Plant Physiol. (1980) 65, 350–354
0032-0889/80/65/0350/05/$00.50/0

Effects of Magnesium on Intact Chloroplasts

I. EVIDENCE FOR ACTIVATION OF (SODIUM) POTASSIUM/PROTON EXCHANGE ACROSS THE CHLOROPLAST ENVELOPE[1]

Received for publication July 2, 1979 and in revised form October 5, 1979

STEVEN C. HUBER[2] AND WENDY MAURY[3]
United States Department of Agriculture, Science and Education Administration, Agricultural Research[2], Departments of Crop Science[2] and Botany[2,3], North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

Exogenous Mg^{2+} (2 millimolar) altered the stromal pH of intact spinach chloroplasts. Without added KCl in the medium, Mg^{2+} decreased the stromal pH in the light by approximately 0.3 pH unit. External KCl (25 millimolar) largely prevented the acidification caused by Mg^{2+}. Effects on the stromal pH were not caused by changes in H^+ pumping across the thylakoid membrane because Mg^{2+} had no effect on the light-induced quenching of atebrin fluorescence by intact chloroplasts. However, Mg^{2+} affected H^+ fluxes across the envelope. Addition of Mg^{2+} to intact chloroplasts in the dark caused a significant acidification of the medium that was dependent on the presence of K^+.

External K^+ or Na^+ also prevented the inhibition of CO_2-dependent O_2 evolution by Mg^{2+}, whereas choline chloride was less effective. The combination of Mg^{2+} and K^+ stimulated O_2 evolution at suboptimal pH, inhibited O_2 evolution at optimal and superoptimal pH, and prevented the inhibition of photosynthesis caused by acetate. In the absence of added K^+, Mg^{2+} was most inhibitory to O_2 evolution at suboptimal pH.

The results suggested that Mg^{2+} activated a reversible $(Na^+)K^+/H^+$ exchange across the chloroplast envelope. It is postulated that changes in the stromal pH may explain the inhibition of photosynthesis caused by the presence of exogenous Mg^{2+}.

Millimolar concentrations of Mg^{2+} have been shown to inhibit CO_2-dependent O_2 evolution by isolated chloroplasts of spinach (8, 9, 13, 17), barley (8, 9, 11), and lettuce (2). Results obtained with spinach and barley chloroplasts suggested that Mg^{2+} inhibits photosynthesis by preventing the light activation of NADP-glyceraldehyde-3-P dehydrogenase, phosphoribulokinase, and fructose-1,6-bisphosphatase (9). It was later postulated that Mg^{2+} inhibits O_2 evolution and the light activation of photosynthetic enzymes by stimulating Pi exchange across the chloroplast envelope (8). The postulate was supported by several lines of evidence. First, Mg^{2+} reduced the optimal Pi concentration required for O_2 evolution (8) and inhibition by Mg^{2+} of both O_2 evolution and the light activation of photosynthetic enzymes was prevented by metabolites which compete with Pi for uptake on the phosphate translocator (11). Second, the activation of photosynthetic enzymes by light in a reconstituted system (stromal proteins plus thylakoid membranes) was inhibited by Pi but not by Mg^{2+} (10). Because the chloroplast envelope is impermeable to divalent cations (3), the above observations suggested that Mg^{2+} stimulated Pi exchange indirectly, perhaps by interaction with some component of the chloroplast envelope.

Recent results from this laboratory have suggested that the Pi dependence of chloroplast photosynthesis is sensitive to pH (8). Specifically, reduction of the stromal pH apparently stimulated Pi exchange (8). The objectives of the present study were to determine whether Mg^{2+} affected the stromal pH, and if so, the mechanism involved.

MATERIALS AND METHODS

Chloroplast Isolation. The spinach (*Spinacia oleracea* L.) plants were grown in soil in a growth chamber with a 12-h photoperiod and 22 C/17 C temperature regime. Intact chloroplasts were isolated by the method of Lilley and Walker (14). The blending medium contained 0.33 M sorbitol, 10 mM $Na_4P_2O_7$, 5 mM $MgCl_2$, and 2 mM isoascorbate, adjusted to pH 7.6. Following centrifugation (200*g*, 90 s), the pellet was washed once and resuspended in 0.33 M sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, and 50 mM Hepes-NaOH (pH 7.6). The final preparation contained 50–70% intact chloroplasts, based on the ferricyanide reduction assay.

