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Regulation of Chloroplast Photosynthetic Activity by Exogenous Magnesium[1]

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STEVEN C. HUBER
United States Department of Agriculture, Science and Education Administration, Departments of Crop Science and Botany, North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

Magnesium was most inhibitory to photosynthetic reactions by intact chloroplasts when the magnesium was added in the dark before illumination. Two millimolar $MgCl_2$, added in the dark, inhibited CO_2-dependent O_2 evolution by *Hordeum vulgare* L. and *Spinacia oleracea* L. (C_3 plants) chloroplasts 70 to 100% and inhibited (pyruvate + oxaloacetate)-dependent O_2 evolution by *Digitaria sanguinalis* L. (C_4 plant) mesophyll chloroplasts from 80 to 100%. When Mg^{2+} was added in the light, O_2 evolution was reduced only slightly. O_2 evolution in the presence of phosphoglycerate was less sensitive to Mg^{2+} inhibition than was CO_2-dependent O_2 evolution.

Magnesium prevented the light activation of several photosynthetic enzymes. Two millimolar Mg^{2+} blocked the light activation of NADP-malate dehydrogenase in *D. sanguinalis* mesophyll chloroplasts, and the light activation of phosphoribulokinase, NADP-linked glyceraldehyde-3-phosphate dehydrogenase, and fructose 1,6-diphosphatase in barley chloroplasts. The results suggest that Mg^{2+} inhibits chloroplast photosynthesis by preventing the light activation of certain enzymes.

It was reported previously (2–4) that Mg^{2+} inhibits photosynthesis by isolated chloroplasts. One report with lettuce chloroplasts (4) suggested that the inhibition of CO_2 fixation by Mg^{2+} was caused by inhibition of carbonic anhydrase. This conclusion seems unlikely because carbonic anhydrase is a stromal enzyme (19) and it has been recently demonstrated that the chloroplast envelope is impermeable to Mg^{2+} (7).

In view of this apparent discrepancy, I have reconsidered the characteristics of Mg^{2+} inhibition of photosynthesis using intact C_3[2] and C_4 mesophyll chloroplasts. Understanding the basis of the Mg^{2+} inhibition may be important because it is likely that the cytoplasm contains free Mg^{2+}, as many enzymes require this cation for activity (16). The results obtained suggest that Mg^{2+} inhibition is a general phenomenon and that Mg^{2+} inhibits photosynthesis by preventing the light activation of (at least) some of the enzymes involved in both the Calvin and C_4 dicarboxylic acid pathways.

MATERIALS AND METHODS

Plant Growth and Chloroplast Isolation. Spinach (*Spinacia oleracea* L.), barley (*Hordeum vulgare*, L., cv. Trophy), and crabgrass (*Digitaria sanguinalis* L., Scop.) were grown in a growth chamber as previously described (10). Spinach chloroplasts were prepared essentially using the method of Lilley and Walker (15) except that the blending medium contained 0.3 M sorbitol, 10 mM $Na_4P_2O_7$, 2 mM isoascorbate, and 20 mM KCl (pH 6.5). After centrifugation at 2,500g for 60 sec, chloroplasts were resuspended in 0.3 M sorbitol, 50 mM HEPES-NaOH (pH 7.6), 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 2 mM EDTA (15).

Barley and crabgrass mesophyll chloroplasts were prepared from enzymically isolated mesophyll protoplasts. Protoplast isolation was as previously described (10) except that the protoplasts were purified by flotation on 0.5 M sucrose (200g, 6 min). Barley protoplasts were ruptured by passage through a 20-μm nylon net in the same medium used to blend spinach leaves. Crabgrass mesophyll protoplasts were ruptured in a mixture of 0.3 M sorbitol and 50 mM Tricine-KOH (7.5), washed once, and resuspended in the same medium.

O_2 Evolution. O_2 evolution was followed polarographically with Clark-type electrodes in 1.8-ml water-jacketed vessels. The basic reaction mixture for barley and spinach chloroplasts contained 0.33 M sorbitol, 50 mM Tricine-NaOH (pH 8.2), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.2 mM sodium phosphate, 4 mM $NaHCO_3$, and with spinach 600 units/ml of catalase. The reaction mixture for crabgrass mesophyll chloroplasts contained 0.3 M sorbitol, 50 mM Tricine-KOH (7.5), 5 mM pyruvate, 0.5 mM OAA, and 1 mM sodium phosphate. In all tables and figures, the given concentration of Mg^{2+} represents the final net concentration (Mg^{2+} concentration minus EDTA concentration). Reactions were run at 25 C for barley and spinach and 35 C for crabgrass chloroplasts. The concentration of Chl was 15 to 30 μg/ml.

