaves, according to the method of Nelson *et al.*[9].

Excitation lifetime fluorescence intensity relationship

Mg^{2+} induce an increase of the Photosystem II fluorescence intensity of chloroplasts. The effect is complete after 10 min in the dark or light. This phenomenon could result either from an increase of the quantum yield of fluorescence or from an increase of the sensitization of Photosystem II. In order to resolve this ambiguity, we measured fluorescence intensity (F) and lifetime (τ) simultaneously during Photosystem II induction, in the presence or absence of $MgCl_2$, using a phase fluorimeter previously described[10,11] The Photosystem II fluorescence induction was followed using the method of Lavorel[12].

The yield of Photosystem II variable fluorescence ρ_f is:

$$\rho_f = \frac{k_f}{k_f + k_p Q + k_c + k_{tl}} \tag{1}$$

where k_f, k_p, k_{tl} and k_c are the rate constants of deactivation, respectively, by fluorescence, reduction of Q, transfer to Photosystem I and other non-radiative processes. Q is the concentration of the oxidized form of Duysens' quencher. The excitation lifetime is.

$$\tau = \frac{1}{k_f + k_p Q + k_c + k_{tl}} \tag{2}$$

so,

$$\tau = \frac{1}{k_f} \cdot \rho_f \cdot \tau \tag{3}$$

Therefore, the intensity of variable fluorescence may be written as followed:

$$F = I \cdot a \cdot k_f \tag{4}$$

where I = incident intensity absorbed and a = fraction of photons absorbed by Photosystem II

Fig. 1 shows that the same linear relationship between τ and F is obtained either in presence or in absence of $MgCl_2$. This result means that the product ($I \cdot a$) of Eqn 4, which determines the slope (τ, F) has not been changed upon $MgCl_2$ addition. Therefore, this experiment points out that $MgCl_2$ induces a real increase of Photosystem II fluorescence yield (*i.e.* of ρ_f) instead of an increase of excitation

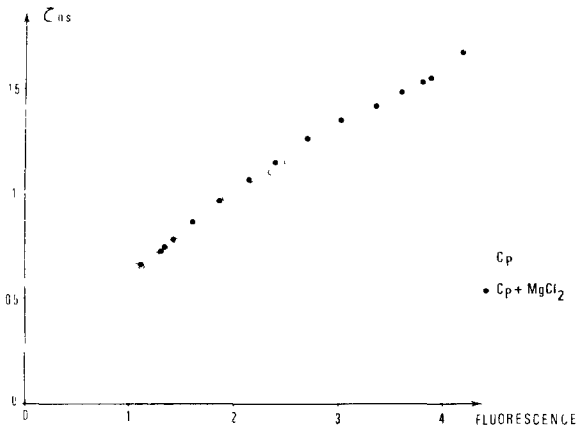

Fig 1 Excitation lifetime (τ)–fluorescence intensity (F) relationship during Photosystem II induction. A phase fluorimeter gave simultaneous measurements of F and τ The frequency of the modulated incident beam was 7 25 MHz The chloroplast suspension, 2-mm thick, contained 30 μg of chlorophyll per ml of a 10^{-2} M Tris–NaCl buffer (pH 7 8) and 0.4 M sucrose The induction was developed in a static way or by flowing By the latter mode, an actinic preillumination, used at various intensities is added 2 ms before fluorescence analysis. This gave different energy states corresponding to various intermediate levels between maximum and minimum fluorescence yields Excitation beam for both analysis and actinic light was blue light (Corning 4-96 filter). Fluorescence emitted is detected through a Corning 2-64 filter $-MgCl_2$, ○–○, $+MgCl_2$ (7 5 10^{-3} M), ●–●.

coming to Photosystem II centers. The increase of ρ_f must result from a change of at least one of the various ways of Photosystem II deactivation. The following experiments were designed to determine which of the deactivation processes are affected.