O_2 Evolution. O_2 evolution was followed polarographically with Clark-type electrodes in 1.8-ml water-jacketed vessels maintained at 25 C. The basic reaction mixture contained 0.33 M sorbitol, 50 mM Hepes-NaOH (pH 7.6), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.5 mM Na_2HPO_4, 6 mM $NaHCO_3$, and 600 units/ml of catalase. The concentration of Chl was 20–50 μg/ml. Illumination was provided by a 75-w floodlamp to give a quantum flux density of 80 nE/cm^2·s between 400 and 700 nm at the face of the cuvette.

Measurement of Stromal pH. The 200-μl chloroplast incubation medium contained 0.33 M sorbitol, 50 mM Hepes-NaOH (pH 7.6), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.5 mM Na_2HPO_4, 600 units/ml of catalase and 5 mM $NaH^{14}CO_3$ (0.75 μCi/μmol). Reactions were run at 25 C and were typically initiated by the addition of chloroplasts (15–30 μg Chl) and terminated after 1 min of illumination by centrifugation through a layer (70 μl) of silicone oil (Wacker AR 200[4]) into a bottom layer of 200 μl of 2.5 N NaOH as previously described (19). Illumination was provided by an overhead 75-w floodlamp that produced 60 nE/cm^2·s (400–700 nm) at the side of the polyethylene centrifuge tube. After centrifugation, a 50-μl aliquot of the top layer was counted in scintillation fluid to determine total dpm in the incubation mixture and the entire bottom layer was excised and placed in scintillation fluid to

[1] Cooperative investigations of the North Carolina Agricultural Research Service and the United States Department of Agriculture, Science and Education Administration, Agricultural Research, Raleigh, North Carolina. Paper No. 6044 of the Journal Series of the North Carolina Agriculture Research Service, Raleigh, North Carolina 27650.

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

determine dpm in the chloroplast pellet. Quench correction was by external standard. The amount of label in the chloroplast pellet was corrected for nonosmotic uptake and absolute volumes were determined by uptake of ^3H_2O and [^{14}C]sucrose as previously described (18). The pH of the stroma was determined in accordance with the relationship (18)

$$\Delta pH = pH_{int} - pH_{ext} = \log \frac{[H^{14}CO_3]_{int}}{[H^{14}CO_3]_{ext}}$$

pH Electrode Measurements. Changes in the pH of the medium were measured with a combination pH electrode at 25 C in the dark. The 2-ml reaction mixture contained 0.33 M sorbitol, 0.5 mM Hepes-NaOH (pH 7.0), 0.5 mM Pi, and chloroplasts (50–75 μg Chl/ml). The buffering capacity of the mixture was determined at the end of each experiment by addition of 0.1 μmol NaOH. The initial pH was adjusted to pH 7.0 to minimize the spontaneous acidification of the medium observed when the initial pH was greater than 7.0.

All experiments were repeated at least three times using different chloroplast isolations.

RESULTS

Effects of Mg^{2+} on Stromal pH. The stromal pH of intact spinach chloroplasts after 1 min of illumination was about pH 8.1 (Table I), which concurs with previous findings (6, 18, 19). $MgCl_2$ decreased the stromal pH in the light by approximately 0.3 pH units (Table I). The stromal pH was not affected by exogenous KCl; however, KCl largely prevented the acidification caused by Mg^{2+} (Table I). Occasionally, the combination of Mg^{2+} + K^+ caused an increase in absolute stromal pH relative to the control pH. The chloroplast stromal volume was also affected by Mg^{2+} (Table I). Mg^{2+} decreased the stromal volume by about 36%. External KCl (25 mM) caused only a slight decrease in volume, a result which may be attributed to the increase in medium osmolarity (50 mM total). The decrease in stromal volume caused by Mg^{2+} was prevented by K^+ (Table I).

The acidification of the stromal pH caused by Mg^{2+} (Table I) could be explained by reduced pumping of H^+ across the thylakoid membrane or by increased permeability of the chloroplast envelope to H^+. In experiments not reported here, exogenous Mg^{2+} (3 mM) had no significant effect on the light-dependent quenching of atebrin fluorescence (12), which reflects acidification of the intrathylakoid space. The results indicated that exogenous Mg^{2+} probably did not affect the stromal pH by affecting H^+ translocation through the thylakoid membrane.