Illumination was provided by a 75-w floodlamp passed through 4 cm of water to give a quantum flux density of 80 nE/cm^2·sec between 400 and 700 nm at the face of the cuvette.

Activation by Light and Enzyme Assays. For the light activation experiments, intact chloroplasts were incubated in the O_2 evolution reaction mixtures with other additions as described. Aliquots were removed at various times and injected directly into the enzyme assay mixtures. Enzyme activity was followed spectrophotometrically at 340 nm at 25 C. NADP-malate dehydrogenase was assayed in a mixture containing 0.1 M Tricine-NaOH (pH 8.0), 1 mM EDTA, 10 mM $MgCl_2$, 0.5 mM OAA, and 0.2 mM NADPH, essentially as described by Johnson and Hatch (12). P-glycerokinase and NADP-glyceraldehyde-3-P dehydrogenase were assayed by the procedure of Latzko and Gibbs (14). The P-glycerokinase reaction medium contained 0.1 M Tricine-NaOH (pH 8.0), 10 mM $MgCl_2$, 5 mM ATP, 0.2 mM NADPH, 0.2 units/ml of glyceraldehyde-3-P dehydrogenase, and 2 mM PGA. NADP-glyceraldehyde-3-P dehydrogenase was assayed in 0.1 M Tricine-NaOH (pH 8.0), 10 mM $MgCl_2$, 5 mM ATP, 2 mM PGA, 0.2 units/ml of P-glycerokinase, and 0.2 mM NADPH. Fructose-1,6-diphosphatase was assayed in a medium containing 100 mM Tris-HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM EDTA, 0.3 mM NADP, 0.6 mM fructose diP, 2

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[2] Abbreviations: C_3 plant: plant having only the Calvin cycle of photosynthesis; C_4 plant: plant having the C_4 dicarboxylic acid pathway of photosynthesis; OAA; oxaloacetic acid; PGA: 3-phosphoglyceric acid.

units of P-glucose isomerase, and 1 unit of glucose-6-P dehydrogenase as described by Kelly *et al.* (13). P-ribulokinase was assayed in a CO_2-free mixture containing 100 mM Tricine-NaOH (pH 8.0), 10 mM $MgCl_2$, 1 mM ATP, 5 mM P-enolpyruvate, 0.4 mM NADH, 0.5 mM ribose-5-P, 6 units of pyruvate kinase, and 9 units of lactate dehydrogenase. Addition of P-ribuloisomerase was not required. For the P-ribulokinase assay, the only bicarbonate present was that introduced by the addition of the chloroplasts (10 μl of chloroplast mixture to give 33 μM $NaHCO_3$ in the assay mixture). With this assay, rates were linear for approximately 5 min, after which rates began to increase, presumably as a result of accumulation of PGA in the mixture. All rates given in the text were calculated from the initial linear phase. This assay was chosen for the preliminary experiments as it does not rely on coupling to any of the other Calvin cycle enzymes. All enzymes and biochemicals were obtained from Sigma Chemical Co.[3]

RESULTS

Effect of Mg^{2+} on O_2 Evolution by Intact Chloroplasts. Free Mg^{2+} was found to inhibit CO_2-dependent O_2 evolution by intact spinach and barley chloroplasts. As shown in Figure 1, trace D, O_2 evolution by barley chloroplasts was completely blocked by 2 mM $MgCl_2$ when the Mg^{2+} was added in the dark prior to illumination. If the Mg^{2+} was added in the dark after the chloroplasts had been illuminated for several min, O_2 evolution was only partially reduced (Fig. 1, trace B). Similarly, if the Mg^{2+} was added in the light, O_2 evolution was reduced only slightly and proceeded linearly for at least 15 min (data now shown).

