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PHOTOCONVERSION KINETICS OF CHLOROPLAST PHOTOSYSTEMS I AND II

EFFECT OF Mg^{2+}

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The effect of divalent cations on the primary photoconversion kinetics of chloroplast Photosystems (PS) I and II was investigated by absorbance difference spectrophotometry in the ultraviolet (ΔA_{320}) and red (ΔA_{700}) regions and by fluorescence at room temperature. Three main chlorophyll (Chl) *a* fluorescence emission components were identified. Addition of 5 mM $MgCl_2$ to unstacked chloroplasts caused a 5–7-fold increase in F_{v_α}, the variable fluorescence yield controlled by the α-centers. The fluorescence yield F_{v_β} controlled by the β-centers and the nonvariable fluorescence yield F_0 were only slightly changed by the treatment. The absolute number of α- and β-centers remained unchanged and independent of divalent cations. The rate constants K_α, K_β and $K_{P\text{-}700}$ determined from the photoconversion kinetics of Q_α, Q_β and P-700 were also unchanged by divalent cations, suggesting a constancy of the respective absorption cross-sections. Evidence is presented that the Mg^{2+} effect on Chl *a* fluorescence is not due simply to unstacking. Conclusion: (1) In the absence of divalent cations from the chloroplast suspending medium, the variable fluorescence yield is not complementary to the rate of PS II photochemistry. (2) A spillover of excitation from PS II to PS I in the absence of Mg^{2+} cannot account for the 7-fold lowering of the variable fluorescence yield F_{v_α} at room temperature. The results are discussed in view of a model of excitation transfer and fluorescence emission in the pigment bed of PS II_α and PS II_β.

Introduction

The effect of divalent cations and especially that of Mg^{2+} on chloroplast structure and function has been investigated in many laboratories [1–9]. The most pronounced structural change in the absence of Mg^{2+} is the unfolding of the grana stacks [1,2], suggesting a specific Mg^{2+}-binding site on the partition region of these thylakoids [10,11]. A well documented functional change observed in the absence of Mg^{2+} involves the lowering of the Chl *a* fluorescence yield controlled by the reaction centers of PS II [3–5].

From the analysis of Chl *a* fluorescence kinetic data in the presence of DCMU, Melis and Homann [12,13] proposed the occurrence of two groups of PS II reaction center complexes, α and β, which differed, among other things, on the effective absorption cross-section or antenna size servicing them [14–18] and the midpoint redox potential of their primary electron acceptor Q [19–21]. A selective Mg^{2+} effect on the photochemical properties of PS II_α prompted the hypothesis that α-centers are exclusively located in

Abbreviations: PS, photosystem; P-700, primary donor of PS I; Q, primary acceptor of PS II; Chl, chlorophyll; LHC, light-harvesting complex; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-tris(hydroxymethyl)methylglycine.

the grana partition regions [22]. It was observed that omission of Mg^{2+} from the chloroplast suspending medium decreased the relative contribution of the α-center kinetic component in the area over the fluorescence induction curve [22] and disrupted the statistical pigment bed organization of PS II_α in favor of smaller aggregates or even separate units [5]. The corresponding parameters of PS II_β were not affected by the treatment. The observed results could be explained by a number of alternative hypotheses: (1) a reversible inactivation of α-centers in the absence of Mg^{2+} [23]; (2) a conversion of PS II_α into PS II_β caused by the uncoupling of the Chl *a*/*b* LHC from the α-centers upon unstacking [24]; (3) a facilitated transfer of excitation energy to PS I in Mg^{2+}-free media [4,5]; or (4) an increase in the rate of the internal conversion of excitation energy at the closed α-centers [25,26].

In the present investigation we examined the merit of each of the above-stated alternatives. We used Chl *a* fluorescence and absorbance difference spectroscopy in the ultraviolet (ΔA_{320}) and red (ΔA_{700}) regions of the spectrum in order to monitor the signal amplitude and photoconversion kinetics of Q_α, Q_β and P-700. Working with chloroplasts suspended in the presence and absence of Mg^{2+}, we verified that the divalent cation effect was exerted mainly on the functional and organizational conformation of PS II_α. Addition of Mg^{2+} to unstacked chloroplasts caused a 5–7-fold increase in F_{v_α}, the variable fluorescence yield controlled by the α-centers [15], while the fluorescence yield F_{v_β} controlled by the β-centers, and the nonvariable fluorescence yield F_0 remained practically unaffected. The absolute number of α- and β-centers remained unchanged by the Mg^{2+} treatment, thus excluding alternatives 1 and 2. The rate constants K_α, K_β and $K_{P\text{-}700}$, determined from the photoconversion kinetics of Q_α, Q_β and P-700 under light-limiting conditions in the presence of DCMU, were also unchanged by the Mg^{2+} treatment, suggesting that a spillover of excitation from PS II to PS I cannot fully account for the lowering of the fluorescence yield in Mg^{2+}-free media (thus excluding alternative 3). The results are explained by the assumption of an Mg^{2+}-dependent rate constant for the nonradiative decay of excitation process at the closed reaction center (P-680*Q^-) of PS II_α. Such Mg^{2+} dependence of the nonradiative decay process is not apparent at the reaction centers of PS II_β.

