Stromal pH and Photosynthesis Are Affected by Electroneutral K\textsuperscript{+} and H\textsuperscript{+} Exchange through Chloroplast Envelope Ion Channels\textsuperscript{1}

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ABSTRACT

Potassium movement across the limiting membrane of the chloroplast inner envelope is known to be linked to counterexchange of protons. For this reason, K\textsuperscript{+} efflux is known to facilitate stromal acidification and the resultant photosynthetic inhibition. However, the specific nature of the chloroplast envelope proteins that facilitate K\textsuperscript{+} fluxes, and the biophysical mechanism which links these cation currents to H\textsuperscript{+} counterflux, is not characterized. It was the objective of this work to elucidate the nature of the system regulating K\textsuperscript{+} flux linked to H\textsuperscript{+} counterflux across the chloroplast envelope. In the absence of external K\textsuperscript{+}, exposure of spinach (Spinacia oleracea) chloroplasts to the K\textsuperscript{+} ionophore valinomycin was found to increase the rate of K\textsuperscript{+} efflux and H\textsuperscript{+} influx. These data were interpreted as suggesting that H\textsuperscript{+} counterexchange must be indirectly linked to movement of K\textsuperscript{+} across the envelope. Studies using the K\textsuperscript{+} channel blocker tetraethylammonium indicated that K\textsuperscript{+} likely moves, in a uniprot fashion, into or out of the stroma through a monovalent cation channel in the envelope. Blockage of K\textsuperscript{+} efflux from the stroma by exposure to tetraethylammonium was found to restrict H\textsuperscript{+} influx, further substantiating an indirect linkage of these cation currents. Further studies comparing the effect of exogenous H\textsuperscript{+} ionophores and K\textsuperscript{+}/H\textsuperscript{+} exchangers suggested that K\textsuperscript{+} uniport through this ion channel likely is the main endogenous pathway for K\textsuperscript{+} currents across the envelope. These experiments were also consistent with the presence of a proton channel in the envelope. Movement of H\textsuperscript{+} through this channel was speculated to be regulated and rate limited by an electroneutral requirement for K\textsuperscript{+} countercurrents through the separate K\textsuperscript{+} uniport pathway. K\textsuperscript{+} and H\textsuperscript{+} fluxes across the chloroplast envelope were envisioned to be interrelated via this mechanism. The significant effect of cation currents across the envelope, as mediated by these channels, on photosynthetic capacity of the isolated chloroplast was also demonstrated.

Light-induced stromal alkalization, and the subsequent maintenance of high stromal pH in the light, is an important regulatory phenomenon that allows for optimal functioning of photosynthetic carbon reduction cycle enzymes (19). Stromal pH in the illuminated chloroplast is known to be linked to internal (i.e., stromal) and external K\textsuperscript{+}. Maintenance of high stromal K\textsuperscript{+} and pH are coincident (2), and K\textsuperscript{+}/H\textsuperscript{+} counterflux is known to occur across the chloroplast envelope (1, 2, 9, 11). Chloroplast photosynthesis, therefore, is greatly affected by regulated K\textsuperscript{+} and H\textsuperscript{+} fluxes across the chloroplast envelope. However, the system of chloroplast envelope proteins that facilitate and regulate K\textsuperscript{+} and H\textsuperscript{+} fluxes is not well characterized.

It has been asserted that a specific envelope protein acts as a K\textsuperscript{+}/H\textsuperscript{+} antiporter (9, 11). However, the apparently nonstoichiometric K\textsuperscript{+}/H\textsuperscript{+} counterexchange documented to occur across the envelope (2) cannot be explained by this mechanism. Another hypothesis focuses on the apparent Donnan system occurring in the stroma of the illuminated chloroplast (2), which was thought to establish a large membrane potential (inside negative) across the envelope. It was postulated that this membrane potential provided the driving force for (monovalent) cation influx. In the absence of external cations, H\textsuperscript{+} influx and stromal acidification were thought to be a result of this driving force. This proposed mechanism, however, does not explain how nonenergetic (15) H\textsuperscript{+} efflux from the stroma (6, 8) can occur. A third proposal regarding the biophysical mechanism(s) driving K\textsuperscript{+}/H\textsuperscript{+} counterfluxes involves the thylakoid electron transport chain, which has been hypothesized to facilitate direct proton and/or monovalent cation flow from the chloroplast to the external medium via thylakoid:inner envelope membrane connections (3, 15). Solid experimental evidence supporting this mechanism is, at present, lacking.

