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Mg^{2+} ACCELERATES PLASTOQUINONE REDUCTION IN CHLOROPLASTS *

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The transient fluorescence quenching induced by the addition of a small amount of an oxidant to illuminated chloroplasts can be used to estimate the rate of electron transported by the oxidant. Using this technique, it is found that the reduction of plastoquinone by the primary acceptor of Photosystem II is sensitive to salt depletion.

Introduction

Under certain conditions, cations stimulate the rate of photosynthetic electron transport, and increase the Chl *a* fluorescence of chloroplasts, suggesting that light reactions are affected. These observations are usually ascribed to a change in the energy distribution among photosystems, and to that of the quantum efficiency of the photochemical reaction of PS II; both types of hypothesis assume, implicitly, that the same cation-induced change is responsible for the increase in the rate of electron transport and for the stimulation of fluorescence yield. These studies have been reviewed extensively, e.g., by Papageorgiou [1], Williams [2] and Lavorel and Etienne [3].

Although Jennings et al. [4–6] obtained evidence indicating that two distinct cation-binding sites may be involved, few authors postulate a direct effect of cations on secondary electron transport of PS II. Recently, Yamamoto and Ke [7] reported some effects of Mg^{2+} on secondary electron transport in isolated PS II particles and postulated that the cation inhibits electron donation to PS II and stimulates the reoxidation of Q^-. Conjeaud and Mathis [8] found that Mg^{2+} may accelerate the reduction of $P\text{-}680^+$ in Tris-washed chloroplasts.

In this communication, we present observations on chlorophyll *a* fluorescence of chloroplasts which indicate that cations are required for electron transport from Q^- to the plastoquinone pool.

Material and Methods

Chloroplasts were isolated from field-grown lettuce by the following methods. Four pieces of leaves (about 10 cm long) were rinsed (with distilled water), deveined, and hand-ground in 40 ml of buffer (25 mM Tricine (pH 7.3), 5 mM $MgCl_2$, 400 mM sorbitol), then filtered through a nylon mesh. Filtrates were centrifuged at a speed equivalent to $4\,340 \times g$ for 5 min, the resulting pellets were washed subsequently with 25 mM Tricine (pH 7.3), 5 mM $MgCl_2$ ($4\,340 \times g$, 5 min), followed by another wash with 400 mM sorbitol ($12\,100 \times g$, 5 min) and resuspended in 400 mM sorbitol as a stock. These stock chloroplasts were diluted 50–100-fold in doubly distilled water for fluorescence measurement.

Fluorescence, isolated with an interference filter (685 nm) and auxiliary red filters, was measured at

Abbreviations: DCIP, 2,6-dichloroindophenol; DCMU, 3-(3′, 4′-dichlorophenyl)-1, 1-dimethylurea; PS II, Photosystem II; P^+-680, oxidized primary electron donor of PS II; Q, primary electron acceptor of PS II; Tricine, *N*-tris(hydroxymethyl)-methylglycine; Chl, chlorophyll.

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90° from the exciting beam. Steady-state fluorescence excited by strong light was measured with an adapted Schoeffel photometer M460. Fluorescence induction of dark-adapted chloroplasts in response to illumination with continuous actinic light (opened by a fast mechanical shutter) was detected with a weak modulated (1 kHz) measuring beam, and the modulated signal was amplified by a lock-in amplifier (Princeton Research 129A) and auxiliary electronics (Princeton Research 181 and 189). Fluorescence excited by the measuring beam alone is defined as dark fluorescence, whereas difference between steady-state fluorescence in the presence of actinic light and the dark fluorescence is defined as variable fluorescence. Intensities of the actinic light are given in the figure legends. The actinic light (used also as a measuring beam in some measurements) was filtered by a broad-band interference filter (500–640 nm), a Corning filter (CS4-96), and a solution of $CuSO_4$. The intensity of the modulated measuring beam (480 nm) was unknown, but the following facts suggest that it was weak: adding 1 μM ferricyanide to chloroplasts suspended in Tricine/$MgCl_2$/sorbitol buffer did not affect the fluorescence yield; upon actinic illumination (28 kerg/cm^2 per s), the fluorescence yield increased more than 5-fold. O_2 evolution was measured with a membrane-covered Clark electrode thermostatically maintained at 26°C. To make sure that all samples had exactly the same concentration of chlorophyll, samples (Figs. 1–3) were taken from a chloroplast stock already diluted with distilled water to the required concentration of the experiment and kept at room temperature (near 29°C). For Fig. 2 the stock suspension had already been kept for 5 h, of which the third and the fourth hours were at 4°C and the rest at 29°C, to avoid a change in the half-times during the experiment: during the first few hours the half-times increased by about 25%.