Materials and Methods

Chloroplasts were isolated from laboratory-grown pea plants by grinding leaves in 50 mM Tricine buffer, pH 7.8, containing 0.4 M sucrose and 10 mM NaCl. The chloroplast isolation procedure has been described previously [12]. Following centrifugation, the unstacked chloroplast pellet was resuspended in a buffer containing 10 mM Tricine, pH 7.8, 0.4 M sucrose and 10 mM NaCl. An aliquot of the chloroplast suspension was supplemented with $MgCl_2$ (5 mM final concentration) and incubated for a minimum of 30 min to yield stacked thylakoid membranes.

Developing chloroplasts were isolated from leaves of pea plants which were germinated in the dark for 7 days followed by 7 days under intermittent illumination as described in Ref. 27. The plastids were isolated and suspended in a 5 mM $MgCl_2$-containing medium.

Chloroplast fluorescence and absorbance difference measurements were performed with a laboratory constructed split-beam spectrophotometer. The optical path length of the cuvette for the measuring beam was 1.4 mm, and for the actinic beam (green light of uniform field transmitted by CS4-96 and CS3-70 Corning filters) it was 1.0 mm. PS I light (far-red) was transmitted by RG 695 Schott filters. Signal averaging was performed with a Tracor Northern NS-570A.

The reaction mixtures contained chloroplasts suspended in the appropriate buffer (chlorophyll concentrations are given in the figure legends). For Q and fluorescence measurements, 50 μM DCMU and 5 mM $K_3Fe(CN)_6$ were added. For the P-700 kinetic measurements, the chloroplast preparation was treated with KCN according to the method described in Ref. 28. Under our conditions, the cyanide treatment inhibited PS I-mediated electron transport by more than 95% (Chain and Melis, unpublished results), effectively preventing any secondary electron donation to $P\text{-}700^+$ at room temperature. The other parameters reported in this work were not affected by the cyanide treatment. The reaction mixture in the P-700

kinetic measurements contained 50 μM DCMU and 0.5 mM $K_3Fe(CN)_6$ as the electron acceptor.

The light-induced absorbance difference spectra were corrected for the particle-flattening effect on absorption bands by the method given in Ref. 29.

Results

Evaluation of the Mg^{2+} effect on PS II

To determine whether the lack of divalent cations caused a reversible inactivation of PS II reaction centers [23], we measured the amplitude of the light-minus-dark difference spectrum of the primary electron acceptor Q of PS II in the near ultraviolet region. Fig. 1 shows the results obtained with chloroplasts suspended in the presence of Mg^{2+} (open circles) and with those suspended in the absence of any divalent cations (solid circles). The signal amplitude at 320 nm is directly proportional to the number of photoconverted PS II reaction centers. With far-red preilluminated samples (to inhibit PS I-related changes), we consistently measured slightly lower amplitudes of ΔA_{320} in the absence of Mg^{2+} than in its presence. The small difference between the two samples (of the order of 10%) is attributed to the different rates of PS II restoration following the far-red preillumination. Therefore, it would not be interpreted as PS II inactivation in the absence of Mg^{2+}. In the presence and absence of Mg^{2+} we estimated the (functional) presence of 4.0 ± 0.5 μmol Q/1000 μmol Chl $(a+b)$.

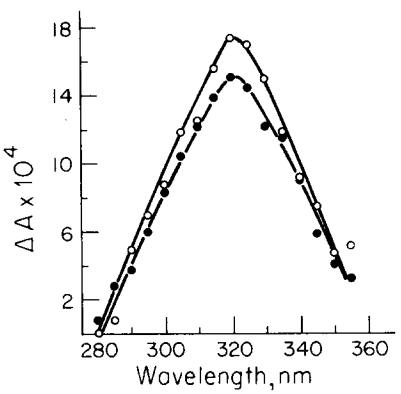

Fig. 1. The light-minus-dark difference spectrum of the primary electron acceptor Q of PS II obtained with chloroplasts suspended in the presence (○) or absence (●) of Mg^{2+}. Each point is the average value of four measurements. The chlorophyll concentration was 230 μg/ml. Samples were preilluminated by far-red light for 30 s prior to the measurement. A small fraction of PS II (15–20%) was also excited by the far-red light.

It was reported earlier [22] that omission of Mg^{2+} from the chloroplast suspending medium decreased the relative contribution of PS II_α in the measurement of the area over the fluorescence induction curve. This could be explained by the reversible transformation of PS II_α into PS II_β in cation-depleted chloroplasts, presumably by dissociation of the Chl a/b LHC from the reaction center core of the α-centers. Alternatively, the variable fluorescence yield F_{v_α} controlled by the α-centers [15] could have decreased significantly in the absence of divalent cations from the chloroplast microenvironment and, therefore, could equally well account for the results. To distinguish between the two alternatives we measured the relative concentrations of PS II_α and PS II_β directly from the kinetics of the absorbance change in the ultraviolet region (ΔA_{310}) and, indirectly, from the kinetics of the area over the fluorescence induction curve in the presence of DCMU. We reasoned that the relative concentrations of PS II_α and PS II_β in the ΔA_{310} measurement would be independent of any fluorescence yield changes occurring as a function of Mg^{2+}.

