Effect of Divalent Cations on Cation Fluxes Across the Chloroplast Envelope and on Photosynthesis of Intact Chloroplasts

B. Demmig and H. Gimmler

Botanisches Institut der Universität Würzburg, Mittlerer Dallenbergweg 64, D-8700 Würzburg

Z. Naturforsch. **34 c**, 233–241 (1979); received December 28, 1978

Intact Chloroplasts, Chloroplast Envelope, Assimilation of CO_2, Magnesium, Light Activation

The effect of divalent cations on cation fluxes across the chloroplast envelope and on photosynthetic reactions of intact spinach chloroplasts was investigated.

In the absence of EDTA, divalent cations inhibited photosynthetic CO_2-fixation and PGA-reduction at low PGA concentrations, but had almost no effect on the reduction of OAA, BQ, and on PGA-reduction at high PGA concentrations. The inhibitory effect of Ca^{2+} was greater than that of Mg^{2+}. Inhibition of photosynthesis was greater when the divalent cations were added in the dark than when added in the light. In spite of its inhibitory effect, Mg^{2+} partially restored the Ca^{2+} inhibited photosynthesis, indicating the involvement of a Mg^{2+}/Ca^{2+} antagonism in the inhibitory effect.

The inhibitory effect of divalent cations is stronger in a medium with low concentrations of K^+ than in the presence of 20–50 mM KCl. Mg^{2+} induced a release of plastidal K^+ and an increase of stromal H^+ concentration.

The results indicate that external Mg^{2+} in the absence of EDTA does not influence neither photosynthetic electron transport nor photophosphorylation, but inhibits the light activation of some enzymes of the carbon reduction cycle. The latter is assumed to be due to an acidification of the stroma pH and the decrease of endogenous K^+ level. Since the chloroplast envelope has only a very low permeability towards Mg^{2+}, possible mechanisms are discussed by which Mg^{2+} changes the properties of the chloroplast envelope and thus secondarily induces the observed effects.

Introduction

Mg^{2+} is an essential cation for photosynthetic CO_2-assimilation because many enzymes of the Calvin cycle require Mg^{2+} as a cofactor. On illumination the concentration of Mg^{2+} in the stroma increases as a result of light-induced H^+/Mg^{2+} exchange across the thylakoid membranes, which leads to an acidification of the intrathylakoid space and an alkalization of the stroma [1–2]. If Mg^{2+} is removed from the stroma by treatments with ionophores, CO_2-fixation is completely abolished. It can be restored by the addition of external Mg^{2+} [1]. Therefore it seems logical that Mg^{2+} must be present in all isolation media and reaction mixtures for intact chloroplasts [3–4]. However, Mg^{2+} and the chelating agent EDTA can be completely omitted from these media without any effect on the integrity of the chloroplast envelope and on the activity of photosynthetic CO_2-fixation [5–6]. This can be explained by the finding that the envelope of isolated spinach chloroplasts is rather impermeable towards Mg^{2+} and thus keeps the endogenous Mg^{2+} level of the chloroplasts high even if the external Mg^{2+} concentration is very low [1, 2, 6].

It is even more surprising that moderate concentrations of external Mg^{2+}, if added in the absence of EDTA, strongly inhibit CO_2-fixation [5–7]. For obvious reasons this effect of external free Mg^{2+} cannot be due to a direct effect of Mg^{2+} on photosynthetic reactions within the chloroplasts. Rather it must be an indirect effect mediated by Mg^{2+} dependent changes in the properties of the chloroplast envelope. These changes could secondarily cause an inhibition of CO_2-fixation. The scope of the following investigation was to give evidence that the latter assumption is true. Our data indicate that Mg^{2+} prevents light-activation of some Calvin cycle enzymes as suggested by Huber [7] and show in addition that the reason for this is acidification of the stroma.

Abbreviations: ABA, abscisic acid; BQ, benzoquinone; Chl, chlorophyll; DHAP, dihydroxyacetone phosphate; FBP, fructose biphosphate; EGTA, ethanedioxy-bis-(ethylamine)-tetraacetic acid; GAP, glyceraldehyde-3-phosphate; OAA, oxaloacetate; PGA, 3-phosphoglycerate; TCA, trichloroacetic acid.

Reprint requests to Dr. H. Gimmler.

0341-0382 / 79 / 0300-0233 $ 01.00/0

Materials and Methods

Material

Intact chloroplasts ("type A") were isolated from young leaves of spinach *(Spinacia oleracea L.)* [3, 8]. The percentage of intact chloroplasts in the

preparations was routinely measured by the ferricyanide method [9] and is indicated in the legends to tables and figures. It varied between 70 and 95%. In some cases the last steps of the isolation procedure were carried out in media containing only 25% of those cation concentrations given in the original procedure [3]. This significantly increased the integrity of the preparations [10]. The capacity to assimilate CO_2 varied between 40 and 120 µmol CO_2 x mg^{-1}Chl x h^{-1}, depending on the spinach material.

