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# HETEROGENEITY IN PHOTOSYSTEM I

## EVIDENCE FROM CATION-INDUCED DECREASE IN PHOTOSYSTEM I ELECTRON TRANSPORT

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**The rate of electron transfer through Photosystem I (reduced 2,6-dichlorophenol indophenol ($DCIPH_2$ → methylviologen) in a low-salt thylakoid suspension is inhibited by $Mg^{2+}$ both under light-limited and the light-saturated conditions, the magnitude of inhibition being the same. The 2,6-dichlorophenol indophenol (DCIP) concentration dependence of the light-saturated rate in the presence and in the absence of $Mg^{2+}$ shows that the overall rate constant of the photoreaction is not altered by $Mg^{2+}$. With *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine or 2,3,5,6-tetramethylphenylenediamine as electron donor only the light-limited rate, not the light-saturated rate, is inhibited by $Mg^{2+}$ and the magnitude of inhibition is the same as with DCIP as donor. The results are interpreted in terms of heterogeneous Photosystem I, consisting of two types, PS I-A and PS I-B, where PS I-A is involved in cation-regulation of excitation energy distribution and becomes unavailable for $DCIPH_2$ → methyl viologen photoelectron transfer in the presence of $Mg^{2+}$.**

### Introduction

It is a well-known phenomenon that when cations (3–5 mM $Mg^{2+}$ or 100–200 mM $Na^+$, for instance) are added to isolated chloroplast thylakoids suspended in low-salt buffer, the rate of Photosystem I electron transfer under limiting intensity of illumination decreases by about 20–30% [1,2]. This observation is generally interpreted as due to redistribution of excitation energy in favour of Photosystem II at the cost of Photosystem I [3–7]. It has been reported recently that the rate of PS I electron transfer (reduced 2,6-dichlorophenol indophenol ($DCIPH_2$) → methyl viologen) under saturated intensity of illumination also decreases by 20–30% upon addition of 5 mM $Mg^{2+}$ or 200 mM $Na^+$ [2,8]. This effect of cations cannot be explained in terms of the well-known cation effect of regulation of excitation energy distribution [8], because the rate at saturating intensity of illumination should not be influenced by alteration in the distribution of excitation energy. Two hypotheses have been proposed to interpret the high-light results: (i) the overall rate constant of the photoreaction $DCIPH_2$ → methyl viologen is decreased or (ii) a fraction of PS I complexes becomes unavailable to the electron donor ($DCIPH_2$).

The first hypothesis has the limitation that it fails to explain the cation effects with limiting light. The second hypothesis is sufficient to ex-

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Abbreviations: DCIP, 2,6-dichlorophenol indophenol; DAD, 2,3,5,6-tetramethylphenylenediamine (diaminodurene); TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; PS I, II, Photosystem I, II; Chl, chlorophyll; LHCP, light-harvesting chlorophyll-protein complex.

plain both the limiting-light and the saturating-light effects of cation; however, it cannot explain further the observations that with TMPD (or DAD) as electron donor only the light-limited rate is decreased by cations [2] and not the light-saturated rate [8]. In this paper data have been presented which exclude the first hypothesis and a model based on the second hypothesis has been proposed to explain the cation effects observed with both low and high intensities of illumination and with various electron donors. This model is an extension of an earlier one which assumes a heterogeneity in PS I and was proposed in the context of different results [9].

## Materials and Methods

Class II chloroplasts were isolated from peas (*Pisum sativum*) as described previously [10], except that $MgCl_2$ was omitted from the isolation medium. The chloroplasts were washed in 10 mM NaCl and resuspended in 20 mM Tris-HCl (pH 7.8)/400 mM sorbitol/20 mM NaCl. PS I electron transport was measured by continuous recording of $O_2$ uptake with a Clark type electrode (Yellow Springs, U.S.A.). The reaction mixture for PS I assay which contained (unless stated otherwise) 20 mM Tris-HCl (pH 7.8), 50 mM sorbitol, 20 mM NaCl, 50 $\mu$M DCIP or TMPD or DAD, 2 mM ascorbate, 2 $\mu$M DCMU, 5 mM $NH_4Cl$, 0.1 mM methyl viologen and chloroplast of 10–20 $\mu$g chlorophyll equivalent was magnetically stirred and thermoregulated by circulating water at 20°C. White actinic light of intensity of $10^6$ erg $\cdot$ cm$^{-2}$ $\cdot$ s$^{-1}$ was passed through 20 cm water before illuminating the sample. Chlorophyll was estimated by extracting with 80% acetone according to Arnon [11].