The work presented in this report focuses on examining K\textsuperscript{+} efflux from the stroma and how this K\textsuperscript{+} flux affects chloroplast metabolism. The overall objectives of the work were to (a) elucidate the nature of the chloroplast envelope protein that regulates K\textsuperscript{+} fluxes and (b) characterize the biophysical mechanism that links K\textsuperscript{+} fluxes to H\textsuperscript{+} countertransport.

MATERIALS AND METHODS

Detailed descriptions of the methods used in this study have been included in recent reports from this laboratory (14, 17, 20) and, therefore, will be only briefly presented here. Spinach (Spinacia oleracea var Melody) was grown in a growth cham-
ber under the same conditions as described before (17). Intact (routinely >90%) chloroplasts were isolated using Percoll step gradients by grinding leaf tissue and subsequent centrifugation in medium containing 0.33 m sorbitol, 50 mM Hepes-NaOH (pH 6.8), 2 mM Na2EDTA, 1 mM MgCl2, and 1 mM MnCl2 (17). All steps of the isolation procedure were carried out at 0°C, and chloroplasts (equivalent to approximately 1 mg Chl/mL) were resuspended in a small volume of grind medium and stored on ice after isolation. Photosynthesis of isolated chloroplasts was determined by measuring CO2-supported O2 evolution with O2 electrodes (17); all studies were done at 25°C.

The silicone oil microcentrifugation technique of Heldt (7) was used for the measurements of stromal pHi, total stromal K+, K+ (36Rb+) uptake, and chloroplast stromal volume (17, 19). Briefly, 20 μL 14% (v/v) HClO4 and, then, 100 μL of silicone oil were layered in 400-μL microfuge tubes. Reaction medium (200 μL) containing chloroplasts was layered on top of the silicone oil. After the chloroplasts were incubated under certain conditions as noted in the text, they were pelleted into the HClO4 fraction by centrifugation. Radionuclide equilibration into the stroma was determined by measuring label centrifuging with the chloroplasts into the HClO4 layer. Stromal pHi was probed by monitoring equilibration of [3H]H2O and [14C]sorbitol and measurement of the relative equilibration of these compounds into the chloroplast. These volume measurements were made for the stromal pHi and [K+] studies; chloroplasts were incubated in media with additions as noted in the text so as to ascertain volumes under the various treatments in each experiment. Radionuclides were measured using a Beckman 3801 liquid scintillation spectrophotometer (Somerset, NJ) with external standards ratio quench correction for [3H]/[14C] experiments; 100% counting efficiency was assumed for [36Rb+] counting. Total stromal K+ was ascertained by measuring K+ in aqueous resuspensions of the HClO4 fraction from silicone oil centrifugation tubes using a Perkin-Elmer 2280 atomic absorption spectrophotometer (Norwalk, CT).

To initiate experiments, aliquots of isolated chloroplasts in grind medium (maintained at 0°C unless otherwise noted) were diluted (25–50 μL/mL) into either normal reaction medium or low salt medium. Normal reaction medium contained 0.33 m sorbitol, 50 mM Hepes-NaOH (pH 7.6 or as indicated in text), 2 mM Na2EDTA, 1 mM MgCl2, 1 mM MnCl2, 0.25 mM NaH2PO4, 5 mM NaHCO3, and 1000 units/mL catalase. Low salt medium has the same contents, except the pH was adjusted with Tris-base instead of NaOH. The normal reaction medium that is used to support optimal photosynthesis of isolated chloroplasts has approximately 45 mM Na+, whereas the low salt medium has only 9.25 mM Na+. Chloroplasts were typically preincubated for various times at 25 or 0°C in either normal reaction medium or low salt medium (as noted in figure legends and table headings) before measurement of photosynthesis or illumination in a water bath at 25°C during silicone oil microcentrifugation studies.