Radiochemicals: [^{14}C]sorbitol, [^{14}C]bicarbonate, ^3H_2O and $^{45}Ca^{2+}$ were purchased from Amersham & Buchler (Braunschweig), [^{14}C]ABA from NEN (Boston). $^{28}Mg^{2+}$ was produced by the Kernforschungsanstalt Jülich (Germany).

Methods

Experimental standard conditions: Experiments were carried out at pH 7.6 and at a temperature of 291 K, if not otherwise indicated. Light-dependent reactions were carried out under saturating light intensities of incandescent or red light and under aerobic conditions. The standard reaction mixture contained 328 mM sorbitol, 10 mM NaCl, 40 mM Hepes/NaOH buffer (pH 7.6) and 0.5 mM KH_2PO_4. It must be especially noted that this medium contained neither Mg^{2+} nor Ca^{2+} nor EDTA. Further details are given in the legends to the tables and figures.

Photosynthetic CO_2-fixation was measured from the incorporation of [^{14}C]bicarbonate into the TCA-soluble fraction of the chloroplasts. Light-dependent O_2-evolution induced by bicarbonate, PGA or OAA were measured polarographically. The *reduction of BQ* in intact chloroplasts was measured spectrophotometrically at 400 nm as mediated by the chemical reduction of external ferricyanide by reduced BQ [20]. Rates were corrected for the ferricyanide reduction of the broken chloroplasts in the suspension.

The *uptake of [^{14}C]ABA, $^{28}Mg^{2+}$ and $^{45}Ca^{2+}$* was measured by the silicone oil layer centrifugation technique [11] as specified by Gimmler *et al.* [12]. [^{14}C]sorbitol served as an impermeable solute and tritiated water as a completely permeable compound in the determination of osmotic spaces. For light samples illumination was continued during centrifugation.

K^+ and Na^+ were determined by flame photometry (type Eppendorf, Netheler & Hinz, Hamburg) or flameless atomic absorption spectroscopy (Zeiss, FMD 3, Oberkochem, Germany). Changes in the content respectively in concentrations of these cations were calculated on the basis of both the appearance of the ionic species in the medium and the disappearance in the chloroplasts.

Radioactive extracts were counted in a scintillation counter (BF 5004, Berthold, Wildbad, Germany). Data were corrected for spillover and quenching.

Results and Discussion

Inhibition of photosynthetic reactions by divalent cations

Mg^{2+} inhibition of CO_2-fixation in intact chloroplasts in the absence of EDTA takes between 0.2 and 10 mM (Fig. 1). The concentration required for 50% inhibition is about 1 mM after 6 min preincubation with Mg^{2+}. The extent of inhibition depends much upon the experimental conditions and the preparations of the chloroplasts. It was much less in many cases than shown in Fig. 1. The effect is not specific

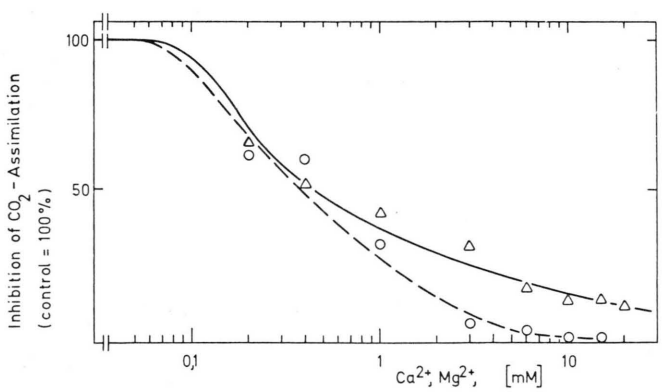

Fig. 1. The effect of Mg^{2+} (△) and Ca^{2+} (○) on photosynthetic CO_2-fixation of intact spinach chloroplasts (integrity 70%). Rate of the uninhibited control: 82 µmol $CO_2 \times mg^{-1}$ Chl $\times h^{-1}$. External K^+ concentration: 0.5 mM. Divalent cations were added in the dark.

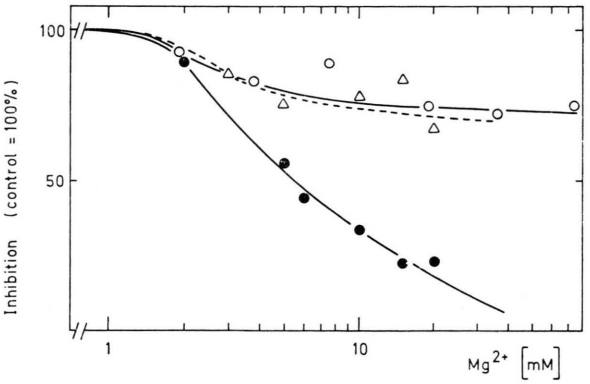

Fig. 2. The effect of Mg^{2+} on PGA reduction (●), OAA reduction (△), and reduction of BQ (○) in intact spinach chloroplasts (integrity 78%). Control rates: 113 μmol PGA × mg^{-1} Chl × h^{-1} (3 mM PGA), 16 μmol OAA × mg^{-1} Chl × h^{-1} (3 mM OAA), 81 μmol BQ × mg^{-1} Chl × h^{-1} (4×10^{-4} M BQ, 2 mM ferricyanide).