The inhibitory effect of Mg^{2+} was completely reversed in the dark by EDTA when the EDTA was added before the Mg^{2+} (Fig. 1, trace A). However, when chloroplasts were incubated in the dark for several min with 2 mM Mg^{2+}, and 2 mM EDTA was added before the light period, the subsequent rate of O_2 evolution was reduced roughly 93% relative to the control (Fig. 1, trace C). The Mg^{2+} inhibition was only partially reversed by EDTA if the chelating agent was added in the light (Fig. 1, trace D).

Figure 2 shows the effect of Mg^{2+} concentration on CO_2-dependent O_2 evolution by barley chloroplasts. Increasing concentrations of Mg^{2+} decreased the maximum rate of O_2 evolution. Although the rate of O_2 evolution in the control was constant up to 20 min, rates in the presence of Mg^{2+} decreased after reaching a maximum (Fig. 2). When 2 mM Mg^{2+} was added in the light, O_2 evolution proceeded at a slightly reduced rate but remained linear for at least 10 min (Fig. 2). In the experiment of Figure 2, Mg^{2+} had no effect on the lag phase of photosynthesis; however, in other experiments Mg^{2+} increased the lag in addition to decreasing the maximum rate.

O_2 evolution in the presence of pyruvate + OAA by crabgrass mesophyll chloroplasts was also inhibited by free Mg^{2+} (Fig. 3). In the absence of Mg^{2+}, O_2 evolution was linear for at least 20 min whereas O_2 evolution tended to be nonlinear with time in the presence of Mg^{2+} (Fig. 3). If 2 mM Mg^{2+} was added in the light, O_2 evolution was reduced only slightly. Mg, when added in the dark, also inhibited uncoupled noncyclic O_2 evolution by crabgrass mesophyll chloroplasts (OAA + 20 mM methylamine, data not shown). However, high rates of O_2 evolution could be induced by subsequent addition of the artificial electron acceptor *p*-benzoquinone. In one experiment, the rate of O_2 evolution in the presence of OAA + methylamine was 130 μmol/mg of Chl·hr and only 3 μmol/mg of Chl·hr when 2 mM Mg^{2+} was added in the dark. Upon the addition of 1 mM *p*-benzoquinone in the light, rates of 610 and 595 μmol/mg of Chl·hr, respectively, were observed.

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

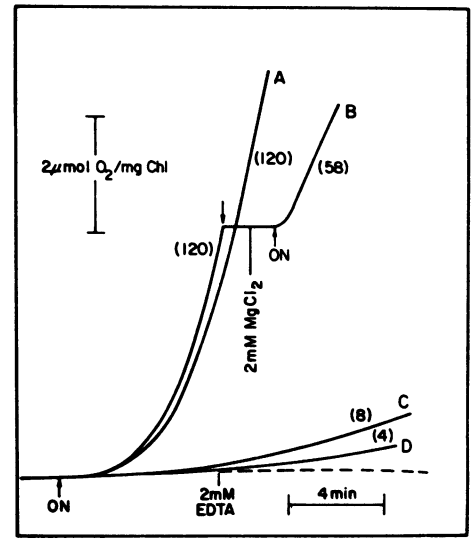

FIG. 1. Effect of time of addition of $MgCl_2$ and EDTA on CO_2-dependent O_2 evolution by barley chloroplasts. In trace A, 2 mM $MgCl_2$ was added in the dark 2 min after addition of 2 mM EDTA. In trace B, 2 mM $MgCl_2$ was added in the dark after 7-min illumination. Trace C initially contained 2 mM $MgCl_2$ and EDTA (2 mM) was added 3 min before illumination. Trace D initially contained 2 mM $MgCl_2$ and EDTA (2 mM) was added in the light. Dashed line of trace D is the curve obtained without addition of EDTA. Maximum rates, expressed as μmol O_2 evolved/mg Chl·hr, are shown parenthetically.