Effect of $MgCl_2$ on Photosystem II and Photosystem I photochemical rates

$MgCl_2$ was found to decrease the rates of Photosystem I-mediated Mehler reactions but increase the rate of Photosystem II-mediated O_2 evolution in either

TABLE I

EFFECT OF $MgCl_2$ ADDITION ON SYSTEM I AND SYSTEM II PHOTOREACTIONS

Stationary rates were measured in limiting light intensity at $\lambda = 647$ nm; (half bandwidth 12.5 nm) and at $\lambda > 695$ nm (Shott RG 695) The rate values are given in μmoles/mg chlorophyll per h Chloroplasts at 40 μg chlorophyll/ml were suspended in 0 01 M Tris–NaCl buffer (pH 7 8) which contained 0 4 M sucrose. When added, $MgCl_2$ concentration was 7 5 10^{-3} M. The System I photoreaction mixture contained DCMU, 10^{-4} M, sodium ascorbate, 10^{-2} M, methylviologen, 10^{-4} M, and sodium azide, 10^{-4} M. Light induced O_2 uptake was measured The System II reaction mixture contained 10^{-4} M dichlorophenolindophenol, O_2 evolution was determined A Clark-type electrode (Gilson Co.) was used to measure O_2 exchanges The magnitude of the error is +3 %. The temperature was maintained at 20 °C

Wavelengths of excitation (nm)	*O_2 evolution*			*O_2 uptake*		
	$+MgCl_2$	*$-MgCl_2$*	*$+MgCl_2/-MgCl_2$*	*$+MgCl_2$*	*$-MgCl_2$*	*$+MgCl_2/-MgCl_2$*
647	17 7	12.5	1 42	104	155	0 67
695	16 1	11.9	1 35	252	296	0 85

Photosystem II or Photosystem I light (Table I). This is in agreement with earlier reports of photochemical activities using Photosystem II illumination[1,13]. It should be noted, however, that the $MgCl_2$ effect on Photosystem I is greatest when Photosystem II sensitizers are preferentially excited. In addition, the ratio of $+Mg^{2+}/-Mg^{2+}$ rates is comparatively greater for Photosystem I at long wavelength than at short wavelengths. In contrast, the ratio of $+Mg^{2+}/-Mg^{2+}$ rates for Photosystem II vary little with the two different lights used.

Effect of $MgCl_2$ on the photochemical rise of Photosystem II fluorescence

As shown in Fig. 2, when all Photosystem II traps are open (F_0 level), a $MgCl_2$ effect occurs but its magnitude is very much smaller than when all Photosystem II traps are closed (F_M level) Although the fluorescence yield F_M is two times larger in the presence of $MgCl_2$, the half-rise of the fluorescence intensity is only slightly changed (27 ms without $MgCl_2$, 24 ms with $MgCl_2$). The half-time of Q reduction (determined by the integral of $(F_M - F_t)$ *versus* time (according to Malkin[14])) is 43.5 ms in the absence of $MgCl_2$ and 30.0 ms in the presence of this salt.

In addition, Fig. 2 shows that the shape of the photochemical fluorescence rise

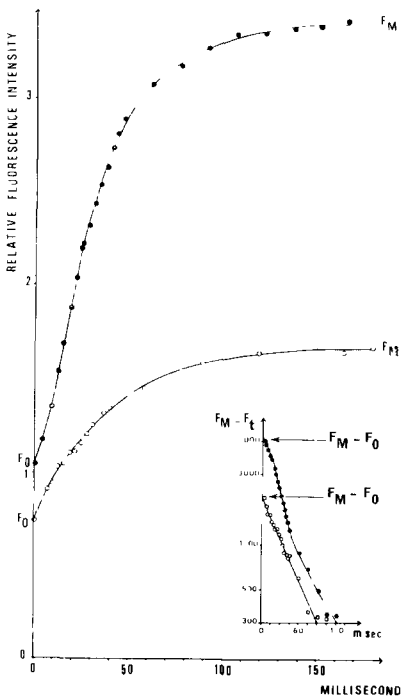

Fig. 2. Fluorescence induction of a chloroplast suspension. The reaction mixture contained chlorophyll (20 μg/ml), $5 \cdot 10^{-5}$ M DCMU, 0.4 M sucrose and 0.01 M Tris–NaCl (pH 7.8) in the presence (●–●) of $5 \cdot 10^{-3}$ M $MgCl_2$, or absence (○–○) of $MgCl_2$. The suspension was 1.5-mm thick. The excitation light (480 nm, $\Delta\lambda = 15$ nm) was both analytic and actinic, its intensity corresponds to 23 photons/center per second The fluorescence (F_t) emitted at 685 nm ($\Delta\lambda = 15$ nm) was measured. F_0, initial fluorescence yield; F_M, maximum yield obtained at the end of the photochemical phase of the induction. The inset corresponds to a log $(F_M - F_t)$ plot, calculated from the induction curves.

is clearly sigmoidal in the presence of $MgCl_2$ and nearly exponential in its absence (see the inset).