for Mg^{2+}. Ca^{2+} also strongly inhibits CO_2-fixation, the latter being even more efficient in most cases. The higher sensitivity of the chloroplasts against free external Ca^{2+} disagrees with observations of Huber [7], who found that the CO_2-assimilation of chloroplasts was less sensitive towards Ca^{2+} than towards Mg^{2+}. La^{3+}, although trivalent, possesses similar chemical properties as Ca^{2+} [13]. It also strongly suppresses CO_2-fixation (50% inhibition at 2×10^{-4} M La^{3+}, not shown). Mg^{2+} inhibits also the reduction of PGA (Fig. 2) as does Ca^{2+} (not shown). However, the inhibition of PGA-reduction by Mg^{2+} depends on the external PGA concentration (Fig. 3): The higher the concentration of PGA in the external medium the less is the inhibition of

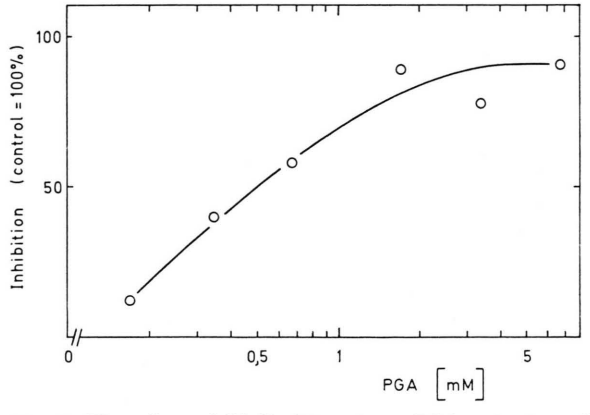

Fig. 3. The effect of Mg^{2+} (10 mM) on PGA reduction of intact spinach chloroplasts (integrity: 75%) as influenced by different PGA concentrations. Maximal control rate (saturation at about 1 mM PGA): 74 μmol PGA × mg^{-1} Chl × h^{-1}.

PGA-reduction by Mg^{2+}. This implies that not the reduction of PGA itself is inhibited by Mg^{2+}, but the supply of the entire reaction with the substrate PGA. It should be noted, that in contrast to PGA-reduction, the degree of inhibition of $^{14}CO_2$-fixation by Mg^{2+} is not influenced by varying the substrate concentrations of CO_2-assimilation, HCO_3^{2-} and "light intensity".

The reduction of OAA and BQ which both can be taken as indicator reactions for noncyclic electron transport are much less influenced by divalent cations (Fig. 2) than is CO_2-fixation. Also photophosphorylation of intact chloroplasts, as qualitatively indicated by the light-induced incorporation of ^{32}P under anaerobic conditions in the presence of ribose-5-phosphate is scarecely influenced by external Mg^{2+} (Kaiser, unpublished). This demonstrates that beside photophosphorylation also ribose-5-phosphate isomerase (E.C. 5.3.1.6.) and ribulokinase (E.C. 2.7.1.16) are not influenced by external Mg^{2+}. It is suggested that neither photosynthetic electron transport to NADP nor photophosphorylation of the intact chloroplasts are primarily affected by the divalent cations. It is rather the activity of certain enzymes of the carbon reduction cycle which are inhibited in an indirect manner by external Mg^{2+} (compare Huber [7]).

Impermeability of the envelope for divalent cations

The manner of inhibition of photosynthesis by divalent cations must be indirect, since the envelope of chloroplasts is very poorly permeable towards Mg^{2+} [6], Ca^{2+} [14] and probably also towards La^{3+}. With the aid of $^{45}Ca^{2+}$ and $^{28}Mg^{2+}$ it could be shown that the uptake of Ca^{2+} and Mg^{2+} is not higher than 1 μmol × mg^{-1} Chl × h^{-1} and probably tenfold less in most cases. With Ca^{2+} it could be demonstrated by the EGTA-technique [15], which permits corrections for the amount of cations externally bound to the envelope, that only about 45% of the measured uptake is due to true uptake, whereas 55% is due to external binding. Similar results might be expected for Mg^{2+} and La^{3+} as well. Taking this into account and on the assumption of an average plastidal osmotic volume of 25 μl × mg^{-1} Chl, maximal possible changes of cation concentrations inside the chloroplasts were estimated to vary between 2×10^{-5} and 5×10^{-4} as the result of the addition of 1 – 5 mM of divalent cations to the external medium (Values indicate concentration changes within

