EFFECT OF DIVALENT CATIONS ON FERREDOXIN-LINKED ELECTRON TRANSPORT IN CHLOROPLASTS

G. HARNISCHFEGER* and N. SHAVIT
Biology Department, Ben-Gurion University of the Negev, Beer-Sheva, Israel

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1. Introduction

The effect of divalent cations on electron transport in chloroplasts has been studied previously and explained mainly in connection with the control of excitation transfer. Murata [1] reported the acceleration by Mg^{2+} of the rate of DPIP reduction and the inhibition of NADP reduction with $DPIPH_2$ as electron donor. Avron and Ben Hayyim [2] observed an increase in quantum requirement in the reduction of NADP upon addition of Mg^{2+}. Rurainski and Hoch [3] compared the rate of reduction of P_{700} and NADP. In the presence of Mg^{2+} they observed an increase in the rate of reduction of NADP, an effect also obtained in the presence of an uncoupler and a decline in the flux of P_{700}. The stimulation of the rate of NADP reduction in uncoupled chloroplasts was recently confirmed by Shoshan [4] and Harnischfeger [5] suggested that these effects of divalent cations might be of physiological significance in the control of photosynthetic activity.

This communication deals with the effect of Mg^{2+} on several partial photoreactions of the photosynthetic electron transport chain, both in the coupled and uncoupled state. The enhancement of NADP and cytochrome *c* reduction by Mg^{2+}, reactions requiring the participation of ferredoxin, might be explained as a stimulation of ferredoxin activity by divalent cations.

* Permanent address: Lehrstuhl für Biochemie der Pflanze, Universität Göttingen, Göttingen, Germany; address for correspondence: Prof. N. Shavit, Biology Department, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

2. Materials and methods

Chloroplasts were isolated from fresh market lettuce leaves by standard procedures. ATP formation and ferricyanide reduction were assayed as described [6,7]. NADP reduction, with water or $DPIPH_2$ as electron donors, and the Methyl viologen mediated oxygen uptake were assayed as described [8]. Cytochrome *c* reduction in chloroplasts [8] was followed spectrophotometrically at 550 nm in a Cary spectrophotometer, Model 15, adapted to measure absorbance changes of suspensions under continuous illumination. Cytochrome *c* reduction, with NADPH as electron donor, mediated by ferredoxin and ferredoxin-NADP reductase was followed at 550 nm as described [9].

3. Results

The effect of Mg^{2+} on several photoreactions in isolated chloroplasts, in the presence of an uncoupler, is summarized in fig. 1. These reactions were all performed in the presence of 20 mM KCl in order to minimize known osmotic and ionic strength effects on the rate of electron transport in chloroplasts [10]. The rates of ferricyanide and methyl viologen reduction were almost unaffected by addition of Mg^{2+}, whereas reactions involving NADP or cytochrome *c* reduction were considerably stimulated. Saturation was obtained at approximately 2–3 mM $MgCl_2$. A similar effect of Mg^{2+} can also be observed on the coupled rates of NADP and cytochrome *c* reduction and their accompanying phosphorylation. Since Mg^{2+} is required also as a cofactor for ATP synthetase activity, its dual