Effect of $MgCl_2$ on the relationship between 715 and 685 nm variable fluorescence

Lavorel[15] found that the emission spectra of variable fluorescence in *Chlorella* shows a 715-nm band, the kinetics of which cannot be differentiated from the fluorescence changes which occur at 685 nm As Fig. 3 shows, $MgCl_2$ addition on chloroplasts decreases the slope of the linear relationship which correlates 715- and 685-nm emission during the induction period. Notice that the two curves, with and without $MgCl_2$, intercept the 715-nm axis at the same point.

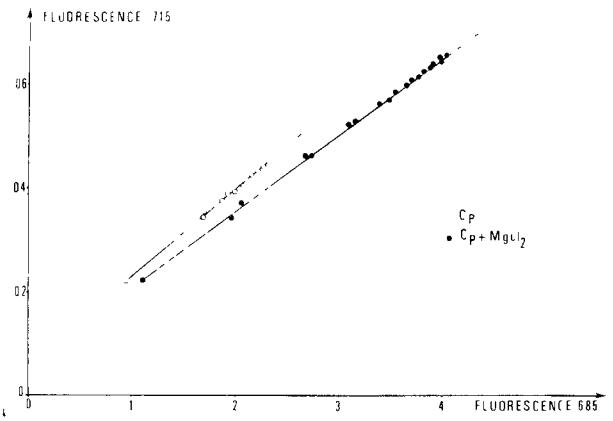

Fig 3 Effect of $MgCl_2$ on the relationship between variable fluorescences measured at 715 and 685 nm. ○–○, in absence of $MgCl_2$; ●–●, in presence of $MgCl_2$ ($7.5 \cdot 10^{-3}$ M). The fluorescence was recorded at 715 nm and 685 nm ($\Delta\lambda = 6.4$ nm). The F_0 level was measured by flowing the suspension in a capillary tubing 1.5-mm in diameter The fluorescence rise curve was obtained by stopping the flow Excitation beam 480 nm ($\Delta\lambda = 10$ nm) The chloroplast suspension was 0.4 M sucrose, 0.01 M Tris–NaCl buffer (pH 7.8) containing 20 μg chlorophyll/ml.

Variation of $MgCl_2$ effect depending upon the chloroplast preparations

A great variability of the magnitude of the magnesium effect has been observed along the preparations tested. In preparations which show the "classical" magnesium effect, the amplitude of the phenomenon varies. For example, representative data from three separate experiments are shown in Table II. It seems likely that at least two independent factors determine the amplitude of the $MgCl_2$ effect on Photosystem II fluorescence: (1) the number of active Photosystem II centers (approximated from the F_M/F_0 ratio) which regulate the effect of Mg^{2+} on the F_0 level and (2) a membrane state which regulates the Mg^{2+} influence on the F_M level. This may relate to our observations that some chloroplast preparations, including isolated grana and stroma lamellae separated after French press disruption[16], do not show a $MgCl_2$ effect on fluorescence. In some cases, these unusual preparations show an opposite effect of $MgCl_2$; *i.e.* a decrease in the 685-nm emission and a correlative increase in the Photosystem I Mehler reaction rate, using 650- or 670-nm lights at a limiting intensity.

TABLE II

COMPARISON OF THE VARIABILITY OF $MgCl_2$ EFFECT IN THREE CHLOROPLAST PREPARATIONS

685-nm fluorescence ($\Delta\lambda = 6$ nm) was measured. F_0 was determined by flowing the suspension, F_M corresponds to the steady state reached during constant illumination. Other conditions are the same as in Fig. 4.

	Prepn 1	*Prepn 2*	*Prepn 3*
$\frac{F_0(+MgCl_2)}{F_0(-MgCl_2)}$	1.17	1.4	1.07
$\frac{F_M(+MgCl_2)}{F_M(-MgCl_2)}$	1.75	2.05	2.10
$\frac{F_M(+MgCl_2)}{F_0(+MgCl_2)}$	3.70	3.30	5.55

DISCUSSION

We have attempted to gather evidence for the mode of action of Mg^{2+} in eliciting a change in the pattern of exciton distribution between Photosystems I and II. It was first shown (Fig. 1) that τ and F change in a parallel relationship with and without $MgCl_2$. Although the data does not show a rigorous linear relationship and extrapolate to a positive τ value at a zero F value, we can conclude that the observed F increase in the presence of divalent cation is a real increase in the fluorescence yield. A change in the partitioning of absorbed photons between the two photosystems or an introduction of Photosystem I to Photosystem II exciton transfer in the presence of $MgCl_2$ would have caused an increase in F without a change in τ. This was not observed.