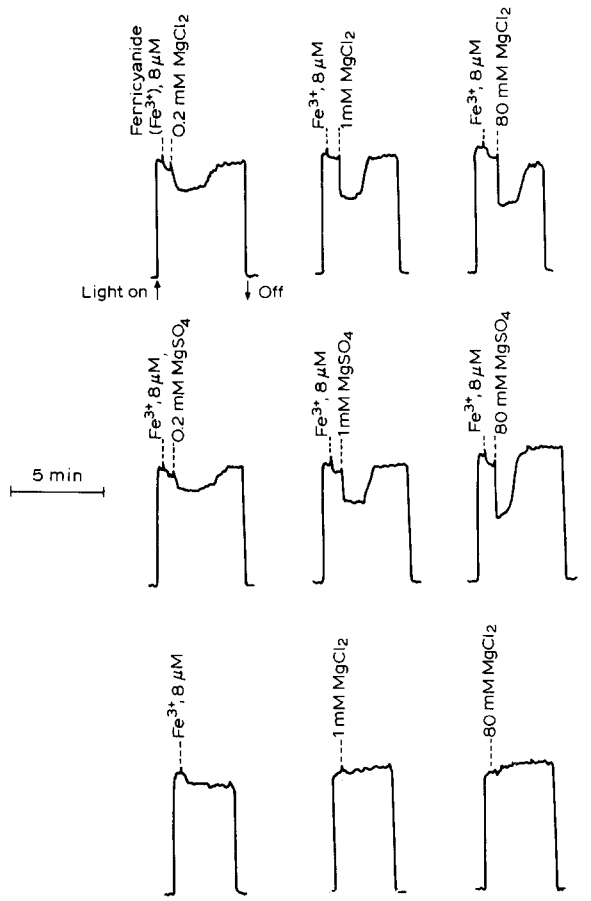

Fig. 1. Dependence on Mg^{2+} of oxidant-induced fluorescence quenching in salt-depleted chloroplasts. Chloroplasts (15 μg Chl/ml) were suspended in H_2O (containing about 8 mM sorbitol carried over from stock) at 29.5°C. Light intensity: 28 kerg/cm^2 per s. Except in the last two traces, 8 μM ferricyanide was added.

Results and Discussion

Malkin and Kok [9] suggested that the quenching of steady-state fluorescence by the addition of a small amount of Hill oxidant and the subsequent fluorescence rise correspond, respectively, to the initiation of a Hill reaction and the completion of the reduction of the substrate and then of Q. Comparative electron-transport and fluorescence studies justified this proposition [10,11]. One can therefore estimate the rate of electron transport by adding a known amount of oxidant and measuring the half-time ($t_{1/2}$) of the fluorescence dip induced by the oxidant.

Fig. 1 shows that ferricyanide, added to chloroplasts suspended in doubly distilled water, induced only a small fluorescence decrease, but subsequent addition of $MgCl_2$ or $MgSO_4$ introduced an instant temporary drop of the intensity of fluorescence, without affecting the steady-state level of fluorescence at high light intensity. At a concentration (1 mM) sufficient to saturate the effect of ferricyanide-dependent fluorescence quenching, $MgCl_2$ pro-