If changes in the stromal pH (Table I) were caused by movement of protons across the chloroplast envelope, changes in the pH of the medium should be observed. Typical results are presented in Figure 1. Addition of 4 mM $MgCl_2$ in the dark to chloroplasts suspended in a medium of low buffering capacity (at pH 7.0) caused a significant acidification of the medium that was dependent on external K^+. Without added K^+, addition of Mg^{2+} usually caused an alkalization of the medium (Fig. 1) and occasionally, a slight acidification (data not shown). No pH changes were observed when chloroplasts were omitted from the reaction mixture. Some of the acidification caused by Mg^{2+} may be attributed to displacement of protons bound to lipid groups in the membrane. Hence, quantitative evaluation of the data may not be justified. However, the increased acidification of the medium by Mg^{2+} in the presence of 40 mM K^+ (Fig. 1) suggested that some of the released protons were obtained from the stromal space (Table I) and that Mg^{2+} affected the envelope rather than the thylakoid membrane.

Reversal of Mg^{2+} Inhibition of O_2 Evolution by Salts. Studies were conducted to determine whether exogenous K^+ would prevent Mg^{2+} inhibition of CO_2-dependent O_2 evolution. Mg^{2+} (4 mM) produced nearly complete inhibition of O_2 evolution, whereas

Table I. *Effects of Mg^{2+} and K^+ on Stromal pH and Volume of Spinach Chloroplasts after 1 min of Illumination at 25 C*

Additions	Stromal pH	Stromal Volume
		μl/mg Chl
None	8.10	27.5
4 mM $MgCl_2$	7.78	17.5
25 mM KCl	8.14	25.0
4 mM $MgCl_2$ + 25 mM KCl	8.02	25.0

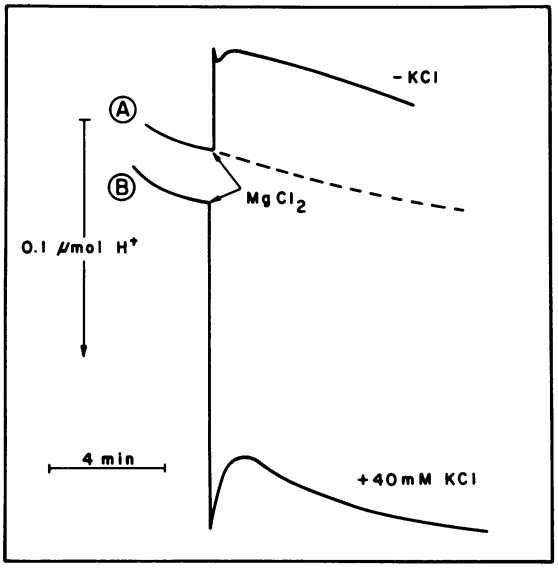

FIG. 1. Changes in pH of external medium by addition of 4 mM $MgCl_2$ in presence and absence of 40 mM KCl. (---): Change in pH without addition of Mg^{2+}.

KCl (30 mM), in the absence of Mg^{2+}, had no effect (Fig. 2). Similar effects of Mg^{2+} and K^+ were reported previously (10). Inhibition of O_2 evolution by Mg^{2+} was completely prevented by K^+ when both were added before illumination and addition of K^+ in the light to Mg^{2+}-inhibited chloroplasts caused a rapid rise in O_2 evolution (Fig. 2).

Other monovalent salts were tested for effects on O_2 evolution in the presence and absence of Mg^{2+}. The chloride salts of K^+, Na^+, and choline did not produce significant inhibition of O_2 evolution but prevented Mg^{2+} inhibition of O_2 evolution to varying degrees (Table II). At a concentration of 50 mM, Na^+ and K^+ almost completely reversed the inhibition by Mg^{2+}, whereas choline did not (Table II). LiCl produced significant inhibition of O_2 evolution in the presence or absence of Mg^{2+}, a result which must be ascribed to inhibition by Li^+. The sulfate salts of the monovalent cations were inhibitory, which is consistent with previous observations (1, 5).