The analysis with chloroplasts suspended in the presence of Mg^{2+} produced results essentially identical to those already reported in detail in previous articles [14–16]. The kinetic traces of ΔA_{310} and fluorescence yield from chloroplasts suspended in the absence of Mg^{2+} are shown in Fig. 2. A first-order analysis of the kinetic data of ΔA_{310} and of the area over the fluorescence induction curve is shown in Fig. 2 (inset). From the semilogarithmic plots of the kinetics of $Q^-[t]$ and of the area over the fluorescence induction curve, we calculated the relative contribution of the α- and β-centers in the two measurements. This is accomplished simply by taking the respective intercepts of the slow β-phase with the ordinate at zero time (dashed lines in Fig. 2, inset).

Table I summarizes the results of such and analysis. In the presence of Mg^{2+} the relative number of α- and β-centers is approx. 65 and 35% of the total, respectively, measured either directly by Q (ΔA_{310}) or by the area over the fluorescence in-

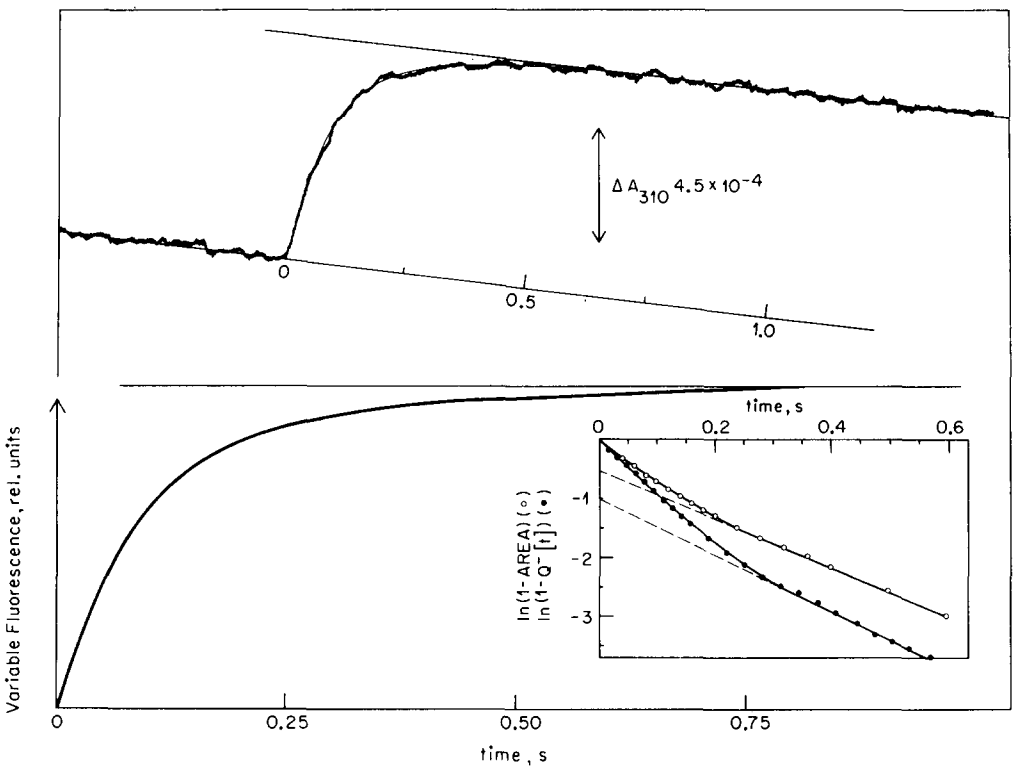

Fig. 2. Light-induced absorbance change (ΔA_{310}) and variable fluorescence kinetics of isolated chloroplasts in the absence of Mg^{2+}. The inset shows a semilogarithmic plot of the kinetic data of ΔA_{310} (●) and of the area over the fluorescence induction curve (○). The intercept of the linear β-phase with the ordinate at zero time (dashed lines) provided a measure of the contribution of the slow component in the two measurements. The ΔA_{310} kinetic curve is the average of 16 measurements, each with a fresh sample. The chlorophyll concentration was 220 μg/ml.

duction curve [14,15]. In the absence of Mg^{2+}, the relative number of α- and β-centers remained practically unchanged in the $Q(\Delta A_{310})$ measurement. A considerable lowering of the value of $AREA_\alpha$ was observed in the absence of Mg^{2+}. However, this change was not compensated for by a corresponding increase in the value of $AREA_\beta$ (see Table I). The results suggest, therefore, that a significant Mg^{2+}-induced transformation of PS II_α into PS II_β must be ruled out as an explanation

TABLE I

THE Mg^{2+} DEPENDENCE OF THE VARIOUS COMPONENTS OF Q (ΔA_{320}), AREA AND FLUORESCENCE YIELD OF CHLOROPLASTS

The absolute concentrations of Q_α and Q_β (in μmol/mmol Chl) were measured spectrophotometrically from the kinetics of ΔA_{320}. The kinetic analysis of the AREA has been presented in Ref. 22. The size (in relative units) of the various AREA components (α and β in $\pm Mg^{2+}$) has been arbitrarily normalized with respect to the $AREA_{max}$ ($+Mg^{2+}$) = 1.0. The fluorescence yield data have been normalized with respect to $F_0 = 1$ for the unstacked chloroplasts ($-Mg^{2+}$).