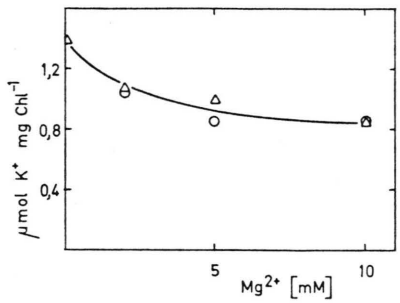

Fig. 4. The effect of Mg^{2+} on the K^+ content of intact spinach chloroplasts (integrity: 93%). Dark, 3 min incubation with Mg^{2+}. K^+ content was calculated on the basis of both the disappearance in the sediments (△) and the appearance in the supernatant (○).

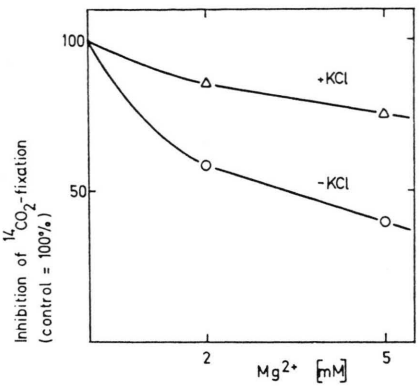

Fig. 5. The effect of 40 mM KCl on the Mg^{2+} inhibition of photosynthetic CO_2-fixation in intact chloroplasts (integrity: 94%). Control rate: 40 µmol $CO_2 \times mg^{-1}$ Chl $\times h^{-1}$.

one minute). The total Mg^{2+} concentration of intact spinach chloroplasts varies between 20 and 30 mM [6]. Free activities of this cation are assumed to be between 1 and 5 mM [1]. Since the inhibition of photosynthesis by external Mg^{2+}, Ca^{2+} and La^{3+} reaches its maximum within a one minute incubation time, these data confirm that the inhibition of photosynthetic reactions inside the chloroplasts by divalent cations cannot be due to direct effects of these cations on reactions in the stroma, but are results of the effects of these cations on the chloroplast envelope.

The effect of Mg^{2+} on the K^+ content

Mg^{2+} induces an efflux of K^+ from the chloroplasts (Fig. 4). Up to 40% of the internal K^+ may be lost, provided the K^+ concentration of the medium is kept low. Similar values are observed also by treatment of chloroplasts with valinomycin [6], which demonstrates the limitation of K^+-efflux into the medium by the Donnan – potential. Mg^{2+} induced also a decrease of the osmotic volume of the chloroplasts (not shown). However, this decrease was less than the decrease in the K^+ content. Thus also an effective decrease of the K^+ concentration in the chloroplasts takes place under the influence of Mg^{2+}. It was also investigated, whether Mg^{2+} does induce a corresponding efflux of Na^+ into a medium of low Na^+ concentration. But the results demonstrated that the Mg^{2+} induced efflux of Na^+ is much smaller than the K^+-efflux. Thus the Mg^{2+} effect exhibits a certain specificity.

If the inhibitory effect of Mg^{2+} on photosynthesis is caused by changes in the activities of certain stromal enzymes due to alterations in the cationic composition of the stroma, then it should be possible to prevent inhibition of CO_2-fixation, *i. e.* to restore CO_2-fixation by increasing the cationic level of the outer medium. This is indeed the case (Figs 5 and 6). The Mg^{2+} inhibition of CO_2-fixation is much more pronounced in the absence of K^+ than in the presence of K^+ and CO_2-fixation can be regained by readdition of K^+ concentrations which are in the

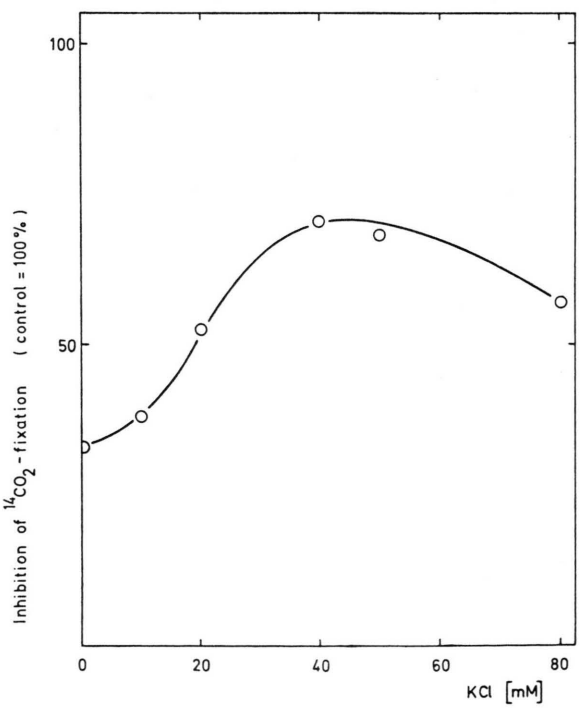

Fig. 6. The effect of KCl on the Mg^{2+} inhibition (5 mM Mg^{2+}) of photosynthetic CO_2-fixation in the intact spinach chloroplasts (integrity: 94%).