The concentration dependence for K^+ and choline reversal of Mg^{2+} inhibition of O_2 evolution is presented in Figure 3. Up to concentrations of 30 mM, K^+ and choline had no effect on the rate of O_2 evolution in the absence of Mg^{2+} (Fig. 3). Without added salt, O_2 evolution was inhibited greater than 90% by 2 mM Mg^{2+} and 97% by 4 mM $MgCl_2$. Relatively low concentrations of K^+ were considerably more effective than equimolar amounts of choline in preventing Mg^{2+} inhibition of O_2 evolution. The action of K^+ was not affected by increasing the concentration of Mg^{2+} from 2 to 4 mM (Fig. 3). The results suggested that prevention of Mg^{2+} inhibition by K^+ was not caused simply by an ionic strength effect, because in that case, choline would be expected to be as effective as K^+ and prevention by the monovalent salt should decrease as the concentration of Mg^{2+} was increased.

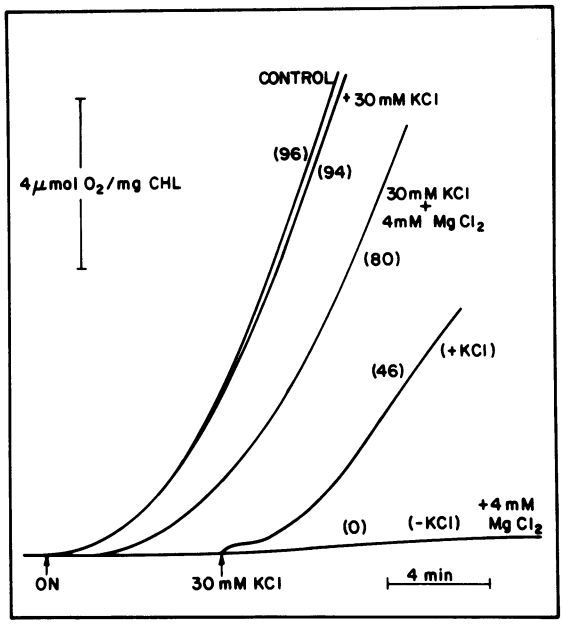

FIG. 2. Typical results showing prevention and reversal of Mg^{2+} inhibition of spinach chloroplast O_2 evolution by KCl. At the arrow, 30 mM KCl was added to a reaction mixture that contained 4 mM $MgCl_2$. All other additions were made in the dark. Maximum rates of O_2 evolution, expressed as μmol O_2/mg Chl·h, are shown parenthetically.

Table II. *Effect of Various Salts on CO_2-dependent O_2 Evolution by Spinach Chloroplasts in the Presence and Absence of $MgCl_2$*

Added Salt (50 mM)	O_2 Evolution	
	$-MgCl_2$	+ 2mM $MgCl_2$
	μmol O_2/mg Chl·h	
None[a]	70	5
LiCl	6	4
KCl	60	65
NaCl	65	59
Choline-Cl	74	35

[a] Reaction mixtures contained about 30 mM Na^+ used to neutralize the Hepes buffer.

Mg^{2+}-dependent Stimulation of O_2 Evolution at Suboptimal pH. If exogenous Mg^{2+} altered the stromal pH, predictable effects should be observed on the pH dependence of O_2 evolution. Heldt *et al.* (6) have shown that the stromal pH varies in response to changes in the pH of the external medium, and that it is the pH of the stroma which controls Calvin cycle activity (19). On this basis, any condition that causes acidification of the stroma should be most inhibitory to O_2 evolution at pH values less than the pH optimum of the control (*i.e.* suboptimal pH). Similarly, alkalization of the stroma would be expected to stimulate at suboptimal pH and inhibit O_2 evolution at pH values above the control optimum (*i.e.* superoptimal pH). The pH dependence of photosynthesis by spinach chloroplasts in the presence of various salts is presented in Figure 4. Rates of O_2 evolution in the absence of added salt were maximal over the pH range 7.7–8.0. The pH dependence was not affected by 30 mM KCl (data not shown). In contrast, Mg^{2+} affected the pH dependence of O_2 evolution by spinach chloroplasts. Inhibition of O_2 evolution was observed over the entire pH range but Mg^{2+} was most inhibitory at suboptimal pH (Fig. 4). The effect of Mg^{2+} was to narrow and increase the pH range over which O_2 evolution was maximal. Similar results were obtained previously with barley chloroplasts (8). The effect of Mg^{2+} was largely reversed by exogenous K^+. As shown by the results presented in Figure 4, Mg^{2+} + K^+ increased O_2 evolution even above control values at suboptimal pH (pH 7.0–7.5) and inhibited O_2 evolution at higher pH (Fig. 4). The results suggested that Mg^{2+} caused acidification of the stroma in the absence of K^+ and alkalization in the presence of exogenous K^+.