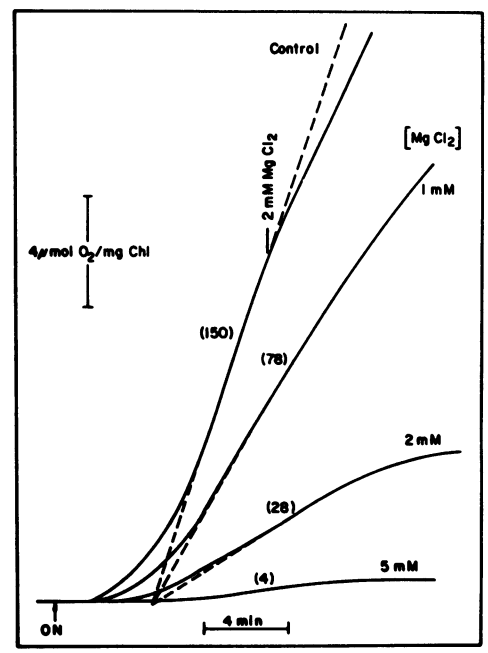

FIG. 2. Effect of $MgCl_2$ on lag phase and maximum velocity of CO_2-dependent O_2 evolution by barley chloroplasts. After approximately 10 min, 2 mM $MgCl_2$ was added to the control trace. In the other traces, $MgCl_2$ was added in the dark before illumination. Maximum rates are shown parenthetically. Dashed lines are tangents to maximum rate portion of curves.

Other divalent cations were tested for inhibition of chloroplast photosynthesis to determine the specificity of the Mg^{2+} inhibition. $MgCl_2$ was more inhibitory to CO_2-dependent O_2 evolution by spinach chloroplasts than either $MnCl_2$ or $CaCl_2$ (Fig. 4). Potassium or sodium (data not shown) chloride were not inhibitory. Similar levels of inhibition by Mg^{2+} were observed with both the chloride and sulfate salts (not shown).

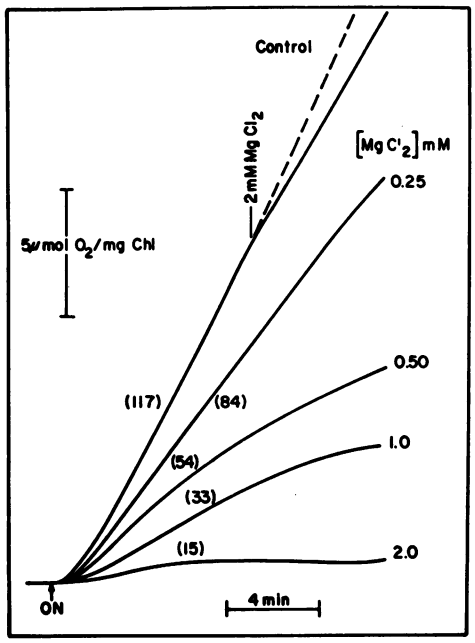

FIG. 3. Effect of $MgCl_2$ on O_2 evolution by crabgrass mesophyll chloroplasts in the presence of 5 mM pyruvate + 0.5 mM OAA. After 8 min in the light, 2 mM $MgCl_2$ was added to the control. In the other traces, $MgCl_2$ was present at concentration indicated prior to illumination. Maximum rates are shown parenthetically.

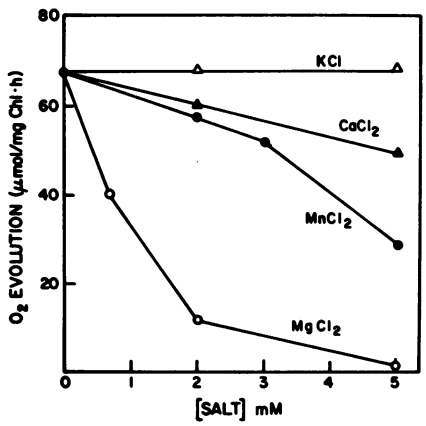

FIG. 4. Effect of various salts on CO_2-dependent O_2 evolution by spinach chloroplasts. Chloroplasts were incubated with the indicated salt for 2 min in the dark before illumination.

Effect of Mg^{2+} on O_2 Evolution in the Presence of PGA. In the presence of 2 mM PGA + 2 mM Pi, barley chloroplasts evolved O_2 in the light almost immediately (Fig. 5). O_2 evolution in this system was less sensitive to inhibition by Mg^{2+}, added in the dark, than CO_2-dependent O_2 evolution (Fig. 4). In the presence of PGA, 5 mM Mg^{2+} resulted in approximately 50% inhibition of O_2 evolution in contrast to complete inhibition of CO_2-dependent O_2 evolution at this concentration (Fig. 4).