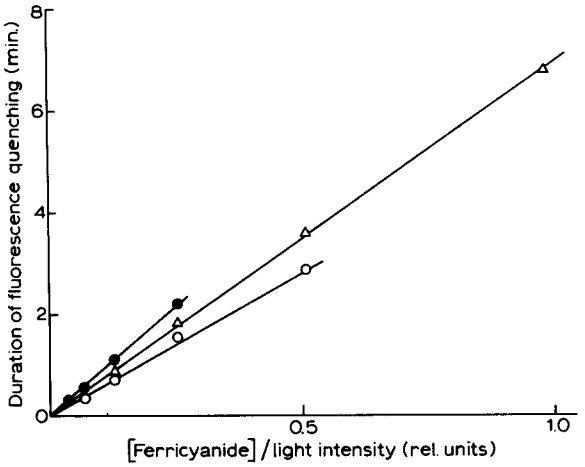

Fig. 2. Dependence of $MgCl_2$-induced fluorescence quenching on light intensity and ferricyanic concentration. The half-time of the fluorescence dip induced by 16 mM $MgCl_2$ is plotted. Chloroplasts (10 μg Chl/ml) were suspended in H_2O with 0.5 mg albumin/ml, at 29°C. Light intensities: 28 (•), 14 (○), and 7 kerg/cm^2 per s (△), adjusted with neutral density filters. Concentrations of ferricyanide, added in light, were 1, 2, 4, and 8 μM.

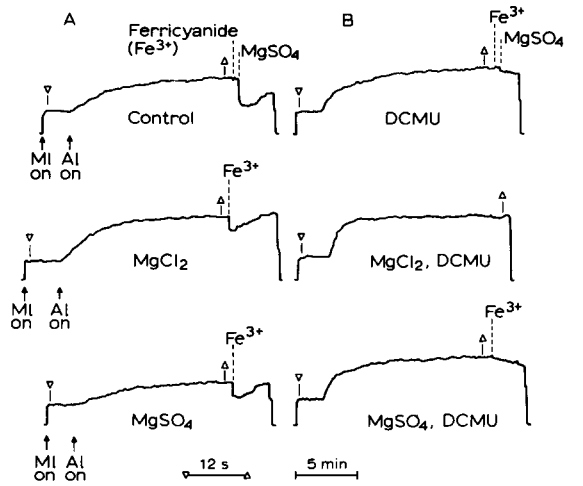

Fig. 3. Effects of $MgCl_2$ and $MgSO_4$ on induction rate and steady-state level of fluorescence under low light intensity in the absence (A) and presence (B) of DCMU. Chloroplasts (7 μg Chl/ml) were presuspended in H_2O supplemented with 0.5 mg albumin/ml at room temperature (29.5°C), and the actinic light intensity was 6 kerg/cm^2 per s. Mg^{2+}, ferricyanide (Fe^{3+}), and DCMU when added had a concentration of 4 mM, 1 μM and 4 μM, respectively. The time constant of the A.C. amplifier was set at 1 s. Between the two triangles (▽———△) the recorder chart was run 25-times faster than outside, MI on, measuring light on; AI on, actinic light on.

duced no appreciable effect on fluorescence in the absence of a Hill oxidant at this light intensity. The maximum rate of ferricyanide reduction in these experiments was 32 μequiv./mg Chl per h and was limited by the light intensity, as shown in Fig. 2.

Fig. 2 shows the duration ($t_{1/2}$) of the fluorescence dip plotted vs. the quotient of ferricyanide concentration and light intensity. The linear relationship between c/I and $t_{1/2}$ shows that the $MgCl_2$-induced quenching of fluorescence is caused by electron transport towards ferricyanide and suggests that salt depletion inhibits the reoxidation of Q^-, thus causing a high fluorescence yield upon illumination [12].

Fluorescence data in Fig. 3 were obtained by using a weak modulated measuring beam, with a low intensity of nonmodulated actinic light. $MgCl_2$ accelerates, but $MgSO_4$ decelerates, the fluorescence induction and the resulting steady-state variable fluorescence is correspondingly affected (Fig. 3A). Presumably oxygen was the electron acceptor in these measurements [13]. It is known that Cl^- is required for the H_2O-splitting system [14,15]. The acceleration of the rate of fluorescence induction by $MgCl_2$ may therefore be due to Cl^-. The slower fluorescence induction with $MgSO_4$ can perhaps be ascribed to an acceleration of Q^- reoxidation by Mg^{2+}, as suggested by the experiments of Figs. 1 and 2.