## Results

When the rate of electron transfer through PS I was measured by $DCIPH_2 \rightarrow$ methyl viologen photoreaction as a function of light intensity, it was observed that the rates both at the limiting and at the saturated light intensities were decreased by addition of $Mg^{2+}$ to a low-salt suspension of pea thylakoids. A double-reciprocal plot of the rate vs. light intensity (Fig. 1A) shows that the slope and the intercept were altered by $Mg^{2+}$ by similar magnitude (Table A in the legend of Fig. 1). With TMPD as donor, only the slope of the double-reciprocal plot was altered by $Mg^{2+}$ by the same magnitude as with DCIP as donor, while the intercept remained insensitive to $Mg^{2+}$ (Fig. 1B).

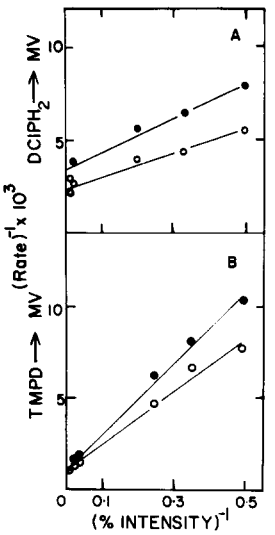


Fig. 1. Effect of $Mg^{2+}$ on PS I rate as a function of light intensity. A double-reciprocal plot between percent light intensity and PS I rate of $DCIPH_2 \rightarrow$ methyl viologen (MV) (A) and TMPD → methyl viologen (B) in the presence (●) and absence (○) of $Mg^{2+}$. The control PS I rate at a saturating light intensity of $DCIPH_2 \rightarrow$ methyl viologen and TMPD → methyl viologen varied between 300 and 400, 400 and 600 $\mu$eq/mg Chlh, respectively. Each point was a mean of data obtained from three experiments. 100% light intensity was $10^6$ erg$\cdot$cm$^{-2}$ $\cdot$s$^{-1}$. The PS I reaction mixture was as given in Materials and Methods. The Table A and B show the correlation coefficient, slope and intercept obtained statistically.

Table A. $DCIPH_2 \rightarrow$ methyl viologen

| | $-Mg^{2+}$ | $+Mg^{2+}$ |
|---|---|---|
| Corr. Coefficient | 0.9856 | 0.9844 |
| Slope | 0.0064 | 0.0093 |
| Int. | 0.0024 | 0.0034 |

Table B. TMPD → methyl viologen

| | $-Mg^{2+}$ | $+Mg^{2+}$ |
|---|---|---|
| Corr. coefficient | 0.9916 | 0.9972 |
| Slope | 0.0144 | 0.0191 |
| Int. | 0.0011 | 0.0012 |

TABLE I

INFLUENCE OF ELECTRON DONORS ON THE EFFECT OF $Mg^{2+}$ ON PS I ELECTRON TRANSPORT AT LIMITING LIGHT INTENSITY

Reaction conditions were the same as in Materials and Methods, except that the light intensity was 0.4%. DCIP, TMPD and DAD were used at a final concentration of 50 $\mu$M.

| Donors | $DCIPH_2 \rightarrow MV$ Rate in $\mu$equiv/mg Chl per h | | Ratio +/ $-Mg^{2+}$ |
|---|---|---|---|
| | $-mg^{2+}$ | $+Mg^{2+}$ | |
| $DCIPH_2$ | 80 | 58 | 0.72 |
| TMPD | 84 | 64 | 0.76 |
| DAD | 83 | 62 | 0.75 |