The alkaline-shifted pH optimum for photosynthesis in the presence of Mg^{2+} (Fig. 4) may reflect both acidification of the stroma (Table I) and the increasing concentration of Na^+ (used to neutralize the buffer) with increased pH. Because Na^+ was equivalent to K^+ in reversing Mg^{2+} inhibition of photosynthesis, a complete evaluation of the effects of Mg^{2+} and monovalent cations will have to be done using a buffer system neutralized with a nonpermeating base. It is apparent that at least at low pH (<8.0), the Na^+ contributed by the buffer was not sufficient to prevent Mg^{2+} inhibition (Fig. 4). At pH 8.5, the rate of photosynthesis in the control was similar to the rate in the presence of Mg^{2+} and Mg^{2+} + K^+ (Fig. 4), which may indicate that the postulated Mg^{2+}-dependent changes in stromal pH do not occur at high external pH.

The postulate predicted that Mg^{2+} + K^+ should reverse inhibition of O_2 evolution caused by weak acids that act by causing acidification of the stroma. Heldt *et al.* (6) have shown that Ac[5] causes acidification of the stroma which was suggested to occur by diffusion of HAc across the envelope followed by internal dissociation to produce H^+ + Ac^-. The interpretation was supported by the demonstration that mM concentrations of NaAc inhibited spinach chloroplast O_2 evolution at suboptimal pH and stimulated O_2 evolution at superoptimal pH (6, 8).

Typical results showing inhibition of CO_2-dependent O_2 evolution by NaAc are presented in Figure 5. Ac inhibited the rate of O_2 evolution about 35% (Fig. 5, trace D). The presence of 30 mM KCl alone caused only a slight inhibition of rate (Fig. 5, trace B), whereas 2 mM $MgCl_2$ produced greater than 95% inhibition (Fig. 5, trace F). In the absence of exogenous K^+, Mg^{2+} accentuated inhibition of O_2 evolution by Ac (Fig. 5, trace G). Inhibition of O_2 evolution by NaAc was not affected by KCl; however, inhibition was completely reversed by Mg^{2+} + K^+ (Fig. 5, trace C).

DISCUSSION

The purpose of this study was to determine the basis for inhibition of chloroplast photosynthesis by Mg^{2+} (2, 9, 13). The results presented herein suggested that exogenous Mg^{2+} altered the pH of the stroma by affecting H^+ movements across the envelope. The direction of the pH change was apparently dependent on the concentration of K^+ in the medium. Admittedly, the effects of Mg^{2+} are complex and not entirely understood. However, as a working model we postulate that Mg^{2+} activated a reversible $(Na^+)K^+/H^+$ exchange across the chloroplast envelope (Fig. 6).

Several lines of evidence indicated that when the concentration of $(Na^+)K^+$ in the medium was low, Mg^{2+} caused acidification of the stroma (Fig. 6A). First, the stromal pH in the light was significantly reduced by Mg^{2+} (Table I), which was probably caused by an influx of H^+ from the medium (Fig. 1). Second, Mg^{2+} was most inhibitory to O_2 evolution at suboptimal pH (Fig. 4 and ref. 8). Previously, Ac (19) and nitrite (16) have been shown to reduce the stromal pH and to inhibit O_2 evolution preferentially at suboptimal pH. Third, it was demonstrated previously with barley chloroplasts that Mg^{2+} inhibition was prevented by NH_4Cl (8) which presumably caused alkalization of the stroma. The stromal content of K^+ has been estimated to be approximately 20–30 mM (3); however, the concentration of free K^+ in the stroma may be considerably less. The mechanism schematically presented (Fig. 6A) suggests that influx of H^+ may be coupled to the efflux of stromal K^+ down its concentration gradient. The resultant

[5] Abbreviation: Ac: acetate.