In the presence of DCMU to avoid the effect on Q^- reoxidation, $MgCl_2$, and to a much smaller extent also $MgSO_4$, accelerate the induction (Fig. 3B). An

TABLE I

Fluorescence induction in the presence of a PS II electron donor couple (400 μM ascorbate and 50 μM hydroquinone). Chlorophyll concentration: 8 μg Chl/ml. Actinic light intensity: 3 kerg/cm^2 per s. Temperature: 30°C. All parameters in relative units.

	Fluorescence induction half-time	Dark fluorescence	Variable fluorescence
Control	1.3	6.5	12.8
$MgCl_2$ (1 mM)	1.9	6	11
$MgCl_2$ (5 mM)	2.4	6	12

unusually large slow induction phase [16–18] is observed in the control (DCMU alone) and with $MgSO_4$, which may be explained by Cl^- depletion [14,15]. The acceleration by $MgSO_4$ may indicate that either Mg^{2+} or SO_4^{2-} can alleviate somewhat the inhibition by Cl^- depletion.

In the presence of an electron donor (hydroquinone) to avoid these effects on the oxidizing side of PS II [15], the measurements of Fig. 3A, i.e., fluorescence induction in the absence of DCMU at rather low light intensity, were repeated. As shown in Table I, $MgCl_2$ increases the half-time of the fluorescence rise. Since the steady-state fluorescence yield finally reached was unaffected, it seems that Mg^{2+} increased the number of electrons stored between the two photosystems, without modifying the rate-limiting step in electron transport. Presumably the equilibrium constant between Q and the plastoquinone pool is shifted towards Q reduction in the absence of cations. The rate-limiting step probably is the reoxidation of the pool by PS I, which in turn may be limited by the rate of the Mehler reaction [13].

Methyl viologen, an acceptor which can be reduced via PS I only, did cause some fluorescence quenching and this quenching was enhanced by $MgCl_2$ (not shown). Benzoquinone, which can accept electrons from the plastoquinone pool [19], was reduced 4-times faster in the presence than in the absence of $MgCl_2$ (Fig. 4). The transient fluorescence quenching by 2 μM benzoquinone lasts much longer in the absence of $MgCl_2$ and is also much smaller, so the acceleration by $MgCl_2$, which was confirmed by measurements of oxygen evolution (not shown), cannot be explained by the Cl^- effect.

Mg^{2+} might also accelerate electron transport from the plastoquinone pool to benzoquinone rather than plastoquinone reduction, especially because the fluorescence quenching by oxidized benzoquinone could in part be due to its direct effect on excited chlorophyll. With DCIP as acceptor this complication is avoided, but the extent of the quenching then is the same in the presence and absence of $MgCl_2$ although the photoreduction of DCIP is also accelerated by $MgCl_2$ (Fig. 5). Apparently, the more rapid oxidation of Q^- in the presence of Mg^{2+} was compensated by its more rapid accumulation in the presence of Cl^-. The fact that DCIP, in the absence of $MgCl_2$, caused a markedly more rapid oxidation of Q^- than benzoquinone may be due to different sites of

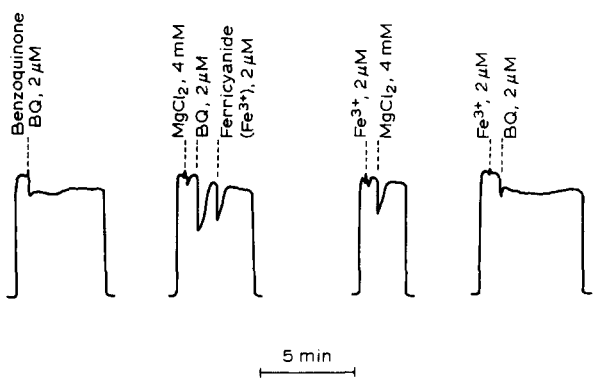

Fig. 4. Fluorescence quenching induced by benzoquinone. Chloroplasts (7 μg Chl/ml) were suspended in H_2O just before measurement. Room temperature was 32°C. Light intensity was 28 kerg/cm^2 per s.