RESULTS AND DISCUSSION

K+ Loss Effects on Photosynthesis

Several researchers (2, 16) have noted that optimal photosynthesis of isolated chloroplasts is facilitated by the addition of 10 to 100 mM K+ (or Na+) to the reaction medium. Although not specifically monitored, the presumption that can be made from these previous studies is that this level of external monovalent cations is required to prevent K+ loss from the stroma, which would inhibit photosynthesis if this K+ flux was linked to H+ import. These relationships are illustrated in the series of experiments shown in Figures 1 and 2. When chloroplasts are suspended in normal reaction medium at 0°C, there is a slow decline in stromal K+ with time (Fig. 1B). At 25°C, K+ loss from the stroma is increased. Temperature-dependent K+ loss from the stroma is further increased when plastids are suspended in media with low monovalent salt concentrations. This rapid K+ loss from the stroma that occurs at 25°C is associated with a rapid loss of photosynthetic capacity (Fig. 1A), which is more pronounced when the suspending medium has a low level of monovalent cations. The relationship between K+ loss and photosynthetic inhibition is illustrated more clearly in Figure 1C. Apparently, a given amount of K+ loss, from chloroplasts suspended in either normal or low salt reaction medium, is associated with a specific extent of photosynthetic inhibition.

K+ efflux from the stroma is associated with a lowered stromal pHi when chloroplasts are subsequently illuminated (Fig. 2). After 10 min incubation at 0°C, there is a slight impairment of light-induced stromal alkalization. A 10-min incubation at 25°C results in a significant decrease in stromal pHi, which is even more pronounced when chloroplasts are incubated at 25°C in low salt medium. A comparison of data presented in Figures 1 and 2 reveals a close association among temperature-dependent K+ loss, impairment of light-induced stromal alkalization, and photosynthetic inhibition.

It should be noted that, in a previous study, Mouriaux and Douce (13) also investigated the nature of photosynthetic activity decline of chloroplasts after isolation. They associated photosynthetic loss with a slow decline in stromal [PO43-]. The loss of photosynthesis over time was found to be partially reversed when 10 mM PO43- was included in the incubation medium. These results are not necessarily in conflict with the data reported here. The rates of both PO43- loss and photosynthetic decline they measured were significantly lower than the rates of K+ loss and photosynthetic decline reported here. K+ and PO43- loss could be occurring concomitantly. Also, the stimulatory effect of the PO43- addition to their incubation medium could have been contributed to by the presence of a monovalent cation (K+ or Na+) in the phosphate salt added to their incubation solution.

Effects of Artificially Increasing K+ Conductance across the Envelope

Exposure of biological membranes to the ionophore valinomycin selectively increases K+ conductance and allows for rapid electrochemical gradient-driven K+ fluxes. Valinomycin was used in a series of experiments to examine the effect of
supplying an exogenous, high-conductance K⁺ channel to the chloroplast envelope. The rapid K⁺ loss that occurs when chloroplasts are incubated at 25°C (Fig. 1B) was found to be even greater when plastids were exposed to valinomycin. In one experiment, chloroplasts incubated in normal reaction medium had stromal K⁺ levels of 193 ± 3, 160 ± 5, and 139 ± 4 mM (mean ± SD), respectively, when incubated at 25°C for 0, 5, and 10 min, respectively. The same chloroplast preparation, when exposed to 0.25 µM valinomycin, has stromal K⁺ levels of 136 ± 5 and 108 ± 5 mM, after 5 and 10 min incubation, respectively, at 25°C. Treatment of chloroplasts with valinomycin was also found to increase the loss of

Figure 2. Incubation time and temperature effects on stromal pH. After the appropriate preincubation times as indicated, chloroplasts were incubated in the light at 25°C for 5 min before silicone oil microcentrifugation (i.e., pH measurement). Data are shown for chloroplasts incubated in normal reaction medium (NRM) and low salt medium (LSM). All data are means ± se (n = 4). This experiment was repeated a total of three times with similar results.