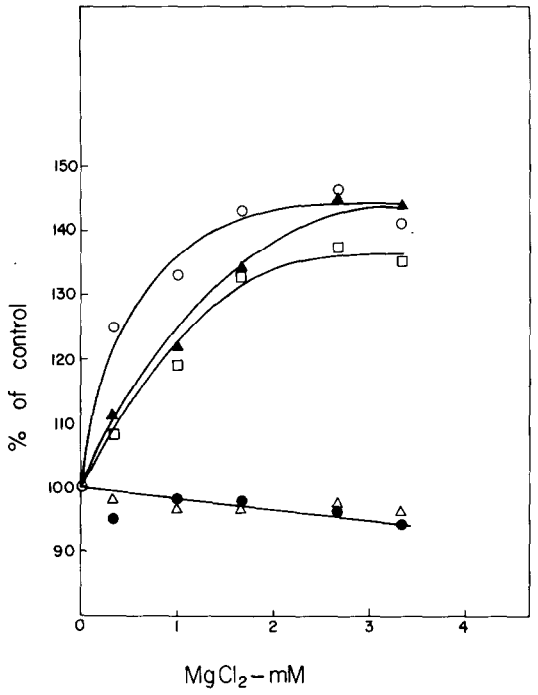

Fig. 1. Effect of Mg^{2+} on the rate of various partial electron transport reactions. All reaction mixtures contained in a final volume of 3 ml: 30 μmol Tricine–NaOH, pH 8.0; 60 μmol KCl; 4 nmol nigericin, chloroplasts containing 15–30 μg chlorophyll and in addition: A – 1 μmol $K_3Fe(CN)_6^{\equiv}$. B – 0.2 μmol methyl violegen (MV); 0.5 μmol NaN_3; 10 μmol ascorbate, pH 7.8; 0.03 μmol 2,6 dichlorophenol indophenol (DPIP) and 3.3 μM 3-(3,4 - dichlorophenyl)-1,1 dimethylurea (DCMU). C – 1 μmol NADP and 4 μM ferredoxin. D – 10 μmol ascorbate pH 7.8; 0.03 μmol DPIP; 3.3 μM DCMU; 1 μmol NADP and 4 μM ferredoxin. E – 0.15 μmol cytochrome *c* and 4 μM ferredoxin. Control activities (100) expressed per mg chlorophyll per hr were: (●) – 800 μmoles $(Fe(CN)_6$ reduced; B (△) – 518 μmoles oxygen consumed; C – (○) 135 μmoles NADP reduced; D – (▲), 80 μmoles NADP reduced and E (□), 270 μmoles cytochrome *c* reduced. Samples were illuminated for 1 min at 20°C.

effect can be separated by comparing the ratio of P/e_2. An increase in the degree of coupling was observed up to 1 mM Mg^{2+}, known to satisfy the requirement for Mg^{2+} as cofactor in the synthesis of ATP. Above this concentration no enhancement in the degree of coupling was observed although the actual rate of phosphorylation was increased. Thus we conclude that the enhanced rates of phosphorylation above 1 mM Mg^{2+} are due solely to its effect on electron transport.

The effect of Mg^{2+} on some electron transport reaction in the uncoupled state is not a specific one. Ca^{2+} proved equally effective. Since monovalent cations are also known to affect the rate of electron transport and the degree of coupling [10], we tested the effect of Mg^{2+} on three partial electron transport reactions in the presence of increasing concentrations of KCl and an uncoupler. As shown in table 1, an increase in ionic strength by KCl brought about an increase in the rate of all three electron transport reactions. However, the addition of Mg^{2+} enhanced only the rate of NADP reduction; the lower the ionic strength the greater the stimulation observed. At about 50 mM KCl the stimulation by Mg^{2+} was obliterated. The rate of ferricyanide reduction was unaffected by Mg^{2+} at KCl concentrations lower than 50 mM and above

Table 1
Effect of Mg^{2+} on the rates of several partial electron transport reactions with and without KCl

KCl (mM)	$H_2O \rightarrow Fe(CN)_6^{\equiv}$		Asc+DPIP → MV		$H_2O \rightarrow$ NADP	
	$-Mg^{2+}$	$+Mg^{2+}$	$-Mg^{2+}$	$+Mg^{2+}$	$-Mg^{2+}$	$+Mg^{2+}$
0	349	342	2992	3036	36	236
16	452	470	4400	3028	252	368
33	455	414	4076	–	396	474
50	507	503	4256	3316	470	434
100	551	465	3428	2416	376	350

Reaction mixtures and assay conditions as in fig. 1 except for: nigericin was replaced by 7.5 μmol NH_4Cl; KCl and 3 μmol $MgCl_2$ were added as indicated. Numbers refer to rates in μeq. electrons/mg chlorophyll/hour.