Table I. The effect of different solutes (40 mM final concentration) on the Mg^{2+} inhibition (5 mM Mg^{2+}) of photosynthetic CO_2-fixation of intact spinach chloroplasts (integrity: 85%).

Experiment	Buffer	Additions [40 mM]	Inhibition of CO_2-fixation (control $-Mg^{2+}$ $+40$ mM $X^{2+}=100\%$)
		—	65
A	40 mM Hepes/NaOH	KCl	92
		Kgluconate	90
		RbCl	73
		CsCl	84
		—	40
B	40 mM Hepes/lysine	KCl	64
		RbCl	50
		NaCl	45

order magnitude of K^+ concentrations in spinach chloroplasts [6]. Now it also became clear to us, why at the beginning of our experiments a large uncontrolled variation in the extent of Mg^{2+} inhibition had occured: It was simply overlooked that although the standard concentration of K^+ in the reaction mixtures was 0.5 mM, the real concentration varied largely because we did not pay attention to the kind of base used for calibration of the pH or the species of salts used in experiments with bicarbonate as substrate. Table I demonstrates that in the restoration effect with K^+ the anionic species is of minor importance. Furthermore K^+ could be replaced only partially by Rb^+, Cs^+ and Na^+. Na^+ especially was not a good substitute for K^+.

The question arises whether the inhibitory effect of Mg^{2+} on the activities of certain stroma enzymes is caused by the K^+-efflux from the chloroplast, only or whether other Mg^{2+} induced changes in the stroma are responsible for the inhibition of enzymes of the carbon reduction cycle. It is obvious that the Mg^{2+} induced K^+ efflux from the chloroplasts must be accompanied for electrochemical reasons by either counter fluxes of other cations or by cofluxes of suitable anions. Since the light-activation of some plastidal enzymes is assumed to be due to light-dependent alkalization of the stroma, it was investigated whether Mg^{2+} induces a decrease of the stroma pH by catalyzing a K^+/H^+ exchange and thereby inhibits stromal enzymes.

The effect of Mg^{2+} on the pH of the stroma

The pH of the chloroplast interior is definitely different from that of the external medium. However, a small but significant effect of the latter on the former can be demonstrated [16]. If a reagent lowers the pH of the stroma then the decrease of the stroma pH should depend also on the stroma pH existing before the addition of the reagent. Consequently it should depend also to a certain extent on the external pH. Therefore it can be predicted that a reagent which inhibits stromal enzymes by lowering the stroma pH should exhibit a larger inhibition at a lower external pH than at a higher external pH. This was shown to be true for HNO_2 [26]. The profile of the Mg^{2+} inhibition of CO_2-fixation is shown in Fig. 7. In the absence of Mg^{2+}, CO_2-fixation exhibits the well known pH dependency of photosynthesis in isolated chloroplasts from spinach with an optimum between pH 7.7 and 8.0 (lower part of Fig. 7). A similar pH dependency is also observed in the presence of Mg^{2+}. However,

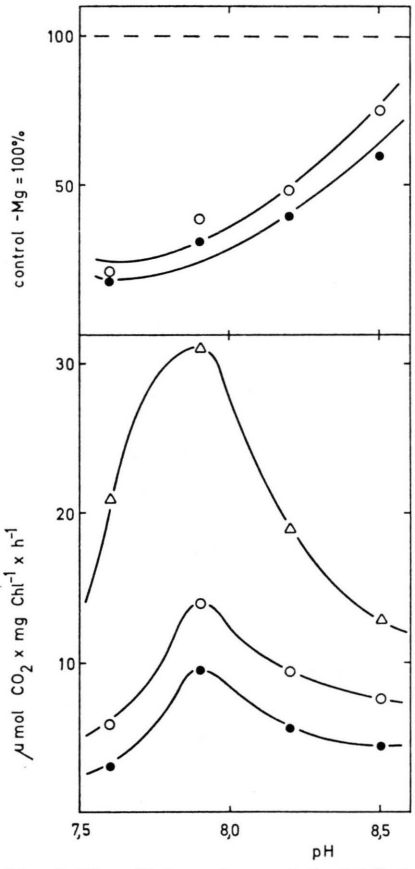

Fig. 7. The pH dependency of the Mg^{2+} inhibition of $^{14}CO_2$-fixation in intact spinach chloroplasts (integrity: 91%). The Hepes-buffer was calibrated with NaOH. Control (△), 5 mM Mg^{2+} (○), 10 mM Mg^{2+} (●).

when the inhibition by Mg^{2+} is plotted as function of the external pH (upper part of Fig. 7) it becomes clear that the inhibition is less at a more alkaline pH. Similar results were observed also with Ca^{2+}. The pH-dependency of the inhibitory effect of Mg^{2+} on photosynthetic CO_2-fixation is independent of the magnitude of the photosynthetic rate, as shown by the comparison of two different pH – values which permit about the same control rate of CO_2-fixation, *e. g.* pH 7.6 and 8.2. Thus our experimental results agree with the assumption that Mg^{2+} decreases the stroma pH, and thereby shifts the activities of certain enzymes of the carbon reduction cycle out of the pH optima. With other word, Mg^{2+} induces a state in the stroma, which in respect to the pH is more comparable to the dark state than to the light state.