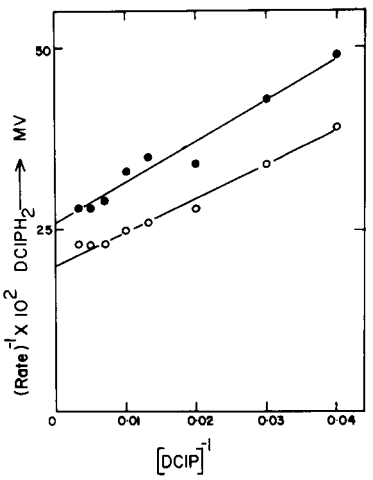


Fig. 2. Double-reciprocal plot between the rate of PS I electron transport ($DCIPH_2 \rightarrow$ methyl viologen (MV) and DCIP concentration in the presence and absence of $Mg^{2+}$. PS I assay conditions were the same as described in Materials and Methods, except that $Mg^{2+}$ concentration, when present, was 5 mM and DCIP concentration varied. (●———●) Plus $Mg^{2+}$; (○———○) minus $Mg^{2+}$.

| $Mg^{2+}$ | Corr. coefficient | $K_m$ |
|---|---|---|
| Minus | 0.99 | 21.71 |
| Plus | 0.98 | 21.74 |

Table I summarizes the results on the effects of $Mg^{2+}$ obtained from parallel experiments on the light-limited rate of PS I electron transfer with different electron donors. With all the three donors tested, a similar magnitude of inhibition by $Mg^{2+}$ was observed. In the same experiment, the light-saturated rate with DCIP as donor was decreased by $Mg^{2+}$ by about a similar magnitude as the light-limiting rates, while that with TMPD was not affected by $Mg^{2+}$ (data not shown), confirming earlier observations [8].

The $Mg^{2+}$-induced decrease of the light saturated rate of $DCIPH_2 \rightarrow$ methyl viologen electron transfer was independent of the DCIP concentration used. A double-reciprocal plot of the rate against concentration (Fig. 2) shows that the $K_m$ values for DCIP with and without $Mg^{2+}$ were the same, suggesting that the overall rate constant of electron transfer was not affected by $Mg^{2+}$. It should be pointed out that several of such experiments were performed, and no significant change in the $K_m$ value was observed upon addition of $Mg^{2+}$ [21]. The plot also shows a non-competitive type of inhibition, suggesting that DCIP and $Mg^{2+}$ did not attack the same site in the membrane.

It has been argued that $Mg^{2+}$ may inhibit the interaction between the negatively charged PS I and positively charged methyl viologen, resulting in a decrease of the light-saturated rate of PS I electron transfer (Barber, J., Good, N.E. and Nakatani, H.Y., personal communication). The influence of this possible effect of the PS I rate was avoided by using saturating concentration of methyl viologen in all the experiments. Nevertheless, to test whether $Mg^{2+}$ inhibits the interaction between PS I and methyl viologen, the PS I rate was measured as a function of methyl viologen concentration. The results (Fig. 3) show that $Mg^{2+}$ inhibited the rate more at limiting methyl viologen concentration than at saturating concentrations. This result indeed suggest an additional effect of $Mg^{2+}$, namely, $Mg^{2+}$ inhibited the interaction between PS I and methyl viologen. But at a saturating concentration of methyl viologen, the inhibition observed with $Mg^{2+}$ was obviously not influenced by this additional effect. Earlier observations that the PS I rate (at saturated intensity of illumination) was unaffected by $Mg^{2+}$ when other donors (TMPD or DAD) were used [8] also indicated that this additional effect of $Mg^{2+}$ did not influence the PS I rate at saturating concentration of methyl viologen. These results indicate that care should be taken in choosing a methyl viologen