	Q_α	Q_β	$AREA_\alpha$	$AREA_\beta$	F_0	F_{v_α}	F_{v_β}	F_m ($F_{v_\alpha} + F_{v_\beta} + F_0$)
$+Mg^{2+}$	2.6	1.4	0.67	0.33	1.05	2.30	0.35	3.70
$-Mg^{2+}$	2.5	1.5	0.22	0.38	1.00	0.34	0.40	1.74

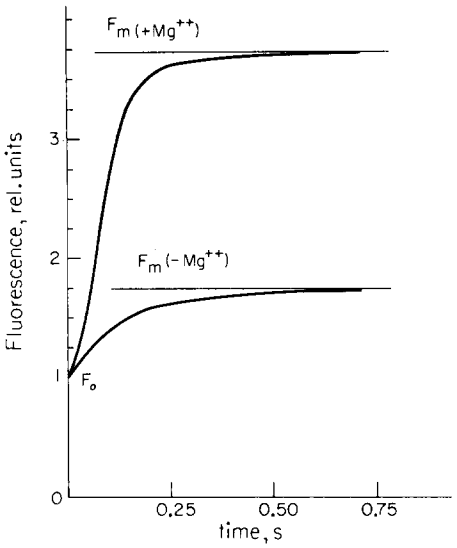

Fig. 3. Kinetics of chloroplast fluorescence in the presence and absence of Mg^{2+}. The reaction mixture contained 50 μM DCMU and chlorophyll at 260 μg/ml.

of the Mg^{2+} effect on $AREA_{\alpha}$.

The above results also suggest a differential effect of Mg^{2+} on the fluorescence yields F_{v_α} and F_{v_β} controlled by the α- and β-centers. We investigated this question quantitatively. Fig. 3 shows the fluorescence induction curves of DCMU-poisoned chloroplasts suspended in the presence and absence of Mg^{2+}. The overall fluorescence yield (F_m) in the absence of Mg^{2+} is less than half of that in its presence, and the difference clearly originates in the yield of the variable fluorescence F_v, since the nonvariable fluorescence level F_0 was only slightly changed by the treatment. To determine the Mg^{2+} dependence of the fluorescence yields F_{v_α} and F_{v_β} controlled by the α- and β-centers, respectively, we applied a first-order kinetic analysis to the variable part of the fluorescence induction curves. The semilogarithmic plots of F_v are shown in Fig. 4 for chloroplasts suspended in the presence (open circles) and absence (solid circles) of Mg^{2+}. From the intercepts of the linear phases with the ordinate at zero time and the respective amplitude of the variable fluorescence yield shown in Fig. 3, we estimated the relative fluorescence yields of F_{v_α} and F_{v_β}. Table I summarizes the results. In the presence of Mg^{2+}, the nonvariable fluorescence emission F_0 was slightly higher (from 1 to 10% in different measurements) while the fluorescence yield F_{v_β} controlled by the β-centers was slightly lower (about 15% of change). Interestingly, the fluorescence yield F_{v_α} controlled by the α-centers showed a significant 7-fold increase upon addition of Mg^{2+}.

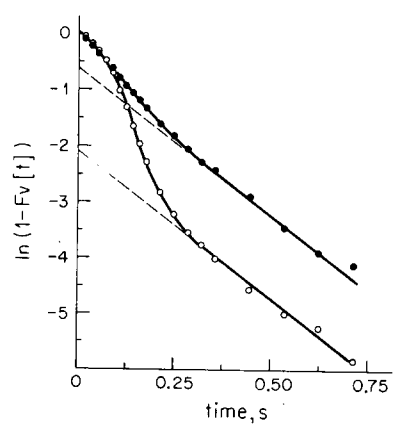

Fig. 4. First-order kinetic analysis of the variable part of the fluorescence induction curve obtained with chloroplasts in the presence (○) or absence (●) of Mg^{2+}. The intercept of the linear β-phase with the ordinate at zero time (dashed lines) gave a measure of the contribution of F_{v_β}, the fluorescence yield controlled by the β-centers. Note the parallel slopes of the β-phase in $\pm Mg^{2+}$ samples.