Table I. Effect of Valinomycin on Photosynthesis of Isolated Chloroplasts

Chloroplasts were preincubated in low salt medium (pH 7.6) at 25°C with or without valinomycin and 40 mM K⁺ before photosynthetic rates were measured. Measurements of photosynthesis were carried out in the same medium at 25°C. Initial rates of photosynthesis before preincubation with and without valinomycin were 115 (−K⁺) and 117 (+K⁺) µmol O₂/mg Chl/h, respectively. Data shown are the means of two measurements (taken on the same chloroplast preparation) for each treatment. This experiment was repeated four times, with similar results.

<table>
<thead>
<tr>
<th>Valinomycin</th>
<th>Preincubation Time</th>
<th>Photosynthesis</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>µM</td>
<td>min</td>
<td>−K⁺</td>
<td>+K⁺</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>41</td>
<td>65</td>
</tr>
<tr>
<td>0.25</td>
<td>10</td>
<td>9</td>
<td>51</td>
</tr>
<tr>
<td>0.50</td>
<td>10</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>1.00</td>
<td>10</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

*All photosynthetic rates are expressed as a percentage of the rate measured in the absence of valinomycin with chloroplasts that were not preincubated at 25°C.
photosynthetic activity that occurs during incubation in low salt medium at 25°C (Table I). As shown in Table I, valinomycin effects on photosynthesis are partially reversed when 40 mM K+ is included in the incubation medium.

This addition of K+ to the incubation medium was also found to partially reverse the photosynthetic inhibition that occurs when chloroplasts are left at 25°C for 10 min in the absence of valinomycin (Table I). It should be noted that the stimulatory effect of K+ (provided as the Cl− salt) addition to the incubation medium in this and other experiments described in this report was not due to the presence of chloride. Control experiments (data not shown) indicated that choline chloride had no effect on photosynthesis under any of the treatment regimens.

The effects of valinomycin on K+ efflux and concomitant photosynthetic inhibition as presented here are not surprising or noteworthy in and of themselves. However, we believe that the data shown in Figure 3 are an important contribution to the understanding of how K+ and H+ fluxes across the chloroplast envelope are linked. Valinomycin specifically allows for only K+ uniport (5). Nonetheless, supplying the chloroplast envelope with an exogenous, high-conductance, (presumably) unregulated K+ uniport pathway results in dramatic reduction in stromal pH (i.e. H+ influx into the stroma), as shown in Figure 3. Notably, this H+ influx is partially ameliorated when 40 mM K+ is supplied to the external medium (Fig. 3); the presence of this level of external K+ should reduce the electrochemical gradient favoring K+ efflux. The answer to the question of how valinomycin-induced K+ efflux can cause H+ influx provides some insights into the biophysical mechanism that links the movement of these cations, in a counterflux fashion, across the envelope. H+ counterflux must be linked indirectly to K+ efflux across the envelope. Whether or not one of the intrinsic envelope proteins is a K+/H+ antiporter as speculated by Huber and Maury (9) it is clear from the data presented here that K+ uniport can result in a substantial extent of H+ counterflux and stromal acidification.

These data are consistent with the possible existence of a proton channel in the chloroplast envelope that allows for H+ flux which is rate-limited by the requirement for electroneutral K+ counterflux through a separate uniport pathway. Increasing membrane conductance for K+ by exposure to the ionophore valinomycin allows for a greater extent of H+ import (Fig. 3). This analysis of driving forces and linkage of H+ and K+ fluxes via electroneutral exchange across an organelle membrane has a theoretical basis in the work of Garlid and coworkers (4, 5), who characterized the mechanisms regulating K+/H+ counterfluxes across the mitochondrial matrix membrane. Further discussion of the mechanism linking K+ and H+ counterfluxes across the plastid envelope will be presented subsequently in this report.