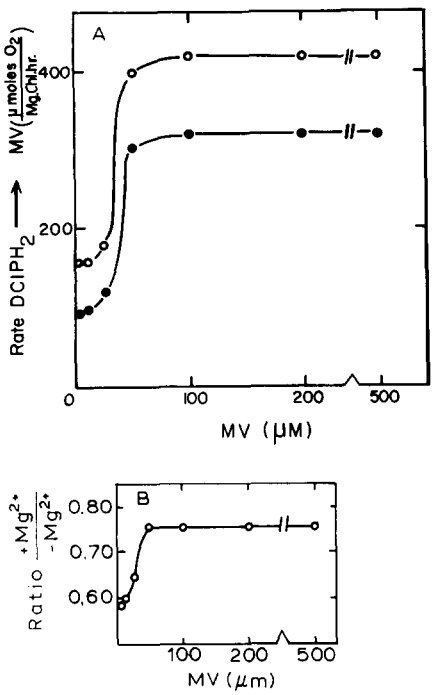


Fig. 3. (A) The rate of PS I electron transport ($DCIPH_2 \rightarrow$ methyl viologen (MV)) as a function of methyl viologen concentration in the presence and absence of 5 mM $Mg^{2+}$ in the reaction medium (B) The ratio between plus $Mg^{2+}$ to minus $Mg^{2+}$ of PS I rates as a function of methyl viologen concentration. Other PS I assay conditions were the same as those given in Materials and Methods. ●———●, Plus $Mg^{2+}$; ○———○, minus $Mg^{2+}$.

concentration to avoid the influence of interaction between PS I and methyl viologen on the $Mg^{2+}$ induced effects on PS I.

## Discussion

The decrease in the light-limited rate of PS I electron transport by addition of $Mg^{2+}$ to a low-salt suspension of thylakoids is generally interpreted as a consequence of decrease in excitation energy transfer to PS I from PS II (spill-over, $T_{II \rightarrow I}$) and from the light-harvesting chlorophyll-protein complex ($\alpha$-change) (see Ref. 12 for a review). The $Mg^{2+}$-induced decrease in the light-saturated rate of PS I with DCIP as donor as observed earlier [2,8] and reported in this paper requires a different mechanism, because the light-saturated rate of a photosystem should not be influenced by changes in the rate of the excitation energy arriving at the photosystem. One possible mechanism could be the decrease in the overall rate constant of the reaction ($DCIPH_2 \rightarrow$ methyl viologen) which is supposed to be located at the electron donation step by DCIP [8]. The double-reciprocal plot of the rate vs. DCIP concentration, however, excluded this possibility, because the $K_m$ value remained unchanged upon addition of $Mg^{2+}$ (Fig. 2). As an alternative mechanism it has been proposed that a fraction of PS I becomes unavailable to $DCIPH_2 \rightarrow$ methyl viologen photoreaction and consequently does not function in transfering electron to methyl viologen. This proposal implies a heterogeneous character of PS I. Heterogeneity in PS I has been proposed earlier from various experimental observations [2,9,13–16]. Hahnel has reported three kinetic components of P-700 behaving differently depending on the presence or absence of $Mg^{2+}$ [16]. From the measurements of chlorophyll fluorescence, NADP reduction and electron flux through P-700, Hoch and Bose [9] have postulated the existence of two types of PS I, namely, PS I-A and PS I-B, where PS I-A receives the excess excitation energy from PS II in the absence of $Mg^{2+}$ and this sensitization is blocked when $Mg^{2+}$ is present.

The proposed mechanism that a fraction of PS I becomes unavailable for electron transport from $DCIPH_2 \rightarrow$ methyl viologen in the presence of $Mg^{2+}$ is sufficient to explain the cation-induced decrease in PS I rates both under limiting and saturated light intensities, not only qualitatively but also quantitatively, because both the slope and the intercept of the double-reciprocal plot of the intensity dependence of the rate changed by the same magnitude upon addition of $Mg^{2+}$ (Fig. 1A). This mechanism, however, is inadequate to explain the cation effects with TMPD (or DAD) as electron donor. In the reaction with TMPD (or DAD), only the light-limited rate decreased, while the light-saturated rate remained unaffected (Fig. 1B). This observation is consistent with the hypothesis of cation-induced regulation of excitation energy distribution ($T_{II \rightarrow I}$ and $\alpha$-change). In fact, the striking similarity in the magnitude of decrease in the light-limited rates for all the three donors used (Table I) suggests a similar, if not the same, mechanism of $Mg^{2+}$ action at least on the light-limited rates.