In order to distinguish structural from ionic effects in the relationship between the area growth over fluorescence induction kinetics with the absorbance change (ΔA_{320}) kinetics, we repeated the above measurements with developing pea chloroplasts. Such chloroplasts synthesize selectively Chl *a*, and are largely devoid of grana and of the Chl *a/b* LHC [27,31,32]. They also contain a larger complement of PS II_β as measured from the kinetics of the area growth [33] and from potentiometric titrations [34]. With developing chloroplasts suspended in the presence of Mg^{2+} we compared the light-induced kinetics of Q^- accumulation (ΔA_{320}) with those of the fluorescence induction curve in the presence of DCMU (Fig. 5). Fig. 5 (inset) shows semilogarithmic plots of the kinetics of the area growth (open circles) and Q^- (ΔA_{320}) accumulation (solid circles). The intercepts of the slow β-phase with the ordinate at zero time defined a 57% relative concentration for PS II_β from the area measurement, a figure similar to that obtained with unstacked ($-Mg^{2+}$) chloroplasts

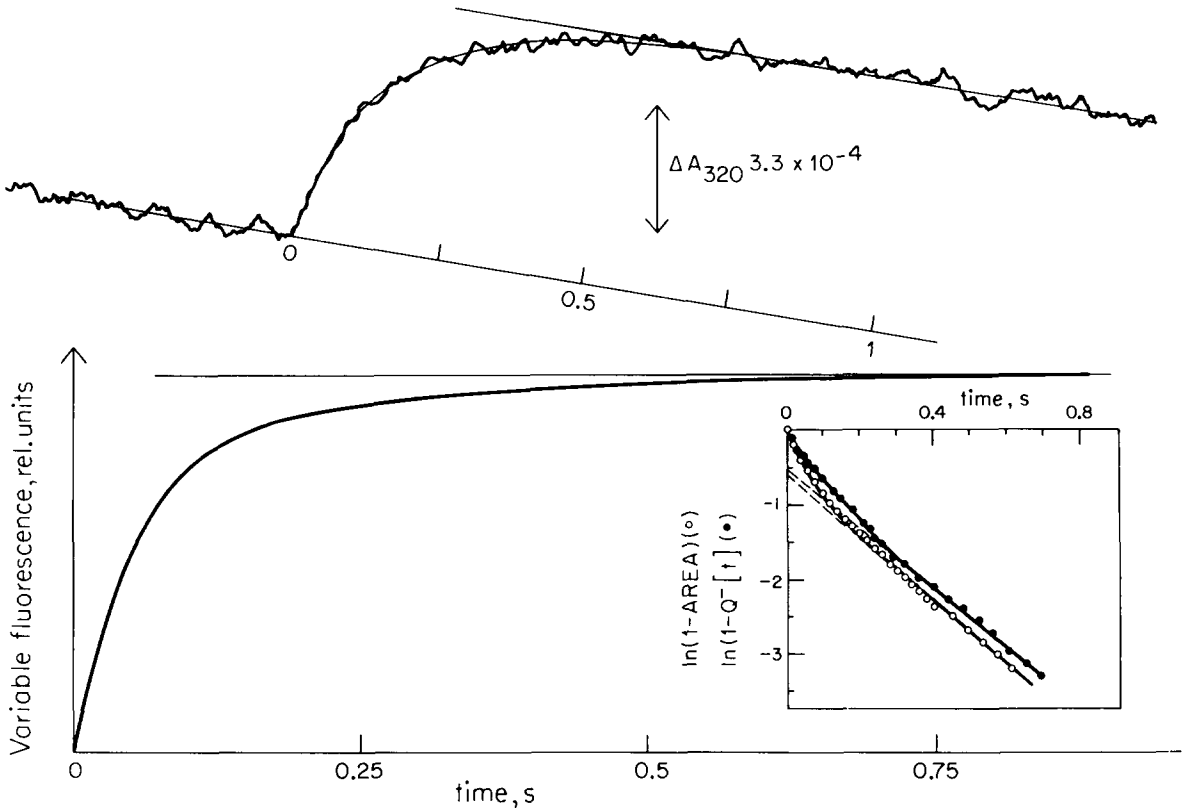

Fig. 5. Light-induced absorbance change (ΔA_{320}) and variable fluorescence kinetics of isolated developing chloroplasts suspended in the presence of Mg^{2+}. The Chl *a*/Chl *b* ratio was 5.2. The inset shows a semilogarithmic plot of the kinetic data of ΔA_{320} (●) and of the area over the fluorescence induction curve (○). The relative concentration of β-centers was estimated to be 61 and 57% from the ΔA_{320} and area, respectively. The ΔA_{320} kinetic curve is the average of 16 measurements, each with a fresh sample. The chlorophyll concentration of the sample was approx. 60 μg/ml.

(Fig. 2). With the developing chloroplasts, however, the biphasic kinetics of the area and the relative concentration of PS II_α and PS II_β strictly correlated with those of Q^- accumulation in the absorbance change measurement (Fig. 5, inset). Furthermore, the omission of Mg^{2+} from the suspension medium of developing chloroplasts had only a negligible effect on the relative expression of PS II_α and PS II_β in the area over fluorescence induction measurement (results not shown). It may be argued, therefore, that the effect of Mg^{2+} on mature chloroplasts is not simply due to unstacking, since in developing chloroplasts that normally contain few grana, kinetic measurements of Q^- and of area accumulation were identical.