Stromal acidification which is facilitated by valinomycin-induced K+ efflux is associated with photosynthetic inhibition (Table I). Data presented in Table II suggest that there is a causal relationship between this stromal acidification and impairment of photosynthesis. At the standard reaction medium pH of 7.6, photosynthetic inhibition caused by valinomycin is dependent on external K+. However, at a substantially higher reaction medium pH, the presence of external K+ no longer has a significant effect on valinomycin-induced photosynthetic inhibition. Also, it should be noted that photosynthesis is less affected by valinomycin at the higher reaction medium pH. Incubation of chloroplasts in pH 8.28 media versus pH 7.6 media should reduce the electrical gradient driving H+ influx. Therefore, these data indicate that (a) photosynthetic inhibition in the presence of valinomycin is not due directly to changes in stromal K+ and (b) flux through the putative H+ channel is likely influenced by the electrochemical gradient across the membrane.

**Identification of an Envelope K+ Channel as the K+ Uniport Pathway**

Rapid and substantial K+ fluxes across the chloroplast envelope have been noted by many previous researchers (1, 2). Chloroplasts were incubated in normal reaction medium titrated to pH 7.6 or 8.28 with or without added valinomycin and 40 mM K+. Photosynthetic rates were measured immediately after the addition of chloroplasts to reaction medium at 25°C. Initial photosynthetic rates (µmol O2/mg Chl/h) under various conditions were 117 (pH 7.6, +K+), 75 (pH 7.6, −K+), 122 (pH 8.28, +K+), and 91 (pH 8.28, −K+), respectively. Data shown are the means of two measurements for each treatment. Similar results were obtained when the experiment was repeated.

![Figure 3. Valinomycin-induced stromal acidification. Chloroplasts were incubated at 25°C in the light for 10 min in low salt medium (LSM) with varying concentrations of valinomycin and in the presence or absence of 40 mM K+. Stromal pH was ascertained after the 10-min incubation. All data are means ± SE (n = 4). Similar results were obtained when the experiment was repeated.](image-url)

### Table II. Interaction of External K+, Reaction Medium pH, and Valinomycin on Photosynthesis of Intact Chloroplasts

<table>
<thead>
<tr>
<th>Valinomycin</th>
<th>pH 7.60</th>
<th>pH 8.28</th>
</tr>
</thead>
<tbody>
<tr>
<td>µM</td>
<td>% of control rate%</td>
<td></td>
</tr>
<tr>
<td>+K+</td>
<td>−K+</td>
<td>+K+</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.25</td>
<td>69</td>
<td>44</td>
</tr>
<tr>
<td>1.0</td>
<td>46</td>
<td>19</td>
</tr>
</tbody>
</table>

*All photosynthetic rates are expressed as a percentage of the rate measured with no valinomycin present.*
### Table III. Effects of TEA and External K⁺ on Photosynthesis of Intact Chloroplasts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Photosynthesis % of control rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47</td>
</tr>
<tr>
<td>+TEA before preincubation</td>
<td>84</td>
</tr>
<tr>
<td>+TEA after preincubation</td>
<td>64</td>
</tr>
</tbody>
</table>

* All photosynthetic rates are expressed as a percentage of the rate measured prior to preincubation.

...stomatal K⁺ due to the addition of TEA was not as great when chloroplasts were incubated in low salt medium with 40 mM K⁺ added (Fig. 5A).

Blockage of the putative envelope K⁺ channel which can apparently facilitate uniport K⁺ flux either into (Fig. 4) or out of (Fig. 5A) the chloroplast was found to have a significant effect on stromal pH (Fig. 5B) when chloroplasts were incubated in low salt media for 10 min at 25°C before illumination. Stomatal pH was higher and was no longer affected by the presence of TEA when 40 mM K⁺ was added to the incubation medium (Fig. 5B). These data further support the contention (based on the experiments with valinomycin) that uniport H⁺ flux through an envelope proton channel is indirectly linked to K⁺ counterfluxes across the envelope. It can be concluded from this series of experiments with TEA that (a) K⁺ uniport in either direction across the chloroplast envelope is facilitated by an envelope K⁺ channel, (b) K⁺ efflux through this channel provides the electrical driving force for H⁺ counterflux (which likely occurs through a distinct, H⁺ uniport pathway), and (c)...