A typical property of the inhibitory effect of Mg^{2+} is that it is much larger when Mg^{2+} is added in the preceding dark period than when added to already illuminated chloroplasts (Table II) [7]. Again this may be related to the different proton concentration in the stroma in the dark and in the light. It should be noted that in spinach chloroplast Mg^{2+} did not extend the lag phase of photosynthetic O_2 evolution (not shown). The same was observed with barley chloroplasts [7].

In Fig. 8 more direct evidence is presented that external free Mg^{2+} increases the H^+concentration of the stroma. ABA, a native plant hormone, which is synthetized in the chloroplasts [17] is lost rapidly from the chloroplasts during the normal isolation procedure [18]. This can be explained by its behaviour as a weak acid (pK = 4.8), which easily penetrates the chloroplast envelope in its protonated form and rapidly equilibrates between the chloroplast interior and the medium. If ^{14}C-labelled ABA

Table II. Effect of Mg^{2+} on CO_2-fixation of intact spinach chloroplasts (integrity: 90%). Control rate: 55 μmol $CO_2 \times mg^{-1}$ Chl $\times h^{-1}$. Mg^{2+} was added either in the dark or the light period preceding the $^{14}CO_2$ incorporation period in the light. Preincubation time with Mg^{2+}: 3 min, incubation time with $^{14}CO_2$: 6 min.

Mg^{2+} Concentration of the reaction mixture [mM]	Mg^{2+} Added in the	Photosynthetic CO_2-fixation (control = 100%)
—	dark	100
5	dark	49
—	light	100
5	light	89

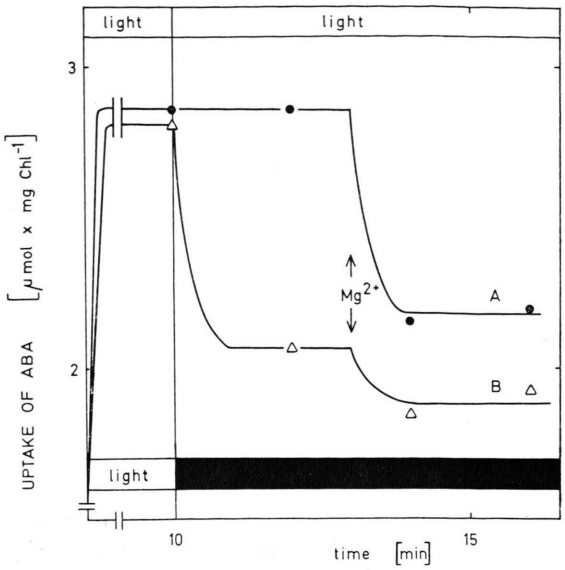

Fig. 8. Distribution of [^{14}C]ABA (1.5×10^{-5} M) between intact spinach chloroplasts (integrity: 90%) and the medium as affected by light and Mg^{2+}. ABA-equilibrium distribution is reached within 3 min [18]. 10 min after the start of [^{14}C]ABA uptake samples of curve B (△) were darkened (black bar), whereas samples of curve A (●) remained in the light. 3 min later 10 mM Mg^{2+} was added to samples of both curves.

(10^{-7} – 10^{-5} M) is added to a chloroplast suspension, it rapidly equilibrates between the chloroplasts and the outer medium according its mass equation and the pH of both the stroma and the external medium [18]. A steady state is reached within a few minutes in the light. Upon darkening a rapid efflux of ABA is observed (Fig. 8, B) due to H^+ back flow from the intrathylakoid space into the stroma. The latter shifts the equilibrium more to the protonated form of ABA, which diffuses out of the chloroplasts to establish a new equilibrium between the medium and the chloroplast interior. If 10 mM Mg^{2+} is added to such chloroplasts in the dark, only a relatively small efflux of ABA is observed, which is indicative for a small decrease in the pH of the stroma However, if Mg^{2+} is added in the light to chloroplasts (Fig. 8, A), which maintain a higher level of ABA than in the dark (indicative for a high pH), a much stronger efflux of ABA is observed under the influence of Mg^{2+}. Since the ABA-level is decreased by this Mg^{2+} concentration to about the ABA-level of the dark control and the normal light-dark difference of the stroma is about one pH unit [16], the stroma pH can be assumed to be decreased in this particular experiment roughly by one pH

Table III. The effect of Mg^{2+} on the uptake of [^{14}C]ABA in intact spinach chloroplasts (compare Fig. 8). Chloroplasts were preincubated for 10 min with 1.5×10^{-5} M ABA in the dark or in light in the presence of different Mg^{2+} concentrations. Values indicate the differential uptake of ABA in light and dark.