stromal acidification may inhibit photosynthesis by increasing Pi exchange (ref. 8; see Fig. 6A) and decreasing the activation of certain photosynthetic enzymes by light (9). Another potential factor limiting photosynthesis is that the photosynthetic enzymes would have to function at suboptimal pH. The extent to which these factors are related remains an open question, although it is clear that stromal pH does not affect apparent Pi exchange by decreasing Calvin cycle activity (Huber, manuscript in preparation). The magnitude of the pH decrease caused by Mg^{2+} (0.32 pH units, Table I) may be sufficient to account for the inhibition of photosynthesis. Heldt *et al.* (6) have shown that a 1 pH unit decrease of the medium pH results in a decreased stromal pH of approximately 0.5 pH unit. Hence, a decrease in stromal pH of 0.3 unit may be analogous to decreasing the pH medium by 0.6 pH units. Such a change in medium pH (*i.e.* from pH 7.6 to 7.0) gave complete inhibition of O_2 evolution with the chloroplasts used in this study (Fig. 4).

When the medium contained high KCl, Mg^{2+} apparently caused alkalization of the stroma (Fig. 6B). The conclusion was supported by the following responses that were dependent on Mg^{2+} + K^+: (*a*) prevention of stromal acidification caused by Mg^{2+} alone and occasionally an increase in the stromal pH above the control values (Table I); (*b*) stimulation of CO_2-dependent O_2 evolution

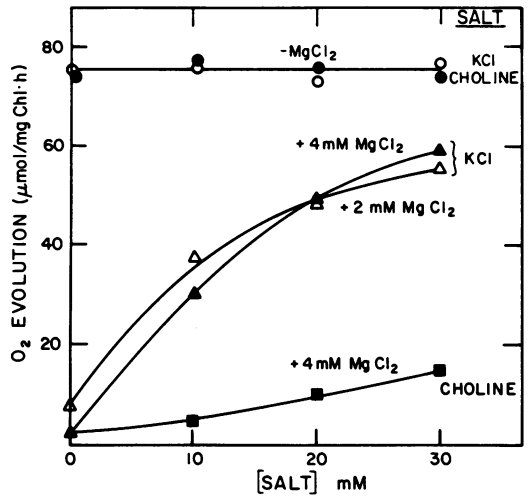

FIG. 3. Effect of K^+ and choline chloride on spinach chloroplast O_2 evolution in the presence and absence of $MgCl_2$.

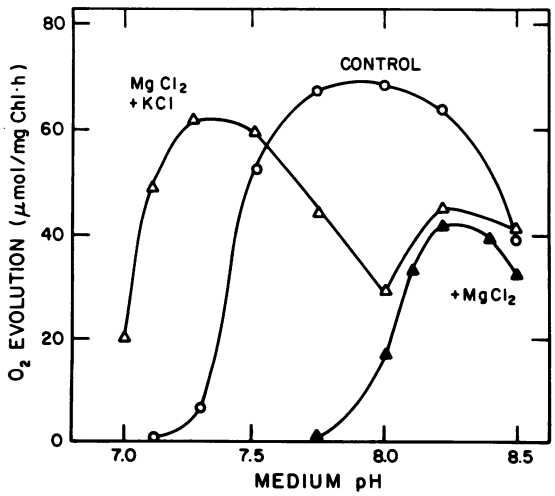

FIG. 4. Effect of pH on O_2 evolution by spinach chloroplasts in the presence and absence of 2 mM $MgCl_2$ and 30 mM KCl. Reaction mixtures were buffered with 50 mM Hepes, adjusted to the indicated pH with NaOH.