We have discussed above the fact that the increase in ρ_f must be due to a reduction in the rate of some deactivation process. The rate constants for these processes were defined in Eqn 1. Each component of the equation will now be discussed with respect to our findings.

The rate constant for fluorescence, k_f, we assume does not change upon addition of $MgCl_2$ since the slope of F versus τ (Eqn 4) was not affected by salt addition. We can also conclude that $k_p \cdot Q$ is not the factor regulating the salt-induced change in the fluorescence yield since $k_p \cdot Q$ approaches zero in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (when the Mg^{2+} effect is maximal).

A decrease of k_c by $MgCl_2$ addition would give an increase of the yields of System II photochemistry and of Intrasystem II transfer, as we have observed. Such a change in k_c would not explain the relative decrease of the 715-nm variable fluorescence nor the inhibition of System I photochemistry which is preferentially sensitized at 647 nm.

The final factor included in Eqn 1 which might be affected by $MgCl_2$ is k_{tt} (excitation transfer from Photosystem II to Photosystem I). Reasons for believing that our data are most consistent with a decrease in this component are as follows.

The data of Table I indicate that there is a greater inhibition by $MgCl_2$ of Photosystem I in short wavelength light, but a uniform effect on Photosystem II

in either 647- or long wavelength light. This suggests that the spillover from Photosystem II to Photosystem I, but not the reverse, is the primary site of excitation distribution regulation.

Another argument indicating that k_{tI} is decreased by $MgCl_2$ addition comes from our finding that the τ–F slope does not change upon addition of the salt. This result means that $MgCl_2$ addition does not change the arrival of excitons to Photosystem II so the salt must not increase a Photosystem I to Photosystem II transfer.

Our data also suggest that there is increased Intrasystem II exciton transfer. The fluorescence induction was quite exponential in the absence of $MgCl_2$ but became sigmoidal when the salt is added (Fig. 2); according to Lavorel and Joliot[17], the sigmoidal kinetics are the result of connections between Photosystem II units.

A decrease in Photosystem II → Photosystem I excition transfer may explain the origin of a 715-nm band in the variable fluorescence, since this band may correspond to a Photosystem I emission produced by the transfer. It would determine that the kinetics of induction of the 715-nm band would follow the 685-nm fluorescence. The decrease of the slope of this linear relationship by $MgCl_2$ would result from the decrease of k_{tI}.

A mechanism by which $MgCl_2$ could elicit a change in k_{tI} must somehow be related to the structural organization of Photosystems I and II within the membrane If the intersystem transfer occurs according to a Förster process, it will occur mainly in the direction Photosystem II to Photosystem I because of the overlap between absorption and emission spectra of the two photosystems. Taking Photosystem I and Photosystem II enriched particles[18] as representative of each photosystem, the transfer Photosystem II to Photosystem I is five times more probable than Photosystem I to Photosystem II transfer on the overlap basis. No change of absorption spectra nor of the position of emission bands was detected on adding $MgCl_2$. therefore, we have to assume that the decrease of the transfer induced by $MgCl_2$ is the result of an increase of the distance between Photosystem I and Photosystem II pigments

Cation addition induces structural changes of chloroplasts[19–22]; glutaraldehyde fixation of chloroplasts suppresses the $MgCl_2$-induced increase in the 685-nm fluorescence[22]. Because many results on chloroplast structures suggest an asymmetrical distribution of the two photosystems in a bilayer membrane[23], we propose that Mg^{2+} increases the distance between the two polar parts of the membrane to which, tetrapyrole rings are linked. The fact that Mg^{2+} may cause a structural change in the membrane may be related to the finding that EDTA-washed chloroplasts show a depressed $MgCl_2$ effect[22]. Mg^{2+} is a cofactor of phosphorylation at the concentration which induces Photosystem II fluorescence yield increase The coupling factor (CF_1) is localized on the outside face of the chloroplast lamellae[23]. Thus we may suggest the following speculative scheme· Mg^{2+} would determine a conformational change of CF_1 and this in turn causes a shrinkage of the polar part of Photosystem I layer and increasing the distance between the two polar regions of Photosystem I and Photosystem II. It has previously been shown that Mg^{2+} reduces chloroplast membrane thickness[21]. This may not be inconsistent with our ideas, however, since salts are also known to induce conformational changes (substructural alteration) within the membrane itself[24].