Whereas PGA-dependent O_2 evolution was relatively insensitive to Mg^{2+}, addition of PGA in the light to chloroplasts that had been incubated with 2 mM Mg^{2+} in the dark did not induce high rates of O_2 evolution. In Figure 6, barley chloroplasts were incubated in the dark with varying concentrations of Mg^{2+} for 2 min; the light was turned on and 2 mM PGA + 2 mM Pi added after 12 min. The rate of O_2 evolution was substantial in the absence of free Mg^{2+}, and was increased roughly 75% by the addition of PGA in the light. Increasing concentrations of free Mg^{2+}, however, reduced the rate of O_2 evolution before and after addition of PGA. Dark addition of 2 mM Mg^{2+} inhibited CO_2-dependent O_2 evolution 95% and O_2 evolution after addition of PGA by 85%.

Effect of Mg^{2+} on Activity of Photosynthetic Enzymes. In an attempt to determine the basis for Mg^{2+} inhibition of O_2 evolution by isolated chloroplasts, the effect of Mg^{2+} on the light activation of photosynthetic enzymes was determined. Intact chloroplasts were incubated in the presence and absence of 2 mM $MgCl_2$ in the dark before illumination. Aliquots were removed at various times and assayed for enzyme activity.

Illumination of intact crabgrass mesophyll chloroplasts resulted in a 19-fold stimulation of NADP-malate dehydrogenase activity (Table I). The light activation of enzyme activity was essentially complete after 3 min of illumination. Incubation of chloroplasts with 2 mM $MgCl_2$ had no significant effect on the dark activity of NADP-malate dehydrogenase, but significantly reduced the light stimulation of enzyme activity (Table I). After 6 min in the light, NADP-malate dehydrogenase activity in Mg^{2+}-treated chloroplasts was only 20% of that in the control chloroplasts.

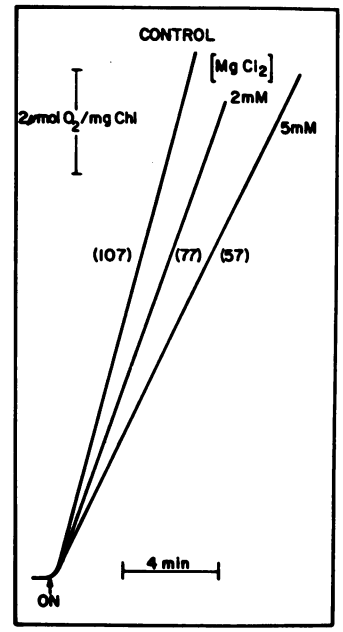

FIG. 5. Effect of $MgCl_2$ on O_2 evolution by barley chloroplasts in the presence of PGA. Chloroplasts were incubated in the basic reaction mixture plus 2 mM PGA + 2 mM Pi. Concentrations of $MgCl_2$ indicated were present in the dark prior to illumination. Maximum rates are shown parenthetically.

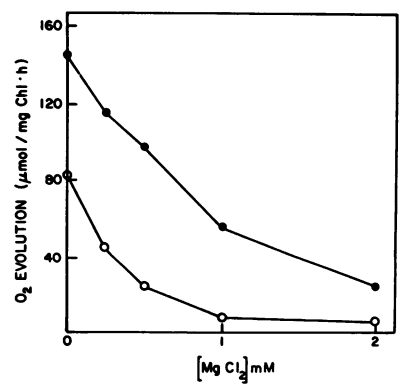

FIG. 6. Effect of $MgCl_2$ on rate of O_2 evolution by barley chloroplasts before and after addition of PGA in the light. Chloroplasts were incubated as described under "Materials and Methods" with the indicated concentrations of $MgCl_2$ in the dark for 2 min prior to illumination. Rates before (○——○) and rates after (●——●) addition of 2 mM PGA + 2 mM Pi. Rates were measured after 10 min of illumination.

Table I. Effect of magnesium on light activation of NADP-malate dehydrogenase in intact mesophyll chloroplasts of D. sanguinalis.

Chloroplasts were incubated at 35 C in a mixture of 0.3 M sorbitol and 50 mM Tricine-KOH (pH 8.0) in the presence or absence of 2 mM $MgCl_2$. At the times indicated, aliquots were withdrawn and assayed immediately for enzyme activity. Data are the averages of two experiments.