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2, 9–11, 16). However, the specific nature of the endogenous K⁺ conductance pathway has not been elucidated in this prior work. A series of experiments undertaken with TEA, which is a well-characterized K⁺ channel blocker (18), was undertaken to test for the presence of a K⁺ channel in the chloroplast envelope.

K⁺ uptake into chloroplasts was monitored by using ⁸⁶Rb⁺ in the experiment shown in Figure 4. A concentration of TEA (10 mM) that has been found to be effective at blocking K⁺ channels in a number of membrane systems (18) was effective at blocking K⁺ transport across the chloroplast envelope (Fig. 4). Exposure of chloroplasts to TEA had a marked effect on chloroplast metabolism under assay conditions that favored K⁺ efflux from the stroma. As noted previously (Fig. 1B), K⁺ loss from chloroplasts incubated in low salt medium was substantial, and resultant photosynthetic inhibition was severe. Data presented in Table III indicate that the addition of TEA to the low salt medium substantially reversed the photosynthetic inhibition that results from a 10-min incubation at 25°C. Addition of TEA after the 10-min preincubation period did not ameliorate the inhibition. Both the photosynthetic inhibition due to incubation in low salt medium and the photosynthetic stimulation due to TEA were less significant when 40 mM K⁺ was included in the incubation media (Table III). These data suggest that K⁺ loss occurring from chloroplasts incubated in low salt medium (which inhibits photosynthesis; Fig. 1) likely is specifically mediated by conductance through a K⁺ channel and that flux through this channel is driven by the electrochemical gradient across the envelope, which is reduced when external K⁺ is added (2).

Further evidence supporting this contention is shown in Figure 5A. After 10 min incubation in low salt reaction medium at 25°C, stomatal K⁺ content was increased when TEA was included in the incubation medium. The relative increase in...

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3 Abbreviations: TEA, tetraethylammonium; CCCP, carbonyl cyanide m-chlorophenylhydrazone.
K⁺ efflux through this channel has a substantial effect on photosynthesis indirectly, due to stromal acidification.

**Elucidation of Partitioned Transport Schemes:**
*Does Endogenous K⁺, H⁺, and/or K⁺/H⁺ Exchange Contribute to Net Fluxes?*

In a landmark analysis, Mitchell (12) proposed that chemosomatic systems such as the intact chloroplast require an electroneutral mechanism for transport of alkali cations. This assertion provided the basis for an analysis and partitioning of endogenous H⁺ and K⁺ transport systems in the mitochondrial matrix membrane by Garlid and coworkers (4, 5).

The relative contributions of endogenous systems facilitating K⁺ uniport, H⁺ uniport, and K⁺/H⁺ antiport to overall flux of these cations into the mitochondrial matrix were partitioned and identified by examining K⁺ flux in the presence and absence of valinomycin, nigericin, and CCCP (5). Several assumptions formulated the basis for this theoretical analysis: (a) ionophore-mediated transport pathways are ion specific and independent of endogenous systems, (b) a given ionophore makes the appropriate conductance across the membrane infinitely large with respect to the other (i.e. endogenous conductances), (c) net K⁺ movement in the direction that will be studied occurs by passive diffusion driven by an electrochemical gradient, and (d) maintenance of electroneutrality impedes free K⁺ exchange (following the chemosomatic model proposed by Mitchell).

We used the analysis developed by Garlid et al. (5) in an experimental system to partition endogenous transport schemes of the chloroplast envelope. K⁺ efflux (i.e. change in stromal K⁺ over time) was monitored; effects of CCCP, nigericin, and valinomycin on K⁺ efflux were compared in a series of experiments similar to the experiment shown in Figure 6. The validity of this experimental analysis is supported by the following: electrochemical gradients favor K⁺ efflux from the chloroplast (Fig. 1B); there is no reason to expect that the ion-selective proton (CCCP), K⁺ (valinomycin) ionophores, or the K⁺/H⁺ ion exchanger (nigericin) used in this study directly interacted with the endogenous envelope transporters; and electroneutrality is likely maintained during fluxes of these cations across the chloroplast envelope. This last assertion is supported by the data shown in Figure 3 and by previous measurements that indicated that a steady-state membrane potential is reached and maintained across the chloroplast envelope under conditions that allow K⁺ fluxes (2, 20).