APPENDIX

Magnitude of the various pathways of Photosystem II deactivation; rate constant values

The ratio of fluorescence yields (ρ_f) with and without magnesium, for instance when all the traps are closed (blue light, DCMU added) is:

$$\frac{\rho_f(+Mg)}{\rho_f(-Mg)} = 2 \quad \text{(average value from several experiments).}$$

The ratio of the photochemical yields (ρ_p) with and without magnesium, for instance when all traps are open (Corning 4-96 blue filter, limiting intensity) is:

$$\frac{\rho_p(+Mg)}{\rho_p(-Mg)} = 1.2 \quad \text{(average value from several experiments)}$$

We assume, in addition, that $MgCl_2$ (10 mM) abolish the transfer Photosystem II → Photosystem I (because such a concentration saturates the effect). In presence of DCMU, $k_p \cdot Q = 0$ ($Q = 0$) so

$$\frac{\rho_f(+Mg)}{\rho_f(-Mg)} = \frac{k_f + k_c + k_{tI}}{k_f + k_c} = 2, \qquad \text{so} \quad k_{tI} = k_f + k_c \tag{5}$$

Now when Q is completely oxidized.

$$\frac{\rho_p(+Mg)}{\rho_p(-Mg)} = \frac{k_p \cdot Q + k_f + k_c + k_{tI}}{k_p \cdot Q + k_f + k_c} = 1.2 \quad \text{so} \quad k_{tI} = 0.2\,(k_p \cdot Q + k_f + k_c) \tag{6}$$

combining (5) and (6)

$$k_p \cdot Q = \frac{1 - 0.2}{0.2}(k_f + k_c)$$

$$k_p \cdot Q = 4k_{tI} \quad (Q \text{ completely oxidized}) \tag{7}$$

from such values, one calculates $\rho_p\,(+Mg) = 0.80$, $\rho_p\,(-Mg) = 0.66$. The importance of the transfer to Photosystem I, ρ_{tI}, will vary, depending upon $Q_{red} \leftrightarrow Q_{ox}$ equilibrium:

When all Q is reduced:

$$\rho_{tI} = \frac{k_{tI}}{k_{tI} + k_f + k_c} = 0.5 \quad \text{because of (5)}$$

When all Q is oxidized.

$$\rho_{tI} = \frac{k_{tI}}{k_{tI} + k_f + k_c + k_p \cdot Q} = 0.166 \quad \text{because of (5) and (7)}$$

The values of rate constants can be calculated assuming the intrinsic lifetime $\tau_i = 15$ ns and a fluorescence yield = 0.027 when all the traps are open[25,26] and 0.108 when both DCMU and $MgCl_2$ are added from $\tau_i = 15\,\text{ns} = 1/k_f$, $k_f = 6.7 \cdot 10^7\,s^{-1}$ is deduced. If $\rho_f\,(+Mg+DCMU) = 0.108 = k_f/k_f + k_c$ then $k_c = 5.53 \cdot 10^8\,s^{-1}$ from

relation (5) $k_{tI} = 6.2 \cdot 10^8\ s^{-1}$ and from relation (7) $k_p \cdot Q = 2.48 \cdot 10^9\ s^{-1} \cdot M$ are reduced.

Value of k_{tII}

Let us consider again the deactivation in one Photosystem II unit. According to the computer calculation of J. Lavorel using Monte–Carlo method, to determine the kinetics of disappearance of open traps in a connected model of Photosystem II units[18], the efficiency of the transfer between two adjacent Photosystem II units is 0.7 in *Chlorella* cells, when all Q is reduced.

So, depending on the importance of k_{tI} in *Chlorella*, (from $0–6.2 \cdot 10^8\ s^{-1}$), k_{tII} would vary from $14.5–29 \cdot 10^8\ s^{-1}$ (values deduced from $\rho_{tII} = k_{tII}/k_{tII} + k_f + k_c \cdot k_{tI} = 0.7$).

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