Condition	NADP-malate dehydrogenase activity	
	Control chloroplasts	Chloroplasts + 2mM $MgCl_2$
	(μmol/mg Chl·h)	
Dark	8	9
Light, 3 min	150	32
Light, 6 min	160	32

When crabgrass mesophyll chloroplasts were incubated in the dark with pyruvate + OAA and varying concentrations of Mg^{2+} (0–2 mM), there was a positive correlation between the rate of O_2 evolution observed in the light and the activity of NADP-malate dehydrogenase (Fig. 7). Enzyme activity, assayed at saturating levels of NADP and OAA, was consistently higher than the rates of O_2 evolution. With 2 mM Mg^{2+}, O_2 evolution was completely inhibited even though there was some detectable enzyme activity (45 μmol/mg of Chl·hr, Fig. 7).

The effect of Mg^{2+} on the light activation of certain Calvin cycle enzymes was also determined with intact barley chloroplasts. Incubation of chloroplasts in the light for 12 min resulted in a 2.7-fold stimulation of P-ribulokinase, a 4.5-fold increase in NADP-linked glyceraldehyde-3-P-dehydrogenase, and a large increase in fructose bisphosphatase activity (Table II). Incubation of chloroplasts in the dark with 2 mM $MgCl_2$ prior to illumination substantially prevented the light activation of these enzymes (Table II). In contrast, there was no significant increase in P-glycerokinase activity by illumination of intact chloroplasts and this activity was not reduced by incubation of the chloroplasts in the dark with Mg^{2+} (Table II).

DISCUSSION

Millimolar levels of Mg^{2+} completely inhibited CO_2-dependent O_2 evolution by spinach (Fig. 4) and barley (Figs. 1 and 2) chloroplasts, and O_2 evolution by crabgrass mesophyll chloroplasts in the presence of pyruvate + OAA (Fig. 3). Spinach and barley chloroplasts contain the Calvin cycle and hence are capable of evolving O_2 with CO_2 as the sole substrate (18). Mesophyll chloroplasts of the C_4 plant crabgrass do not contain the Calvin cycle (6, 12) but instead contain some of the enzymes of the C_4 dicarboxylic acid pathway. With crabgrass mesophyll chloroplasts, O_2 evolution requires the addition of pyruvate + oxaloacetate (11). The OAA, by reduction to malate, serves to keep the pyridine nucleotides oxidized, and conversion of pyruvate to P-enolpyruvate utilizes the ATP generated by noncyclic electron flow. The chloroplast enzymes involved are NADP-malate dehydrogenase and pyruvate Pi kinase, respectively. With both chloroplast types, Mg^{2+} inhibited O_2 evolution when present in the dark before illumination, but not when added in the light. This suggests that Mg^{2+} inhibition is a general effect and that light, in some fashion, protects the chloroplast from Mg^{2+} inhibition. That the observed inhibition of O_2 evolution was not caused by inhibition of electron flow was indicated by the fact that Mg^{2+} did not inhibit O_2 evolution in the presence of *p*-benzoquinone.

The simplest system tested for inhibition by Mg^{2+} was O_2 evolution by crabgrass mesophyll chloroplasts in the presence of OAA + methylamine. O_2 evolution in this system requires noncyclic electron transport, NADP-malate dehydrogenase activity, and transport of OAA and malate across the chloroplast envelope. Crabgrass mesophyll chloroplasts have a dicarboxylate translocator that exchanges malate for OAA and is not inhibited by Mg^{2+} (Huber, unpublished). By process of elimination, it appears that in this system, Mg^{2+} must interfere with NADP-malate dehydrogenase. It is clear that Mg^{2+} does not inhibit the enzyme *per se*, because 5 mM $MgCl_2$ is normally included in the enzyme extraction mixture (13). NADP-malate dehydrogenase was shown to be activated by light in maize leaves (13) and in isolated pea chloroplasts (1). I have confirmed the activation of this enzyme by light in chloroplasts from crabgrass mesophyll protoplasts and my evidence shows that exogenous Mg^{2+} blocks this activation (Table I and Fig. 7).