The current hypothesis for the interpretation of the lowering of the chloroplast fluorescence yield in divalent cation-free media involves the concept of spillover, i.e., the transfer of excitation energy from the pigment bed of PS II to that of PS I reaction centers [4,35]. Such a phenomenon, it was argued, emanates from the decrease in light energy available to PS II and will result in a concomitant lowering of the rate of photochemistry and fluorescence yield at PS II. Since the excitation energy lost by PS II is transferred to PS I (spillover), the hypothesis predicted a corresponding increase in the rate of photochemistry at PS I reaction centers. In the absence of Mg^{2+} we measured a 7-fold decrease in the fluorescence yield F_{v_α} controlled by the α-centers. Under the same experimental conditions, however, the rate constants K_α and K_β for the photoconversion of Q_α and Q_β, under continuous weak illumination, remained practically unchanged by the Mg^{2+} treatment [22], suggesting a constancy of the antenna size for the two types of

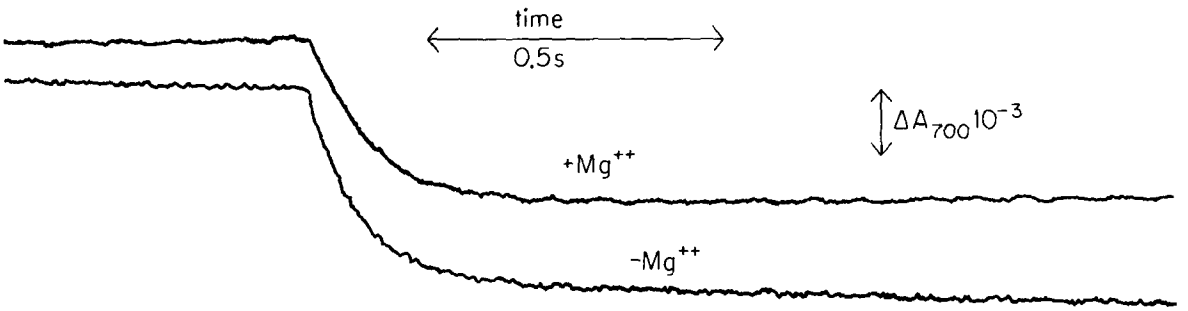

Fig. 6. Kinetics of P-700 photoconversion of cyanide-treated chloroplasts suspended in the presence and absence of Mg^{2+}. The kinetics represent the average of 16 measurements, each with a fresh sample. The reaction mixture contained 240 μg/ml Chl, 50 μM DCMU and 0.5 mM $K_3Fe(CN)_6$.

PS II. This is also evidenced in Ref. 5 where the half-rise of the fluorescence intensity was only slightly changed, although the fluorescence yield F_m was 2-times larger in the presence of Mg^{2+}. To obtain more information on this apparent contradiction we measured the rates of PS I (P-700) primary photoconversion in the presence and absence of Mg^{2+}.

Evaluation of the Mg^{2+} effect on PS I

The rate of PS I primary photoconversion depends on the incident light intensity and the effective absorption cross-section of its reaction center complexes. The latter parameter defines the number of chlorophyll molecules that transfer excitation to each P-700 molecule. We hoped to evaluate the Mg^{2+} effect on the effective absorption cross-section of PS I by defining the rate constant $K_{P\text{-}700}$ for P-700 photoconversion in the presence and absence of divalent cations. The correct determination of $K_{P\text{-}700}$ requires the elimination of all secondary electron-transfer steps from cytochrome *f* and plastocyanin to $P\text{-}700^+$, and also the elimination of any back-reactions between the primary photoproducts. Under such conditions, the rate constant $K_{P\text{-}700}$ is directly proportional to the incident light intensity and to the absorption cross-section of the PS I light-harvesting pigments. The elimination of all secondary electron transfer to $P\text{-}700^+$ is accomplished either at low temperature [16] or in KCN-treated chloroplasts which are apparently inhibited at the plastocyanin level [28,36]. In the present work we used the latter approach in our effort to eliminate any secondary electron transport to $P\text{-}700^+$. Back reactions at PS I were prevented by using an efficient electron acceptor ($K_3Fe(CN)_6$ or methyl viologen). With cyanide-poisoned chloroplasts suspended in the presence or absence of Mg^{2+}, we measured the rate of P-700 photoconversion (ΔA_{700}) upon a weak actinic illumination. Fig. 6 shows the kinetic traces obtained with $K_3Fe(CN)_6$ as the terminal electron acceptor. The same traces were observed with methyl viologen as the terminal electron acceptor. Under both experimental conditions ($\pm Mg^{2+}$), the photooxidized P-700 was restored very slowly (minutes) in the dark in the presence of DCMU and when ferricyanide or methyl viologen were used as the terminal electron acceptor. With cyanide-inhibited chloroplasts, the slow restoration pattern was independent of the duration of the actinic illumination (from a 10 μs flash to a 1 s continuous illumination).