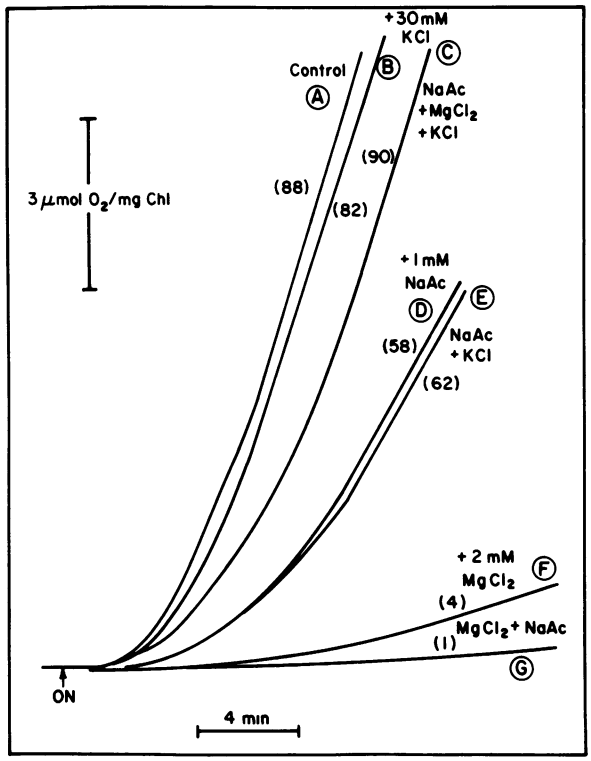

FIG. 5. Prevention of Ac inhibition of spinach chloroplast O_2 evolution by Mg^{2+} + K^+. The indicated salts were added before illumination. Maximum rates of O_2 evolution, expressed as μmol O_2/mg Chl·h are shown parenthetically.

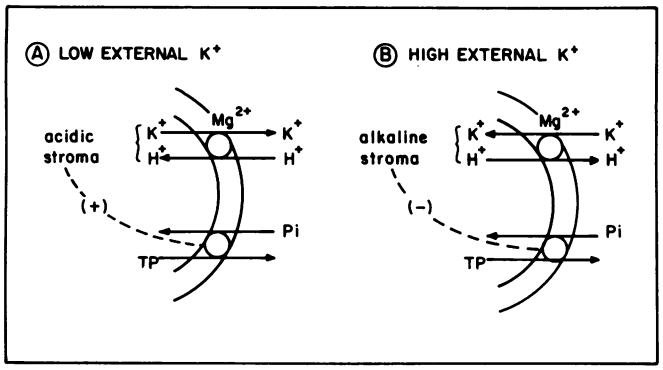

FIG. 6. Schematic diagram of effect of Mg^{2+} induced K^+/H^+ exchange on stromal pH at (A) low and (B) high concentration of external K^+ and suggested effects of stromal pH on phosphate translocator. It is postulated that the K^+/H^+ exchange shown occurs only in the presence of exogenous Mg^{2+}. (+): activation; (−): inhibition.

at suboptimal pH (Fig. 4); (*c*) prevention of Ac inhibition of O_2 evolution (Fig. 5); and (*d*) acidification of the medium (Fig. 1). The results may be explained on the basis that influx of K^+, driven by the existing concentration gradient, was coupled to an efflux of H^+, thereby causing alkalization of the stroma and reduced phosphate exchange (Fig. 6B).

Because the chloroplast envelope is impermeable to divalent cations (3), it may be adduced that Mg^{2+} binds to some "site" on the inner membrane of the envelope. It was important to determine whether exogenous K^+ simply reversed the effect of Mg^{2+} by causing an ionic strength displacement of Mg^{2+} from the envelope. An ionic strength effect seemed unlikely because choline was less effective than K^+ or Na^+ in reversing Mg^{2+} inhibition of O_2 evolution (Table II and Fig. 3). Further, K^+ did not simply reverse

the effect of Mg^{2+}, but actually caused additional effects. For example, at suboptimal pH, the combination of Mg^{2+} + K^+ stimulated O_2 evolution well above the control rates (Fig. 4) and prevented inhibition of O_2 evolution by acetate (Fig. 5) which causes acidification of the stroma.

The $(Na^+)K^+/H^+$ antiporter postulated to be presented in the chloroplast envelope (Fig. 6) may be mechanistically similar to the Na^+/H^+ antiporter in the inner membrane of rat liver mitochondria (15) and the $(Na^+)K^+/H^+$ antiporter of plant mitochondria (7). The mechanism postulated in the present study seemed to account for many of the experimental observations concerning the effects of Mg^{2+} on isolated chloroplasts. Experiments are underway to correlate H^+ and K^+ fluxes across the chloroplast envelope and to determine whether the envelope ATPase is involved.

Because many cytoplasmic enzymes require Mg^{2+} for activity, it is likely that the chloroplast *in situ* must function in an environment containing this cation. From the results presented herein, it appears that whether Mg^{2+} is inhibitory to chloroplast photosynthesis is directly dependent on pH and the concentration of K^+ and indirectly on factors affecting the phosphate translocator (11). The potential may exist for the control of both chloroplastic and extrachloroplastic processes, such as sucrose formation, by the concentration of cytoplasmic Mg^{2+}.