K⁺ efflux rates (V) in the absence (Vo) and presence of valinomycin (Vc), CCCP (Vv), and nigericin (Vn) were used in equation 16 from the work of Garlid et al. (5):

\[
f^2 = [(V_d/V_o - 1)(V_o/V_d - 1)]/[1 - V_d/V_o(1 - V_d/V_o)]
\]

where f is the fraction of total K⁺ flux across the (chloroplast) envelope that is contributed by K⁺ uniport. Flux rates were calculated from a series of four separate experiments similar to the one shown in Figure 6 (with four replications per stromal K⁺ measurement at each time point in each experiment). Mean flux rates (normalized to the V₀ rate in each experiment) were: V₀ = 1, Vc = 1.56 ± 0.06, Vn = 0.77 ± 0.02, and Vd = 2.53 ± 0.10. These parameters, when used in the above equation, yield a value of 1.11 for f. K⁺ uniport accounts for 111% (i.e. essentially all) of the K⁺ flux across the chloroplast envelope. This analysis is consistent with the previous results presented in this report. The data suggest that a specific envelope protein capable of facilitating K⁺/H⁺ antiport does not contribute to net flux across the chloroplast envelope. Rather, the data are consistent with the following model: uniport of K⁺ and H⁺ across the envelope occur through intrinsic ion channels, and these fluxes comprise the sum of net K⁺ and H⁺ passive diffusion. A last point regarding this model involves the assertion that H⁺ flux through the H⁺ channel is rate limited by electroneutral K⁺ counterfluxes. This point is suggested by the data in Figure 3 and the fact that addition of CCCP to the chloroplast envelope does not increase the rate of K⁺ efflux (Fig. 6). A final experiment demonstrating the indirect linking of K⁺ and H⁺ counterfluxes and the interrelationship among K⁺ flux, stromal pH, and photosynthesis is shown in Figure 7. In this experiment, photosynthetic inhibition due to CCCP (which is directly the result of stromal acidification; data not shown) was partially reversed under the following conditions: (a) an increase (from 7.6 to 8.3) in the external pH (which would have the effect of reducing the electrical driving force for passive proton movement through a membrane channel), (b) an increase in external K⁺ (which would reduce the electrochemical gradient for K⁺ counterflux), and (c) the presence of TEA (which would block electroneutral K⁺ counterflux).

**CONCLUSION**

The interrelationship between K⁺ and H⁺ fluxes across the chloroplast envelope and the influence they have on photosynthetic capacity of the isolated intact chloroplast were investigated in this report. Many excellent studies of these interrelationships have already been published (1, 2, 8-11, 15, 16, 19). The important contributions of these previous studies notwithstanding, we believe that the work reported here is significant in that substantial new evidence has been presented to characterize both the physiochemical nature of
K+-H+ counterflux and the nature of the chloroplast envelope proteins that facilitate this regulated transport.

It should be noted that data presented in this report clearly indicate that Na+ can replace K+ in the external medium, in terms of modulating chloroplast metabolism. For example, incubation at 25°C of chloroplasts in normal reaction medium versus low salt medium causes less K+ efflux, concomitant stromal acidification, and resultant photosynthetic inhibition (Figs. 1 and 2). Normal and low salt reaction medium differ as to the Na+ content. This point has been demonstrated previously in the literature (9, 10) and, therefore, has not been focused on in this report. This finding is consistent with the model, developed in this study, of envelope proteins that regulate K+ and H+ fluxes across the chloroplast envelope. Na+ can affect chloroplast metabolism similarly to K+ if the putative K+ channel has an affinity for Na+ or Na+ fluxes across the envelope are facilitated by a separate Na+ channel. In either case, it can be postulated that Na+ flux is linked to H+ counterflux indirectly in the same manner as K+.

LITERATURE CITED