Table 2
Stimulation of NADP reduction by Mg^{2+} with varying amounts of ferredoxin

Ferredoxin (X 10^7 M)	μmoles NADP reduced/mg chlorophyll/hr $-Mg^{2+}$	$+Mg^{2+}$	% Stimulation
4	21.6	48	222
20	84	155	184
40	157	195	124

Reaction mixtures and assay conditions as in fig. 1, except that 7.5 μmol NH_4Cl replaced nigericin and 9 μmol $MgCl_2$ were added as indicated.

this concentration inhibition was obtained. Methyl violegen reduction was found to be inhibited by Mg^{2+} in the presence of KCl.

The degree of stimulation of NADP reduction by Mg^{2+} was dependent on the amount of ferredoxin present (table 2). At sub-saturating ferredoxin concentration the largest increase in rate was observed. In view of the apparent involvement of ferredoxin in the effect of Mg^{2+}, we used the assay of cytochrome c reduction with NADPH as electron donor in the presence of ferredoxin-NADP reductase and ferredoxin. As shown in table 3, this activity was also enhanced by Mg^{2+}, saturation being attained at about 5 mM $MgCl_2$. At higher Mg^{2+} concentration or in the presence of KCl above 20 mM, a decrease in rate was observed. The

Table 3
Stimulation of cytochrome c reduction by Mg^{2+}

Mg^{2+} (mM)	Δ_{550}	% of control	
0	0.460	100	
1	0.465	101	
2	0.562	122	
5	0.679	148	100
10	0.652	142	
5+20 mM KCl	0.531		78
5+50 mM KCl	0.326		48

Reaction mixtures contained in a final volume of 1 ml : 0.07 μmol cytochrome c (horse heart, type III); 0.49 μmol NADPH, 0.25 nmol ferredoxin, 50 μmol Tricine–NaOH, pH 8.0, and limiting amounts of flavoprotein in order to measure the rate of reaction. Activity is expressed in ΔOD 550/3 min.

stimulation of the rate of this reaction was similar to that observed in NADP reduction, e.g., the largest stimulation was observed at limiting ferredoxin concentrations.

4. Discussion

The experiments described above indicate that the effect of divalent cations on electron transport activity are not simply an ionic strength effect, since divalent cations are more effective than monovalent cations. Among the partial electron transport reactions tested, those affected by magnesium ions involve components as ferredoxin, ferredoxin-NADP reductase, P_{430} [11] and FRS [12]. An effect on the ferredoxin-NADP reductase alone can be ruled out, since the reduction of cytochrome *c* which does not involve this enzyme is thus ruled out, the data presented (table 3) may be the result of increased activity of the ferredoxin–flavoprotein complex in the presence of Mg^{2+}. The possible control function of this complex has been considered earlier [13–15]. Nelson and Neumann [14] suggested the participation of this type of complex in photosynthetic electron transfer on the basis of the inhibition of NADP reduction by salts which decompose the complex. Nakamura and Kimura [15] in a careful kinetic study of the interaction between the reductase and ferredoxin, found that the type of complex formed depended upon the ionic strength of the medium. Catalytically most effective was a comparatively loosely bound complex, formed at an intermediate ionic strength of about 0.1 M NaCl. Since only electron transport reactions involving ferredoxin show the observed stimulation by Mg^{2+} and the degree of enhancement is dependent on the concentration of this protein (table 2) it is suggested that the effect of Mg^{2+} on electron transfer reactions involving ferredoxin is an expression of the degree of association of this protein and the multienzyme complex of the electron transport chain. Rurainski and Hoch [3] interpreted the stimulation of NADP reduction by Mg^{2+} as the result of the activation of unused reaction centers. On the basis of the data presented above it is likely that a large part of the observed stimulation of NADP-reduction is due to the enhancement of ferredoxin activity by divalent cations.

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