Note. During review of this manuscript, we became aware of a paper by B. Demming and H. Gimmler (Z Naturforsch 34c: 233–241) in which they have independently demonstrated acidification of the stroma and decrease in stromal K^+ caused by Mg^{2+}. These authors also reported prevention of Mg^{2+} inhibition of O_2 evolution by K^+ and derived conclusions similar to our own.

LITERATURE CITED

1. Baldry CW, W Cockburn, DA Walker 1968 Inhibition, by sulfate, of the O_2 evolution associated with photosynthetic carbon assimilation. Biochim Biophys Acta 153: 476–483
2. Bamberger ES, M Avron 1975 Site of action of inhibitors of carbon dioxide assimilation by whole lettuce chloroplasts. Plant Physiol 56: 481–485
3. Gimmler H, G Schäfer, U Heber 1974 Low permeability of the chloroplast envelope towards cations. *In* M Avron, ed, Proc 3rd Int Cong Photosynthesis. Elsevier, Amsterdam, pp 1381–1392
4. Gimmler H, G Schäfer, H Kraminer, U Heber 1974 Amino acid permeability of the chloroplast envelope as measured by light scattering, volumetry and amino acid uptake. Planta 120: 47–61
5. Hampp R, I Ziegler 1977 Sulfate and sulfite translocation via the phosphate translocator of the inner membrane of chloroplasts. Planta 137: 309–312
6. Heldt HW, K Werdan, M Milovancev, G Geller 1973 Alkalization of the chloroplast stroma caused by light-dependent proton flux into the thylakoid space. Biochim Biophys Acta 314: 224–241
7. Hensley JR, JB Hanson 1975 The action of valinomycin in uncoupling corn mitochondria. Plant Physiol 56: 13–18
8. Huber SC 1978 Effect of pH on chloroplast photosynthesis. Inhibition of O_2 evolution by inorganic phosphate and magnesium. Biochim Biophys Acta 545: 131–140
9. Huber SC 1978 Regulation of chloroplast photosynthetic activity by exogenous magnesium. Plant Physiol 62: 321–325
10. Huber SC 1978 Substrates and inorganic phosphate control the light activation of NADP-glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase in barley (*Hordeum vulgare*) chloroplasts. FEBS Lett 92: 12–16
11. Huber SC 1979 Effect of photosynthetic intermediates on the magnesium inhibition of O_2 evolution by barley chloroplasts. Plant Physiol 63: 754–757
12. Krause GH, SW Thorne, GH Lorimer 1977 Glycolate synthesis by intact chloroplasts. Studies with inhibitors of photophosphorylation. Arch Biochem Biophys 183: 471–479
13. Lilley R McC, JD Schwenn, DA Walker 1973 Inorganic pyrophosphatase and photosynthesis by isolated chloroplasts. II. The controlling influence of orthophosphate. Biochim Biophys Acta 325: 596–604
14. Lilley R McC, DA Walker 1974 The reduction of 3-phosphoglycerate by reconstituted chloroplasts and by chloroplast extracts. Biochim Biophys Acta 368: 269–278
15. Mitchell P, J Moyle 1969 Translocation of some anions, cations and acids in rat liver mitochondria. Eur J Biochem 9: 149–155
16. Purczeld P, CJ Chon, AR Portis Jr, HW Heldt, U Heber 1978 The mechanism of the control of carbon fixation by the pH in the chloroplast stroma. Studies with nitrite-mediated proton transfer across the envelope. Biochim Biophys Acta 501: 488–498
17. Walker DA 1976 CO_2 fixation by intact chloroplasts: photosynthetic induction and its relation to transport phenomena and control mechanisms. *In* J. Barber, ed, The Intact Chloroplast. Elsevier/North Holland Biomedical Press, Amsterdam, pp 235–278
18. Werdan K, HW Heldt 1972 Accumulation of bicarbonate in intact chloroplasts following a pH gradient. Biochim Biophys Acta 283: 430–441
19. Werdan K, HW Heldt, M Milovancev 1975 The role of pH in the regulation of carbon fixation in the chloroplast stroma. Studies on CO_2 fixation in the light and dark. Biochim Biophys Acta 396: 276–292