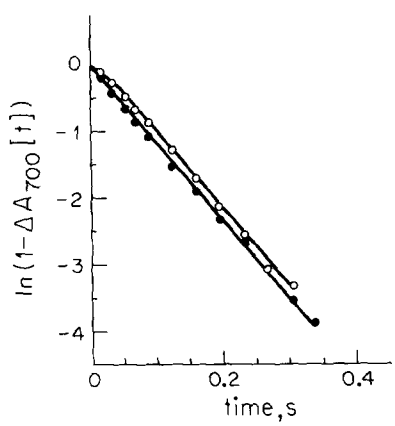

Fig. 7. First-order kinetic analysis of the kinetics of P-700 bleaching. Chloroplasts were suspended in the presence (○) or absence (●) of Mg^{2+}. Other conditions as in Fig. 6.

Determination of the rate constant $K_{P\text{-}700}$ required a first-order kinetic analysis, the semilogarithmic plot of which is shown in Fig. 7. The kinetic analysis of Fig. 7 revealed a small initial deviation from exponentiality in the rate of P-700 photoconversion of the $+Mg^{2+}$ sample. This initial deviation from linearity could be explained by the spillover hypothesis. An alternative explanation, however, is that of a secondary electron donation to $P\text{-}700^+$ from plastocyanin, a reaction that was not fully inhibited in the presence of Mg^{2+}. This contention was supported by the observation of the extent of nonlinearity which depended on the concentration of cyanide and time of incubation. At lower cyanide concentrations the nonlinearity was observed in the absence as well as in the presence of Mg^{2+}. Fig. 7 also shows that the main part (60%) of the photoconversion curve in the presence of Mg^{2+} occurred with monophasic exponential kinetics, in agreement with earlier results [16]. The slope of the two lines ($\pm Mg^{2+}$) in the semilogarithmic plot (which define the rate constant $K_{P\text{-}700}$) were identical, within a 10% experimental error, suggesting that the size of the light harvesting antenna of PS I remained unchanged in the presence or absence of Mg^{2+}.

Discussion

The results from our work identify three room-temperature components of fluorescence emission from the pigment bed of isolated chloroplasts (see Table I). The nonvariable fluorescence yield F_0 and the variable fluorescence yield F_{v_β} were found to be essentially independent of divalent cations. The well documented dependence of the yield of chloroplast fluorescence on divalent cations [3–11] is entirely accounted for by the changes observed in the yield of the variable fluorescence F_{v_α} controlled by the α-centers: upon addition of Mg^{2+} to a chloroplast suspension we detected a 7-fold increase in the yield of F_{v_α}. This phenomenon will adequately explain the dependence of $AREA_\alpha$ on Mg^{2+}, since the fluorescence induction area size is proportional to the corresponding variable fluorescence yield [13,26] (see Table I).

The 7-fold increase in the yield of F_{v_α} upon addition of Mg^{2+} was not accompanied by quantitatively similar changes in the yield of photochemistry either at PS II_α or at PS I. Therefore, there is no direct relationship or proportionality between Mg^{2+}-induced fluorescence yield changes and the yield of primary photochemistry at room temperature. The same evaluation was offered earlier by Butler and Kitajima [37] who concluded that a large part of the variable fluorescence yield change by Mg^{2+} could not be related to energy transfer between the two photosystems. In consequence of this conclusion, the applicability of room-temperature Chl *a* fluorescence in monitoring spillover must be reassessed.

The Mg^{2+}-independent rates of primary photoconversion at PS II and PS I reaction centers contradict numerous other indirect measurements involving rates of electron transport to artificial electron acceptors like ferricyanide, dichlorophenolindophenol and methyl viologen [3–8,38]. In such indirect measurements, the rates of linear electron flow through several components of the electron-transport chain were involved, and as such, they can be criticized for underestimating side effects of Mg^{2+} [39]. Examples may include the effect of Mg^{2+} on the interaction of P-700 with complexed plastocyanin [40], the Mg^{2+} dependence of the rate of electron transport through the plastoquinone pool (Melis, unpublished results) and the Mg^{2+} dependence of thylakoid membrane accessibility to various electron acceptors [30]. The approach used in this work is free of such criticism because it involved direct measurements of the rate of photochemistry at the reaction center complexes of PS II and PS I. The experimental conditions were carefully chosen to suppress secondary electron transfer to and/or from the reaction center, thus eliminating the possibility of interference from any Mg^{2+}-dependent secondary electron-transfer steps.