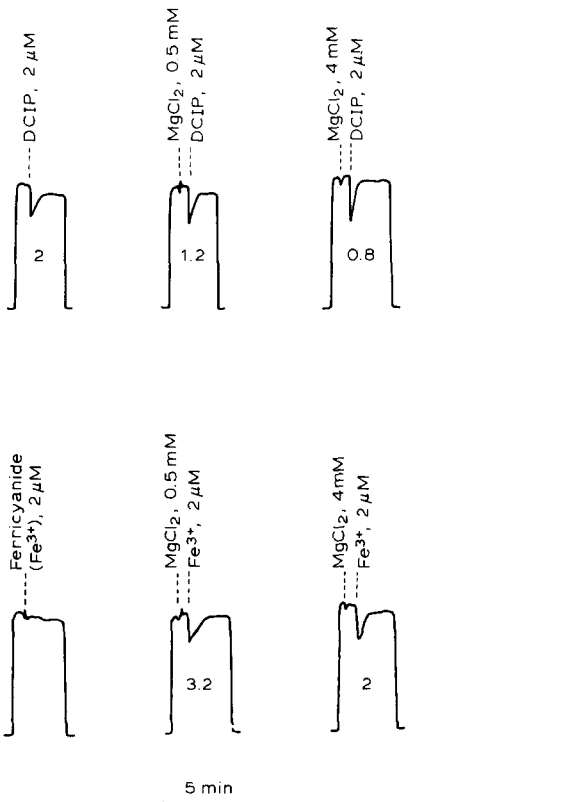

Fig. 5. Fluorescence quenching induced by DCIP and by ferricyanide. Concentration of chloroplasts was 9 μg Chl/ml. Room temperature was 24.5°C. Light intensity was 28 kerg/cm^2 per s. The number under each recording is the half-time of the dip in relative units.

action. DCIP probably is reduced at a site between Q and the plastoquinone pool [20], while benzoquinone reduction under these conditions possibly proceeds via the plastoquinone pool only.

It is noteworthy that the reduction of ferricyanide was more strictly dependent on Mg^{2+} than that of methyl viologen and benzoquinone. Probably Mg^{2+} is also required for accessibility of the membrane to the anionic ferricyanide. In the presence of $MgCl_2$ electron transport towards ferricyanide was 4-times slower than that towards DCIP (Fig. 5), and had about the same rate as benzoquinone reduction (Fig. 4) (note that DCIP and benzoquinone are two-electron acceptors).

If electron transport between Q and the pool is cation dependent, addition of $MgCl_2$ alone should cause a transient fluorescence quenching until a higher steady-state reduction level of the pool is reached. Using a low light intensity, so that the pool was largely in the oxidized state, Mg^{2+} was indeed found to quench fluorescence transiently (Fig. 6). Apparently, an endogenous acceptor pool is filled upon addition of $MgCl_2$. A 2-fold increase in light intensity shortened the duration of the dip, though not proportionally, suggesting that already at this intensity (4.5 kerg/cm^2 per s, green light) a significant fraction of the pool was in the reduced state. The initial extent of the quenching (depth of the dip, which depends on the reduction level of the pool) was saturated already at 2 mM $MgCl_2$. An increase in the concentration of $MgCl_2$ decreased the half-time ($t_{1/2}$) of the dip, and thereby also the 'void area' bound by the kinetics of the dip and the steady-state fluorescence level. Since it seems unlikely that the number of electrons stored in the pool decreased again at higher Mg^{2+} concentrations, the data suggest that more than 16 mM $MgCl_2$ was required to saturate the enhancing effect of Cl^- on the quantum efficiency of plastoquinone reduction.