$MgCl_2$ [mM]	Light-dark uptake of ABA [nmol ABA × mg^{-1} Chl]	Control $-Mg^{2+}=100\%$
—	0.59	100
2	0.60	102
5	0.42	71
10	0.25	42

unit. Table III confirms that the light-dark uptake of ABA is diminished by different Mg^{2+} concentrations.

Mg^{2+}/Ca^{2+} antagonism

Calcium is known to act in an antagonistic manner to Mg^{2+} in many biochemical reactions, *e. g.* a less severe inhibition of photophosphorylation by Ca^{2+} occurs in the presence of Mg^{2+} [19]. 5 mM Mg^{2+} was able to essentially eliminate the Ca^{2+} inhibition of photophosphorylation in broken chloroplasts. Also, the permeability of the envelope may be controlled by a Mg^{2+}-Ca^{2+} antagonism (Fig. 9). Although Mg^{2+} in moderate concentrations itself is inhibitory, it can counteract the much stronger inhibition of CO_2-fixation by Ca^{2+}. Table IV demonstrates additionally

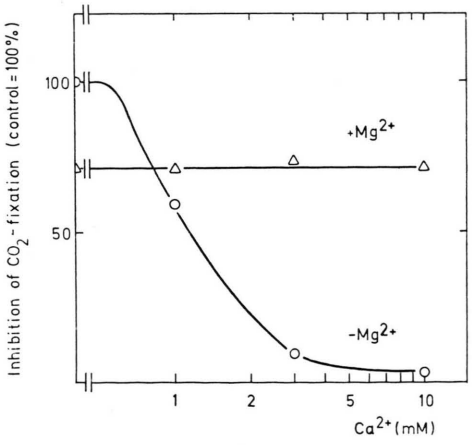

Fig. 9. Photosynthetic CO_2-fixation of intact spinach chloroplasts (integrity 90%) as affected by Ca^{2+} and Mg^{2+}. The Ca^{2+} concentration was varied, whereas the Mg^{2+} concentration was kept constant (5 mM). Control rate: 44 μmol $CO_2\times mg^{-1}$ Chl $\times h^{-1}$.

Table IV. The effect of Mg^{2+} and Ca^{2+} on photosynthetic CO_2-assimilation in intact spinach chloroplasts (integrity: 91%). Control rate: 30 μmol $CO_2\times mg^{-1}$ Chl $\times mg^{-1}$. Incubation time with $^{14}CO_2$: 6 min.

Cation concentration of the medium [mM]		Treatment with cations preceding the light $^{14}CO_2$-incorporation period	CO_2-fixation (control = 100%)
Ca^{2+}	Mg^{2+}		
—	—	none	100
—	5	7 min + Mg^{2+} in the dark	69
0.5	—	7 min + Ca^{2+} in the dark	47
0.5	5	5 min + Ca^{2+} in the dark, then 2 min + Mg^{2+} in the dark	44
0.5	5	7 min + Mg^{2+} + Ca^{2+} in the dark (added at the same time)	98

that this counteraction is only possible if Mg^{2+} and Ca^{2+} are added at the same time. Then K^+-efflux and the acidification of the stroma is prevented. If Ca^{2+} is added first and the damage occurs, subsequent addition of Mg^{2+} does not restore CO_2-fixation. This may indicate that Ca^{2+} and Mg^{2+} compete for the same binding sites at the chloroplast envelope but have different effects in the bound state. It could be experimentally demonstrated that indeed Mg^{2+}, Sr^{2+} and La^{3+} compete with Ca^{2+} for the same binding sites at the envelope, because these cations inhibited the binding of ^{45}Ca to the envelope in a competitive fashion as revealed by Lineweaver-Burk plots (not shown here). The inhibitor constant K_i increased in the sequence $La^{3+} \gtrsim Sr^{2+} \gtrsim Mg^{2+}$. In summary, Mg^{2+} and Ca^{2+} are suggested here to be involved in an antagonistic manner in the regulation of the permeability of the chloroplast envelope just as has been postulated for the mitochondrial membranes [21].