The Mg^{2+} dependence of F_{v_α} can be accommodated by a model of PS II_α based on the considerations of Butler and Kitajima [26] (see Fig. 8), in which incoming excitation will predominantly be converted to photochemical work when the reaction centers are open. The origin of the nonvariable fluorescence emission F_0 is excitation energy in the pigment bed before it has reached the reaction center P-680. Such emission will ap-

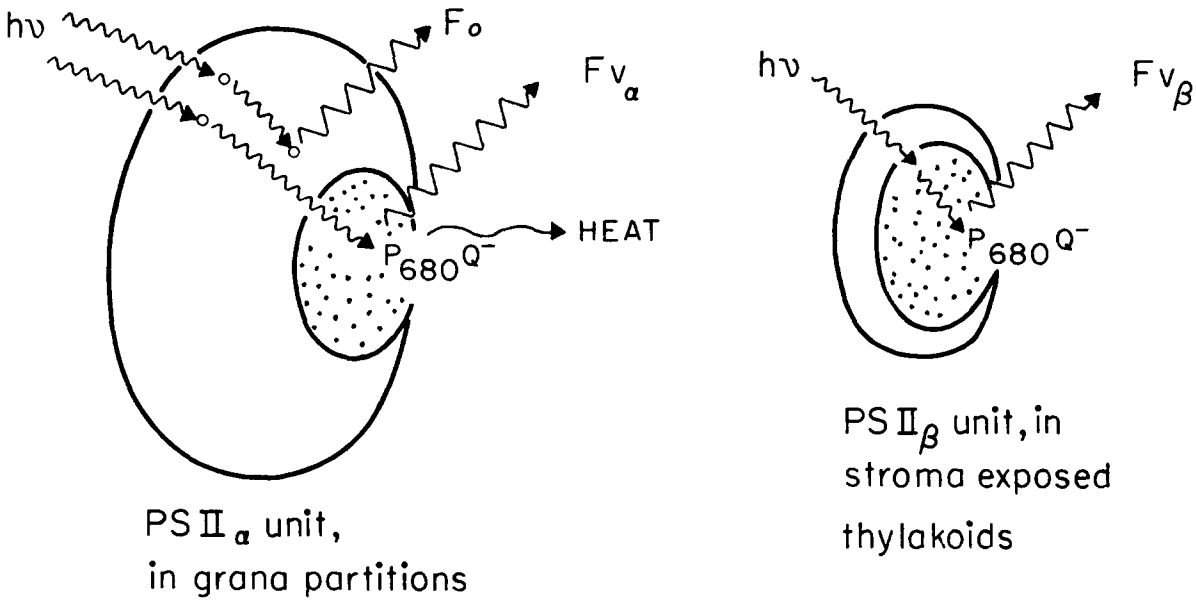

Fig. 8. Schematic presentation of the antenna composition and size of PS II$_\alpha$ and PS I$_\beta$. The dotted area represents the core Chl *a* pigment adjacent to the reaction center, while the unshaded area represents the Chl *a*/*b* LHC. Shown also is the excitation pathway in the pigment bed of PS II$_\alpha$ and PS II$_\beta$ and the origin of the nonvariable (F_0) and variable (F_{v_α} and F_{v_β}) fluorescence emissions from these photochemical units. The nonvariable fluorescence emission F_0 from the pigment bed of PS II$_\alpha$ and the variable fluorescence emission F_{v_β} from the pigment bed of PS II$_\beta$ are not Mg^{2+} dependent. The nonvariable fluorescence emission from the β-centers is negligible [14]. PS II$_\alpha$ occurs in the partition regions of grana in aggregates of four to six units, while PS II$_\beta$ occurs in stroma-exposed thylakoids as isolated units [14].

parently occur mostly from the light-harvesting pigment bed of PS II$_\alpha$ [14]. When the reaction centers are closed (Q in the reduced state), excitation energy trapped by the reaction center [41] will be unable to perform photochemical work and will be returned to the pigment bed where part of it will be emitted as variable fluorescence F_{v_α}. Since divalent cations have a small effect on the yield of F_0 but a profound effect on the yield of F_{v_α}, it may be argued that the amount of excitation returning from the closed α-center to its pigment bed is Mg^{2+} dependent. In reference to the photochemical model of Butler and Kitajima [26], the value of the rate constant K_d for the nonradiative decay process at the closed reaction center (P-680*Q$^-$) would be Mg^{2+} dependent (greater in the absence of Mg^{2+}, lower in its presence). Such a phenomenon would explain the Mg^{2+} dependence of the fluorescence yield F_{v_α}. In addition, the assumption of a photochemical rate constant K_p such that $K_p \gg K_d$ [26] will suffice to explain the apparent lack of Mg^{2+} effect on the rate of photochemistry at PS II$_\alpha$.

The results presented here cannot exclude the possibility that a small fraction of the excitation returning from a closed α-center to its pigment bed is subsequently transferred to the pigment bed of PS I centers. The physiological importance of this phenomenon is questioned, however, since it does not have any significant effect on the yield of trapping at either photosystem.

A similar excitation pathway and mechanism for fluorescence emission would occur in the pigment bed of the β-centers (see Fig. 8). In this case, the yield of the variable fluorescence emission F_{v_β} is largely Mg^{2+} independent. The small but consistent increase in the yield F_{v_β} observed in the absence of Mg^{2+} may actually indicate a limited transformation of PS II$_\alpha$ into PS II$_\beta$. This interpretation has not been investigated further in this work.

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