In chloroplasts of C_3 plants, several enzymes of the Calvin cycle are light-activated (1, 3, 14) including NADP-linked glyceraldehyde-3-P dehydrogenase, P-ribulokinase and fructose bisphosphatase. Activation of these enzymes by light was confirmed *in vitro* with intact barley chloroplasts (Table II) and Mg^{2+}, when present in the dark, almost completely blocked the light-dependent increase in activity. In contrast P-glycerokinase did not increase significantly in activity due to illumination of intact chloroplasts (17), and $MgCl_2$ did not inhibit the observed activity (Table II). These results suggest that Mg^{2+} specifically blocks the process of activation of photosynthetic enzymes by light. This mode of action is consistent with the observation that Mg^{2+} was most inhibitory when present in the dark prior to the first illumination period (Figs. 1–3). The mechanism by which Mg^{2+} blocks the light activation process is unclear, but it seems likely that a membrane interaction is involved because the chloroplast envelope is not considered to be permeable to Mg^{2+} (7). It may be significant that PGA, when present in the dark, could apparently protect the chloroplasts from Mg^{2+} inhibition (Fig. 5), but could not reverse the inhibition when added in the light (Fig. 6). Protection of chloroplasts by other metabolites is currently being studied.

The characteristics of the Mg^{2+} inhibition presented in this paper are reminiscent of earlier work (5) which demonstrated that light prevented the inhibition by arsenite of CO_2 fixation by intact spinach chloroplasts. Subsequent work (1) established that arsenite

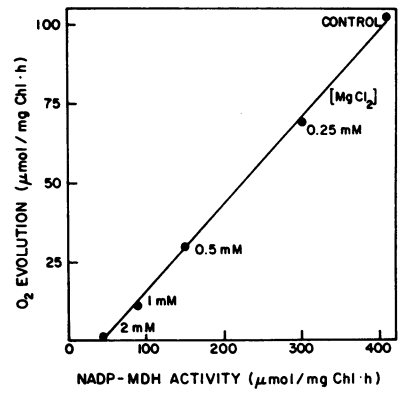

FIG. 7. Correlation of O_2 evolution by crabgrass mesophyll chloroplasts observed in presence of pyruvate + OAA with NADP-malate dehydrogenase (NADP-MDH) activity. Chloroplasts were incubated with the indicated concentration of $MgCl_2$ in the dark before illumination. After 6 min in the light, rates of O_2 evolution were measured and aliquots were assayed for enzyme activity.

Table II. Effect of magnesium on the activity of certain Calvin cycle enzymes.

Intact barley chloroplasts were incubated as described in Materials and Methods either with or without 2 mM $MgCl_2$. After preincubation in the dark for 2 min, and after illumination for 12 min, aliquots were assayed for enzyme activity. Data are averages of three experiments.

Chloroplast treatment	Enzyme Activity			
	Ribulose-5-P kinase	NADP-glyceraldehyde-3-P dehydrogenase	P-glycerate kinase	Fructose bisphosphatase
	(μmol/mg Chl·h)			
Dark	393	108	648	<2
Light	1064	486	800	34
Dark, +2 mM $MgCl_2$[b]	385	112	730	N.D.[a]
Light, +2 mM $MgCl_2$[b]	378	131	717	4

[a]Not determined

[b]The $MgCl_2$ was added in the dark

blocks the activation of several of the dark-inactivated Calvin cycle enzymes. Whereas these authors tentatively concluded that arsenite caused inhibition by reacting with membrane-based sulfhydryl groups, an effect caused simply by membrane perturbation could not be ruled out. Similarly, the inhibition by Mg^{2+} might be explained on the basis of membrane perturbation.

The Mg^{2+} inhibition of photosynthesis described in this paper may reflect a regulatory mechanism whereby the level of cytoplasmic Mg^{2+} controls the photosynthetic capacity of the chloroplast. It is clear that the cytoplasm of a C_3 mesophyll cell contains many enzymes that require free Mg^{2+} for activity, *e.g.* all of the kinases involved in glycolysis as well as uridine diP glucose pyrophosphorylase (8), an enzyme involved in sucrose synthesis. In the case of the C_4 mesophyll cell, P-enolpyruvate carboxylase, a Mg^{2+}-requiring enzyme, also appears to be free in the cytosol (9). Hence, in both cell types, changes in the level of free Mg^{2+} in the cytosol may modulate both the activity of cytoplasmic enzymes as well as the photosynthetic activity of the chloroplast.

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