The void area induced by $MgCl_2$ could be calibrated by comparison to that induced by known amounts of DCIP. The area created by 16 mM $MgCl_2$

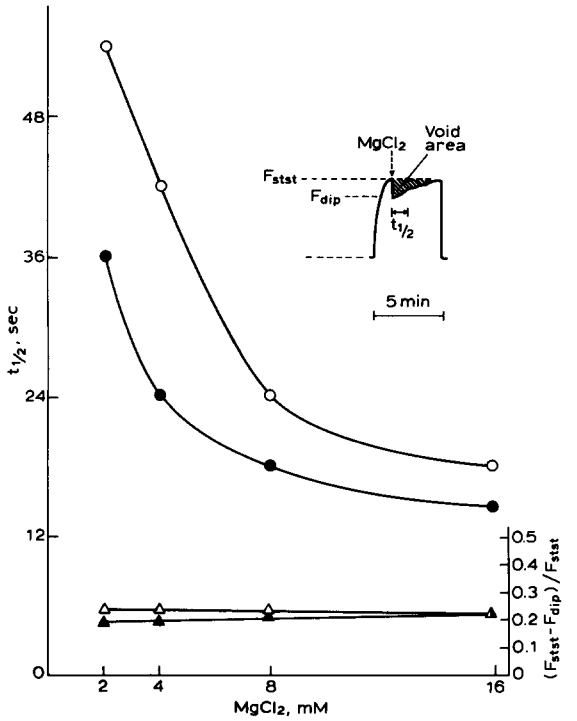

Fig. 6. $MgCl_2$-induced transient fluorescence quenching. Half-time ($t_{1/2}$; ○, ●) and extent of the fluorescence dip (△, ▲) as indicated in the insert. Chloroplasts were suspended in H_2O (7 μg Chl/ml) at 30°C. Light intensity was 4.5 (●, ▲) or 2.25 kerg/cm^2 per s (○, △). Room temperature was 30°C.

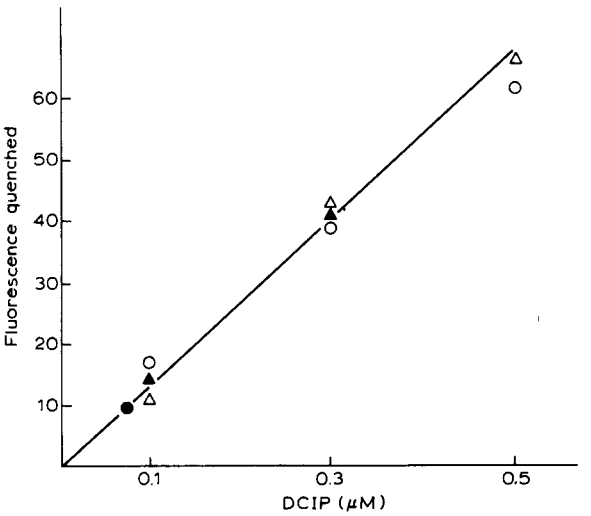

Fig. 7. The size of void area induced by DCIP vs. the concentration of the oxidant. Chloroplasts (8 μg Chl/ml) were suspended in H_2O at 30°C. Fluorescence quenched was measured by the void area induced by DCIP added in light to a sample either without $MgCl_2$ (△), or with 4 mM $MgCl_2$ (▲), or with 16 mM $MgCl_2$ (○) added before illumination. The solid circle (●) is the value of void area induced by 16 mM $MgCl_2$ with no Hill oxidant added, which corresponds to 1 electron/50 Chl.

was used for the estimation to ensure that electron transport on the donor side of PS II was fast so that fluorescence titrated only the oxidant pool on the acceptor side of PS II. From a plot of the concentration of DCIP vs. the void area created by the oxidant (Fig. 7) we estimate that $MgCl_2$ creates an area corresponding to 8 electrons per 400 Chl molecules at a light intensity of 6 kerg/cm^2 per s. At the lowest intensity used, 2.25 kerg/cm^2 per s, about 13 electrons were stored, which is nearly the total capacity of the plastoquinone pool [21].

On the basis of the data reported in this paper we conclude that cation depletion has a direct inhibitory effect on electron transport from Q^- to the plastoquinone pool.

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