All the observations described above (with different electron donors and light intensities) can be explained in terms of the heterogeneity in PS I by postulating further that it is the PS I-A fraction (which, according to Hoch and Bose [9], is involved in cation regulation of excitation energy transfer) that becomes unavailable to $DCIPH_2$ for electron donation in the presence of cations. The scheme illustrated in Fig. 4 explains the results. The essential features of the scheme are: (1) $Mg^{2+}$ blocks excitation energy transfer from PS II ($T_{II\rightarrow I}$ and from LHCP ($\alpha$-change) to PS I-A only, and (b) $Mg^{2+}$ blocks electron donation to PS I-A when $DCIPH_2$, but not TMPD or DAD, is the electron donor. Thus, either in the case of low intensity of illumination with any of the three electron donors or in the case of high intensity of illumination with $DCIPH_2$ as electron donor, it is the PS I-A that is not available for electron transfer to methyl viologen in the presence of $Mg^{2+}$, resulting in a decrease by similar magnitude in the rate of electron transfer.

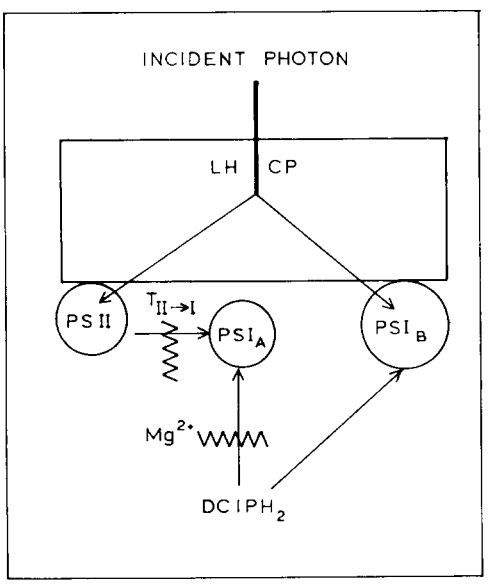


Fig. 4. Schematic illustrations of the effects of $Mg^{2+}$ on PS I electron transport. PS I-A: a PS I fraction which receives excess excitation ($T_{II\rightarrow I}$) from PS II in absence of $Mg^{2+}$; $Mg^{2+}$ blocks $T_{II\rightarrow I}$. $DCIPH_2$ donates electrons to PS I-A in absence of $Mg^{2+}$. $Mg^{2+}$ blocks electron donation to PS I-A. PS I-B: the other PS I fraction which is not involved in spill-over, and donation of electrons by $DCIPH_2$ to this photosystem is not influence by $Mg^{2+}$.

It would be interesting to examine whether the functional heterogeneity in PS I could be attributed to some kind of structural heterogeneity. It is well known that the distribution of the two photosystems in the unstacked (under low-salt condition) membranes is distinctly different from that in the stacked (high-salt condition) membranes (see Ref. 17–19 for reviews). It is generally believed that a minor fraction of PS I remains in the appressed region and in the end membranes of the grana, while the major fraction of PS I remains in the stroma membranes [17,19,20]. From the observation that the $Mg^{2+}$-induced decrease in PS I rates correlates with the corresponding increase in the degree of stacking of the thylakoid membranes [8], it is tempting to equate the PS I in the grana to PS I-A in the proposed scheme. A structural organisation of this nature would allow TMPD and DAD to be better electron donors as compared to $DCIPH_2$, as TMPD and DAD will have better access to all PS I complexes because of their higher lipophillicity than that of $DCIPH_2$.

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