Concluding Remarks

The effect of external Mg^{2+} and Ca^{2+} (in the absence of EDTA) on photosynthetic reaction inside the chloroplasts are indirect effects because both cations are unable to enter the chloroplast interior as quickly as inhibition of photosynthesis occurs. The primary effect of these cations on the chloroplast envelope rather induces a decrease in the K^+ content of the chloroplasts and an increase of the H^+ concentration in the stroma. Both alterations may be contribute to the observed inhibition effect. Although nothing is known about a regulation of

Calvin cycle enzymes by mM concentrations of K^+, such effect cannot be excluded *a priori*. However, more probable is that the decrease of the stroma pH by Mg^{2+} prevents the light activation of some of these enzymes. Our results demonstrate that the following enzymes are not inhibited by external Mg^{2+}: Ferredoxin-NADP-reductase, ATP-synthetase, ribose-5-phosphate isomerase, ribulose-5-phosphate kinase, phosphoglycerat kinase, glyceraldehyd-phosphat-dehydrogenase and malate dehydrogenase. This indicates that these enzymes are not light-activated by the light-induced alkalization of the stroma. A possible candidate for the Mg^{2+} dependent prevention of light-activation is fructose biphosphatase which was shown to be regulated by light-induced changes of the stroma pH [26]. In this respect it may be noteworthy that in earlier experiments Mg^{2+} increased the photosynthetic ^{14}C-labelling of fructosebiphosphate and dihydroxyacetonephosphate, whereas the labelling of sugarmonophosphates and of PGA was inhibited [22]. Similar changes in the ^{14}C-distribution pattern were suggested at low external pH. In principal, our suggestion that Mg^{2+} prevents light-induced activation of certain enzymes of the carbon reduction cycle, *e. g.* that of FBPase, is in agreement with similar suggestions of Huber [7], but differs with respect to the enzymes concerned. Only with respect to the FBPase is there agreement that such an irreversible reaction can be influenced indirectly by free external Mg^{2+} concentrations. The restoration of Mg^{2+}-inhibited photosynthesis by K^+ is explained by a proton extrusion from the stroma due to a K^+-influx, leading to an realkalization of the stroma pH. In this respect it is of interest that if external K^+ concentrations are too high photosynthesis is suppressed again (Fig. 6). This effect is especially pronounced at high external pH-values and may indicate that also superoptimal pH values in the stroma do inhibit photosynthesis.

The inhibitory effect of Mg^{2+} on PGA-reduction at low substrate concentrations could lead to the wrong assumption that the activity of GAPDH itself is influenced indirectly by external Mg^{2+}. However, the noninhibition of this reaction at higher PGA concentrations demonstrate that this reaction itself is not inhibited by Mg^{2+}, rather the supply of the reaction with PGA is affected. This agrees with the observation that GAPDH has been shown to be almost insensitive to pH changes of the stroma between pH 7.0 and 8.5 [16]. The phosphate translocator catalyzes for electrochemical reasons the transport of PGA only as PGA^{2-}. The consumed species during PGA-reduction is PGA^{3-} [27, 28]. Thus an increase in the pH of the stroma should stimulate the uptake of PGA, whereas a decrease should inhibit it. The latter effect indeed was observed. However, high external PGA concentration could overcome this inhibition of PGA transport.

Regarding the primary mechanism of Mg^{2+} at the envelope, our experiments are not conclusive and further work has to be done. The results up to now neither favour the involvement of the Mg^{2+} dependent ATPase [23 – 25], which could catalyze an exchange of H^+ against K^+, nor do they present evidence against it. The importance of the envelope membran potential should also be mentioned here. The envelope bears fixed negative charges at the outer side, which may may be neutralized by divalent cations. As a result the general membrane conductivity and selectivity may change. It is known that many biomembranes of intact cells and organelles exhibit a K^+ conductivity which is selectively controlled by Ca^{2+} and Mg^{2+} [21]. Nevertheless, the exchange of K^+ against H^+, the stoichiometry of which has still to be demonstrated, is up to now only a phenomenological description of what goes on after free external Mg^{2+} is bound to unknown negatively charged sites of the envelope. That the envelope indeed is able to catalyze a K^+/H^+ exchange with a stoichiometry of at least 0.5 molecules K^+ against one proton was demonstrated earlier [6]. If energized by light the direction of this exchange is against the chemical concentration gradient of K^+, whereas in the nonenergized state (dark) it follows the diffusion gradient, as shown in this paper.

Finally our experiments demonstrate again the importance of the chemical composition of isolation and incubation media for the regulation of photosynthesis in intact chloroplasts. To get a true picture of *in vivo* photosynthesis and not only to get high rates of photosynthesis, one must again emphazise the necessity of approaching as close as possible the chemical composition of the cytoplasm not only in respect to osmotic values but also in respect to electrolytes. By doing that our present understanding about properties and permeabilities of the envelope (and its importance for intracellular regulations) may have to be partially revised.

We are most grateful to Prof. U. Heber and Miss U. Enser for stimulating discussions and to Dr. J. Ten Hunen for improving the English of the manuscript. The technical assistance of Miss G. Carl and Miss C. Steinhübel is acknowledged. The investigations were supported by the Deutsche Forschungsgemeinschaft.

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